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CONTENTS

Evaluation of tourism climate comfort in order to attract more tourists - Case study: Sanandaj city in Iran

Mohammad Azad Ahmadi
Sama technical and vocational college, Islamic Azad University, Sanandaj Branch, Sanandaj, Iran
Tel: 0098-918-8711988 E-mail: mo.aahmadi@yahoo.com
Post Code: 66177-66869, 1st unit, no. 1320, Havarinasab St., Villashahr area, Sanandaj, Iran

Abstract: Sanandaj is ready for tourism industry considering all aspects by having ancient and enduring culture consists of tribes, ethnic groups, and climate variability, historical, cultural and natural attractions. But one of the required information for tourists to travel is climate conditions of the destination, namely tourist chooses a time for travelling when the climate conditions are favorable. The climatic conditions that the tourist is interested in are temperature, humidity, radiation and air flow and these climatic conditions factors provide an index called comfort in relation to the reaction of human to thermal environmental conditions that all these factors must be considered altogether. The study area has the ability to attract and attend tourist in national and international level due to the Zagros Mountains, vegetation, local winds and latitude. Therefore, in this article, using climate data from weather stations, first meteorological parameters, hydrological phenomena, summary of regional climate based on various climatic methods (Blair, Gorsczynski, modified koppens, Goussen, silyaninof, Domarton, Barat, Emberger and the best climate in the region were studied and determined, and then climatic conditions of Sanandaj city were evaluated based on tourism comfort climate, to identify the best times and provide the tourists. According to the conducted studies in this area, the best time for tourists in which they can be physically and mentally in comfort is from late May to late October. Generally, it can be said that 6 months of the year has comfort climate conditions for recreational programs in the city of Sanandaj.
[Mohammad Azad Ahmadi. **Evaluation of tourism climate comfort in order to attract more tourists, Case study: Sanandaj city in Iran**. *Life Sci J* 2012;9(3):623-629] (ISSN:1097-8135). http://www.lifesciencesite.com. 87

Keywords: Tourism, climate, Sanandaj city, tourism comfort

1. Introduction

Tourism is one of the world's largest industries and is affiliated to a major part of economy and global. Such a phenomenon is difficult to be defined with simple words, because this phenomenon has been integrated with human life and states in terms of economic, social, culture, environment. Tourism has always been considered and praised for having high potential in creating and promoting components of national, regional, urban and rural development. Increasing urbanization and approach to leisure time geography in recent decades has caused that attention to tourism industry as the largest and most diverse industry as well as an attainable goal in the process of sustainable development be considered in many countries. Many countries consider this dynamic industry as the main source of income, job creation, private sector growth, human and cultural exchanges and infrastructure development. There is no doubt that one of the required information for tourists is climatic conditions of the destination; and many tourists consider weather conditions for selecting tourist destination. To evaluate the effect of climatic elements of human thermal comfort conditions, it is required that human comfort indices be used.

Tourism comfort climate index is an index that will systematically determine the influence of climatic elements on tourism. In this index, climatic elements of temperature precipitation, humidity, radiation and wind are used. Therefore, using data from weather stations and statistical analysis, the suitable time for the presence of tourists are specified with the help of this index (Mieczkowski, 1985).

The first directional climate categories by the weather experts who were eager to classify weather based on human perspective were in 1931 by Koppen, in 1931 and 1948 by Thornth Waite and in 1954 by Trewartha. In 1954, Brazol provided a map of convenience and the same in Argentina. In 1962, Maunder classified weather in 22 states of New Zealand based on individuals; and Burnet classified climate regions by the sea in France in 1963. In 1966, Tehang developed and implemented climatic classification related to weather based on the individuals in the U.S. In 1968, Davis declared that Great Britain has the best climate. In 1973, Gates introduced and determined regions where had the best state in terms of temperature and humidity and people witnessed the study. In 1974, Kandror invented physical-climate zoning system in the Soviet Union based on similarity and frequency of various weathers. In 2001, Perry investigated the tourism climate condition in hot and dry areas especially

Mediterranean areas. In 2001, Maureen et al. investigated climate and international tourism. Daniel Scott and Ceoff mc Boyle (2001) found that given the global trend of climate change up to 2050 and 2080, the condition of tourism comfort climate index will be better than current condition in most areas of Canada. Jacqueline et al. (2007) concluded that in the coming years, tourist attraction will move slowly toward the north in Britain and Ireland, and the Germany, due to warmer weather and creation of more favorable conditions in the inner coastal areas, tourism attraction process will be toward the south.

Some studies have been conducted in the field of tourism in Iran by Kaviani (1993), Jahanbakhsh (1998), Feyzi and Mohammadi (2008), Farajzadeh et al. (2009), Ziayee and Bakhtiari (2009), Shayan et al. (2010), Farajzadeh (2011).

2. Research methodology

In this study, in order to evaluate tourism climatic conditions and climatic attractions of Sanandaj city, first various climatic parameters and also different climates were studied and determined in order to identify the overall climate of the region and use the obtained results in different parts including water resources, vegetation, environment, agriculture, tourism, etc.

In this study, using Baker's index in the following equation which is used to calculate the cooling power of the environment, climate and comfort of the region were studied.

$$CP = (0.26 + 0.34V^{0.672})(36.5 - T)$$

In the above equation

CP: cooling power in terms of micro-calorie in square centimeter per second

V: average wind speed in terms of meter per second

T: average daily temperature terms of degrees Celsius

Map of depth view

Based on Baker's index, when CP is less than 5 or more than 20, bioclimatic power will appear. Generally, the obtained result of Baker's index for evaluating environmental comfort conditions can be stated as:

- CP value below 10 indicates unfavorable bio-climatology condition (warm) in the environment (A)

- CP value 10 to 20 indicates natural favorable bio-climatology in the environment (B)

- CP value 20 to 30 indicates unfavorable bio-climatology conditions (cold) in the environment (C)

- CP value more than 30 indicates unfavorable bio-climatology conditions (very cold) in the environment (D).

3. Climate and tourism comfort

As we know, one of the factors affecting life, health and comfort of human is climate and atmospheric conditions. Hence, studying the effect of climate and atmospheric conditions has an important role in human's life and health. Today, the study of climate and atmospheric conditions on life, health, comfort and behavior of human is investigated and examined in terms of one of the scientific branches namely human bioclimatology or biometeorology. This branch of science is in close connection with meteorology, climatology and physiology. Overall, it can be stated that almost all atmospheric elements affect human's comfort and feeling but some of them are clearer and more prominent, while others are mild and invisible. However, temperature, humidity, wind and radiation have the most impact. Considering that each of the four elements above has impact together, their combined effects should be considered. For example, radiation at low temperature is favorable while it's unfavorable and annoying at high temperature.

Urban structure and its different activities cause air pollution and noticeable changes in the level of atmospheric elements. Among the atmospheric elements, the overall radiation reached the earth can be mentioned which is completely influenced by the amount of atmosphere darkness.

3.1. Atmospheric factors affecting the tourism industry

Climate is considered as one of basic and ground factors in natural tourism planning. Studies have shown that climate is the most important source of tourism in natural environments (Hozuri, 1381). In fact, time and type of natural tourism activity depends on the climate type and its governing conditions. Therefore, the identification of climatic factors is very essential. Several climatic factors in different seasons of the year in the process of implementing programs affect various activities including the tourism industry.

4. Results

4.1. Climatic view of Sanandaj city

Several factors are effective in the formation of climatic view of the desired region, that the most important one is altitude after altitude, other factors such as humidity resources, air masses, weather systems and latitude are evaluated as main factors affecting the region's climate. Besides the four factors above, some secondary factors such as

vegetation and agricultural activities affect the formation of varied climates in a more limited scale.

In the field of climate classification especially in arid areas, many efforts have been made. Climate classification is carried out in some methods by formula and in others by chart. Climate formulas are functions of two or more meteorological parameters that by substituting in these functions, numbers called climatic coefficients have been obtained and these coefficients are basis of the classification. In the appendix of this study, a summary of the results of climate determination based on the type of classification, the calculated climate and the obtained coefficients of the desired method have been provided, and they have been mentioned in detail as follows:

Blair method is the most simple climate classification based on annual rainfall of the regional climate, according to this method, Sanandaj station with 459.1 mm rainfall has been estimated a semi-arid region. Domarton believes that the amount of evaporation is proportional to the mean annual temperature; and various climates can be specified using drought coefficient calculation, accordingly, based on Domarton method, 6 types of climates can be specified. Therefore given the 19.58 drought coefficient, this is a semi-arid region. Based on Gorsczynski classification, continental coefficient calculated for this region is 22.36%; and based on this classification, Sanandaj has continental climate with a relatively cold and semi-arid winter. But in the classification system of modified coupon, five climate groups can be distinguished. In this method, climate of the region can be determined based on annual temperature (^{0}C), annual rainfall (mm), the amount of evaporation (mm) (Faraji, 1995). Therefore this region has a dried dessert climate with 459.1 mm annual rainfall and 1602.4 mm annual evaporation.

Since there is no evaporation measuring station near Sanandaj city, evaporation must be achieved by meteorological data. Two common methods in this regard are Thornth Waite and Ivanov methods. In Thornth Waite's method, evaporation and transpiration potential which has been considered based on monthly mean temperature and day length mean is calculated. Thornth Waite suggested a special classification for arid climates by comparing the potential evapotranspiration and rainfall. Climatic classification is this way that after calculating monthly evapotranspiration, rainfall changes curve and potential evapotranspiration than different months of the year is drawn in a coordinate axis. By comparing the two curves, it can be determined that whether rainfall exceeds evapotranspiration or not, and if exceeds, when does this happen (Figure 1).

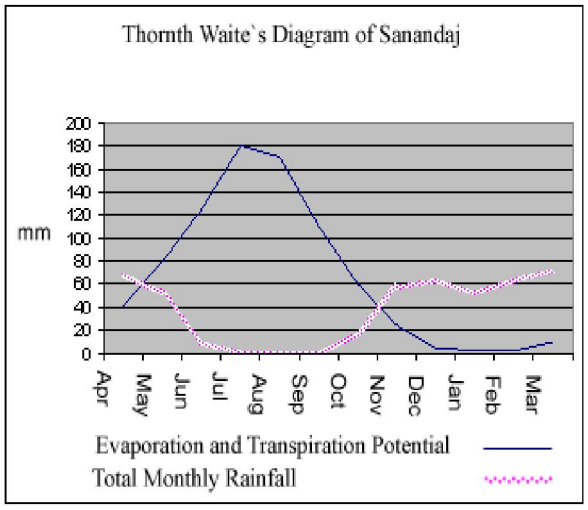

Figure 1. Thornth Waite's diagram for Sanandaj city

Ivanov method is based on the comparison of precipitation and evaporation. In this method, it is necessary that first monthly evaporation and then annual evaporation be calculated according to the relative humidity and temperature. After calculating humidity coefficient of Ivanov, climate classification is done according to the six climate areas. According to this method, humidity coefficient of Ivanov was calculated 0.81243 which is forest steppe climate type. Ivanov evaporation and Torrent White evapotranspiration has been compared (Figure 2).

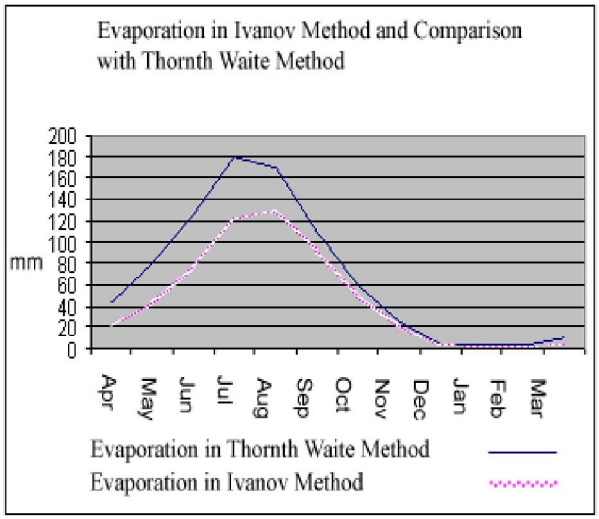

Figure 2. Comparison Ivanov evaporation with Torrent White in Sanandaj city

In Barat method climate classification system is based on aqueous of the region. According to this method, Barat's coefficient determines 4 climates; in this region I=1.98943 which represent the semi-humid climate. In Emberger method of classification, determining factors of each region's

climate include average maximum temperature in the warmest month of the year, average minimum temperature in the coldest month of the year, and average annual rainfall. Emberger provided an exponential climate based on his experiences which are divided into different parts each of which specifies particular climatic conditions. Coordinates of each point in terms of minimum temperature in the coldest month of the year and Emberger's coefficient is located in one of these regions. Based on old formula of Emberger, this region is a semi-arid cold climate. But based on new formula of Emberger it is a semi-humid cold climate. In Goussen method, parameters of number of raining days, relative humidity of the air, number of foggy or dew days are used, which is based on drawing embrothermic curves and calculating xerothermic coefficient. Considering that there is no information about number of foggy or dew days, therefore, it is not possible to calculate xerothermic coefficient. But the embrometric curve has been presented. Thus it's possible to calculate dry days. The level related to drought introduces the drought severity. Finally, in the last method, Silyaninov has used the coefficient related to this method in Russia. These coefficients are defined based on the relative humidity to the heat. This method has a good application in terms of agriculture; because largely shows specified and obvious differences of various regions in this regard; accordingly Sanandaj has a severe semi-arid climate.

4.2. The effect of altitude on the climate of Sanandaj

Sanandaj city is a mountainous area and this feature has led that climatic conditions of Sanandaj is a function of mountainous and high conditions in a large scale. Since the studied region in an elevation range from 1600 to 2400 from sea level, as a result we are faced with climatic in different altitudes. Therefore, map of the region's altitude in Arc view software was divided into 5 categories after DEM preparation. Rainfalls in high altitudes (2400 and above) has cold winters, and is often as snow; and in low altitudes mostly as rain. Generally, the resulting materials from the mountains and the land are influenced by climate, topography and other environmental factors.

4.3. Effects of air masses and meteorology systems on the climate of Sanandaj

The regional climate is mainly influenced by the Mediterranean and high-pressure Siberian flow. Atlantic air flow carries Mediterranean vapors that cause rain. So they provide more or less. Adequate rainfall in autumn, winter and early spring in the province; and on the other hand, Siberian polar air masses enter the region in late autumn to early spring through the northeast of the country and affect it which create a cold high-pressure, strong and dry front known as anti-cyclone; winds of this front has been known as Zalan wind in the region, and its direction is usually north and northwest which is the messenger of the onset of winter and cold.

4.4. The effect of latitude on the climate of Sanandaj

since Kurdistan is the first region of the West Country and subjected to the influence of. Adjacent air masses, therefore, the first rainfalls of western rain-bearing systems take place on it; and also resulted in significant difference between the hours of sunshine during the day and sunshade percentage, etc.

4.5. The effect of vegetation on the climate of Sanandaj

Mountainous location, expansion direction of altitude, rainfalls, and the amount of solar radiation are factors affecting vegetation of the region. Generally, climatic heterogeneity is the result of temperature difference, rainfall season and drought level. Therefore, identification of vegetation (grass, bush, shrub, and tree) and how these species are deployed adapted according to their needs, in harsh conditions that naturally have high flexibility, would be possible in determining the type of climate. Among the main factors, altitude, latitude and air masses, and the secondary factor of vegetation, the two factors of altitude and air masses have the greatest role in the formation of the region's climate.

4.6. The number of frost days in Sanandaj

In Sanandaj, frost occurs more than three months of the year, and the average annual number of frost days in this city is 110 days, there is frost during autumn, winter and the first two months of spring. Among different months of the year, January has the most frost days with 27 days and next December and February with 24 days. Given the frost information, it is essential that the impact of this phenomenon in a variety of activities and programs of the province be considered by provincial officials and planners.

4.7. The effect of temperature on the human comfort in Sanandaj

In order to establish thermal equilibrium between himself and his surroundings, human has always attempted; and providing this equilibrium is of his certain requirements for achieving comfort and case. To create this equilibrium, it is necessary that skin temperature remain constant or change so little despite large changes in the ambient temperature. Establishing this equilibrium depends on several factors such as metabolic characteristics of human,

physical activities, type of coating used, and how people adapt and adjust to the ambient temperature, that climatic factors such as temperature, solar, radiation, humidity, air flow are effective in turn in this case. Heat exchange occurs in any environment between human's body and its surrounding temperature; that in case of lack of thermal balance, various complications appear. In normal conditions, internal temperature of human's body is 37 and skin temperature is 32 degrees Celsius. If the body is placed in an environment warmer than the skin, it will absorb heat, and if in a colder environment than the skin, it will gradually lose heat. The effect of temperature on human comfort is completely affected by other atmospheric conditions, physical and mental conditions and his blood circulation. Given that human comfort is in relation to maintaining his core body temperature which is approximately 37^0C, being in climates with temperatures between 25 and 30 degrees, will be the best living conditions (Eshghi zadeh, 1382). In order to achieve thermal comfort situations, two conditions are necessary. First, loss of skin and core body temperature leads to a state in which the person neither feels cold nor heat; second, body energy balance is established, i.e. there is balance between the heats of metabolism with the heat wasted from the body (Russell et al, 1997). In addition to temperature, the time of human presence in those thermal conditions is also important so when the ambient temperature is higher or lower human body temperature, cooling or heating equipment should be used to maintain the body temperature.

Following diagrams show months of year in which cooling and heating equipment must be used (Figure 3, 4).

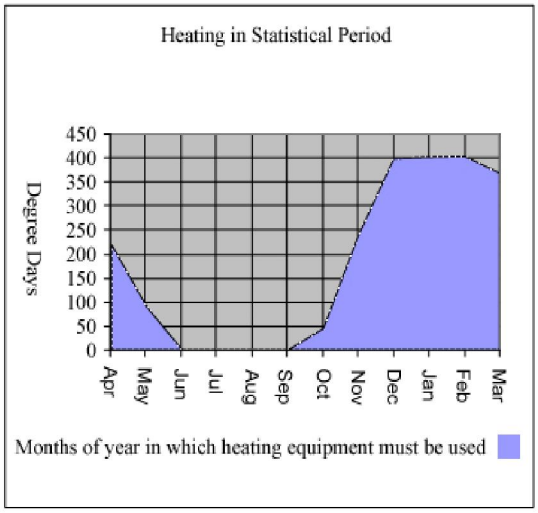

Figure 3. Months in terms of degree days in which heating equipment must be used.

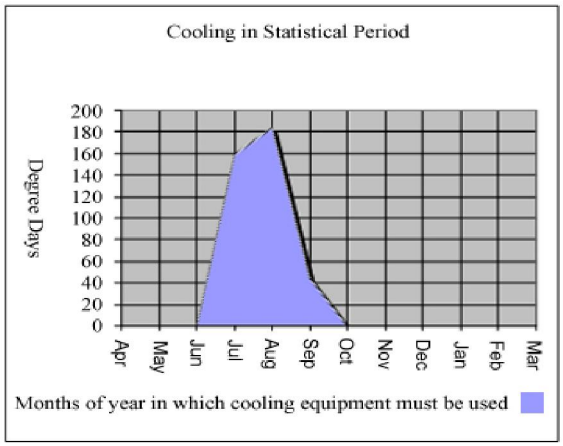

Figure 4. Months in terms of degree days in which cooling equipment must be used

Baker's index: in this study, climate and comfort of the region were investigated and the results were specified using Baker's index.

Following diagrams show months of year in which climatic comfort conditions are favorable and unfavorable (Figure 5, 6).

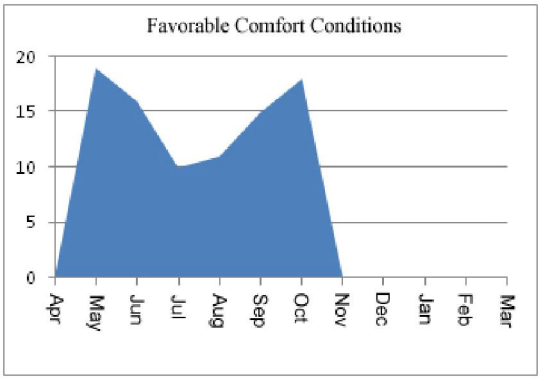

Figure 5. Months of year in which climatic comfort conditions are favorable.

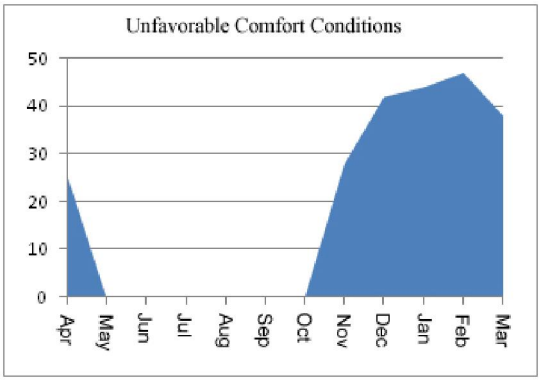

Figure 6. Months of year in which climatic comfort conditions are unfavorable

In terms of spatial dispersion in suitable months, by providing welfare-comfort services, we can be a god host for tourists (Figure 7).

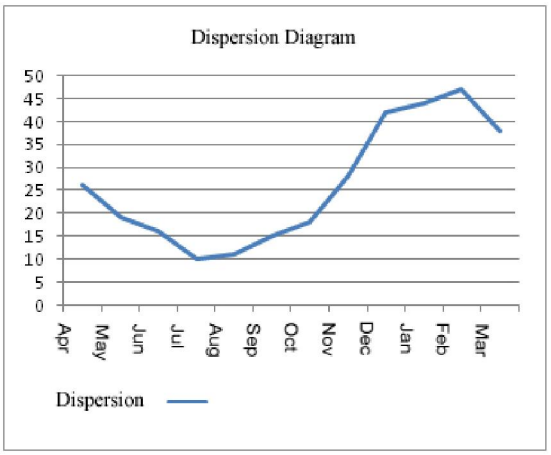

Figure 7. Dispersion diagram's index of comfort climate tourism in Sanandaj city

5. Conclusion

Considering that Sanandaj city is considered a semi-arid region given the experiences and existing statistics, features of semi-arid regions i.e. extreme temperature fluctuations and scattered rainfall are seen inadequately and heterogeneously. Due to the different methods that have been studied for climatic classification, Domarton method is suggested because it has a good application in phytogeography studies, and high importance in forest and pasture sectors, and is largely consistent with vegetation conditions of the region; and Silyaninov method is suggested as well because it is consistent with agricultural expansion and diversification of its products. It also became clear that both factors of altitude and air masses have the greatest role in shaping the climate of this region. As mentioned in the article, all atmospheric elements involved in the natural tourism have meaning together and their combined impact should be considered; only then climatic conditions of the environment can be evaluated accurately. However, considering some important climatic factors and indices such as temperature, humidity and wind, climatic comfort condition of regions can be clarified to a large extent. In general, with regard to climatic factors of temperature and humidity, it can be concluded that May, June, October, September, July and August respectively has resort priority in terms of climatic parameters affecting resort and tourism in the region. Based on tourism comfort climate index (Baker); the results of this study show that in the city of Sanandaj, in the months of May, June, July, August, September and October comfort condition is the best and completely favorable for the presence of tourists, but in other months the condition is slightly unfavorable and tends to cold. In April and November the condition is unfavorable and cold but in January, February, March and April the condition is completely unfavorable and very cold, in which there are no recreational comfort conditions. In general, it can be said that 6 months of the year has climatic comfort conditions for recreational planning in the city of Sanandaj. It should be noted that this does not mean impossibility of recreational planning in the cold months of the year. But given the potentials of winter recreational planning in the region, the possibility of establishing recreational activities appropriate for cold climatic conditions is also provided.

6. Suggestions

1. Conducting comprehensive climatic studies for the region based on suitable tourism conditions
2. Creating ecotourism calendar based on climatic conditions of seasons and months of the year
3. Introducing the region's ecotourism effects and potentials by experts
4. Fundamental planning on sustainable ecotourism
5. Creating appropriate infrastructure in sustainable tourism
6. Creating virtual tourist networks
7. Creating nature tours in the province in order to understand its ecotourism aspects more
8. Further activity of nature tour committee and more coordination with sports committee of the province
9. More coordination of organizations involved in nature tour and leisure time.

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Designing, manufacturing and evaluating microwave –hot air combination drier

Amin Hazevazife[1*], Parviz Ahmadi Moghadam[2], A. Mohammad Nikbakht[3], Farough Sharifian[4]

[1]MSc in Mechanics of Agricultural Machinery, Urmia University, Iran
[2]Assistant professor of mechanic agriculture machinery urmia University, Iran
[3]Assistant professor of mechanic agriculture machinery urmia University, Iran
[4]Phd student in mechanic agriculture machinery, Urmia University, Iran
Amin.hazervazife@gmail.com

Abstract: In this paper, one microwave-hot air drier was designed and manufactured and then was evaluated. In the manufactured drier, one circuit was employed for feeding Magnetron lamp with nominal power of 1.3 kW and frequency of 2.45 GHz in order to produce microwaves. Hot air was produced using six 700 W heaters and a 175 rpm fan. The drier container volume was 30625 cm^3andhot airways blown into the container through its bottom face and microwaves were injected inside through its side face. This drier is capable of controlling microwaves power and temperature and flow rate of inlet air. Also during drying process, changes in mass and moisture of the product, inlet and outlet air temperature and total consumption power can be simultaneously measured. In order to evaluate operation of the manufactured drier, apple slices were dried up to their 20% moisture content using both microwaves and hot air. The results showed that increasing microwaves power causes the drying time to considerably reduce and drying rate to increases. On the other hand, increasing inlet air temperature had a significant effect on increasing drying rate while inlet air flow rate had a negligible effect on drying rate. Comparing operation of the device in two conditions indicated that comparing to hot air flow drying; drying rate can be increased up to approximately 10-fold by using microwaves.

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Key Words: Drier, Microwaves, hot air flow, drying time, drying rate, apple

Introduction

Drying is one of the most important processing operations of agricultural products which reduces speed of undesired chemical reactions such as oxidation and browning by decreasing moisture content of product and increases storage time by maintaining the appearance of products well (Goksu et al. 2204). Another advantage of drying products is easier use of dried foods in processes such as chopping, grinding and mixing. Also since foods weight and volume is reduced due to drying, their packaging, transportation and storage will be much easier (Li et al. 2011).

Human has used solar energy to dry agricultural products for many years. Despite advantages of solar energy in drying process, this method has its own disadvantages including lack of enough control on drying process, long drying time, occupation of high areas, high labor costs and insanitary dried products. These disadvantages provide reasons for development of industrial drying. The most common industrial drier is hot air flow drier in which hot air exposed to product causes moisture content to evaporate and product to dry. This dries have also some disadvantages such as singing product, high shrinkage in dried product, long drying time and high consumption energy (Das et al, 2003). Disadvantages of hot air flow driers and also needs for increasing efficiency of energy considering world energy challenges, encouraged researchers to find more efficient and appropriate methods for drying agricultural products. In recent years, many research shave been done on vacuum, freezing, infrared- hot air flow and microwave-hot air flow driers.

In microwave driers, microwave radiation is used for drying products. Microwave is an electromagnetic wave (combination of electric and magnetic fields) with a frequency in the range of 300 to 300000 MHz and a wavelength in the range of 1 m to 1 mm, respectively. These waves are capable of rotating bipolar molecules and due to high friction produced by changing polarity of molecules (for about several billion times per second), they produces heat in bipolar materials such as water (Kuchakzade and Shafer, 2010). Food and agricultural products since containing water are heated when exposed to microwaves and this heat cause evaporation of water molecules and drying the products.

In drying products using microwaves, in contrary to hot air flow method direction of heat and moisture movement is the same and is outwards. This increases moisture gradient in the product and accelerate mass transfer (Hu et al. 2006). On the other

hand, using microwaves in drying process causes energy consumption to decrease and quality of dried products to increase (Andres et al., 2003). Moreover, since rotation of molecules is stopped when radiation of waves is removed, quick control of product temperature is feasible (Li et al, 2001).

Despite of mentioned advantages of using microwaves in drying process, this method can unevenly heat the product depending on thermal and dielectric properties of the product (Abbasisouraki and mowla, 2008). Also because of fast mass transferring this method, removal of emerging moisture from product is difficult and this result in condensation of steam inside the container (sharmaa et al., 2009). To overcome this difficulty combinedmicrowaves- hot air flow drying, pulsed microwaves and also combined microwave –vacuum drying can be used in drying process (Gunasekaran, 1999).

Despite abundant performed researches on designing and manufacturing driers and also drying agricultural products by different methods including design and manufacture of solar driers (Gatea, 2010), designing a solar drier for date (Ampratwum, 1998), kinetics of drying strawberry by hot air flow (Doymaz, 2008), kinetics of drying tomatoe by microwaves (Al-Harahsheh et al., 2009), drying carrot by combination of microwaves and halogen lamp (Sumnu et al, 2005) and drying apple and mushroom by combination of microwaves and hot air flow (Funebo and ohlsson, 1998 and Andres et al., 2004), yet sufficient information about manufacturing microwaves-hot air flow combination driers has not been available for researchers. The main purpose of this research is designing, manufacturing and evaluating one combined microwaves- hot air flow drier. For evaluating this device, apple fruit was dried separately under hot air flow and microwaves and drying parameters of product such as drying time, moisture ratio and drying rate were studied.

Materials and Methods

Design and manufacture of the device

The manufactured drier (Figure 1) has a microwave generator, inlet and outlet hot air flow channels, product container and device control board.

One Magnetron lamp with nominal power of 1.3 kW and frequency of 2.45 GHz was used in the drier for producing microwaves (Figure 2). Magnetron lamp which actually acts as an electron accelerator is fed by a high voltage circuit (Figure 3). Designed circuit consists of one transformer with voltage gain of 10 and also an inductive-capacitive circuit with voltage gain of 2through which 220 volts electrical current passes and the voltage rises to about 4500 volts which is threshold voltage of magnetron lamp. Since some heat is produced during operation of magnetron lamp, one fan is used to cool it down. Also because of using hot air flow in this drier and proximity of high voltage circuit to product container through which hot air is flowed, two other fans is used for cooling the circuit.

Figure1. A schematic of manufactured drier

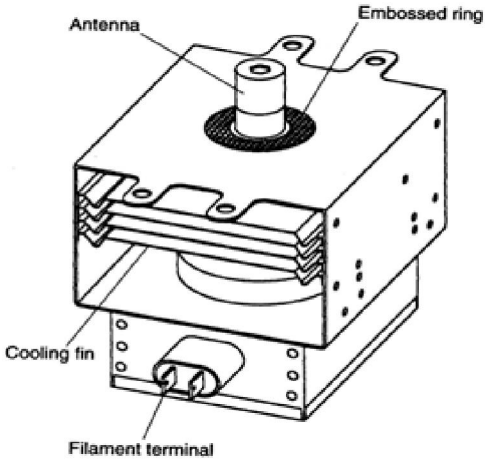

Figure 2.Schematic drawing of a magnetron lamp.

In order to adjust microwave generator, one control board is used by which magnetron lamp operation time and microwaves applied power can be controlled (Figure 4).

Product container (Figure 5) corresponding to employed magnetron lamp in the drier was designed as a rectangular cube with dimensions (38cm×35cm×22 cm) .

Since microwaves cannot pass through metals, product container was made of galvanized plate. Also because of polarity of metal molecules

and therefore, being sensitive to microwaves, internal wall of container was coated by paint. On the other hand, product tray was perforated in order that hot air can easily pass on the product (Figure 5).

Figure 3.A schematic of high voltage circuits.

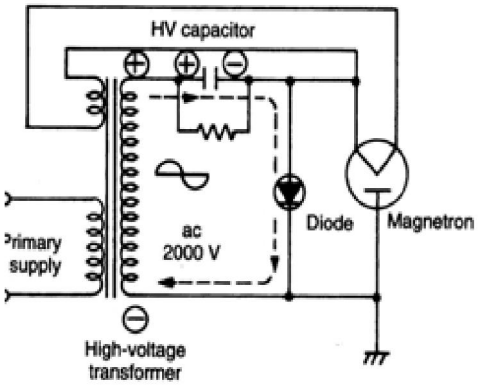

Figure 4.Control board of microwave generator

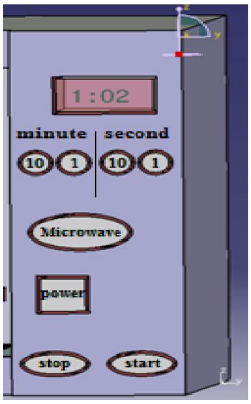

Figure 5. Container and perforated tray of product

For simultaneous measurement of product mass during drying process at desired time intervals, one load cell (sewhacnm, AB120) with accuracy to 1gr was used (Figure 6). Product tray was hanged from load cell by a connector that passes through a hole above the container. One inherent disadvantages of microwave is its inconsistent distribution inside the drier container. To overcome this problem, one engine was employed to rotate the product tray with constant speed to avoid heating some part of product too much.

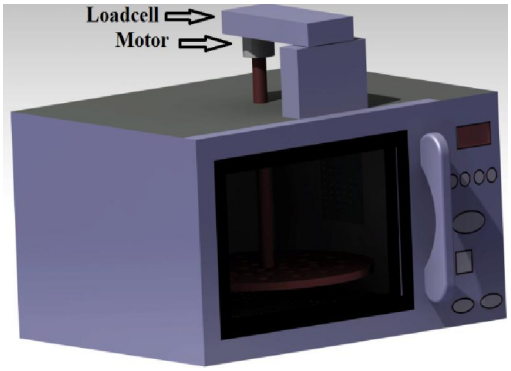

Figure 6. Load cell and the engine rotating product tray

In order to produce hot air flow, six 700 W heaters and one 1750 rpm fan were employed. Produced air flow was directed into product container by a channel (Figure 7). For controlling temperature of inlet air, a thermal sensor (PT100) inside air flow channel, a thermostat and contactor wereused. Consequently after measuring the temperature by the sensor and sending out a signal to thermostat which is adjusted at a desired temperature, a signal based on which the contactor decides to switch on or off the electric current is sent to contractor.

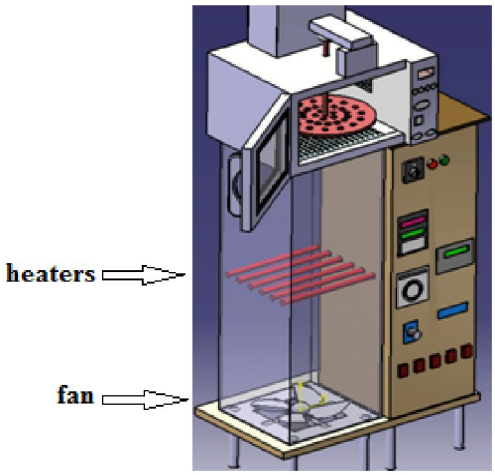

Figure 7. Hot air flow channel, heaters and fan

In order to prevent turbulence in inlet air and outgoing of microwaves and to make air flow uniform, one perforated plate with 2mm diameter holes was used at the bottom of product container (Figure 8). One dimmer was employed to change electric current into fan. Changing electric current of

fan corresponding to different levels of air velocity (0.5, 1, 1.5, 2 m/s) rpm was also changed. Mentioned air velocities were measured using an anemometer located inside the air flow channel. It is noteworthy that external surface of the channel was insulated by fiberglass to prevent heat exchange between environment and air flow channel.

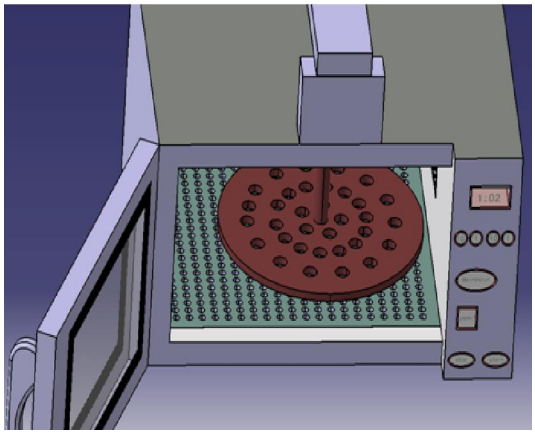

Figure 8. Perforated plate at the bottom of product container

Air which is flowed into the container should be removed after heating the product; therefore one channel is embedded at the top of container allowing air flow to outgo (figure 9). Inside this channel a moisture and temperature sensor (SHT75) is placed for measuring simultaneously the temperature and relative moisture of outlet air.

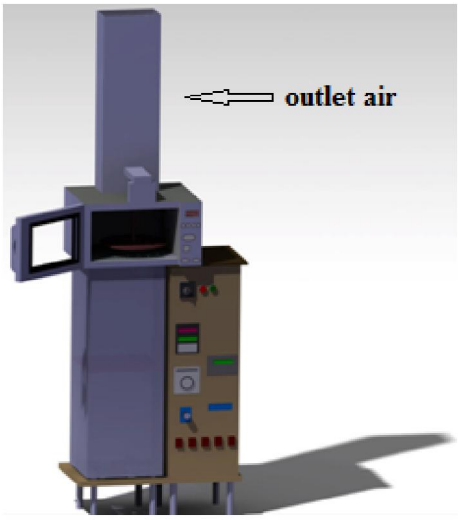

Figure 9. Outlet air flow channel

This drier is provided with a control board on which there are on and off switches of heaters, microwave, cooling fans and inlet air flow fan. Also inlet air temperature monitor and controller, product mass monitor and outlet air moisture and temperature monitor which simultaneously show the measured values during drying process are installed on this board. Product mass monitor and outlet air moisture and temperature monitor are connected to computer by a data logger and at the same time measured data are transferred to the computer.

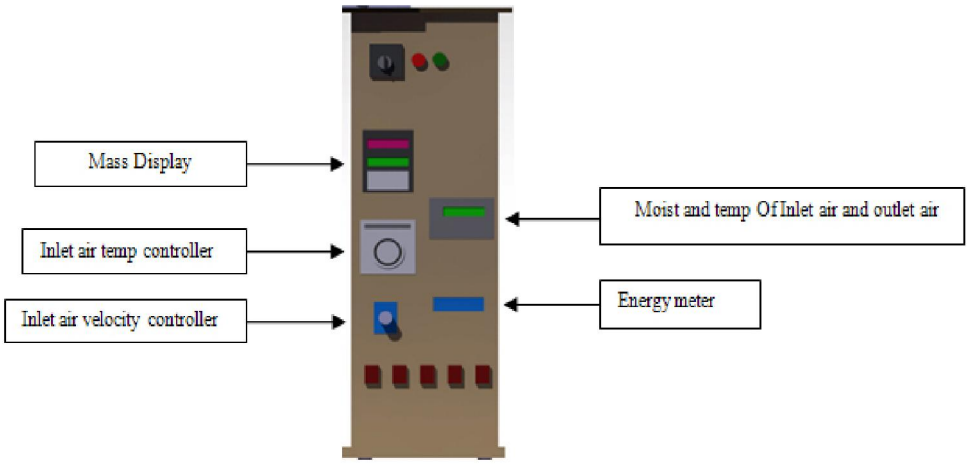

Figure 10. The drier control board and its elements

Preparing samples for evaluating the devise

For evaluating operation of manufactured drier, slices of golden delicious apple were used in experiments. Fruits were gathered from an orchard in Azarbayejan gharbi and transferred to a refrigerating room. Before the experiments, initial moisture of the product was calculated according to standard (AOAC, 1980) by a vacuum oven having fan and it was found to be 74.82 percent. Initially fruit peels were removed by a sharp knife and then 4mm thick cylindrical slices were prepared using a slicer and a specific mass of fruit were put on the product tray.

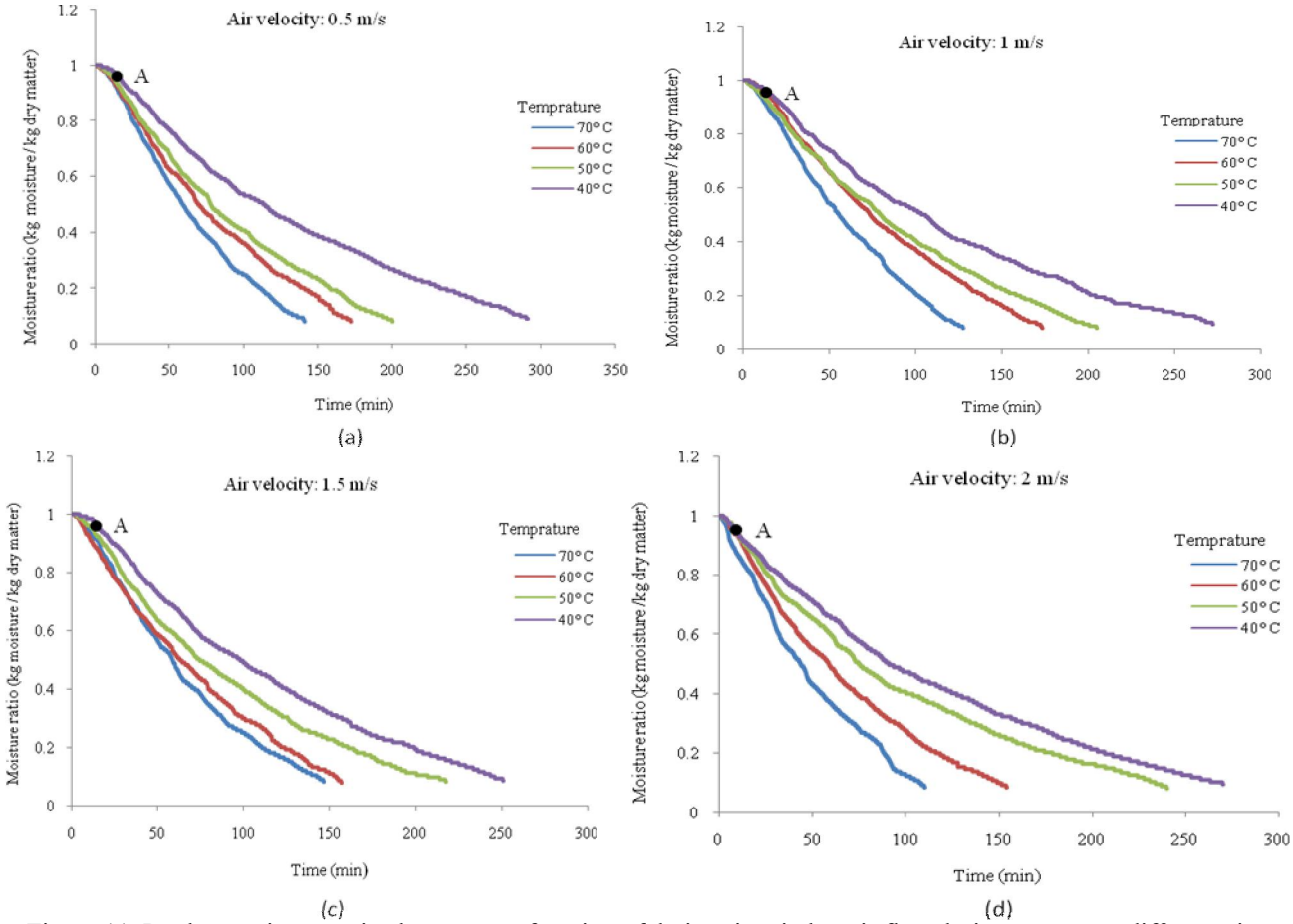

Figure 11. Product moisture ratio changes as a function of drying time in hot air flow drying process at different air velocities and temperatures.

Relations and theories of drying

In this paper, both functions of the drier (microwave and hot air flow) were evaluated separately. In the first experiment, only microwaves with powers of 500, 1000, 1500 and 2000 watt were applied. In the second experiment only hot air flow was used at different temperatures and velocities. In this study air temperature and velocity respectively were varied for 40, 50, 60 and 70 degrees of centigrade and 0.5, 1, 1.5 and 2 m/s.

Two parameters of moisture ratio and drying rate were studied for both experiments. Moisture ratio is an important factor in controlling drying process. Drying rate is also defined as the amount of moisture emerges from product per unit time and is an important factor in description of drying process. Dimensionless moisture ratio (MR) and drying rate (DR) in terms of (kgr moisture/kgrdry product ×second) are obtained by below equations (Kaya andAydın, 2009):

(2)

$$MR = \frac{M - M_e}{M_o - M_e}$$

(3)

$$DR = \frac{M_{t+\Delta t} - M_t}{\Delta t}$$

Where M ،M_o ،M_e ،$M_{t+\Delta t}$ and M_t represents respectively instantaneous moisture, initial moisture, equilibrium moisture and moisture at the moments of $t+\Delta t$ and t in terms of (kgr moisture/ kgr dry product) and t is drying time in terms of second.

Results and discussion

In order to describe and compare drying process in two methods of microwave and hot air flow and also to investigate effects of microwave power, air temperature and velocity on drying process of apple, moisture ratio and drying rate changes were represented as a function of time in different treatments.

Figure 11 shows that in all experiments moisture ratio gradually decreases at the beginning of the process (until point A). At this step, the product is being heated, then moisture ratio decreases with a sharper slope. This step is the decreasing step of drying which is continued until achieving the desired moisture content (end of drying process) but the slope gradually decreases. Most of the drying process occurs at the decreasing step of drying. This is in agreement with findings of Kaya et al. (2007) for apple, Doymaz (2007, 2006) for tomato and grapes. As it is seen in plots of figure 11, increasing air temperature has a significant effect on decreasing the drying time. For example, by increasing air temperature from 40°C to 70°C at a constant flow rate of 0.5 m/s, drying time decreases from 296 min to 141 min (Figure 11-a). Koyuncu et al. (2007) for cherry and Prabhanjan et al. (1995) for carrot reported similar results. However, at a constant air temperature drying time does not considerably decrease by increasing the air flow rate. For example, at constant temperature of 70°C, with increasing air flow rate from 0.5 m/s to 2 m/s drying time only decreases from 141 min to 111 min (figure 11-a). Similar results were obtained by Sacilikand Elicin (2006), Ramaswamy and Nieuwenhuijzen (2002) and Wang and Chao (2002) for apple.

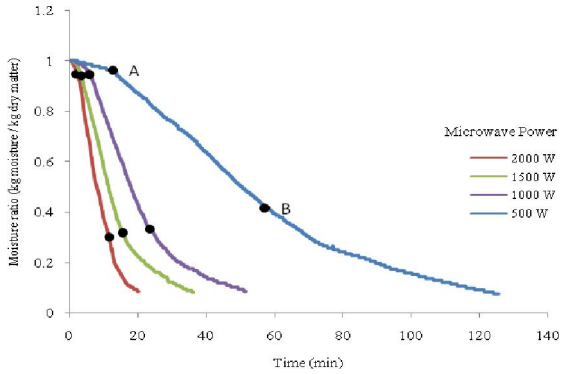

Figure 12.Changes of moisture ratio of product as a function of drying time in microwave drying process with different powers

Product moisture ratio Changes as a function of drying time in microwave drying can be divided into three steps (figure 12). Initially moisture ratio of product decreases slowly (until point A) while the product is being heated. Then, moisture ratio decreases progressively (until point B). This step, during which a part of drying process occurs, is the drying process with constant rate. Then decreasing step of drying process begins during which moisture ratio decreases with a decreasing slop until the end of process. This result is in agreement with results obtained by Al-Harahsheh et al. (2009) in drying process of tomato. Based on Figure 12, it is obvious that increasing microwave power has a significant effect on reduction of drying time. For example, when power is increased from 500 w to 2000 w, drying time reduces from 125 min to 20 min (figure 12). Consequently, process time until desired moisture content is decreased to one-sixth.

Drying rate of the product in different treatments of hot air flow is shown in figure 13 as a function of drying time. At the beginning of the drying process, rising drying rate is marked in all experiments. As noted during this step the product is being heated. Then the decreasing step begins and it is found that drying rate falls until the end of drying process. With increasing air temperature, drying rate decreases progressively and drying time decreases. This indicates the significant effect of air temperature on drying rate. However, increasing air flow rate has no considerable effect on drying rate of process (figure 13).

Considering Figure 13, it is observed that when the product is being heated, drying rate increases until it reaches a maximum point and then drying process continues with a constant rate slope during a small time interval. Next step is the decreasing step of drying process during which a reduction in drying rate continues till the desired moisture content is achieved. Effect of increasing microwave power on decreasing drying time and increasing drying rate is clearly observable such that increasing microwave power causes drying rate tohave higher slopesin all steps of drying. Thiscan be attributed an increase in internal temperature of the product due to rising microwave power and consequently increasing moisture gradient. In drying apple slices by hot air flow, the decreasing step of process beginsafter the product was heated whilein microwave drying process, a constant drying rate step,during which some part of drying process occurs,begins after this step. In microwave drying process, the decreasing step of drying process has a less contribution in total drying process because some moisture content of the product is dried during constant rate step. Drying by microwave takes less time than drying by hot air flow (compare figures 11 and 12). Also drying rate significantly increases in microwave method comparing to hot air flow method (compare figures 13 and 14).

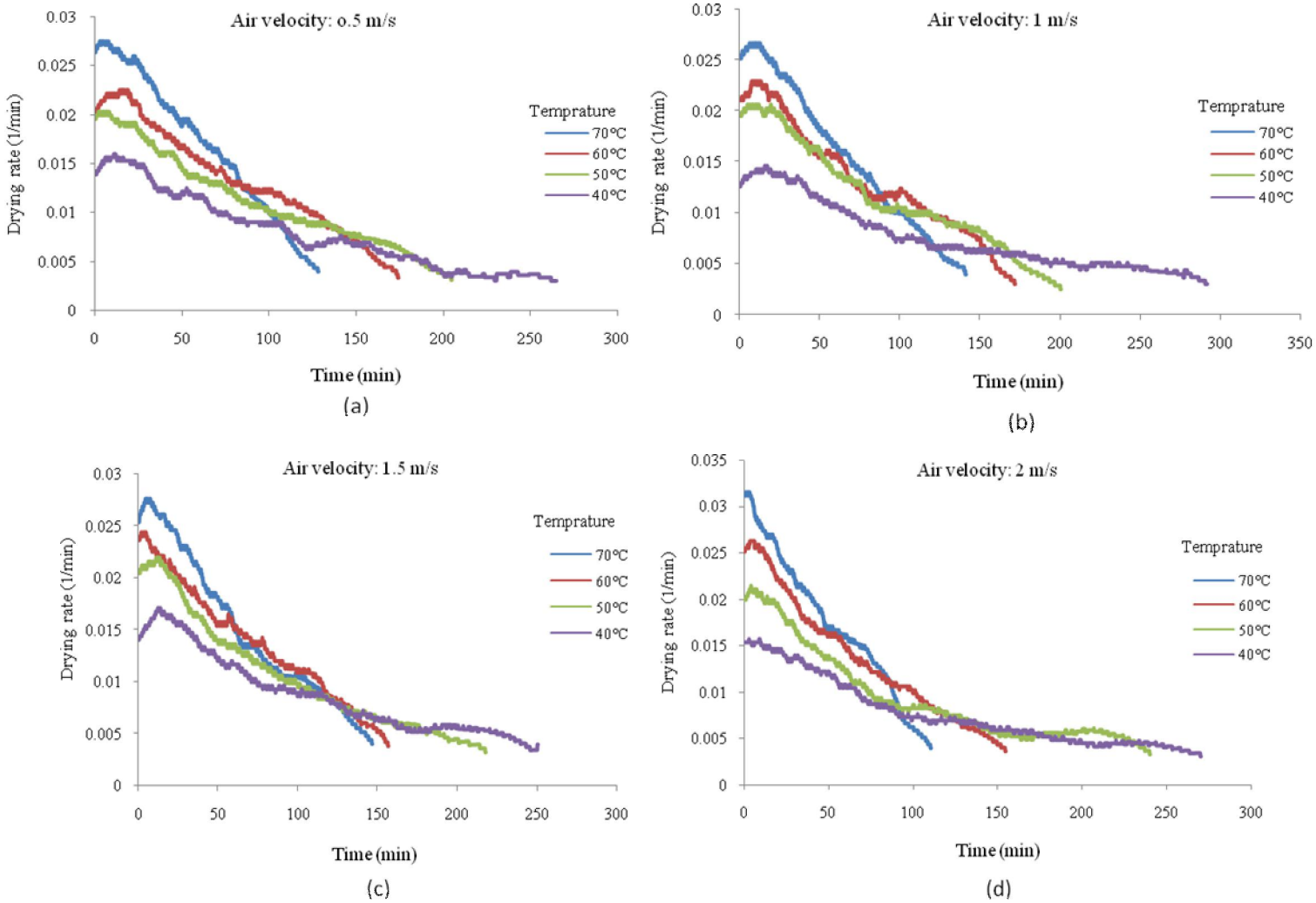

Figure 13. Drying rate versus time in hot air flow drying process at different temperatures and flow rates

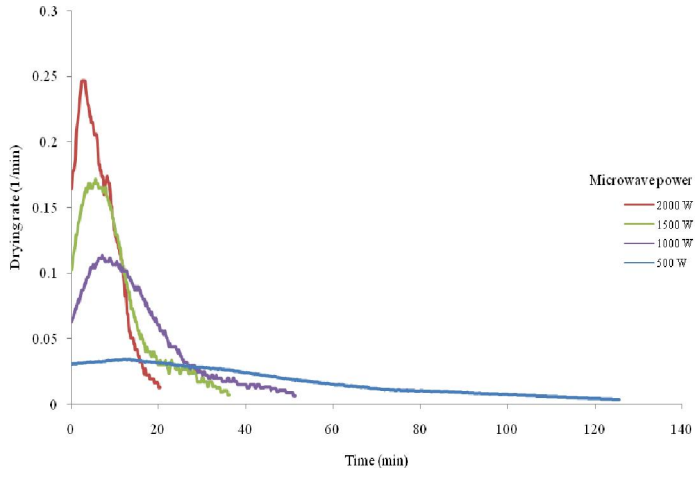

Figure 14.Changes of drying rate versus drying time in microwave drying method with different powers

Conclusion

In this paper, one microwave hot air flow drier was designed and manufactured. Evaluation results revealed that drying by microwave was more efficient than hot air flow drying in significantly reducing drying time and increasing drying rate. In hot air flow method, increasing air temperature had a significant effect on rising drying rate and reducing drying time. However, the effect of flow rate of inlet air on drying rate was negligible. It was observed that rising microwave power obviously caused a reduction in drying time and an increase in drying rate. This devise is capable of drying thin slices of different fruits and depending on optimum drying conditions for various fruits microwave can be used as pretreatment or post-treatment. Microwave-hot air flow combination drying is also possible using this drier.

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6/29/2012

The association of environmental fluoride, trace elements and urine fluoride in adults living in endemic fluorosis villages in Henan Province

Ruirui Cui[1], Lijun Ren [2], Liuxin Cui [1], Shihong Li[2], Xuemin Cheng[1], Yu Xi[3], Mingxu Zhao[1], Liju Duan[1], Jiaxiang Hou[1], Jie Liu[1], Yue Ba[1*]

[1]Department of Environmental Health, School of Public Health, Zhengzhou University, Zhengzhou, Henan 450001,China; [2] Center for Disease Prevention and Control, Kaifeng, Henan, China; [3] Center for Disease Control and Prevention of Tongxu county, Kaifeng, Henan, China.
bayue1963@hotmail.com

Abstract: Objective To analyze the relationship of environmental fluoride level, urine fluoride concentration and trace elements in adults living in endemic fluorosis villages. Methods Fluoride concentrations in drinking water, vegetables, crops, soils and urine were determined using fluorine ion selective electrode method. The concentrations of the Calcium (Ca), magnesium (Mg) in drinking water and serum in adults were detected using the flames atom absorption method. Results The concentrations of fluoride in drinking water were 2.15±1.97mg/L, 0.46±0.05mg/L and 0.38±0.15mg/L in the endemic fluorosis villages (EFV), villages conducted defluoridation project (DFPV) and control villages (CV) respectively. The fluoride concentration in drinking water of EFV was significantly higher than that of CV and DFPV (P<0.05 respectively). The concentration of fluoride in the plough layer in the high fluoride village was higher compared with control villages (P<0.05). There were no statistical differences of fluoride concentrations in vegetables, grain and plow pan layer of soil among EFV, DFPV and CV (P>0.05). The concentrations of the fluoride in the urine were 2.50±1.50 mg/L, 1.42±0.97mg/L and 0.98±0.50 mg/L in adults from EFV, DFPV and CV respectively. There were statistical differences between any two of the three groups (P<0.05). There were no significant differences among the concentration of Ca^{2+} and Mg^{2+} in drinking water of EFV, DFPV and CV. There was negative correlation between blood Ca^{2+} and urine fluoride (r=-0.183, P=0.022). Conclusion Individuals who have higher urine fluoride level tend to have lower blood Ca^{2+} concentration.
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Key words: fluoride, urine fluoride, calcium, magnesium

1 Introduction

Fluorosis is a public health problem in some areas resulting from long-term consumption of water with high fluoride (F^-) level. Most obvious sign of this disease is mottled enamel on the teeth of the local people (Oruc, 2008) Fluoride accumulates over time because of its long biological half-life in bone and this accumulation can cause skeletal fluorosis, a painful condition. Radiological reports of people who have fluoride toxicity show the bone to be affected by osteosclerosis osteopenia and/or osteomalacia(Tamer et al, 2007; Wang et al, 2007) Endemic fluorosis areas are still exist in many provinces in China such as Henan, Shanxi, Yunnan and so on though the prevalence of this disease has decreased considerably after controlling the fluoride ingestion by defluoridation project and improvement of stove. Fluoride, calcium (Ca), magnesium (Mg) and other trace elements play important roles in the occurrence and development of endemic fluorosis. In the study of endemic fluorosis, the distributions of some chemical elements in the internal and external environment and their relationships with fluorosis have attracted more and more attention in this field. Some researches in China about the relationship between fluoride and relative elements have made a great progression especially in the aspect that antagonistic or synergistic effect of trace elements to the toxic function of fluoride (Wang et al, 2007). Previous studies showed that dental fluorosis index would be decreased in children living in endemic fluorosis areas with higher calcium in drinking water compared to those areas with relatively lower calcium in drinking water even if their fluoride level in drinking water was similar (Ba and Cui, 1995). At present many studies focus on the relationship of environmental fluoride, urine fluoride and trace elements, but the results can not reach an agreement. Here, we conducted a cross sectional study in Henan province to investigate the association of environmental fluoride, trace elements and urine fluoride in adults living in endemic fluorosis villages.

2 Materials and Methods

2.1 Location and subjects:

A cross sectional study was conducted in six

villages of Tongxu County in Henan province, China in 2011 by using simple cluster sampling method. It included three endemic fluorosis villages and three non-endemic fluorosis villages. Endemic fluorosis villages (EFV) were defined as villages with fluoride levels exceeding 1.0 mg/L (Chinese water quality standard) in drinking water. Two of the three villages were conducted defluoridation project (DFPV) of drinking water at the end of 2008. Non-endemic fluorosis villages were defined as control villages (CV) with fluoride levels of less than 1.0 mg/L in drinking water. There were no significant differences in the natural environment, socioeconomic status, life styles, dietary habits, and other demographic characteristics among the six villages. Local residents who were aged between 18 and 50 years for male, 18-48 for female and who were born and raised in the six villages were considered eligible for the study. By questionnaire and healthy physical examination, we excluded subjects who had received drug treatment in forms of bisphosphonates, calcitonin, fluoride, or hormone replacement therapy and⁄or who had hip fractures. A total of 894 participants met the inclusion criteria in this study composed of 287men and 607 women with the participation rate of 90.30% (894/990). Each subject provided two 5 ml fasting blood samples and 50 ml of instant urine sample. Blood was collected in red top vacuum tubes, and placed immediately on ice. After centrifugation, serum and white blood cells were separated and frozen at -70℃ for subsequent analyses. All procedures were approved by the Institutional Review Board at Zhengzhou University, China.

2.2 Detection of Fluoride levels

The fluoride concentrations of drinking water and each of the urine samples from each of the subjects were determined in duplicate using a fluoride ion selective electrode (Shanghai Exactitude Instrument Company, China) connected to a digital pH/mV meter. The fluoride concentrations of collected vegetables, crops, and soils were determined by the combination ion-selective fluoride electrode after adequate pre-treatment of the samples which included sample drying, weighing, grinding and so on. The dried vegetables were dissolved with 6 mL of 0.1 N HCl and then shaken in the constant temperature shaker for one hour. The supernatants were collected after centrifuging with 5000 rpm for 15 minutes and repeated this step with 6 ml deionized water. All the collected supernatants were moved into 25 ml volumetric flask and brought to volume by deionized water and TISAB Ⅰ (10:1 v/v). Fluoride concentration was measured in 50ml beaker with 25ml sample, 12.5ml TISAB Ⅰ and 12.5ml deionized water by using a combination fluoride electrode method. The final result was corrected for blank F concentrations assessed in parallel and determined through a standard addition method.

2.3 Detection of calcium and magnesium levels

The concentrations of calcium and magnesium in drinking water were determined by atomic absorption spectrophotometry (Hitachi 10 Z-5000, Japan) (Lu et al, 2008). The serum concentrations of calcium, magnesium were also measured by atomic absorption spectrophotometry after mixed-acid digestion with nitric acid and perchloric acid (4:1 v/v) The final result was corrected for blank value concentrations assessed in parallel and determined through a calibration curve method.

2.4 Statistical analysis

The data was analyzed by the SPSS software, version 12.0 (SPSS Inc, 2003). Continuous parameters between groups were compared with Student's t test, and continuous parameters among 3 or more groups were compared with One-way ANOVA if all the numeric materials belong to normal distribution. However, if the data didn't meet normal distribution, it should be transformed to achieve approximately normal distribution using logarithmically-transformed method, and the transformed values were used in data analyses. Pearson correlation analysis was made between drinking water fluoride and the trace elements. So did between urine fluoride and blood Ca, Mg. A P value <0.05 was considered statistically significant.

3 Results

3.1 The comparison of fluroride, Ca, and Mg concentrations in drinking water in different groups (Table 1)

The fluoride concentration in drinking water of EFV was significantly higher than those of CV and DFPV. (P ＜ 0.05 respectively). No significant differences were observed between DFPV and CV (P>0.05). The concentrations of Ca^{2+} and Mg^{2+} in drinking water were also shown in table 1, from which we can see that there were no significant differences of Ca^{2+} concentrations among EFV, DFPV and CV, but the concentrations of Mg^{2+} in drinking water of any two groups have significant differences (P＜0.05 respectively).

Table 1. The comparison of Ca, Mg and fluoride concentrations（mg/L, $\bar{\chi}\pm s$） of EFV, DFPV and CV in drinking water.

group	n	Ca^{2+}	Mg^{2+*}	Fluoride##
EFV	13	77.84±72.48	41.61±17.59	2.44±1.88
DFPV	8	44.98±4.57	6.10±1.46	0.36±0.30
CV	16	96.34±27.13	31.34±6.01	0.37±0.15
F		2.532	22.542	12.289
P		0.094	**0.000**	**0.000**

* There was significant difference between any two of the three groups.
The fluoride concentration in drinking water of EFV was significantly higher than those of CV and DFPV.

The comparison of fluoride in crops, vegetables and soils in different investigated villages were shown in Table 2. Due to the irrigation water in DFPV is still the water which from the wells before defluoridation project; we merged the vegetables, grain and soil samples from EFV and DFPV as the samples of endemic fluorosis villages (EFV+DFPV). As we can see from it that there were no significant differences of fluoride concentration in vegetables, crops and plow pan layer of soil between EFV+DFPV and the CV ($P > 0.05$), but the water-soluble fluorine and total fluorine in plough layer were higher in EFV+DFPV than those in CV ($P<0.05$).

3.2 The comparison of fluoride in foods, vegetables and soils of different villages (Table 2)

Table 2. The comparison of fluoride in food, vegetable and soil in different villages (mg/kg, $\bar{\chi}\pm s$)

Group	Food (n=35)	Vegetable (n=51)	water-soluble fluoride in soil (n=9)		total fluorine in soil (n=9)	
			Plough layer	plow pan layer	plough layer	Plow pan layer
EFV+DFPV	1.77±0.25	0.58±0.34	126.09±18.64	120.91±28.71	37.90±20.45	39.97±14.90
CV	1.89±0.21	0.66±0.92	100.03±18.64	109.65±35.71	34.64±9.08	37.90±20.45
t	1.552	0.455	-3.084	-0.742	-2.564	0.236
P	0.130	0.651	**0.007**	0.469	**0.021**	0.817

3.3 The comparison of urine fluoride level, serum Ca^{2+} and Mg^{2+} level in different groups. (Table 3)

Fluoride levels in urine in different groups were: EFV>DFPV>CV, and there were significant differences in any two of the three groups ($P<0.05$ respectively). There were no significant differences of serum Ca^{2+} level among of the three groups ($P>0.05$). As for the serum Mg^{2+} level, there was significant statistical difference between CV and DFPV ($P<0.05$)

Table 3. The comparison of the concentrations of urine fluoride and serum Ca^{2+} and Mg^{2+} (mg/L, $\bar{\chi}\pm s$) of adults of EFV, DFPV and CV

Group	n	Urine fluoride*	Blood Ca^{+}	Blood $Mg^{+\#}$
EFV	157	2.50±1.50	89.62±13.74	22.51±4.18
DFPV	245	1.42±0.97	90.07±17.00	21.81±7.43
CV	492	0.98±0.50	87.84±20.56	23.31±5.84
F		173.605	1.383	5.090
P		0.000	0.251	0.006

* There was significant difference between any two of the three groups.
# There was significant difference between DFPV and CV.

3.4 Correlation analysis between fluoride and Ca^{2+}, Mg^{2+} concentrations both in drinking water and in peripheral blood. (Table 4, 5)

Correlation analysis between fluoride and Ca^{2+}, Mg^{2+} concentrations in drinking water was shown in table 4, from which we can see that there was negative correlation between fluoride and Ca^{2+} concentration in drinking water of EFV（r=-0.509，P=0.044 ）; fluoride concentration was negative correlated with Mg^{2+} concentration in drinking water in CV（r=-0.720， P=0.005）.

Correlation analysis between the urine fluoride and the concentrations of Ca^{2+}, Mg^{2+} in peripheral blood of adults in different investigated villages was shown in table 5, the result showed that there was negative correlation between urine fluoride and blood Ca^{2+} in EFV (r=-0.183，P=0.022).

Table 4. Correlation analysis of concentrations of fluoride and Ca^{2+}, Mg^{2+} in drinking water (mg/L)

Group	n	Mg^{+}		Ca^{+}	
		r	*P* value	r	*P* value
EFV	157	-0.442	0.087	-0.509	**0.044**
DFPV	245	0.168	0.691	0.347	0.400
CV	492	-0.720	**0.005**	-0.190	0.535

Table 5. Correlation analysis between urine fluoride concentrations and the blood Ca^{2+} and Mg^{2+} concentrations of adults in EFV, DFPV and CV (mg/L)

Group	n	Mg^{+}		Ca^{+}	
		r	*P* value	r	*P* value
EFV	157	0.058	0.469	-0.183	**0.022**
DFPV	245	-0.031	0.626	-0.012	0.850
CV	492	0.020	0.664	0.086	0.058

4 Discussions

Endemic fluorosis is a major public health concern in China due to the excessive consumption of fluoride in drinking water, and this issue is not limited to China. India, one of the two most populous country of the world, is also the worst affected by fluoride. India is plagued with numerous water quality problems due to prolific contaminants mainly of geogenic origin and fluoride stands first among them. The weathering of primary rocks and leaching of fluoride-containing minerals in soils yield fluoride rich groundwater in India which is generally associated with low calcium content and high bicarbonate ions (Ayoob and Gupta , 2006).

Endemic fuorosis was caused by choronic persistent fluoride exposure due to the excessive consumption of fluoride through drinking water, brick tea, and coal-burning. In view of the influence of tea and coal consumption on chronic fluorosis, the fluoride levels in indoor and outdoor air, vegetables and crops were determined in the six villages. We found that most of the families cooked meals using wheat straw and cornstalk as the sources of energy both in endemic fluorosis villages and control villages because of their relatively undeveloped. The fluoride levels in indoor and outdoor air were all lower than the national standard and also no significant differences were found in different villages. Fluoride levels in grain, vegetables and plow pan layer of soils also had no significant differences in all six villages, which showed that with the exposure level, there were no accumulations in vegetables and crops and so on. But the water-soluble fluorine and total fluorine in plough layer were higher in endemic fluorosis villages than those in control villages. The results above showed that consumption of fluoride in drinking water is the major exposure pathway in the investigated villages. The high fluoride in plough layer maybe related with irrigation water with high fluoride level because the improved water was just used for drinking and cooking. Urine fluoride level represents the internal burden of individual exposed to fluoride. In current study, the fluoride concentrations in urine were higher in adults both from EFV and DFPV than those from CV. The fluoride level has been maintained at relatively higher level in local residents from DFPV even if the defluoridation project has been implemented for two years. It suggested that the excretion of accumulated fluoride from body is a long process. As we know, about half of the absorbed fluoride is quickly incorporated into developing bone and teeth, where nearly all of the body's fluoride is found (Padula and Macmillan, 2005). The absorbed fluoride by the skeleton is most efficient in children and decreases with age(Maudsley et al, 2004), but this process can continue up to age 55 (Merviel et al, 2005).

Major influences in fluoride metabolism, however, result from the simultaneous ingestion of fluoride and inorganic ions, e.g. calcium, magnesium and aluminum. It's well known that these ions, when present at higher levels in diet or water, will decrease fluorine absorption (Büttner, 1963). Previous study showed that fluoride can interfere in metabolism of trace elements, which maybe caused by the moderate to severe level of water fluoride concentration (Bian, 1993). In current study, there were no statistical significances of serum Ca^{2+} level among three groups; but serum Mg^{2+} level was lower in adults from DFPV than that from CV. On the other hand, urine fluoride was negative correlated with blood Ca^{2+} in EFV(r=-0.183, P=0.022). It suggests that fluoride can affect on calcium metabolism and may combine blood Ca^{2+} to form indissolvable CaF_2 which can explain why individuals with high urine fluoride concentration have lower blood Ca^{2+} concentration.

Conclusion

In this study, it was found that the fluoride level has been maintained at relatively higher level in local residents even if the defluoridation project has been implemented for two years. It suggested that the excretion of accumulated fluoride from body is a long process. Individuals who have higher urine fluoride concentration tend to have lower blood Ca^{2+} concentration in EFV. It may suggest that fluoride can interfere in metabolism of calcium.

Acknowledgments

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Corresponding author:
Fax:+86-371-67781868;
E-mail address: bayue1963@hotmail.com

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A Design of Fault Tolerant Reversible Arithmetic Logic Unit

Parisa Safari [1], Majid Haghparast [2], Asgar Azari [3]

[1.] Young Researchers Club, Ahar Branch, Islamic Azad University, Ahar, Iran
[2.] Department of Computer Engineering, Shahre-Rey Branch, Islamic Azad University, Tehran, Iran
[3.] Department of Electrical Engineering, Tabriz Branch, Islamic Azad University, Tabriz, Iran
p-safari@iau-ahar.ac.ir, haghparast@iausr.ac.ir , azari@iaut.ac.ir

Abstract: Since the Arithmetic Logic Unit (ALU) is one of the essential components of the Central Processing Unit (CPU), its well performance is the most important factor in obtaining the high reliability. The reversible logic has also found emerging attention in nanotechnology, optical computing, quantum computing and low power CMOS design. In this paper we are going to propose and analyze a basic model of fault tolerant reversible ALU and show that the realization of an efficient fault tolerant reversible ALU is possible with both minimum constant inputs and garbage outputs. The proposed fault tolerant reversible ALU is a versatile approach to the implementation of quantum computing with having both a remarkable low power consumption and nano scaling.
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Key words: Reversible Logic, Parity Preserving, Fault Tolerant, Arithmetic Logic Unit, Quantum Computing, Nanotechnology based systems, Nanometric Circuits.

Introduction

This paper proposes an efficient fault tolerant reversible arithmetic logic unit. Traditional irreversible hardware computation inherently leads to the energy losses due to the missing bit information, where the energy dissipation is proportional to the number of missing bits [1]. Bennet showed that to avoid this energy loss in a logic circuit is to use reversible logic gates [2]. A gate is reversible if there is a one-to-one mapping of the input/output. That is the relationship between input/output has to be an injective one. For this main reason, reversible logic has received significant attention and proven to have applications in areas such as optical computing, low power electronic design, DNA, quantum computing, and nanotechnology based systems to name a few [3],[4],[5]. It should be noted that the non existence of both any fan out and feed back (loop) are two major problems with the reversible logic synthesis. Thus, the synthesis and implementation of the reversible logic circuit becomes more complex than the conventional one [4], [5]

If a system is made up of fault tolerant components, then it will be able to continue operating properly when the failure occurs in some of its components. The detection and correction of faults in such fault tolerant systems are easier. We can achieve fault tolerance in many systems by using parity bits. Thus, parity preserving reversible circuit design will be very important for development of fault tolerant reversible systems in nanotechnology which is an emerging technology [6]. It is worth to note that the parity preserving gates can be used to make fault tolerant reversible logic circuits.

2. Novel Design

2.1. Reversible, Quantum Gates and Circuits

A gate, a circuit or a function is reversible if and only if there is a one-to-one mapping between its input and output. Therefore, a reversible gate has an equal number of inputs and outputs. There is a number of commonly used reversible logic gate such as Feynman Gate, FG [7], Toffoli Gate, TG [8] and Peres Gate, PG [9].

A 2×2 Feynman Gate, also known as controlled NOT (1-CNOT), is depicted in Fig.1a. The 3×3 reversible Peres and Toffoli gates are also shown in Fig. 1b and Fig 1.c, respectively with both of them being universal gates. On the other hand consider a 3*3 Toffoli gate, we know that the two of the inputs are mapped to the first two outputs and if both of the first inputs are one then the result will be complemented, otherwise the third output will be the same as the third input.

Peres gate (PG), is equivalent to the transformation produced by a Toffoli gate followed by a Feynman gate. The Feynman gate and the Peres gate are one-through gates, (i.e. one of the input lines is also output). The Toffoli gate is two-through gate, that is two of the input lines are also outputs.

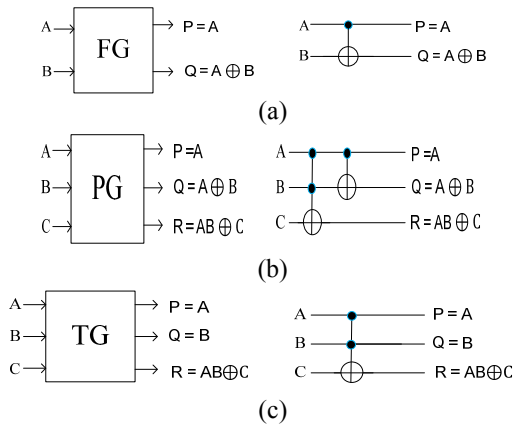


Fig. 1: commonly used reversible logic gates. a) Feynman gate; b) Peres gate; c) Tofolli gate

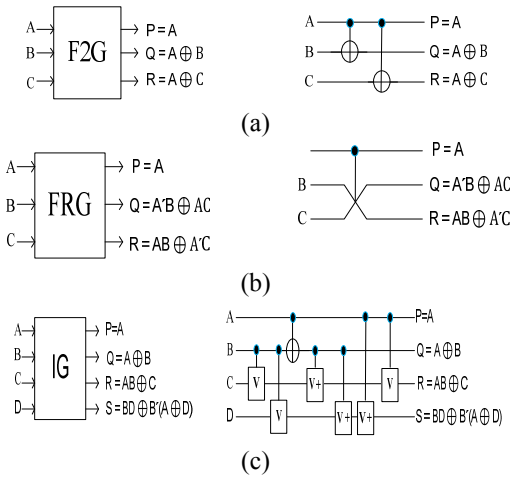
2.2. Parity Preserving Reversible Gates and Circuits

Between reversible logic gates, those with their input parity being the same as their output parity are called "parity preserving reversible gates (circuits)". Most of arithmetic and other processing functions do not preserve the parity of the data. Parity checking is one of the most widely used methods for error detection in digital logic systems [12], [13], [14]. Therefore it is important to construct parity preserving reversible gates and circuits. There are some problems using standard methods of error detection in reversible circuits, since fan-out is not allowed, and it may increase the number of gates being used along with the number of garbage outputs being produced. Given that reversible logic gates have the equal number of inputs and outputs, a sufficient requirement for parity preservation of a reversible circuit is that each gate to have a parity-preserving characteristics. Thus, a sufficient condition for having a parity preserving reversible logic gates is the implementation of the reversible circuit with each gate being parity preserving. In figure 2, three different parity preserving reversible gates along with truth table is shown [6], [12], [15].

3. The proposed Fault Tolerant Reversible Arithmetic Logic Unit

An ALU unit is a multi-functional circuit that conditionally performs one of several possible functions on two operands say, A and B, depending on a control unit. Mathematician John Von Neumann proposed the ALU concept in 1945, where he laid the foundations for a new computer called the "EDVAC" [16]. Knowing that ALU performs all arithmetic logic operations, it is considered to be the fundamental building block of the central processing unit (CPU).

In general there are two design considerations for serial and parallel ALU with reversible circuits [17].To optimize the speed and cost we have used a fault tolerant reversible ALU with combinations of both parallel and serial structures. To implement any one of four basic logical operations, AND, OR, EX-OR and ADD a reversible fault tolerant 4×1 multiplexer along with three FRG gates which is expandable to $2^n \times 1$ and shown in figure 3 , is used. The important property of this circuit structure is that by using a fault tolerant full adder(FTFA) block for computations of EX-OR and ADD the gate number and the quantum cost both are decreased by a factor of one. In figure 4, the two different design representations are also shown.



A	B	C	P	Q	R
0	0	0	0	0	0
0	0	1	0	0	1
0	1	0	0	1	0
0	1	1	0	1	1
1	0	0	1	1	1
1	0	1	1	1	0
1	1	0	1	0	1
1	1	1	1	0	0

(d)

Fig 2: a) Feynman Double gate; b) Fredkin Gate; c) IG Gate; d) the FRG truth table.

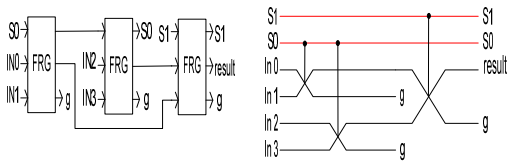
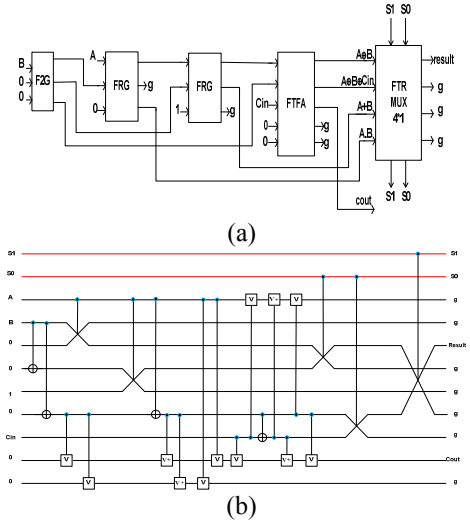


Fig. 3: proposed fault tolerant reversible 4×1 multiplexer.



(a)

(b)

Fig 4: a) fault tolerant reversible ALU with fault tolerant reversible gates.
b) Equivalent quantum representation of fault tolerant reversible ALU.

Before doing any type of computation we define the constant input as being zero or one, the garbage output being the one which is not used for further computations , and the quantum cost is considered to be as the number of reversible logic gates which are either 1×1 or 2×2 reversible.

With the four main factors of circuit complexity defind as:

α = A two input EX-OR gate calculation
β = A two input AND gate calculation
δ = A NOT calculation
T = Total logical calculation

The table 2 shows the result of the computations for n×1 multiplexer and n-bit ALU:

Table 2

	Constant Input	Garbage Output	Total Number of Gates	Quantum Cost	Total Logic Calculation
Fault tolerant reversible multiplexer 2^n*1	$0^{\dagger}$	2^n-1	2^n-1	10^n-5	$(2^n-1)\times(2\alpha+4\beta+2\delta)$
Fault tolerant reversible ALU(nbit)	6n	7n	7n	41n	$n\times(20\alpha+26\beta+12\delta)$

†: Optimum value

4. Conclusion

We have shown that a fault tolerant reversible basic arithmetic logic unit and a fault tolerant reversible 4×1 multiplexer as a block controller is possible. To do this we have used a parity preserving reversible gates for circuit design. Since the ever increasing demand for communication will soon exceed today's performance limit, it is interesting to study ways of reducing cost and increasing speed along with increasing operations. Knowing that all the circuits have nano dimensions, it is clear that the nanotechnology will plays an important role in the future developments.

Corresponding Author:
Parisa Safari [1], Majid Haghparast [2], Asgar Azari [3]
[1.] Young Researchers Club, Ahar Branch, Islamic Azad University, Ahar, Iran
[2.] Department of Computer Engineering, Shahre-Rey Branch, Islamic Azad University, Tehran, Iran
[3.] Department of Electrical Engineering, Tabriz Branch, Islamic Azad University, Tabriz, Iran
p-safari@iau-ahar.ac.ir,
haghparast@iausr.ac.ir ,
azari@iaut.ac.ir

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Diagnostic Value of Flow Cytometry in Cases with Myelodysplasia

Islam M. Hussien[1], Samia F. El-Belbessy[1], Shereen M. El-Maghraby[1], Amani F. Sorour[2] and Nahla Farahat[2]

[1]Hematology Department, Medical Research Institute, Alexandria University
[2]Clinical Pathology Department, Faculty of Medicine, Alexandria University
shereenmaghraby36@yahoo.com

Abstract: Background: Myelodysplastic syndrome (MDS) is a term used to encompass a spectrum of clonal (neoplastic) myeloid disorders The combination of obvious marrow dysplasia and clonal karyotypic abnormalities is considered diagnostic for MDS, with each technology confirming the other. However, not all patients with MDS will have this combination of findings. In this study, we evaluated the utility of flow cytometric immunophenotyping in the diagnosis of MDS. **Material and Methods:** We studied 20 patients with MDS , two of them were chronic myelomonocytic leukemia (CMML) (as diagnosed by morphologic evaluation of the initial bone marrow specimen) and compared results with those obtained in healthy controls subjects. All patients and controls were subjected to full history taking , Clinical examination, complete blood count, Bone marrow aspirate ,iron stain and immunophenotyping using a panel of antibodies CD13, 33, 34, 38, 16, 14,45 ,56 and CD11b to analyze dyspoiesis by quantifying the expression of each monoclonal antibodies (MoAb) on blasts ,granulocytes and monocytes with respect to controls. Bone marrow biopsy was done in some cases. **Results:** The results are classified according to the gate into blast, granulocytes and monocytic gates. On blast gate, we found statistically significant increase in expression and percentage of CD34 + cells , also decrease in CD 38 expression on CD34 + cells in cases of MDS in comparison to control group. Granulocytic gating revealed statistically significant increase of CD13 expression and decrease in CD56 expression in cases in comparison to control group, while the differences in expression of CD45, CD14, CD33 and CD11B were statistically insignificant. Monocytic gating revealed statistically significant decrease of CD38 expression in cases of RA and increase of CD14 & CD11b expressions in cases in comparison to control group, while the differences in expression of CD45, CD13, CD33 and CD56 were statistically insignificant. **Conclusion:** We emphasis on the role of flow cytomerty in MDS for accurate blast count and identification of abnormal myeloblasts on the basis of antigenic profiles, even in the marrow with less than 5% of myeloblasts. Also recognition of immunophenotypic dysplastic changes in mature myeloid cells and monocytes. No one single simple immunophenotypic parameter has been proved to be diagnostic of MDS.

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Keywords: MDS, Flow cytometry

1. Introduction

The myelodysplastic syndromes (MDSs) are characterized by bilineage or trilineage dysplasia. Although diagnostic criteria are well established for MDS, a significant number of patients have blood and bone marrow findings that make diagnosis and classification difficult. The diagnosis of MDS is based on a combination of clinical history, the morphological features of the peripheral blood (PB) and BM (e.g., percentages of blasts and dysplasia of cells), cytogenetic data, and ruling out other diseases.(1)

However, clonal cytogenetic abnormalities are typically found in less than 50% of these disorders, while morphologic evaluation is intrinsically subjective. Because reproducible patterns of antigen expression are identified in both normal myeloid maturation and benign/reactive settings such as marrow regeneration following injury, significant deviations from these benign maturational patterns can provide objective evidence supporting the presence of MDS or chronic myelomonocytic leukemia .(2)

A diagnostic challenge exists in low-grade MDS that lack conventional, specific diagnostic markers, ringed sideroblasts and karyotypic aberration. The diagnosis of this category (called *low-grade MDS*) largely relies on the presence of dysplasia, and therefore experienced examiners (hematologists/hematopathologists) are required to make the diagnosis. On the other hand, the dysplastic features of myeloid cells do not in themselves establish a diagnosis. Conditions other than MDS can induce dysplastic myeloid cells (e.g., deficiencies of vitamin B_{12} and folate, viral infections, ethanol or lead), and thus such conditions should be ruled out by careful history taking and physical and laboratory examinations. (3)

Flow cytometric immunophenotyping is an accurate method for quantitative and qualitative evaluation of hematopoietic cells, and several groups have used flow cytometry in the study of MDS. MDS patients have been found to have abnormal expression of several surface antigens, as indicated by either the

intensity of fluorescence or the percentage of positive cells. No one single simple immunophenotypic parameter has been proved to be diagnostic of MDS.(4).

The aim of this study is to diagnose and categorize cases of myleodysplastic syndrome by applying more accurate and objective techniques such as flow cytometry to detect abnormal maturation patterns.

2. Material and Methods

This study was conducted on twenty (20) patients diagnosed with myelodysplastic syndrome or myelodysplastic syndrome /myeloproliferative disease (MDS/MPD) at presentation at the department of Clinical Pathology, main University hospital of Alexandria and at Hematology department, Medical Research Institute, University of Alexandria. Ten (10) healthy subjects age and sex matched will be recruited as a control group whom were subjected for bone marrow aspiration for hypersplenism

Methods

All patients and controls were subjected to full history taking, clinical examination, complete blood count, Bone marrow aspirate, Prussian blue stain and immunophenotyping. Bone marrow biopsy was done for some cases.

Bone marrow sampling:

Bone marrow aspiration was done, between 2 and 3 mls were aspirated and the sample was used to prepare bone marrow films, and the remaining was used for immunophenotyping. The diagnosis and classification of myelodysplasia was primarily based on the morphologic characteristics of different lineages in PB and BM according to WHO 2008 classification. (5)

PB and BM films were stained with leishman stain, and were used for the morphological identification of various cell types. Prussian blue stain was also performed on BM films for proper classification. Morphological analysis of marrow specimens was performed by two hematologists. A total of 500 bone marrow nucleated cells per sample were assessed. In the myeloid lineage, the following abnormalities were considered: bizarre nuclear shape, hypo- or agranularity, nuclear/cytoplasmic asynchrony, and pseudo-Pelger anomaly. The evaluation of the erythroid lineage was based on the detection of megaloblastic changes, nuclear lobulation, multinuclearity, internuclear bridges and cytoplasmic granules/inclusions. . Micromegakaryocytes, small binucleated megakaryocytes, megakaryocytes with small round separated nuclei, and megathrombocytes were considered signs of megakaryocytic dysplasia.

Immunophenotyping of the cells

Analysis was performed on total nucleated bone marrow cells after erythrocytes lysis. All samples were processed and analyzed within 24 hours. Samples should be stored at room temperature until processed in the laboratory. The detailed characterization of hematopoietic cells is obtained by analyzing the expression of a given set of antigen in a cell population. Peripheral blood and / or BM cells from patients in the present study were analyzed by immunophenotyping with panel of M_OAbs. (CD 45, 14,16,38,33,13,34, 11b,56) (DAKO, Denmark)

In the present study, the direct immunofluorescence technique was employed using labeled antibodies. Immunofluorescence on the viable cells in suspension was analyzed using Becton Dickinson, FACS calibur flow cytometer equipped with cellquest software. Isotopic antibodies were used as negative control .

Gating:

We quantify the expression of each MoAb on blast, granulocytes and monocytes gates..

Statistical analysis

Data were fed to the computer using the Predictive Analytics Software **(PASW Statistics 18).** Quantitative data were described using median, minimum and maximum as well as mean and standard deviation.The distributions of quantitative variables were tested for normality using Kolmogorov-Smirnov test, Shapiro-Wilk test. D'Agstino test was used if there was a conflict between the two previous tests. If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used.

For normally distributed data, comparison between two independent population were done using independent t-test. For abnormally distributed data, Mann-Whitney Test (for data distribution that was significantly deviated from normal) were used to analyze two independent population**.** Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

3. Results

The present study was conducted on twenty new diagnosed patients with MDS or MDS/MPD and ten healthy subjects of matched age and sex as control. The patients were 8 (40%) females and 12 (60%) males with a mean age of 54.70±14.02 years while the control group included 5 (50 %) males and 5 (50%) females of mean age 40.67±11.00 years.

The distribution of the studied patients among the WHO subtypes was as follows; five patients were Refractory anaemia (RA) (25%), three were Refractory anaemia with multilineage dysplasia (RAMD) (15 %),

seven were Refractory anaemia with excess blasts type 1 (RAEB 1) (35 %), three were Refractory anaemia with excess blasts type 2(RAEB 2) (15 %) and two were CMML (myelodysplastic syndrome /myeloproliferative) (10 %).

Figure (1a,1b) shows dysmegakaryopoiesis in a patient with refractory anemia , Figure (2a,2b) shows abnormal localization of immature precursors in bone marrow biopsy in a patient with refractory anaemia with excess blast type 1 whereas figure (3) shows dysplastic changes in a case of CMML.

There is statistically significant higher CD 34 percentage on blast gate of cases (total), RAEB1 & RAEB2 cases in comparison to control group. (Table 1). Figure (4) shows CD34 expression in the control while figure (5) shows increased CD34 expression in a case with refractory anaemia with excess blast type 1. There's statistically significant lower CD 38 expression on CD34 + cells of cases (total), RA, RAMD & RAEB1 cases in comparison to control group.(Table 2)

There is statistically significant higher mean fluorescent intensity (MFI) of CD 13 expression on neutrophils in cases (total), RAMD, RAEB1 & RAEB2 in comparison to control group. (Figures 6,7).The difference of the mean fluorescence intensity of CD33 expression on neutrophils between cases & subgroups and the difference of the mean fluorescence intensities of CD14 & CD45 expressions on neutrophils in cases of MDS in comparison to control was statistically insignificant.The decrease of the mean fluorescence intensity of CD38 expression & increase of CD16 expression on neutrophils in cases of MDS in comparison to control was statistically insignificant. There was decrease in mean fluorescence intensity of CD 11B expression on neutrophils in cases (total) of MDS in comparison to control but was statistically insignificant. There's statistically significant lower MFI of CD 56 expression on neutrophils in cases (total) in comparison to control group (Table 4, Figures 8 and 9).

The difference of the mean fluorescence intensity of CD 45 expression on monocytes in cases of MDS in comparison to control was statistically insignificant. There is statistically significant higher MFI of CD 14 expression on monocytes of cases (total), RA, RAMD & RAEB2 in comparison to control group.(Table 5).There is statistically significant lower MFI of CD 38 expression on monocytes in RA cases in comparison to control group. There is statistically significant higher MFI of CD 16 expression on monocytes in RAEB 1 cases in comparison to control group. (Table 6)

The difference of the mean fluorescence intensity of CD 13 and CD 33 expressions on monocytes in cases of MDS in comparison to control was statistically insignificant.There is statistically significant higher MFI of CD 11B expression on monocytes of cases (total) & RAMD in comparison to control group.(Figures 10,11). The difference of the mean fluorescence intensity of CD 56 expressions on monocytes in cases of MDS in comparison to control was statistically insignificant. (Table 7)

Table -1- Comparison between control and cases group & subgroups according to CD 34 percentage

	Control	Cases (Total)	Cases subgroups				
			RA	RAMD	RAEB 1	RAEB 2	MDS / MPN
CD 34 percentage							
Range	4.0 – 14.0	2.0 – 26.0	2.0 – 12.0	5.0 – 23.0	6.0 – 25.0	12.0 – 26.0	4.0 – 14.0
Mean ± SD	6.20 ± 2.90	12.15 ± 7.87	5.40 ± 4.10	11.57 ± 6.37	15.67 ± 9.50	18.67 ± 7.02	6.20 ± 2.90
Median	5.50	11.0	5.0	10.0	16.0	18.0	5.50
p	0.035*		0.453	0.053	0.047*	0.017*	0.063

p: p value for Mann Whitney test between control and other groups; *: Statistically significant at $p \leq 0.05$

Table -2- Comparison between control and cases group & subgroups according to CD 38 expression on CD 34 + cells

	Control	Cases (Total)	Cases subgroups				
			Refractory anemia	Refractory anemia with lineage dysplasia	RAEB 1	RAEB 2	MDS / MPN
CD 38 / CD 34							
Range	45.0 – 243.0	4.0 – 126.0	42.0 – 126.0	30.0 – 91.0	4.0 – 116.0	22.0 -110.0	58.0 – 94.0
Mean ± SD	138.30 ± 62.34	61.20 ± 35.08	73.40 ± 32.11	62.0 ± 30.61	42.71 ± 37.69	73.33 ± 45.80	76.0 ± 25.46
Median	131.50	61.50	66.0	65.0	28.0	88.0	76.0
p	0.001*		0.037*	0.043*	0.003*	0.091	0.133

p: p value for Mann Whitney test between control and other groups *: Statistically significant at $p \leq 0.05$

Table -3 Comparison between control and cases group & subgroups according to CD 13 and CD 33 MFI on neutrophils

	Control	Cases (Total)	Cases subgroups				
			RA	RAMD	RAEB 1	RAEB 2	MDS / MPN
CD 13							
Range	97.0 – 260.0	45.0 – 1151.0	50.0 – 623.0	213.0 – 631.0	102.0 – 632.0	183.0 – 1151.0	45.0 – 299.0
Mean ± SD	175.30 ± 62.58	349.35 ± 270.40	244.0 ± 225.27	414.0 ± 209.46	319.29 ± 177.78	648.67 ± 485.04	172.0 ± 179.61
Median	159.50	277.0	205.0	398.0	278.0	612.0	172.0
p	0.048*		1.000	0.042*	0.040*	0.042*	1.000
CD 33							
Range	47.0 – 385.0	88.0 – 181.0	78.0 – 178.0	100.0 – 252.0	47.0 – 385.0	65.0 – 319.0	135.0 – 170.0
Mean ± SD	150.85 ± 94.19	125.40 ± 23.55	111.60 ± 40.99	169.0 ± 76.96	167.29 ± 128.91	158.67 ± 139.51	152.50 ± 24.75
Median	121.0	121.0	91.0	155.0	118.0	92.0	152.50
p	0.930		0.270	0.397	0.845	0.498	0.085

p: p value for Student t-test between control and other groups; *: Statistically significant at $p \leq 0.05$

Table -4-Comparison between control and cases group & subgroups according to CD 11 B and CD 56 MFI on neutrophils

	Control	Cases (Total)	Cases subgroups				
			RA	RAMD	RAEB 1	RAEB 2	MDS / MPN
CD 11 B							
Range	392.0 – 3074.0	499.0 – 2160.0	392.0 – 3064.0	1088.0 – 2756.0	801.0 – 3019.0	495.0 – 3074.0	45.0 – 299.0
Mean ± SD	1524.50 ± 917.26	1045.80 ± 478.54	1286.0 ± 1075.91	1978.0 ± 839.62	1622.86 ± 897.61	1523.33±1366.54	172.0 ± 179.61
Median	1161.0	1052.50	970.0	2090.0	1218.0	1001.0	172.0
p	0.244		1.000	0.128	0.130	1.000	0.667
CD 56							
Range	26.0 – 439.0	78.0 – 311.0	26.0 – 420.0	224.0 – 274.0	133.0 – 439.0	109.0 – 352.0	135.0 – 170.0
Mean ± SD	233.40 ± 114.52	140.40 ± 83.07	217.20 ±157.63	250.0 ± 25.06	246.86 ± 125.07	261.0 ± 132.49	152.50 ± 24.75
Median	207.0	113.50	201.0	252.0	201.0	322.0	152.50
p	0.026*		0.327	0.091	0.057	0.091	0.519

p: p value for Mann Whitney test between control and other groups; *: Statistically significant at $p \leq 0.05$

Table -5-Comparison between control and cases group & subgroups according to CD 45 and CD 14 MFI on monocytes

	Control	Cases (Total)	Cases subgroups				
			RA	RAMD	RAEB 1	RAEB 2	MDS / MPN
CD 45							
Range	44. – 542.0	97.0 – 811.0	114.0 – 743.0	141.0 – 407.0	97.0 – 811.0	113.0 – 360.0	111.0 – 340.0
Mean ± SD	195.90 ± 152.64	307.95 ± 230.92	362.0 ± 260.88	282.0 ± 133.72	348.29 ± 307.88	204.67 ± 135.25	225.50 ± 161.93
Median	151.0	219.50	328.0	298.0	128.0	141.0	225.50
p	0.428		0.269	0.234	0.845	1.000	1.000
CD 14							
Range	50.0 – 1079.0	129.0 – 3008.0	519.0 – 2901.0	1245.0 – 3008.0	129.0 – 2901.0	2240.0 – 3008.0	255.0 – 499.0
Mean ± SD	275.10 ± 326.57	1792.95±1186.53	2127.80 ± 953.98	2420.33±1017.87	1293.71±1329.61	2716.33 ± 415.97	377.0 ± 172.53
Median	99.50	2284.50	2329.0	3008.0	417.0	2901.0	377.0
p	0.001*		0.003*	0.011*	0.057	0.011*	0.282

p: p value for Mann Whitney test between control and other groups; *: Statistically significant at $p \leq 0.05$

Table 6: Comparison between control and cases group & subgroups according to CD 38 and CD 16 MFI on monocytes

	Control	Cases (Total)	Cases subgroups				
			RA	RAMD	RAEB 1	RAEB 2	MDS / MPN
CD 38							
Range	35.0 – 250.0	4.0 – 830.0	27.0 - 106	24.0 – 830.0	4.0 – 229.0	51.0 – 369.0	111.0 – 142.0
Mean ± SD	122.20 ± 54.89	140.10 ± 182.74	69.40 ± 29.84	297.0 ± 461.64	101.43 ± 73.61	200.33 ± 159.88	126.50 ± 21.92
Median	119.50	88.50	77.0	37.0	95.0	181.0	126.50
p	0.244		0.020*	0.498	0.353	0.397	0.830
CD 16							
Range	91.0 – 1688.0	110.0 – 1989.0	142.0 – 1782.0	110.0 – 1727.0	419.0 – 1989.0	215.0 – 1396.0	150.0 – 1613.0
Mean ± SD	523.10 ± 466.49	894.50 ± 642.09	635.60 ± 667.35	1115.67 ± 877.67	1083.14 ± 541.72	673.33 ± 633.32	881.50 ± 1034.50
Median	491.50	687.0	307.0	1510.0	1148.0	409.0	881.50
p	0.113		0.713	0.236	0.032*	0.735	0.667

p: p value for Mann Whitney test between control and other groups; *: Statistically significant at $p \leq 0.05$

Table 7: Comparison between control and cases group & subgroups according to CD 11 b and CD 56 MFI on monocytes

	Control	Cases (Total)	Cases subgroups				
			RA	RAMD	RAEB 1	RAEB 2	MDS / MPN
CD 11 b							
Range	104.0 – 1262.0	190.0 – 3116.0	302.0 – 1467.0	994.0 – 1704.0	190.0 – 1932.0	651.0 – 3116.0	45.0 – 299.0
Mean ± SD	495.10 ± 476.60	1069.15 ± 690.77	860.20 ± 444.78	1437.67 ± 386.79	1001.71 ± 612.80	1515.67±1387.43	172.0 ± 179.61
Median	181.50	882.50	830.0	1615.0	876.0	780.0	172.0
^{MW}p	0.016*		0.141	0.042*	0.070	0.127	0.517
CD 56							
Range	64.0 – 588.0	0.0 – 595.0	129.0 – 470.0	52.0 – 180.0	0.0 – 470.0	374.0 – 595.0	135.0 – 170.0
Mean ± SD	350.80 ± 193.81	258.65 ± 173.25	252.60 ± 131.71	107.33 ± 65.74	224.86 ± 180.80	469.67 ± 113.45	152.50 ± 24.75
Median	324.50	237.0	202.0	90.0	279.0	440.0	152.50
p	0.197		0.330	0.061	0.196	0.342	0.760

^{MW}p: *p* value for Mann Whitney test between control and other groups

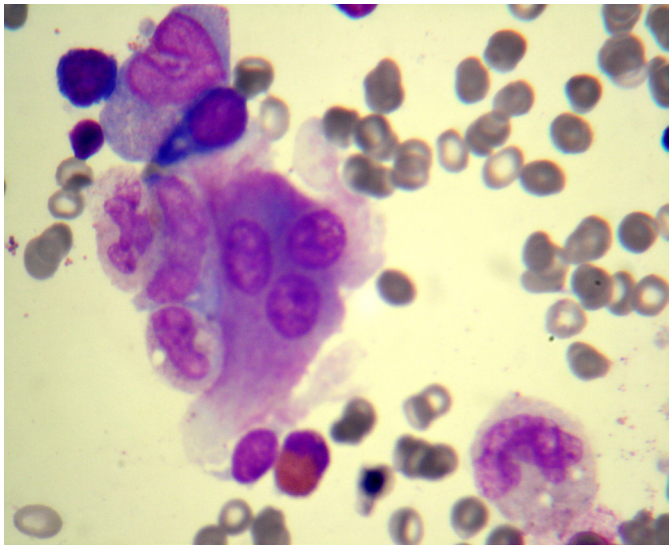

Figure (1a) :Shows dysplastic megakaryocyte in a patient with refractory anaemia

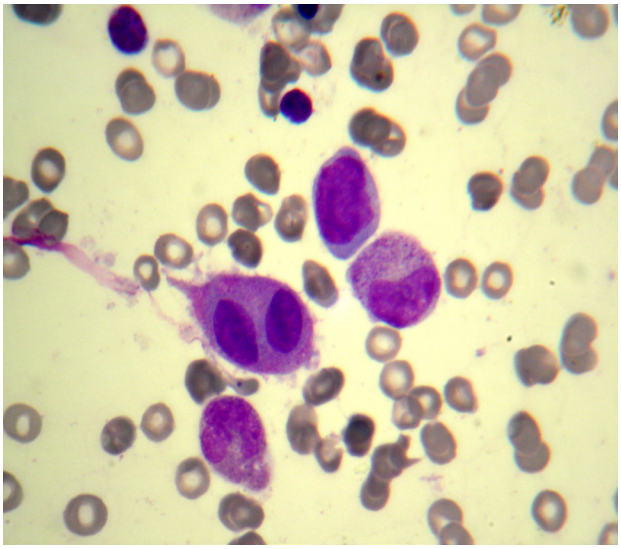

Figure (1b):Shows binucleated megakaryocyte in a patient with refractory anaemia

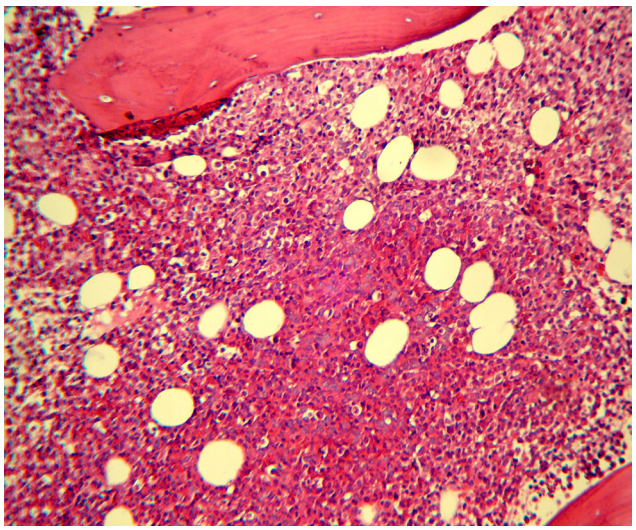

Figure (2a): Shows abnormal localization of immature precursors in a patient with refractory anaemia with excess blasts type 1 (by low power.)

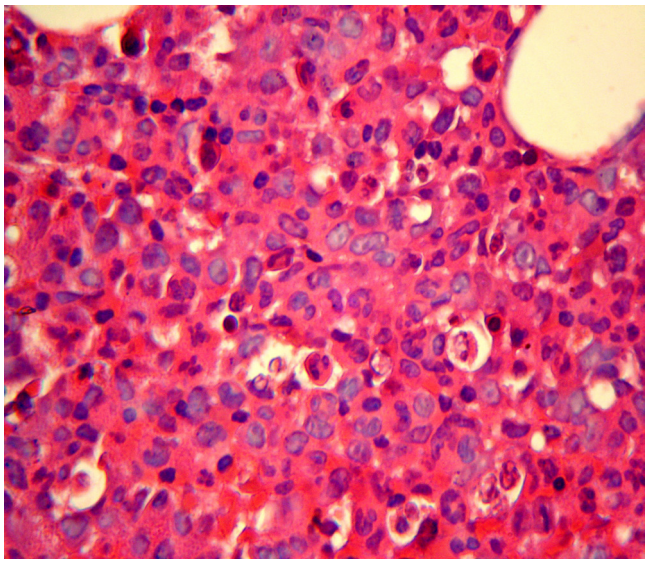

Figure (2b):Shows abnormal localization of immature precursors in a patient with refractory anaemia with excess blasts type 1(by high power)

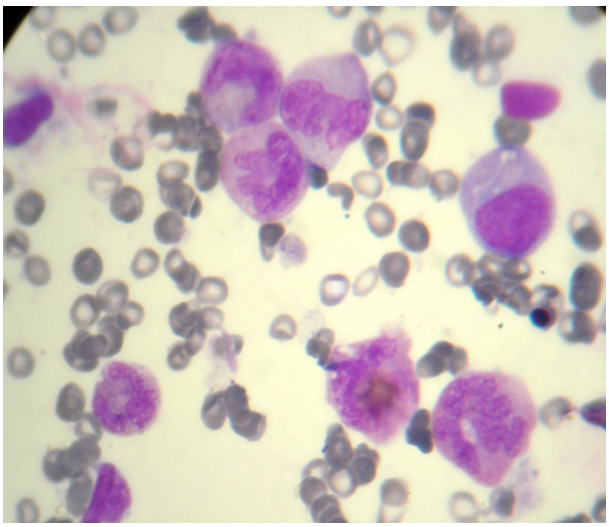

Figure (3):Shows dysplastic feature in a patient with CMML

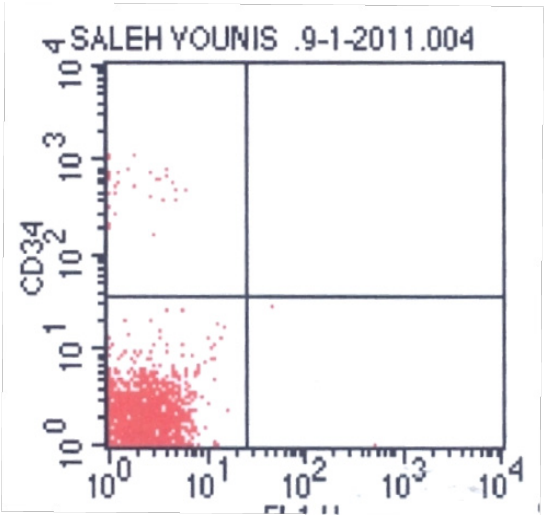

Figure 4: Normal CD 34 expression in a control

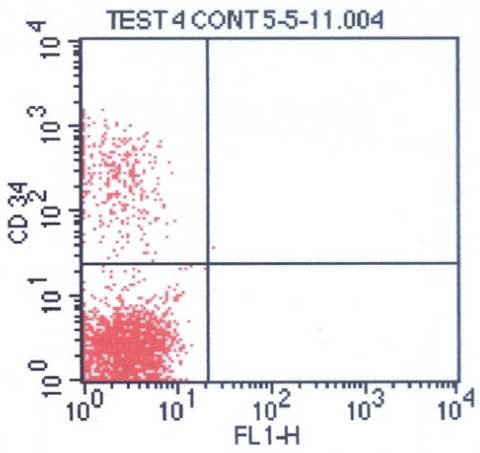

Figure 5: Higher percentages of CD 34 in a patient with refractory anaemia with excess blast type 1

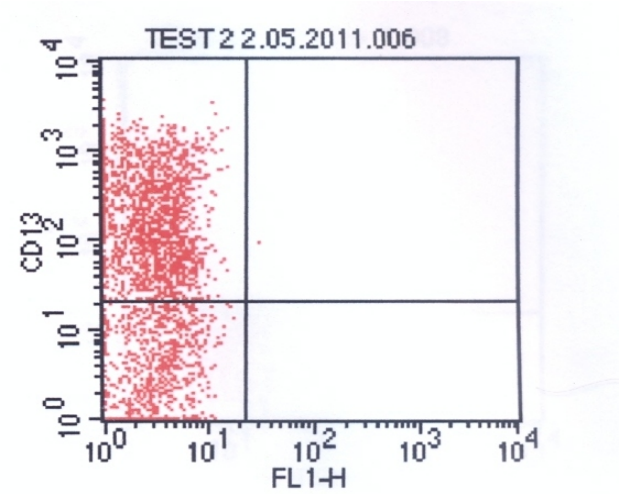

Figure 6: Higher CD 13 expression on granulocytes in a patient with refractory anemia with multilineage dysplasia

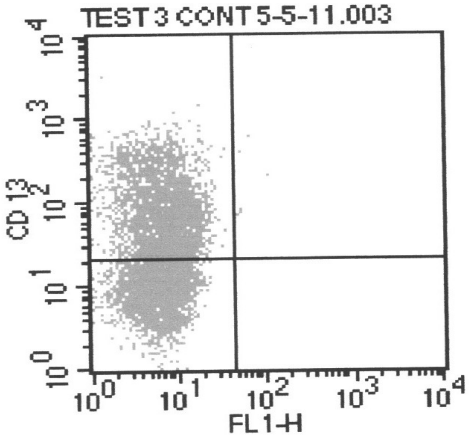

Figure 7: CD13 Expression on granulocytes in a control

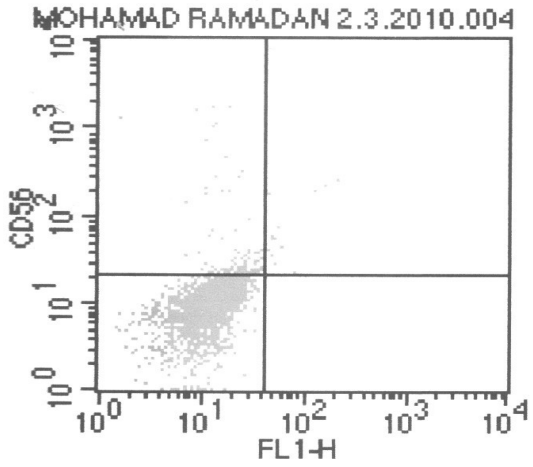

Figure (8): Low CD 56 expression on granulocytes in a case with myelodysplasisa

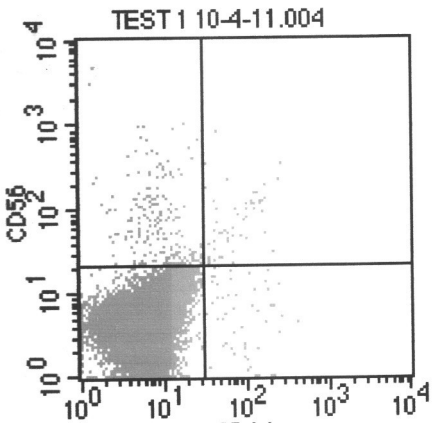

Figure (9): CD 56 expression on granulocytes in a control

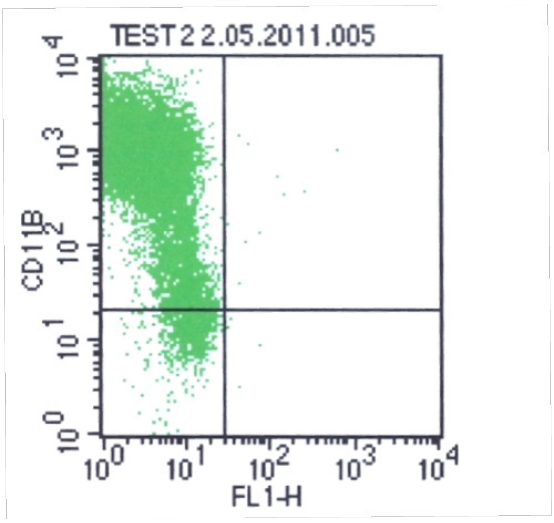

Figure 10: Higher expression of CD11B on monocytes in a case with refractory anaemia with multilineage dysplasia

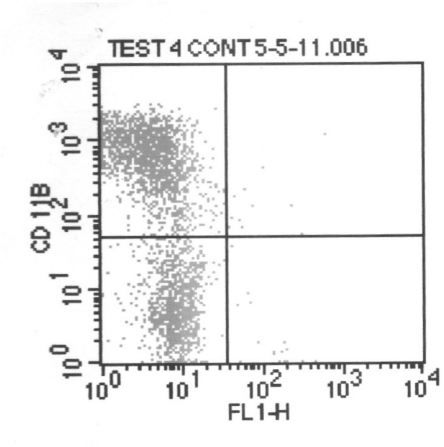

Figure (11): Expression of CD11B on monocytes in a control

4. Discussion

Myelodysplastic syndromes (MDS) are malignant disorders of haematopoietic cells. The bone marrow(BM) in MDS is composed of clonal myeloid cells showing various degrees of differentiation in each case. MDS usually exhibit cytopenia, mainly due to the early death of partially or fully differentiated myeloid cells and insufficient differentiation capacity of the progenitors to transform into mature blood cells. Manifestations caused by cytopenia and transformation to acute myeloid leukaemia(AML) due to further loss of the ability of clonal cells to differentiate are the major causes of death in MDS.(6)

The diagnosis of MDS is straightforward if clearly objective abnormalities, such as increase in blasts and/or ringed sideroblasts and/or presence of chromosomal aberration as evidence of clonal myelopoiesis are detected. A diagnostic challenge exists in low-grade MDS without ringed sideroblasts with normal karyotype For this reason laboratory scientists have investigated the use of flow cytometry (FC) to increase the sensitivity and specificity of diagnosis in such cases. (7)

Recently, many laboratories have been working to develop MDS FCM and are still struggling to determine suitable flow parameters .Flow cytometry can detect minimal aberrancies in the differentiation of myelomonocytic cell populations by changes in antigen expression in BM that are otherwise not detected by morphology.

The aberrancies in hematopoiesis that can be observed are the expression of lymphoid antigens on myeloid cells, over, under and/or loss of antigen expression on mature cells and vice versa and abnormal differentiation patterns as compared to antigen expression levels from normal hematopoietic cells.(8)

Flow cytometric role in MDS is based upon the knowledge that maturation and differentiation of hematopoietic cells is a tightly controlled process, leading to highly conserved levels of antigen expression at different stages of development. In myelodysplastic syndromes (MDS), progenitor cell formation is affected resulting in deviation from the normal level of antigen expression in the mature and immature myelo-monocytic, erythroid and megakaryocytic cell lineages. [9]

Both flow cytometric immunophenotyping and morphologic evaluation were more sensitive than cytogenetics in detecting MDS .As more antibodies useful in studying erythroid and megakaryocytic maturation are developed , the sensitivity of flow cytometric testing may increase.(1)

In this study, we evaluated the utility of flow cytometric immunophenotyping in the diagnosis of MDS. We studied 20 patients with MDS (as diagnosed by morphologic evaluation of the bone marrow specimen) and compared results with those obtained in healthy controls subjects using combination of antibodies CD13, 33, 34, 38, 16, 14,45,56 and CD11b to analyze dyspoiesis by quantifying the expression of each MoAb on granulocytes and monocytes with respect to controls. The results are classified according to the gate into blast, granulocytes and monocytic gates.

On blast gate, we found statistically significant increase in the expression and percentage of CD34+ cells and decrease in CD38 expression on CD34 + cells in MDS cases in comparison to the control group.

In between cases there was higher CD 34+ cells percentage in cases of RAEB1 & RAEB2 subtypes in comparison to other subtypes. This difference between the subtypes of MDS showed a good correlation with the number of bone marrow blast cells assessed by morphology.

In Consistence with this finding of our study; Fuchigami *et al.* [10] scored absolute numbers of CD34-positive cells using CD34 monoclonal antibody and found that the total CD34+ cells were decreased in RA patients, but increased in patients with RA with excess of blasts (RAEB) as compared to normal.

Similarly, Ogata *et al.* [11] and Matarraz *et al.* [12] focused on blast immunophenotype and found that quantifying CD34+ cells in blast compartment is useful in diagnosing patients with low grade MDS with or without karyotype abnormalities. Also increased CD34 expression was associated with a poor international prognostic scoring system, a poor cytogenetic risk factor, and a high blast cell count on bone marrow smears.

We conclude that this method allows distinguishing RA from other MDS subtypes more reliably than by morphology alone and providing early signs of progression to acute leukemia. Also CD34 expression could be significant as a prognostic marker rather than as a diagnostic marker of MDS. On blast gate, we found statistically significant decrease in CD38 expression on CD34+ cells in cases of MDS in comparison to control group.

Goardon *et al.* [13] investigated whether reduced mean fluorescence intensity (MFI) of CD38 expression on CD34+ cells could be used as a surrogate marker for abnormalities in the MDS CD34+ compartment, and whether this may provide a single simple useful flow cytometric measurement diagnostic of MDS. They found that the examined immunophenotypic parameter diagnosed low-risk MDS with 95% sensitivity and 92% specificity, and concluded that it may be of value in the routine clinical diagnosis of MDS, especially in cases with a low blast count and normal karyotype.

The present work revealed that the difference of the mean fluorescence intensity (MFI) of CD33 expression on granulocytes and monocytes in MDS cases in comparison to control were statistically insignificant while there is statistically significant higher MFI of CD 13 expression on neutrophils in

cases (total), RAMD, RAEB1 & RAEB2 in comparison to control group.

Some studies on peripheral blood neutrophils in MDS found no abnormality in CD33expression while others found an increased number of CD33-positive neutrophils, particularly in RAEB and RAEB-t. Several mechanisms may be involved in the abnormal expression of surface antigens in MDS including defective granulopoiesis, defective intracellular storage pool, abnormal membrane of cytoplasmic granules, and the effect of high levels of marrow cytokines such as tumor necrosis factor alpha and transforming growth factor-beta(5). Other studies have reported a much higher CD13 expression in high-risk groups than low-risk patients.(14) .Decreased or absent CD 33 expression in MDS has been reported by others. (15)

While Maynadié *et al.* ([16]) studied the immunophenotypic abnormalities that could be defined in MDS and the data obtained from granulocytes showed that the most discriminating markers were CD11b, CD13, CD33, CD38 and HLA-DR also the increased mean fluorescence intensities of CD38, CD13, and CD33 were associated with more advanced MDS stages (refractory anemia with excess blasts and RA with excess blasts in transformation).

The present study showed insignificant increase of MFI of CD16 expression on granulocytes and there was decrease of CD11b expression on granulocytes in the cases (total) in comparison to controls but that decrease was statistically insignificant. This might be explained by low sample size in the study.

Bowen and Davis [17] studied the pattern of CD16 and CD11b expression on maturing granulocytes in the bone marrow of patients with MDS and healthy controls. There was a highly consistent normal pattern of CD11b and CD16 expression in the granulocytic series in healthy subjects, but in MDS patients there was an increased percentage of granulocytic cells with low CD16 or both low CD16 and low CD11b.

In the present study we found decrease in CD56 expression on neutrophils in cases in comparison to control group whereas there was increase of 11b expression on monocytes in cases(total and RAMD) in comparison to control group that were not reported by others.

Also there's a statistically significant lower MFI of CD 38 expression on monocytes of RA cases in comparison to control group and higher MFI of CD 16 expression on monocytes in RAEB 1 cases in comparison to control group.

Van de Loosdrecht, *et al.* (18) **found** the following aberrancies in maturing monocytes and considered them relevant: decreased or increased proportion of monocytes as compared to lymphocytes, abnormal intensity of CD13 or CD33, an abnormal CD11b/HLA-DR pattern, abnormal intensity of CD14, CD36 or CD64, over-expression of CD56 and expression of lineage infidelity markers CD2, CD7 or CD19.

Del Canizo *et al.* [错误！未定义书签。] found abnormally low CD45 expression on monocytes more frequent in MDS RAEB patients as compared to lower risk MDS patients and normal controls. This was in disagreement to the present study as the difference of mean fluorescence intensity of CD 45 expression on neutrophils and monocytes in cases of MDS in comparison to controls was statistically insignificant. This might be explained by low sample size in the study.

The results of the present study show significant increase of CD14 expression on monocytes between cases (total), RA, RAMD & RAEB2 and controls. On the other hand there was an insignificant difference of CD56 expression on monocytes between cases of MDS and normal controls.

As regarding CD56 expression which is a normal marker on natural killer cells, Lacronique-Gazaille *et al.* [20] and Xu Y *et al.* [21] used the flow cytometric analysis of monocytes to detect the phenotypic abnormalities in cases of CMML and found a significantly higher expression of CD56 and CD14 on marrow monocytes in CMML than in reactive monocytosis and normal marrow samples. Our result showed higher expression of CD 14 on monocytes in CMML compared to controls but the result was statistically insignificant as we have only two cases .

Also a study by Van de Loosdrecht [22] detect aberrancies in the myelo-monocytic lineage in cases of MDS patients in the form of over expression of CD 56 on monocytes in cases and the expression of CD56 was only scored as aberrant when its intensity exceeded that of CD56 expression as detected on monocytes in some of the normal samples by 1 log.

Our results as regarding CD56 expression in cases of CMML was insignificant ,this might be explained by the few CMML cases included in this study.

In conclusion, flow cytometry is of value in diagnosis of RA especially when the morphologic and cytogenetic evaluations are equivocal or non informative as it can detect accurate blast count and identification of abnormal myeloblasts on the basis of antigenic profiles, even in the marrow with less than 5% of myeloblasts. Also we were able to detect immunophenotypic dysplastic changes in mature myeloid cells and monocytes.

No one single simple immunophenotypic parameter has been proved to be diagnostic of MDS and because the panels needed for complete immunophenotypic analysis of all 3 lineages are extensive as well as costly, we do not recommend flow cytometric evaluation as a screening procedure for all cases of MDS.

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Correspondence author name:
Shereen M. El-Maghraby
Hematology Department, Medical Research Institute, Alexandria University
shereenmaghraby36@yahoo.com

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Is hyperuricaemia one of the cardiovascular risk factors clustering in type 2 diabetic patients?

Sabry Shoeib[1]; Ehab Abdel-Atti[1]; Ashraf G. Dala[1] Mohamed El-Noamany [2]; Samar M. Kamal[3] and Hala M Gabr[4]

[1.] Internal Medicine , [2]Cardiology, [3]Clinical Pathology and [4]Community Departments, Faculty of Medicine, Menofiya University hospital, Egypt. ehab_abdelatty@hotmail.com.

Abstract: Background & Aim: The prevalence of hyperuricaemia (HU) in type 2 diabetic patients (T2DM) and its relation with diabetic micro- and macro-vascular complications has been conflicting. The aim of the present study was to investigate the relationship between HU and both micro and macroangiopathies (IHD and diabetic nephropathy and neuropathy) in patients with type 2 diabetes mellitus. **Methods:** The cohort of this cross-sectional study was sixty T2 diabetic patients (26 men and 34 women, aged 52.4±8.6 years). They have been recruited from the Outpatient Department of Menofia University Hospital between January and June, 2010. In addition to comprehensive clinical examination, they were subjected to laboratory check-up for serum uric acid, fasting blood glucose (FBG) and postprandial blood glucose (PPBG), glycated hemoglobin A1c (HbA1c), serum lipids, 24-hours urine collection for microalbuminuria (μA), stress ECG and coronary angiography as indicated. **Results:** HU was detected in 18 out of out 60 (30%) type 2 diabetic patients. The frequency of hypertension (HT), ischaemic heart disease (IHD), peripheral neuropathy (PN) and μA were significantly higher in diabetic patients with (78%, 67%, 78% and 78%, respectively) than in those without HU (48%, 5%, 38% and 33% respectively) (*P*=0.04, 0.0001, 0.01 and 0.001, respectively). We also observed a significantly higher FBG, PPBG and HbA1c in the diabetic patients with compared to those without HU (*P*=0.02, 0.01 and 0.01 respectively) have. Likewise, total cholesterol, triglyceride (TG) and creatinine levels in diabetic patients with HU were again significantly (*P*=0.02, 0.001 and 0.001, respectively) above their counterparts values in diabetics without HU. **Conclusion:** The cheap, basically available and modifiable serum uric acid level we observed to prevail in T2 diabetic patients would be a useful investigational tool to prompt a cost-effective search for other cardiovascular risk factors known to cluster in them.
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Keywords: Type 2 diabetes mellitus, hyperuricemia, microangiopathy and ischaemic heart disease.

1. Introduction

Serum uric acid (SUA) is produced by xanthine oxidase from xanthine and hypoxanthine, which are in turn produced from purine (1). It has been shown at population-based epidemiological surveys to be associated with an increased risk of hypertension (HT), cardiovascular disease (CVD) and chronic kidney disease (CKD) (2, 3). Also, an elevated level of uric acid (hyperuricemia; HU) was remarkably associated with insulin resistance (4) as well as markers of metabolic syndrome (MS) such as dyslipidemia, glucose intolerance, high blood pressure, and central obesity, which are accepted as risk factors for developing CVDs. In fact, it's not only the development but also the progression of cardiovascular events and even kidney dysfunction on long-term scale (5, 6). Further, SUA was also elevated in the individuals with impaired glucose tolerance (7) and even has recently been reported to be associated with the onset of diabetes mellitus and metabolic syndrome (8, 9). Hyperuricemia is probably associated with glucose intolerance due to various mechanisms (8, 10) including among others reduced urinary excretion of uric acid due to activation of its proximal tubular transporter by hyperinsulinemia (11, 12).

Diabetes mellitus is a chronic disorder that is not only associated with cardiovascular complications of which the MS plays a prominent role (13) but also a well-known major risk factor for atherosclerotic disease and CKD (14).

Although several studies have reported a relationship between HU and both diabetic micro- and macro-angiopathies, such as coronary heart disease (CHD), and peripheral neuropathy, yet the conclusions have been fragmented and controversial (15, 16). Moreover, the influence of HUA on the renal functions has received little attention and has been insufficiently investigated in patients with T2DM.

The aim, therefore, of the present study was to investigate the relationship between HU and both micro and macroangiopathies (IHD and diabetic nephropathy and neuropathy) in patients with type 2 diabetes mellitus.

2. Patients and Methods:

A total of 60 patients with type 2 DM (26 men and 34 women) aged between 38 and 68 years were randomly selected as every other patient from those attending Outpatient Clinic of Internal Medicine Department, Menoufiya University Hospitals during the first half of 2010 and were asked to participate in

the present study. An informed consent was signed by all contributors and the study was approved by the local Medical Research and Ethics committee.

Inclusion criteria were type 2 DM diagnosed after the age of 30 years and absence of ketones at diagnosis. All patients with severe hypertension, end-stage renal disease receiving maintenance dialysis and patients with orthopaedic or neurological disabilities which may interfere with stress ECG testing were excluded from the study. Pregnancy was also an exclusion criterion.

All patients were interviewed and asked about the age of diabetes diagnosis, antihypertensive treatment, family history of IHD, smoking and their knowledge of the presence of any of the diabetic complications. They also underwent comprehensive physical examination including: measurements of height, weight to calculate their body mass index (BMI; weight (Kg) divided by the square root of height in meter), blood pressure measurement, cardiac examination and peripheral neuropathy (PN) examination. Diabetic PN was diagnosed by the presence of two or more components among clinical symptoms (bilateral spontaneous pain, hypoesthesia, or paraesthesia of the legs), the absence of ankle tendon reflexes and decreased vibration sensations using a C128 tuning fork according to the guidelines published by the Japan Diabetes Society (17).

Fasting venous blood was sampled from an antecubital vein for the measurement of FBG, total cholesterol (TC), high density lipoprotein (HDL) cholesterol, low density lipoprotein (LDL) cholesterol, serum triglycerides (TG), serum creatinine, serum uric acid and HbA1c. Another venous blood was withdrawn after 2 hours for PPBG estimation. Twenty four hours urine sample was collected for determination of urinary-albumin excretion (UAE) rate (18).

Patients were considered to have arterial hypertension if systolic blood pressure was ≥140 mmHg and diastolic blood pressure ≥90 mmHg or were currently receiving antihypertensive treatment. Hyperuricaemia (HU) was defined by the presence of SUA levels more than 7 mg/dl in males and more than 6 mg/dl in females (19) while µA was present when UAE ranged from 30 mg to 300 mg/24 hrs (20).

Resting ECG and stress ECG was done for all patients and coronary angiography was done for those who showed ischaemic changes in their stress ECG for detection of significant coronary artery disease.

Statistical Analysis:

Results are presented as mean ± standard deviation (SD) unless otherwise stated. Comparison between groups was performed using unpaired *t* test and non-parametric Mann-Whitney test. Fisher Exact analysis was also applied to compare proportions between groups. The correlation coefficient analysis was employed to detect the significance of relationships between two qualitative variables using Spearman's correlation. A p of ≤ 0.05 was considered statistically significant. All statistical analyses were performed using the Statistical Package for Social Science (SPSS) version 10.

3. Results:

Hyperuricaemia (HU) was detected in 18 patients out of 60 patients with type 2 DM. The frequency of HUA was 30%. Table 1 shows a comparison between diabetic patients with and without HU. There was no significant difference between diabetic patients with and without HU with regard to age, gender, smoking, family history of IHD and LDL level. The frequency of HT, IHD, PN and µA were significantly higher in diabetic patients with HU (78%, 67%, 78% and 78% respectively) than in diabetic patients without HU (48%, 5%, 38% and 33%, respectively) (*P*=0.04, 0.0001, 0.01 and 0.001, respectively) (Figure 1).

Diabetic patients with HU have significantly higher FBG, PPBG and HbA1c% than diabetic patients without HU (*P*=0.02, 0.01 and 0.01, respectively). Total cholesterol, TG and creatinine levels are significantly higher (*P*=0.02, 0.001 and 0.001, respectively) while the level of HDL was significantly lower in diabetic patients with HU than in those without HU (*P*=0.001). Diabetic patients with HU were of significantly (*P* =0.001) higher BMI than diabetic patients without HU (BMI=26.8 and 25.1 kg/m^2, respectively) although both groups were below the defining figure for obesity. Again, DM duration was significantly longer in diabetic patients with than in those patients without HU (*P* =0.01).

Table II shows the comparison between T2DM patients with and without IHD. There was no significant difference between diabetic patients with and without IHD with regard to gender and smoking. The frequency of HT, PN, µA and positive family history for IHD were significantly (*P* =0.02, 0.001, 0.0001 and 0.02 respectively) higher in diabetic patients with IHD (86%, 86%, 100% and 57%, respectively) than in diabetic patients without IHD (48%, 39%, 30% and 22%, respectively). Diabetic patients with IHD have significantly higher FBG, PPBG and HbA1c% than diabetic patients without IHD (*P* =0.001, 0.0001 and 0.0001, respectively). Total cholesterol, TG, LDL and creatinine levels were significantly higher in diabetic patients with IHD than diabetic patients without IHD (*P* =0.0001, 0.0001, 0.001 and 0.0001 respectively). Duration of DM was significantly longer in diabetic patients with IHD than diabetic patients without IHD (*P* =0.0001). Diabetic patients with IHD are significantly more obese and

older than diabetic patients without IHD (*P* =0.001 for both). Level of HDL is significantly lower in diabetic patients with IHD than diabetic patients without IHD (*P* =0.001). Diabetic patients with IHD have significantly higher serum uric acid level than diabetic patients without IHD (*P* =0.0001) (Figure 2).

Table III shows a comparison between DM patients with and without µA. There was no significant difference between diabetic patients with and without µA with regard to gender, smoking and family history of IHD. The frequency of HT and PN are significantly higher in diabetic patients with µA (79% for both) than in diabetic patients without µA (38% and 25%, respectively) (*P* =0.001 and 0.0001, respectively). Diabetic patients with µA have significantly higher FBG, PPBG and HbA1c% than diabetic patients without µA (*P*=0.03, 0.0001 and 0.0001, respectively). Total cholesterol, TG and LDL levels are significantly higher in diabetic patients with µA than in diabetic patients without µA (*P* =0.02, 0.0001 and 0.0001, respectively). Duration of DM was significantly longer in diabetic patients with MA than diabetic patients without µA (*P* =0.01). Diabetic patients with µA are significantly more obese and older than diabetic patients without µA (*P* =0.001 for both). Level of HDL is significantly lower in diabetic patients with MA than diabetic patients without µA (*P* =0.02). Diabetic patients with µA have significantly higher SUA and creatinine levels than diabetic patients without µA (*P* =0.04 and 0.0001 respectively) (Figure 2).

Table IV shows a comparison between DM patients with and without PN. There was no significant difference between diabetic patients with and without PN with regard to gender, smoking, family history of IHD and total cholesterol and LDL levels. The frequency of HT is significantly higher in diabetic patients with PN (80%) than in those without PN (33% & *P* =0.0001). Diabetic patients with PN have significantly higher FBG, PPBG and HbA1c% than diabetic patients without PN (*P* =0.001, 0.0001 and 0.0001, respectively). Duration of DM was significantly longer in diabetic patients with PN than in diabetic patients without PN (*P* =0.0001). Diabetic patients with PN have significantly higher SUA and creatinine levels than diabetic patients without PN (*P* =0.04 and 0.0001, respectively) (Figure 2). Diabetic patients with PN are significantly older than diabetic patients without PN (*P* =0.0001). Diabetic patients with PN have significantly higher TG level and lower HDL level than diabetic patients without PN (*P* =0.001 and 0.0001, respectively). Diabetic patients with PN are comparable in terms of body weight to those without PN.

Interestingly, non-parametric correlation coefficient between the evaluated micro- and macro-angiopathic complications in our T2 diabetic patients with HU was significant. Precisely, the exact level of significance between PN and µA and PTCA were 0.015, and 0.048, respectively while the level of significance between µA and PTCA was 0.007 as seen in Table V of correlation matrix.

Table I. Comparison between DM patients with and without hyperuricemia:

VARIABLE	DM with Hyperuricaemia (n=18)	DM without Hyperuricaemia (n=42)	P-value
Age (years)	54.6 ± 9.3	51.3 ± 8.3	0.1
Gender (Male %)	39% (n = 7)	45% (n = 19)	0.9
Smoker %	22% (n = 4)	43% (n = 18)	0.2
DM duration (years)	11.1 ± 5.1	7.7 ± 5.6	0.01*
Arterial hypertension%	78% (n = 14)	48% (n = 16)	0.04*
BMI (kg/m2)	26.8 ± 2.2	25.1 ± 2.1	0.001*
Family history of IHD %	44%(n=8)	24%(n=10)	0.1
IHD %	67%(n=12)	5%(n=2)	0.0001*
Peripheral neuropathy %	78%(n=14)	38%(n=16)	0.01*
Microalbuminuria %	78%(n=14)	33%(n=14)	0.001*
FBG (mg/dl)	148.1 ± 32.4	131.9 ± 44.7	0.02*
PPBG (mg/dl)	252.3 ± 78.6	204 ± 55.5	0.01*
HbA1c (%)	7.5 ± 0.6	7.1 ± 0.5	0.01*
Total Cholesterol (mg/dl)	206.4 ± 44.4	176.2 ± 62.2	0.02*
HDL (mg/dl)	29.3 ± 7.3	37.2 ± 8.7	0.001*
LDL (mg/dl)	143.3 ± 38.5	135.8 ± 47.9	0.2
Triglycerides (mg/dl)	291.3 ± 108.3	221.9 ± 86.7	0.001*
Creatinine (mg/dl)	2 ± 1.3	1.1 ± 0.7	0.001*

DM= diabetes mellitus BMI=body mass index FBG=fasting blood glucose
PPBG=post-prandial blood glucose HDL=high-density lipoprotein L=low-density lipoprotein
IHD=ischaemic heart disease n=number *=significant.
Data are expressed as mean±SD, unless otherwise stated.

Table II: Comparison between DM patients with and without IHD:

VARIABLE	DM with IHD (n=14)	DM without IHD (n=46)	P-value
Age (years)	57.3 ± 9.9	50.4 ± 7.7	0.001*
Gender (Male %)	43% (n = 6)	43% (n = 20)	1
Smoker %	43% (n = 6)	35% (n = 16)	0.7
DM duration (years)	14.4 ± 6.7	6.8 ± 3.7	0.0001*
Arterial hypertension%	86% (n = 12)	48% (n = 22)	0.02*
BMI (kg/m2)	27.2 ± 2.1	25.1 ± 2	0.001*
Family history of IHD %	57%(n=8)	22%(n=10)	0.02*
Uric acid (mg/dl)	7.3 ± 0.9	5.1 ± 1.3	0.0001*
Peripheral neuropathy %	86%(n=12)	39%(n=18)	0.001*
Microalbuminuria %	100%(n=14)	30%(n=14)	0.0001*
FBG (mg/dl)	154.8 ± 30.4	132.5 ± 44.6	0.001*
PPBG (mg/dl)	271.6 ± 57.2	205.6 ± 67.6	0.0001*
HbA1c (%)	7.8 ± 0.5	7.1 ± 0.4	0.0001*
Total Cholesterol (mg/dl)	227.9 ± 50	172.2 ± 54.8	0.0001*
HDL (mg/dl)	28.3 ± 7.2	36.7 ± 8.8	0.001*
LDL (mg/dl)	159.2 ± 40.6	132.3 ± 45.1	0.001*
Triglycerides (mg/dl)	331.3 ± 87.5	217 ± 86.2	0.0001*
Creatinine (mg/dl)	2.4 ± 1.2	1 ± 0.6	0.0001*

DM= diabetes mellitus BMI=body mass index FBG=fasting blood glucose
PPBG=post-prandial blood glucose HDL=high-density lipoprotein
LDL=low-density lipoprotein, IHD=ischaemic heart disease n=number
*=significant. HbA1c= Glycated haemoglobin
Data were expressed as mean±SD

Table III. Comparison between DM patients with and without µA:

VARIABLE	DM with microalbuminuria (n=28)	DM without microalbuminuria (n=32)	P-value
Age (years)	55.4 ± 9.1	49.6 ± 7.3	0.001*
Gender (Male %)	50% (n = 14)	37% (n = 12)	0.4
Smoker %	43% (n = 12)	31% (n = 10)	0.4
DM duration (years)	11.3 ± 6.8	6.5 ± 3.2	0.01*
Arterial hypertension%	79% (n = 22)	38% (n = 12)	0.001*
BMI (kg/m2)	26.6 ± 2.1	24.8 ± 2	0.001*
Family history of IHD %	36%(n=10)	25%(n=8)	0.4
Uric acid (mg/dl)	6 ± 1.7	5.2 ± 1.2	0.04*
Peripheral neuropathy %	79%(n=22)	25%(n=8)	0.0001*
FBG (mg/dl)	144.1 ± 32.4	130.4 ± 48.1	0.03*
PPBG (mg/dl)	254.8 ± 65.5	186.8 ± 49.5	0.0001*
HbA1c (%)	7.6 ± 0.5	7 ± 0.3	0.0001*
Total Cholesterol (mg/dl)	204.5 ± 57.5	168.6 ± 55.5	0.02*
HDL (mg/dl)	32.3 ± 9.3	37.1 ± 8.3	0.02*
LDL (mg/dl)	160 ± 45.4	118.9 ± 35.5	0.0001*
Triglycerides (mg/dl)	298.9 ± 98.3	193.6 ± 67.5	0.0001*
Creatinine (mg/dl)	1.9 ± 1.2	0.9 ± 0.3	0.0001*

DM= diabetes mellitus BMI=body mass index FBG=fasting blood glucose PPBG=post-prandial blood glucose HDL=high-density lipoprotein LDL=low-density lipoprotein IHD=ischaemic heart disease n=number *=significant
HbA1c= Glycated haemoglobin µA=microalbuminuria
Data were expressed as mean±SD.

Table IV. Comparison between DM patients with and without PN:

VARIABLE	DM with peripheral neuropathy (n=30)	DM without peripheral neuropathy (n=30)	P-value
Age (years)	56.4 ± 7.3	48.2 ± 7.9	0.0001*
Gender (Male %)	47% (n = 14)	40% (n = 12)	0.8
Smoker %	47% (n = 14)	27% (n = 8)	0.2
DM duration (years)	11.9 ± 6.1	5.5 ± 2.6	0.0001*
Arterial hypertension%	80% (n = 24)	33% (n = 10)	0.0001*
BMI (kg/m2)	26.2 ± 2	25.1 ± 2.4	0.06
Family history of IHD %	27%(n=8)	33%(n=10)	0.8
Uric acid (mg/dl)	6 ± 1.6	5.2 ± 1.3	0.04*
FBG (mg/dl)	145.5 ± 32.1	128.1 ± 48.6	0.001*
PPBG (mg/dl)	252.9 ± 66.4	184.2 ± 46.3	0.0001*
HbA1c (%)	7.5 ± 0.5	7 ± 0.3	0.0001*
Total Cholesterol (mg/dl)	194.5 ± 59	176.2 ± 58.2	0.2
HDL (mg/dl)	31.1 ± 8	38.6 ± 8.5	0.0001*
LDL (mg/dl)	146.1 ± 46.5	130.1 ± 43	0.2
Triglycerides (mg/dl)	284.7 ± 100.7	200.7 ± 76.1	0.001*
Creatinine (mg/dl)	1.7 ± 1.1	1 ± 0.7	0.0001*

DM= diabetes mellitus BMI=body mass index FBG=fasting blood glucose
PPBG=post-prandial blood glucose HDL=high-density lipoprotein LDL=low-density lipoprotein,
IHD=ischaemic heart disease n=number *=significant.
PN=peripheral neuropathy HbA1c= Glycated haemoglobin
Data were expressed as mean±SD, unless otherwise stated.

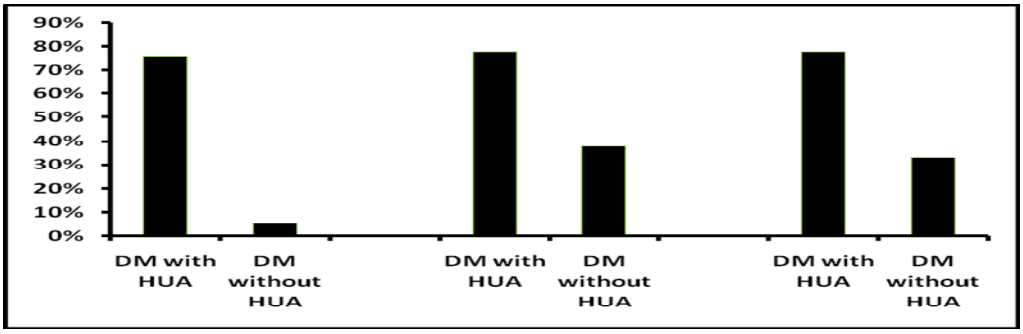

Figure 1: Frequency of ischaemic heart disease (IHD), microalbuminuria (µA) and peripheral neuropathy (PN) in diabetic patients with and without HU.

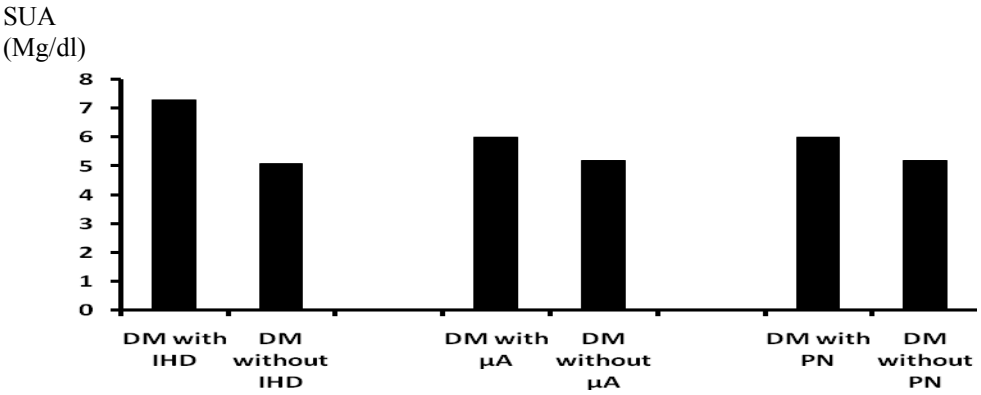

Figure 2: Level of uric acid in diabetic patients with and without the observed micro- and macro-angiopathies; ischaemic heart disease (IHD), microalbuminuria (µA) and peripheral neuropathy (PN).

Table V: Spearman's correlation matrix for micro- and macro- angiopathic Complications among hyperuricaemic T2 diabetic patients.

VARIABLES	Spearman's Correlation	PN	µA	STRESS ECG	PTCA
Peripheral Neuropathy	Correlation Coefficient	1.000	.564	.189	.472
	Sig. (2-tailed)	.	.015	.453	.048
	N	18	18	18	18
Microalbuminuria	Correlation Coefficient	.564	1.000	.614	.614
	Sig. (2-tailed)	.015	.	.007	.007
	N	18	18	18	18
STRESS ECG	Correlation Coefficient	.189	.614	1.000	.500
	Sig. (2-tailed)	.453	.007	.	.035
	N	18	18	18	18
PTCA	Correlation Coefficient	.472	.614	.500	1.000
	Sig. (2-tailed)	.048	.007	.035	.
	N	18	18	18	18

PTCA=Percutaneous transarterial coronary angiography N=number

4. Discussion

In the present study, we noticed an appreciable frequency of hyperuricaemia (HU) in our T2 diabetics and the same observation was reported by others (21, 22). Serum uric acid (SUA) may inhibit glucose-induced insulin secretion via binding to an essential arginine residue in pancreatic β-cells (23). By contrast, some studies reported that serum uric acid is inversely associated with diabetes mellitus (24, 25). Moreover, Wen and co-workers (2010) described a negative correlation between SUA and the blood glucose level in an approximately half a million population in Taiwan (26). A plausible mechanism for the reported conflicting results may either be related to the inhibition of uric acid reabsorption in the proximal tubule by high glucose levels in diabetic individuals (7) or the difference in the studied population.

Markers of poor glycemic control evaluated in this investigation such as FBG, PPBG and HbA1c were remarkably higher in patients with than in those without HU. This would be explained by the ability of high SUA to augment insulin resistance and to promote the impaired insulin secretion by its pro-oxidative capacity as recently reported (27).

Our results showed that hyperuricemic individuals had higher rates of coexistence of other cardiovascular risk factors such as hypertension, hypercholesterolemia, hypertriglyceridemia, and hypo-HDL cholesterolemia than those of non-hyperuricemic individuals. Our findings were not different from that noticed by Nagahamas and associates (2004) who reported that subjects with HU had increased rates of coexisting CV risk factors such as HT, hypertriglyceridemia, and dyslipidemia than did subjects without hyperuricemia (28). Insulin resistance may constitute a common pathophysiologic feature of obesity, hypertension, dyslipidemia, and hyperuricemia. We conjectured that insulin resistance might have affected the interaction between hyperuricemia and the cardiovascular risk factors and there is evidence that the association between SUA concentration and insulin resistance may be mediated by increased triglyceride (29).

Although the measurements of BMI have not reached the defining level of obesity in our study cohort, yet those with HU had significantly higher values than those without HU. Some previous studies that focused on general populations reported that the SUA levels were positively correlated with BMI correlated independently (30) in general as well as with both the visceral the subcutaneous fat areas; particularly, the serum uric acid levels were more closely correlated with the visceral fat (31, 32).

Our study investigated the association between diabetic macro-vascular complications and the existence of HU. Our data suggested that IHD was clearly more prevalent in the patients with compared with those without HU and that T2DM patients with IHD had significantly higher SUA than those without IHD. This is in concordance with the work done by Rathmann and colleagues (1993) as they reported significant association between HU coronary heart disease (CHD) in their 4,047 type 2 diabetic patients (33). The discrepancy between our findings and that observed by Ong and coworkers is merely apparent as they were interested in studying the CV and all-cause mortality in their 1,268 type 2 diabetic in Western Australia (16).

The frequency of hypertension (HT) was significantly higher in the patients with HU than in those with normal SUA in the current study. The correlation between hypertension and HU has been well established in clinical studies as several groups have reported that higher levels of SUA might be a marker of susceptibility or an intermediate step in the pathways leading to hypertension (34) and independently increase not only the risk for the development of hypertension (30) and but also strongly predicted BP progression (35). In a series of elegant experiments in rats, it was demonstrated that HU trigged HT and impaired nitric oxide (NO) generation in the macula densa and both HT and renal injury were reduced by the NO precursor l-arginine treatment (36). On the other hand, another study found that the proportion of hypertensive and non hypertensive patients with HU was comparable and there was no association between SUA and blood pressure readings (37). Such difference could be attributed to fact that they studied Chinese patients who are definitely different from our Egyptian population.

In the present study, the frequency of µA is significantly higher in the patients with HU compared with those without HU and T2diabetics patients with µA have significantly higher SUA level than diabetic patients without µA. Studies conducted in the past few years provided new evidence to support the observation that elevated SUA is a novel yet well-established risk factor for renal dysfunction (38). A more recent report confirmed the fact that HU is an independent risk factor for the progression of renal dysfunction (5).

Tseng and associates (2005) (39) and Fukui and colleagues (2008) (40) reported that the SUA level was elevated, along with increased UAE and reduced glometrular filtration rate (GFR) in their studied population with type 2 diabetes using a cross-sectional design. The relationship between renal function and uric acid has been known for a long time and the renal lesion consists of variable degrees of arteriolosclerosis, glomerulosclerosis, and interstitial fibrosis, often with focal deposition of urate crystals in the outer medulla. It is, in fact, an interrelationship, as excess uric acid impairs renal function and insufficient kidneys filter uric acid out of the circulation to a lower degree. The significance of HU is further emphasized by studies showing that reducing its concentration with allopurinol results in a slower development of renal failure in a parallel reduction of CV risk (41).

Of significance was our observation in the present study that SUA levels were increased in the presence of peripheral neuropathy (PN) among patients with T2DM. The presence of HU and dyslipidemia in our T2diabetic patients with PN is in concordance with what has previously been shown that peripheral neuropathy is associated with increased TGs and TC, but not HDL-C, in type 2 DM (42). Despite some minor differences in the lipid fractions involved, these findings highlight the importance of dyslipidaemia in diabetic neuropathy. Our results are in agreement with experimental evidence that elevated TG is neurotoxic via oxidative stress, independently of hyperglycemia (43). Others have also implicated hyperlipidaemia in diabetic PN and identified elevated serum lipids as a potential additional therapeutic target in the management of this micro-vascular complication (44, 45).

Importantly, we observed a remarkable association between PN, µA and CHD which represent the three facets of cardiovascular complications detected among the studied T2 diabetics with HU. The mechanisms that could possibly link HU to the observed macro- and micro-vascular complications might include its deleterious effects on platelet adhesiveness and aggregation, on endothelial dysfunction which is thought to be an important early step in the development of atherosclerosis (46), oxidative stress through the xanthine oxidase pathways (47) as well as its capacity to increase in reactive oxygen species (ROS) by activation of the NADPH oxidase, and sustained inflammation (27) and recently, a close association between elevated SUA and numerous markers of inflammation such as white blood cells, TNF-α, CRP (48) or interleukin-6 (49).

Therefore, since HU is potentially a modifiable factor, measuring SUA that is cheap and logistically easily available, it might provide a cost-beneficial investigational tool allowing identification at risk T2 diabetic patients who may benefit from a full work up for the risk factors associated with micro- and macro-angiopathies. Conversely, comprehensive and meticulous clinical examination is mandatory to detect the vascular complications at bed-side level when HU is found in patients with type 2 diabetes mellitus. Collectively, these findings provide support for aggressive management of cardiometabolic risk factors including HT and dyslipidaemia along with lifestyle modifications in T2 diabetic patients with hyperuricemia. Although allopurinol administration to lower SUA concentrations has been shown to be beneficial in conditions such as post coronary artery bypass surgery where it reduced ischemic events and produced less ST

segment depression (50) As well as in improving endothelial dysfunction and reducing oxidative stress burden in patients T2 DM (51) yet it should not be prescribed except for those with clear-cut indications to avoid the serious allopurinol toxicity.

In conclusion, the cheap, usually available and modifiable serum uric acid elevations we observed to prevail in T2 diabetic patients would be a useful investigational tool to prompt a cost-effective search for other cardiovascular risk factors known to cluster in them as well as meticulous clinical examination for a possible presence of diabetic micro- and macroangiopathies such as ischaemic heart disease, renal dysfunction and peripheral neuropathy.

Limitations:

Of the limitations to be declared are the small sample size of this study and its performance in a single centre besides absence of control group. Further, exclusion of those with chronic disabilities as well as major psychiatric disorders for sure would limit the generalization of our finding to the wider Egyptian population.

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RASSF1A Gene Hypermethylation in Tissue and Serum Together with Tissue Protein Expression in Breast Cancer Patients

Heba H. Gawish [1], Hoda A. Hagrass[1]andEman H. Abdel Bary[2].

[1]Clinical **P**athology **D**epartment,Faculty of **M**edicine, Zagazig University, Egypt
[2]**P**athology **D**epartment, Faculty of **M**edicine, ZagazigUniversity ,Egypt
hebagawish@yahoo.com

Abstract: Background: Recently genetics and epigenetics alterations have been found to be characteristic of malignancy and hence can be used as targets for detection of neoplasms. RASSF1A genehypermethylation has been a subject of interest in recent researches on cancer breast patients. **Design and methods:** We investigated 30 breast cancer patients and 10 control subjects diagnosed with benign lesions of the breast for RASSF1A methylation status in paired tissue and serum samples using MSP and we evaluated RASSF1A protein expression in tissues by IHC .Results were studied in relation to known prognostic clinicopathplogical features in breast cancer. **Results:** We evaluated 30 breast cancer patients mean age (50.9±7.7) years and 10 control patients mean age (38.4±8.6 **years**). Frequency of RASSF1A methylation in tissues, serum were 73% and 63.3% respectively and RASSF1A protein expression showed frequency of 46.7%. There was an association between RASSF1A methylation in tissues, serum and loss of protein expression in tissues with invasive carcinoma, advanced stage breast cancer, L.N metastasis, ER/PR negativity and HER2 positivity.RASSF1A methylation in serum showed high degree of concordance with methylation in tissues (Kappa =0.851, P <0.001). **Conclusion:** RASSF1A hypermethylation in tissues and serum and its protein expression may be a valid, reliable and sensitive tool for detection and follow up of breast cancer patients.
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Key words:RASSF1A;hypermethylation;MSP;cancer breast.
Abbreviations: RASSF1A, RAS association domain family protein 1A; PCR,Polymerase chain reaction; CIS, carcinoma in situ;IHC, immunohistochemistry.

1-Introduction:

Breast cancer is the most common cancer and the second most common cause of death from cancer in women. Every year more than one million women are diagnosed with breast cancer and approximately 400,000 die[(1)].Breast cancer is the most common malignancy among Egyptianwomen[(2)],.For successful treatment and outcome, early detection of breast cancer is a necessity. Despite the availability of mammography and prevalence of self-examination, there is still additional benefit to be gained from additional screening methodologies.

The genetic and epigenetic alterations that initiate and drive tumorgenesis can be used as targets for detection of neoplasms in body fluids[(3)],because they may precede clinically obvious cancer, can be detected at sensitive levels, may be specific for tumor cells, and can potentially provide information about the prognosis and treatment of the disease[(4,5)]. CpG islands located in promoter regions of genes are normally unmethylated. In cancer cells, aberrant hypermethylation of these promoter regions is associated with transcriptional silencing. Hypermethylation is therefore an alternative mechanism for inactivation of tumor suppressor genes[(6,7)].

Also It has been found that gene hypermethylation is a common and early alteration in many tumor types[(8-10)], including breast[(11,12)], hence it is considered as a promising target for detection strategies in clinical specimens[(4,5)].

RASSF1 encodes several isoforms, including *RASSF1A*, *RASSF1B*, and *RASSF1C*, which are derived from alternative mRNA splicing and promoter usage[(13)].

RAS association domain family protein 1A (*RASSF1A*) methylation status has been examined in different tumors[(13,14,15)] and breast cancer[(3,14)]. *RASSF1A* identified at 3p21.3 was suggested as the major target tumor suppressor on the basis of its frequent epigenetic silencing[(13)].It was reported previously that *RASSF1A* is epigenetically inactivated in 40 –72% of primary lung tumors by *de novo* methylation at the CpG island in the promoter[(16,17,14)]. Methylation-associated inactivation of *RASSF1A* was also observed in a considerable proportion of breast, ovarian, and nasopharyngeal cancer cell lines and primary tumors[(14,17-20)]. In small cell lung cancers, allelic deletion at 3p21.3 is associated with *RASSF1A* methylation, suggesting that both genetic and epigenetic steps are crucial for *RASSF1A* inactivation in some tumor types. The tumor suppressor function of RASSF1A has been suggested by observations that exogenous expression of RASSF1A decreases *in vitro*-colony formation, suppresses anchorage-independent growth, and dramatically reduces tumorigenicity*in*

vivo[16,17]. With these tumor suppression effects, the presence of a RAS association domain suggests that RASSF1 proteins may function as effector molecules in Ras or related growth inhibitory signaling pathways.

Aim of the Work:

In this study we aim to study the methylation status of RASSF1A gene in paired serum and tissue samples in cancer breast patients together with immunohistochemical analysis of RASSF1A protein. Results will be studied in relation to prognostic clinicopathological features in a trial to reveal RASSF1A gene role in prognosis.

2-Materials and Methods

2.1Specimen Collection:

Thirty consecutive patients diagnosed with breast cancer who were admitted to Zagazig University hospitals, in the period from January 2011to December 2011, were enrolled in this study. Patients ages ranged from 34 to 62 years. There were 5 cases of ductal CIS, 2 lobular CIS, 20 invasive ductal, and 3 invasive lobular carcinomas. Matched preoperative serum and tissue specimens were obtained from breast cancer patients and from control group that included 10 patients with benign breast lesions (7 fibroadenomas; 3 fibrocystic changes).

As regards tissue samples, Four µm thick sections from formaline-fixed, paraffine-embedded tissue blocks were stained with hematoxylin–eosin for morphological assessment. Tumors were evaluated for tumor grade using the Elston and Ellis grading system for invasive carcinoma, and the criteria of the European Breast Screening Group for DCIS, and tumor stage based on TNM, according to the 2003 WHO classification of breast tumors[20].

Ethical consideration: A written consent was taken from all of the participants after explaining details, benefits as well as risks to them.

2.2 Immunohistochemistry

Immunohistochemical staining was carried out using streptoavidin-biotin immunoperoxidase technique (Dako-cytomation, Glostrup, Denmark). Three µm thick sections, cut from formalin fixed paraffin embedded blocks, were deparaffinized in Xylene and rehydrated in graded alcohol. Sections were boiled in citrate buffer (pH 6.0) for 20 min for antigen retrieval and then washed in phosphate buffer saline (pH 7.3). Blocking of endogenous peroxidase activity by 3% H_2O_2 in methanol was attained. The slides were then incubated over night with the monoclonal antibodies: anti-RASSF1A (mouse monoclonal IgG, clone 3F3, code number AB23950), anti-ER(mouse monoclonal IgG, code number sc-56833, Santa Cruz Biotechnology, CA), anti-PR (rabbit polyclonal IgG, code number sc-539, Santa Cruz Biotechnology, CA) and anti-HER2 (mouse monoclonal IgG, code number sc-33684, Santa Cruz Biotechnology, CA). Incubation with secondary antibody and product visualization was performed employing (DakoCytomation, Glostrup, Denmark) method with Diaminobenzidine (DAB) substrate chromogen. Slides were finally counterstained with Mayer's haematoxylin. The primary antibody was replaced by phosphate buffer solution (PBS) for negative controls.

RASSF1A protein expression appeared as yellowish brown staining in the cytoplasm of the cells. Positivestaining in more than 10% of tumor cells in the examined area was considered.we calculated a score (intensity x % area) for each tumor as follows: weak <100, moderate 100–200, and strong >200. Then a score equal or over 100 was considered positive expression, and bellow 100 considered as significant loss of expression[22].

2.3 DNA Extraction: DNA was extracted from fresh frozen tissue or from blood using a standard technique according to the manufacturer's instructions(QIAampDNAMinikit,QIAGEN GmbH, Hilden, Germany).

2.4 Methylation Analysis: Specimen DNA was modified with sodium bisulfite, converting all unmethylated, but not methylated, cytosine to uracil followed by amplification with primers specific for methylated versus unmethylatedDNA[23]*by* using a commercial kit (EpiTect Bisulfite,QIAGEN GmbH, Hilden, Germany) according to the manufacturer's instructions in brief the procedure comprises a few simple steps: bisulfite-mediated conversion of unmethylatedcytosines; binding of the converted single-stranded DNA to the membrane of an EpiTect spin column; washing; desulfonation of membrane-bound DNA; washing of the membrane-bound DNA to remove desulfonation agent; and elution of the pure, converted DNA from the spin Column then kept at -20°C for further using.

2.5 Methylation-specific PCR analysis:

PCR was performed with methylation specific primers RASSF1A (U)
F(5′TGGTTTTTTTTAGTTTTTTTTTGTT-3′)
R(5′ACTACCATATAAAATTACACACA-3′)
RASSF1A (M)
F(5′GGTTTTTTTTAGTTTTTTTTCGTC-3′)
R(5′- CTACCGTATAAAATTACACGCG -3′)
using 200 ng of the bisulfite-modified genomic DNA as templates and EpiTect MSP kit (QIAGEN GmbH, Hilden, Germany) kit ,the cycling conditions consisted of an initial denaturation step at 95°C for 5 min, followed by 35 cycles of 94°C for 30 s, 57 °C for 30 s, and 72°C for 45 s the PCR products (15 µl) were resolved on a 2% agarose gel.

2.6 Statistical analysis:

Data was analyzed using SPSS win statistical package version 17 (SPSS Inc., Chicago, IL). Chi-square test or Fisher's exact test was used to examine the relation between qualitative variables. For not-normally distributed quantitative

data, comparison between two groups was done using Mann-Whitney test. Odds ratio (OR) with it 95% confidence interval (CI) were used for risk estimation. A p-value < 0.05 was considered significant.

3- Results:

In the current study we evaluated 30 breast cancer patients mean age (50.9±7.7)years and 10 patients diagnosed as benign breast lesions (7 fibroadenomas and 3 fibrocystic change) used as a control group. The mean age was 38.4±8.6 years. All patients were subjected to clinical and histopathologicalevaluation .Both patients and control groups were evaluated for RASSF1A gene hypermethylation in paired tissue and serum samples, furthermore RASSF1 protein expression in tissues was evaluated by Immunohistochemistry.

Demographic and clinicopathologic data of breast cancer patients and their frequencies as regards RASSF1A methylation status in tissues and serum and RASSF1A protein expression are shown in table (1).

	RASSF1 in tissue		RASSF1 in serum		RASSF1 IHC score	
Frequency	M	U	M	U	<100	≤ 100
N (%)	31 (70%)	9 (30%)	19(63.3%)	11(36.7%)	16(53.4%)	14(46.6%)
Age: < 50 y n= 13 (43.3)	8 (61.5)	5 (38.5)	7 (53.8)	6 (46.2)	6 (46.2)	7 (53.8)
≤ 50 y n= 17 (56.7)	13 (76.5)	4 (23.5)	12 (70.6)	5 (29.4)	10 (58.8)	7 (41.2)
***P*- value**	**0.376**		**0.346**		**0.491**	
Type:						
carcinoma in situ n=7 (23.3)	3(42.9)	4(57.1)	2(28.6)	5(71.4)	3(42.9)	4 (57.1)
Invasive carcinoma n=23 (76.7)	18(78.3)	5(21.7)	17(73.9)	6(26.1)	13(56.5)	10 (43.5)
***P*- value**	**0.153**		**0.068**		**0.675**	
Low grade (I, II) n=14 (46.7)	9(64.3)	5(35.7)	8(57.1)	6(42.9)	5 (35.7)	9 (64.3)
High grade (III) n= 14 (46.7)	12(85.7)	2(14.3)	11(78.6)	3(21.4)	10 (71.4)	4 (28.6)
***P*- value**	**0.385**		**0.42**		**0.058**	
Early stage (0& I) n=12 (40)	5(41.7)	7(58.3)	4(33.3)	8(66.7)	4 (33.3)	8 (66.7)
Advanced stage (II& III) n= 18 (60)	16(88.9)	2(11.1)	15(83.3)	3(16.7)	12 (66.7)	6 (33.3)
***P*- value**	**0.006**		**0.005**		**0.073**	
ER						
-ve n=9 (30)	9(100)	0(0)	9(100)	0(0)	8 (88.9)	1 (11.1)
+ve n=21 (70)	12(57.1)	9(42.9)	10(47.6)	11(52.4)	8 (38.1)	13 (61.9)
***P*- value**	**0.019**		**0.006**		**0.017**	
PR						
-ve n=13 (43.3)	13(100)	0(0)	13(100	0(0)	10 (76.9)	3 (23.1)
+ve n=17 (56.7)	8(47.1)	9(52.9)	6(35.3)	11(64.7)	6 (35.3)	11 (64.7)
***P*- value**	**0.002**		**< 0.001**		**0.024**	
HER2						
-ve n=15 (50)	7(46.7)	8(53.3)	7(46.7)	8(53.3)	7 (46.7)	8 (53.3)
+ve n=15 (50)	14(93.3)	1(6.7)	12(80)	3(20)	9 (60)	6 (40)
***P*- value**	**0.014**		**0.058**		**0.464**	
Lymph node						
-ve n=20 (66.7)	12(60)	8(40)	10(50)	10(50)	8 (40)	12 (60)
+ve n=10 (33.3)	9(90)	1(10)	9 (90)	1(10)	8 (80)	2 (20)
***P* -Value**	**0.091**		**0.032**		**0.038**	

3.1 *Comparison among different clinicopathological groups as regards RASSF1A:*

There was near significant difference (***P***=0.153) between in situ and invasive carcinoma when compared as regards RASSF1A methylation in tissues , similarly it showed near significant difference (***P***=0.068) when compared as regards methylation status in serum, as there was association of RASSF1A methylation with invasive breast cancer. while there was no statistical difference when compared as regards RASSF1A protein expression in tissue.

Comparison between low grade and high grade tumors (cut off point was grades I& II versus

Grade III). patients showed none significant difference when compared as regards methylation status in tissues and serum, while it showed near significant difference when compared as regards RASSF1A protein expression (***P***=0.058) .with higher frequency of loss of protein expression in tissues of high grade patients.

We found that when comparing early stage to advanced stage patients (cut of point was stage 0 & I versus stage II & III) as regards RASSF1A methylation in tissues and serum , it showed significant difference (***P***=0.005, 0.006, respectively) between both groups with association of RASSF1A methylation and advanced tumor stage, while when compared as regards RASSF1A protein expression in tissues it showed near significant difference (***p***=0.073) with higher frequency of loss of protein expression in tissues with advanced stage patients.

Patients without lymph node metastasis were compared to patients with LN metastasis as regards RASSF1A methylation in tissue and serum and protein expression in tissues , near significant difference was found between the two groups (***P***=0.091) when compared in tissue . A statistical significant difference was found when compared in serum or protein expression in tissues (***P***= 0.032, 0.038 respectively), as there was an association between methylation in tissue and serum on one hand and lymph node metastasis on the other, moreover lymph node metastasis was associated with loss of protein expression.

3.2 Comparison according to hormone receptors and HER2 status as regards RASSF1A:

In our study all patients were evaluated according to their hormone receptor status, we found that there was a significant difference between ER-ve and ER+ve patients as regards RASSF1A methylation in tissue, serum and protein expression in tissue (***P***= 0.019 , 0.006, 0.017, respectively) as there was higher frequency of methylation in tissues and serum in ER-ve patients, moreover there was an association between ER negativity and loss of protein expression.

There was a significant difference between PR-ve and PR+ve patients as regards RASSF1A methylation in tissue, serum and protein expression in tissue (***P***=0.002 ,<0.001, 0.024 respectively), there was an association between methylation in tissue and serum ,also loss of protein expression and PR-ve patients.

HER2-ve patients showed statistically significant difference from HER2+ve patients as regards RASSF1A methylation in tissue (***P***=0.014) while it showed near significant difference as regards methylation in serum (p=0.058) with higher frequency of methylation in HER2 +vepatients , while there was no significant difference as regards RASSF1A protein expression in tissues. Moreover Triple negative patients(ER-ve,PR-ve,HER2-ve) showed methylation in both tissue and serum and loss of protein expression in all 4 cases.

3.3 Case-control comparison and risk estimate:

10 patients diagnosed as benign lesions of the breast, there was a highly significant statistical difference between patients group and control group when compared as regards age(***P***<0.001) with the older age incidence in cancer breast patients.

Comparison between Breast cancer patients and control group as regards RASSF1A methylation in tissue and serum ,showed highly statistical significant difference(***P***<0.001) with risk estimate (odd's ratio 2.1, 1.9 respectively) 95% confidence interval (1.3-3.4) and (1.3-2.9), while it showed significant difference when both groups where compared as regards RASSF1 protein expression by IHC (***P***=0.003) with risk estimate (odd's ratio: 1.7), (95% confidence interval 1.2-2.4).

3.4 Measurement of agreement for RASSF1A in tissue, serum and protein expression by IHC:

In the present study, we evaluated the concordance (measurement of agreement)between RASSF1A methylation in tissue and serum it showed a highly significant agreement (Kappa=0.851, ***p***<0.001) with a sensitivity of serum testing 90.5% and a specificity 100%, while the positive predictive value of serum was 100% the negative predictive value was 81.1% in reference to RASSF1A methylation in tissue.

As for symmetric measures for both RASSF1A protein expression by immunohistochemistry compared to RASSF1A methylation in tissue showed significant measurement of agreement(kappa=0.521, p=0.004),while it showed non significant agreement between RASSF1A protein expression and methylation in the serum.

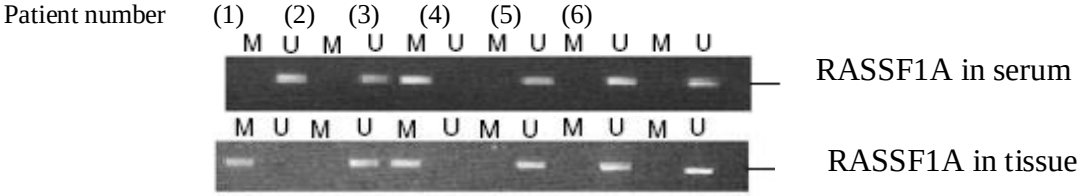

Fig.1. Representative samples of methylation specific PCR assays of RASSF1A in tissue and serum Methylated alleles (M) 269 bpunmethylated alleles (U) 271bp

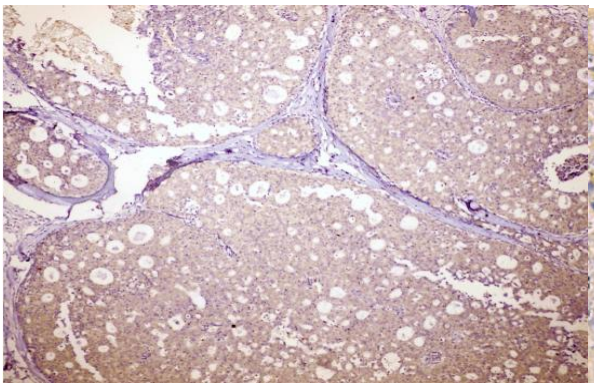

Fig. (2A): A case of ductal carcinoma in situ (UM) showing strong RASSF1A immunoreactivity (original magnification X 200)

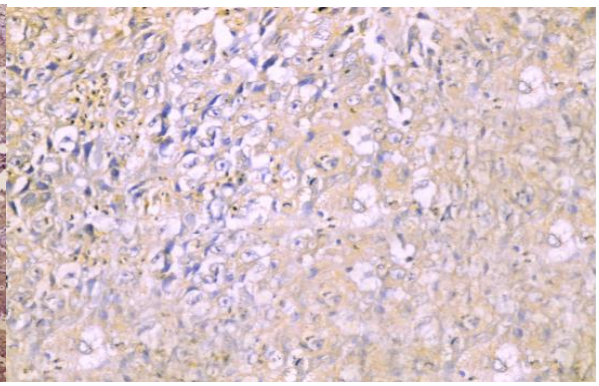

Fig. (2B): A case of invasive duct carcinoma (M) showing moderate RASSF1A immunoreactivity (original magnification X 400)

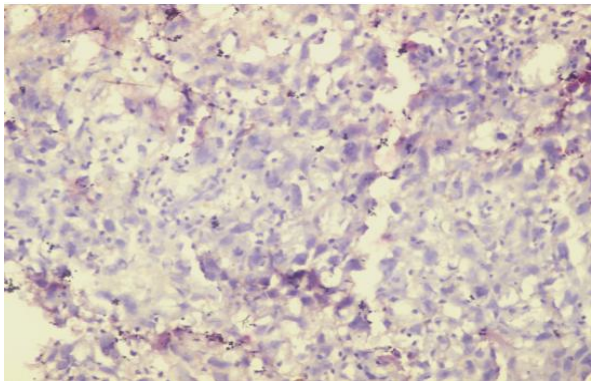

Fig. (2C): A case of invasive duct carcinoma (M) showing negative RASSF1A immunoreactivity (original magnification X 400)

4- Discussion:

Alteration in the methylation status of DNA are amongst the most frequent molecular changes associated with human cancers[24,4,25]. Aberrant promoter methylation has been described for several genes in various malignancies and the wide spectrum of genes involved suggest that specific tumors may have their own distinct methylation profile[25,26].

RASS1A gene has been a common factor in recent studies using a panel of genes to study hypermethylation in cancer breast patients[27-29].They tried to explore the role of RASSF1A and other genes epigenetics in the prognosis, early detection and differentiation between malignant and non malignant lesions.

Similarly, we conducted our study to explore the importance of RASSF1A gene methylation and protein expression in breast cancer patients and study the link with clinicopathological characteristics in an attempt to assess its role in prediction of prognosis.Moreover, we tried to assess the sensitivity of non invasive , accessible serum samples as a potential tool for follow up of patients.

In the current study, we investigated 30 breast cancer patients with mean age (50.9±7.7years) and 10 benign breast lesions(38.4±8.6years) ,as control group for RASSF1A methylation status in tissues ,and serum together with RASSF1A protein expression in tissues. We also studied clinicopathological features and hormone receptor status of cancer breast patients.

We found that there was no statistical significant difference between patients of different age groups (>,<50) as regards methylation status in tissue or serum or protein expression in tissues (***P***= 0.376,0.346,0.491 respectively). This is similar to previous studies[27,30]who didn't find any correlation between age and gene promoter methylation or protein expression. While it is different from another Tunisian study that found an association of age at diagnosis and methylation of RASSF1A gene (***P***=0.048) and they concluded that silencing of tumour suppressor gene by abnormal methylation is a prevalent event in tumors from younger patients[31]also other previous studies found association between age and methylation[32,33]. The discrepancy among studies may be explained by the fact that methylation profile of cancers is ethnicity specific[34,35].

Frequency of methylation of RASSF1A gene in tissues and serum was 70% and 63.3%respectively). In tissues it is lower than Karray –chouayekh***et al.***[31]who found that frequency of methylation in breast cancer patients is 87% and somehow it is comparable to another study by Park ***et al.***[28] who found that frequency of

methylation in tissues was 76% , ours was higher than another study[22] who found that methylation frequency among breast cancer patients was 67%.As regards serum , hypermethylation frequency in another study was 65%[3]. Regarding RASSF1A protein expression, 53.4% of our cases showed weak or absent expression. This is lower than the incidence in the work of Li ***et al.***[30] (72.2%). These differences may be attributed to different selection criteria and difference in sensitivity of MSP technique and anti-RASSF1A antibodies.

Comparison between in situ and invasive breast cancer as regards RASSF1A methylation in tissue, serum and protein expression revealed near significant difference between the groups with association of hypermethylation and invasive tumors. This is similar to a previous study[3] who found an association of hypermethylation of RASSF1A and invasive tumors .this can be explained by the fact that RASSF1A modulates multiple apoptotic cell cycle checkpoints pathways and hence its methylation may lead to progression of the disease[36,37].

As for protein expression in tissues, we found no significant difference between in situ and invasive carcinomas. However, Alvarez ***et al***.[22] found a significant decrease in protein expression in cases of in situ carcinoma which is contradicting our study this can be explained by the difference in genetic behavior among ethnic populations.

There was no statistical significant difference between low and high grade tumors as regards RASSF1A methylation in tissues and serum this is similar to a recent study[31]. While there was near significant difference (***P***=0.058) when compared as regards protein expression in tissues with association between loss of expression and high tumor grade.

These findings are different from Alvarez ***et al.***[22] who did not find any correlation between RASSF1A and protein expression this can be explained by difference in sample size and selection criteria.

In the present study we found that there was a statistical difference between patients diagnosed with early and advanced stages. this is similar to another study[31] who found an association between RASSF1A methylation and advanced tumor stage.(***P***=0.03).

On the other hand comparison between the same groups as regards protein expression in tissues showed near significant difference with association between loss of protein expression and advanced tumor stage, this is similar to a previous study[22]. This can be explained by the fact that promoter hyper methylation is a relevant molecular mechanism in inhibiting protein expression

Comparing patients with lymph node metastasis to patients without L.N metastasis as regards RASSF1A methylation in tissues showed near significant difference, and there was a statistical significant difference when compared as regards RASSF1A methylation in serum or protein expression in tissues, as there was an association between L.N metastasis and methylation in tissues and serum also an association with loss of protein expression in tissues. This is similar to a study by Muller ***et al.***[38]who found that L.N metastasis had a trend of high prevalence of methylation compared to the primary breast carcinoma which suggests that RASSF1A methylation may be a participant of key molecular pathways in tumor progression and aggressive tumor behavior.

In our study there was a significant association between RASSF1A methylation in tissue, serum and loss of protein expression and ER/PR negativity .This is similar to Sunami***et al.***[39]who found a strong correlation between double negative marker and hypermethylation. Similarly a recent study[36] found a strong correlation between ER/PR/HER2 triple negative and hypermethylation, this may have been explained by the possibility that RASSF1A methylation is associated with bad prognosis and poor clinical outcome, but the findings by previous studies[40,22,41,29,30] contradicted with our results as they found an association between ER/PR positivity and RASSF1A methylation , we recommend further studies in this context with larger number and more sensitive MSP techniques.

On the other hand a significant association between HER2 positivity and RASSF1A methylation in tissues and serum, but not with protein expression. Previous studies[31,40] found non significant correlation between methylation and Her2 status. The contradiction can be explained by the difference in distribution of grades and stages among patients.

In the present study all cases with RASSF1A methylation showed loss of protein expression in tissues, this is in agreement with Alvarez et al***et al.***[22] who found a highly significant association (***P***= 0.0063) between RASSF1A promoter hypermethylation and loss of protein expression, and they explained that promoter hypermethylation is a relevant molecular mechanism in inhibiting protein expression. furthermore, Li ***et al.***[30] suggested that methylation may be responsible for alleles silencing. The silencing of gene expression may also be explained by gene deletion or point mutation, tumors having deletion of RASSF1 and presenting M and UM PCR products ,show a significant loss of protein expression[22].

In the current study we compared our breast cancer patients to a control group(n=10) diagnosed as fibroadenomaand fibrocystic disease, they were all negative for RASSF1A methylation and strongly expressing RASSF1A protein.

We found that there was a highly significant statistical difference between patients and controls

as regards age, with older age incidence in cancer breast patients. This is similar to a recent study by Cho ***et al.***[29],this can be explained by the fact that cancer breast occurs in older age

Comparison between patients and control groups as regards RASSF1A methylation in tissue and serum showed highly significant difference with risk estimate (odd's ratio 2.1,1.9) respectively while it showed a significant difference as regards protein expression by IHC with risk estimate (odd's ratio1.7) .

This means that RASSF1A methylation and protein expression could be valuable tests in discrimination of malignant from non malignant breast lesions. This is consistent with a previous study[4] who stated that RASSF1A methylation could be used as a cancer molecular marker.

Also we are in agreement with several previous studies[42,43,44,38]who demonstrated that the acquisition of high level methylation at RASSF1A gene promoter and other studied genes is relevant for breast tumorgenesis, enabling their use as a specific breast cancer marker.

Aberrant promoter methylation needs to be used as a routine clinical test for breast cancer detection which obligates the use of more accessible samples,less painful and less intruding with female privacy.

In a trial to evaluate how serum samples can be trusted with suspecting, diagnosis and follow up of cancer patients , we studied the degree of concordance between RASSF1A methylation in tissues and serum ,we found that measurement of agreement showed high degree of concordance(Kappa=0.851, ***P*** <0.001)

Moreover we found that sensitivity of serum testing of RASSF1A was 90.5%, specificity 100% ,positive predictive value was 100% and negative predictive value was 81.1%. This is in context with Dulaimi***et al.***[3] who confirmed that hypermethylation can be detected by MSP in serum DNA and it can be considered as a screening method which may enhance early detection of breast cancer.

Moreover a recent study by Yamamoto ***et al.***[45] evaluated paired serum and tissue samples from breast cancer patients for detection of hyper methylation in a panel of genes including RASSF1A and concluded that the use of more sensitive MSP technique is promising for enhancing the sensitivity for diagnosis of metastatic breast cancer and moreover this can be used as a potential tumor marker for early detection of cancer breast. They also evaluated RASSF1A gene methylation before and after surgery and they found that it turned to be negative after surgery which confirms that the origin of serum DNA is the tumor itself.

Conclusion:

RASSF1A gene hypermethylation in tissue and serum together with loss of RASSF1A protein expression are associated with clinicopathological features of bad prognosis in breast cancer patients. RASSF1A hypermethylation in serum shows high concordance with hypermethylation in tissue and shows reasonable sensitivity and specificity. In this context RASSF1A may be used in prediction , early diagnosis , follow up in breast cancer patients.

Recommendations:

More researches should be done on gene hypermethylation including larger number of patients, and different panels of genes should be tried to come up with a panel that can be used as routine investigation for diagnosis and follow up of breast cancer patients. Indeed, researches on more accessible body fluids specially serum and blood plasma should be addressed for better screening procedures. Moreover, more sensitive MSP techniques should be enhanced and developed for more accurate detection.

Conflict of interest: None

Corresponding author
Heba H. Gawish
Clinical **P**athology **D**epartment,Faculty of **M**edicine, Zagazig University, Egypt
hebagawish@yahoo.com

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Modulatory Effects of Pomegranate Juice on Nucleic Acids Alterations and Oxidative Stress in Experimentaly Hepatitis Rats

Amal, A. Fyiad; Monira, A. Abd El-Kader and Abeer, H. Abd El-Haleem

Biochemistry Dept. Genetic Engineering and Biotechnology Division, National Research Centre,Cairo, Egypt.
amalfyiad@yahoo.com

Abstract : The present study was designed to test whether the pre-treatment with pomegranate juice could attenuate the nucleic acids alterations and oxidative stress that produced in D-Galactosamine /lipopolysaccharide induced hepatitis in rats. Animals were dosed with D-Galactosamine / lipopolysaccharide (300 mg kg^{-1} b.wt , i.p / 30 µg kg^{-1} b.wt , i.p) with or without pretreatment of pomegranate juice. The protective role of pomegranate juice was evaluated on the aspects of the release of hepatic enzymes into serum, the nucleic acids alterations**,** the formation of malondialdehyde, and the histopathological changes in hepatic tissues. Obtained results revealed that D-Galactosamine / lipopolysaccharide led to increase in the activities of serum marker enzymes such as aspartate transaminase, alanine transaminase and alkaline phosphatase, while there was a significant inhibition in deoxyribonucleic acid, ribonucleic acid and protein contents in liver tissues. Oxidative stress was also increased in hepatic tissue represented by increased malondialdehyde and decrease of antioxidants (superoxide dismutase, catalase and reduced glutathione). which accompanied with histopathological changes in the hepatic tissue.In addition; pretreatment with pomegranate juice (20 ml kg^{-1} b. w. day^{-1} for 14 days) effectively hindered the adverse effect of D-Galactosamine / lipopolysaccharide and protect against hepatic damage via suppression of oxidative stress. Histopathological studies of the liver of different groups also support the protective effects exhibited by pomegranate juice through restoring the normal hepatic architecture. In conclusion, pomegranate extract could afford a significant protection in the alleviation of D-Galactosamine / lipopolysaccharide –induced hepatiis.
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Key words: lipid peroxidation; antioxidants; D-galactosamine; nucleic acids; histopathology.

1. Introduction

Hepatitis is a major public health problem worldwide, responsible for considerable morbidity and mortality from chronic liver disease [1]. Endotoxin from gram negative bacteria (lipopolysaccharide: LPS) induces septic shock and finally wide variety of several organ disorders in human and animals [2]. On the other hand, D-galactosamine (GalN) highly sensitizes animals to develop lethal liver injury mimicking fulminant hepatitis when given together with a sublethal dose of lipopolysaccharide (LPS) [3,4]. A growing body of evidence is emerging which suggests that reactive oxygen-derived free radicals play a crucial role in the pathogenesis of LPS/D-GalN induced liver injury [5,6].

There is an urgent need for the clinical development of safe and non-toxic cytoprotective agents for the adequate management of hepatitis. Hence crude drugs or natural food diet which possess antioxidant or free radical scavenging activity has become a central focus for research designed to prevent or ameliorate tissue injury and may have a significant role in maintaining health [7]. .*Punica granatum*, commonly known as pomegranate, is a shrub or a small tree native, to the Mediterranean region. Edible parts of pomegranate fruit (about 50% of total fruit weight) comprise 80% juice and 20% seeds. Fresh juice contains 85% water, 10% total sugars, and 1.5% pectin, ascorbic acid, and polyphenolic compounds such as anthocyanins, punicalagin, ellagic and gallic acid. The soluble polyphenol content in pomegranate juice (PJ) varies within the limits of 0.2% to 1.0% depending on the variety [8]. The antioxidant activity of pomegranate associated with its phytochemicals, such as, polyphenols, flavonoids, and anthocyanidins has gained importance [9]. Fruits are globally consumed fresh, in such processed forms as juice, jam, wine and oil and in extract supplements [10]. PJ has anti-arthrogenic [11]; anti-carcinogenic [12], chondroprotective [13], anti-nephrolithiasis [14], anti-bacterial [15] and anti-gastric ulceration effects [16]. In addition pomegranate could modify the risk of hypercholesterolemia [17] and has photoprotective properties on skin [18]. The aim of the present study was to evaluate the efficacy of pre-supplementation with PJ against nucleic acid alterations and oxidative stress in hepatitis induced by D- GalN / LPS in rats.

2. Materials and Methods

Pomegranate juice preparation

The fresh pomegranate fruits, free of blemishes or obvious defects were washed and stored at 4°C until use. The fruits were manually peeled, without separating the seeds. PJ was obtained by squeezing using a commercial blender (Braun blender, Germany) and was filtered to remove the residue. The juice was used within 1 h after squeezing and filtration [13].

Animals:

The study was conducted on forty adult male Sprague –Dawley rats with body weights 120- 150 g, were obtained from the Animal House in National Research Centre, Giza, Egypt. Animals were housed in plastic cages at an environmentally controlled room (constant temperature 25-27°C, with 12h light / dark cycle) for one week prior to starting the experiments and they were fed on standard laboratory diet and water *ad libitum*.

Experimental protocol:

The animals were divided into four groups of rats each of ten.

G 1: served as vehicle control and received distilled water through oral route (20 ml kg^{-1} b. w, day^{1}) for 14 days.

G 2: rats were given PJ orally by gavages at a dose of 20 ml kg^{-1} b. w, day^{-1} for 14 consecutive days. The used dose was selected on the basis of the previous studies [13]. **G3**: rats were induced with hepatitis by giving i.p injections of D-GaIN and LPS (300 mg kg^{-1} b.w and 30 µg/ kg^{-1} b.w, respectively) which well known to induce hepatitis damage [19].

G 4: rats were pretreated with PJ for 14 days prior to the induction with D- GaIN / LPS as in G3.

Blood collection and tissue homogenate:

After the end of the treatment period, rats were fasted overnight and the blood samples were collected using capillary tubes from the retro-orbital plexus of the individuals of all groups. Samples were left to clot then centrifuged at 3000 rpm for 15 min to separate the sera, which were stored at −20ºC until analysis could be completed. The serum was used for the assay of aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP). After blood collection, all animals were rapidly sacrificed and the liver of each animal was dissected,weighted and portion of it was preserved in 10% formalin (pH7.2) and subjected to histopathological examination. The remaining part then divided into two parts, the first part for the determination of hepatic antioxidants and malondialdehyde (MDA) as marker for lipid peroxidation. The second part for the determination of nucleic acids and total proteins. The first part of liver of each animal immediately homogenized in 50mM ice –cold phosphate buffer (pH 7.4) to give 20% homogenate (W/V) [20]. The homogenate was centrifuged at 1700 rpm and 4ºC for 10 min. and the supernatant was stored at -70ºC to the next day until analysis. This supernatant (20%) was used for the determination of hepatic MDA and it was further diluted with phosphate buffer solution to give 2% and 0.5% dilutions for the determination of hepatic catalase (CAT), superoxide dismutase (SOD) activities and reduced glutathione (GSH) level. The second parts of the samples fresh or frozen was taken, blotted using filter paper, weighted and homogenized in 0.9% sodium chloride solution for 5 minutes at 0ºC for the determination of nucleic acids and total proteins.

Biochemical assays

Determination of serum AST, ALT and ALP

Serum AST and ALT were assessed spectrophotometerically by the dinitrophenylhydrazene method [21], using spectrophotometer **(Shimadzu – Model UV 2401).** ALP was assayed by spectrophotometric method described by [22]. Commercially available reagent kits for assays AST, ALT and ALP were purchased from Roche Diagnostic kits (Germany).

Determination of nucleic acids and proteins:

Nucleic acids were extracted from liver by using trichloracetic acid (TCA) and ethanol according to the methods of [23,24]. Hepatic tissue were homogenized (10%) in Tris-EDTA; 0.01 M using a Potter-Elvehjem homogenizer with a Teflon pestle. 5.0 ml of 10% TCA was then added to the homogenate (1 ml) and kept in ice for 30 minutes, to allow complete precipitation of proteins and nucleic acids. The mixture was centrifuged and the precipitate obtained was washed thrice with ice cold 10% TCA. It was then treated with 95% ethanol to remove lipids. The final precipitate was suspended in 5.0 ml of 5% TCA and kept in a water bath, maintained at 90ºC for 15 minutes with occasional shaking which facilitated the quantitative separation of nucleic acids from proteins. The supernatant after centrifugation was used for the estimation of DNA and RNA. Tissue DNA content was measured by the method described by [25]. The blue colour developed was read spectrophotometrically at 640 nm. The value was expressed as mg/g tissue. Tissue RNA content was measured by the method of [26]. The reading was taken at 655 nm and was expressed as mg/g tissue.

Protein measurement:

The total protein content in liver samples were assayed according to [27], and the level was expressed as mg protein /g tissue.

Determination of hepatic antioxidants and lipid peroxidation

Malondialdehyde was estimated as index for lipid peroxidation by spectrophotometric method [28] using Oxis Research™ Co. Kit (USA) and was expressed as nmol/g tissue. The assay for hepatic CAT activity was

carried out spectrophotometerically by the modified method of [29] using 50 μL diluted liver homogenate using kit purchased from Oxis Research™ Co., USA. Hepatic SOD activity was determined spectrophotometerically by red formazan dye reduction procedure [30] using 50 μL diluted liver homogenate using Ransod kit from Randox Laboratories Co., UK. Hepatic GSH concentration was assayed spectrophotometerically by the modified method of [31], using Ransel kit obtained from Randox Laboratories CO., UK. The specific activity of hepatic CAT and SOD was expressed as units/g tissue, where the concentration of GSH was expressed as mmol/g tissue.

Histopathological examination was evaluated according to the method described by [32].

Statistical analysis:

Results were expressed as the mean values ± the standard error, using Microcal Excel™ for windows (Microcal Software, 2000), and statistical differences between groups were assessed by Student's t –test. Values of P<0.05 were considered significantly different.

3. Results:

Biochemical assessments

As shown in **table (1);** rats intoxicated with D-GalN/LPS (G3) developed a state of hepatic damage as evident from a significant (P < 0.001) elevation in the serum marker activities of AST and ALP when compared with control group. Pretreatment with PJ (G4) afforded a significant protection against D-GalN/LPS – induced hepatitis, where the activities of AST, ALT and ALP were significantly (P< 0.05) decreased as compared to G3.

As shown in **table (2);** the toxicity of D-GalN/LPS was accompanied with significant (P< 0.001) decrease in the levels of DNA, RNA and protein contents as compared with G1. Also, there was a significant (P<0.05) increase in tissues DNA, RNA and protein contents as compared with G3.

With regard to MDA content , CAT & SOD activities and GSH concentration , the results in **Table (3)** showed that treatment of rats with D-GalN/LPS caused a significant (P < 0.001) increase in hepatic MDA content in comparison to G1, whereas pre-treatment with PJ (G4) resulted in a significant reduction in MDA content as compared with G3. Also, the treatment with D-GaIN/LPS resulted in a significant (P<0.001) decrease in CAT, SOD and GSH values in comparison to G1. Pre-treatment with PJ resulted in a significant (P<0.05) increase in the activities of CAT, SOD and GSH content in comparison with G3.

Table1. Effect of PJ on liver enzymes activities in D-GalN/LPS -induced hepatitis in rats.

Groups	AST U/ml	ALT U/ml	ALP IU/L
G1	38.6 ±1.3	37.3±1.4	124.5±3.83
G2	40.3±0.83	37.1±1.12	126.5±2.73
G3	50.4±2.64 [b]**	59.4±5.38 [b]**	301.6±14.5 [b]**
G4	46.9±1.3 [a]*	45.4±1.4 [a]*	274±7.1 [a]*

[a]: indicates significantly from G3 ; [b]: indicates significantly from G1 ; *:P< 0.05 and **: P<0.001 .

Table 2. Effect of PJ on Tissue DNA, RNA and Protein contents in D-GalN/LPS -induced hepatitis in rats.

	DNA	RNA	Protein
Groups		mg / whole liver	
G1	7.45 ± 0.16	8.96 ± 0.23	201.41 ± 4.22
G2	7.95 ± 0.27	8.39 ± 0.30	199.77 ± 5.25
G3	5.64 ± 0.22[b]**	6.34 ± 0.35[b]**	171.05 ± 6.14[b]**
G4	6.06 ± 0.14[a]*	7.45 ± 0.17[a]*	188.12 ± 5.06[a]*

[a] : indicates significantly from G3 ; [b]: indicates significantly from G1 ; *:P< 0.05 and **: P<0.001 .

Table 3. . Effect of PJ on Tissue MDA and antioxidants in D-GalN/LPS -induced hepatitis in rats.

Groups	MDA (nmol/g liver)	CAT (U/g liver)	SOD (U/g liver)	GSH (mmol/g t liver)
G1	4.4± 0.37	1.72±0.04	7.06±0.17	0.18±0.02
G2	4.32±0.35	1.7±0.05	7.14±0.06	0.19±0.01
G3	15±1.20[b]**	0.51±0.04[b]**	6.4±0.13[b]**	0.07±0.01[b]**
G4	9.5±0.46[a]**	0.81±0.03 [a]*	6.67±0.09[a]*	0.12±0.01[a]*

[a] : indicates significantly from G3 ; [b]: indicates significantly from G1 ; *:P< 0.05 and **: P<0.001 .

Histopathological investigation of liver

The histological evidence authenticated the injury caused by D-GalN/LPS and the protection offered by PJ to hepatocytes. **Fig. 1** Illustrated a section of control rat liver showing normal architecture. In **Fig. 2,** a section of liver of PJ treated rat showing normal liver parenchyma with central vein and cords of hepatocytes. Microscopical examination revealed loss of architecture and cell necrosis with inflammatory collections in the central zone in D-GalN/ LPS - induced rats (G3, **Fig.3**). Pre-treatment with PJ(G4) prevented the histopathological changes in liver induced by D-GalN/LPS **(Fig .4).**

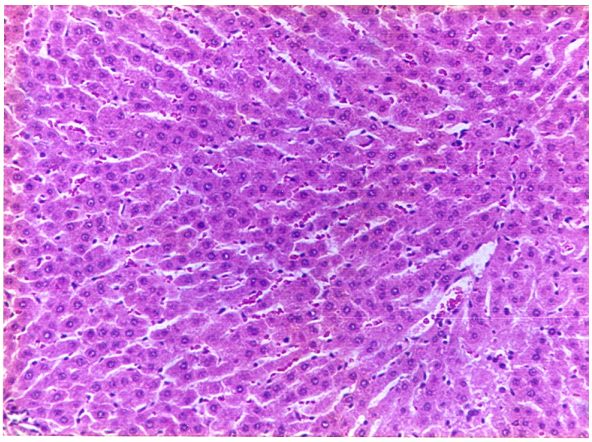

Fig. 1

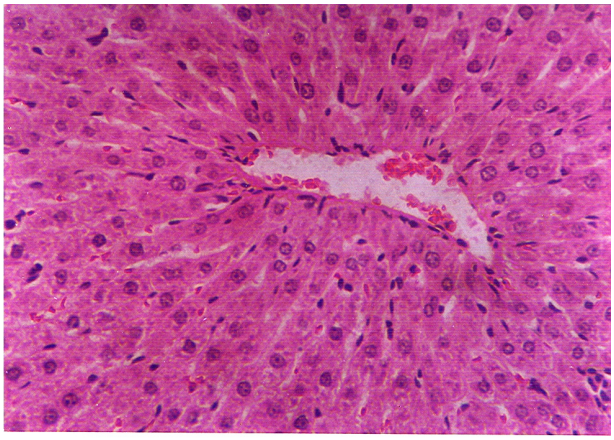

Fig. 2

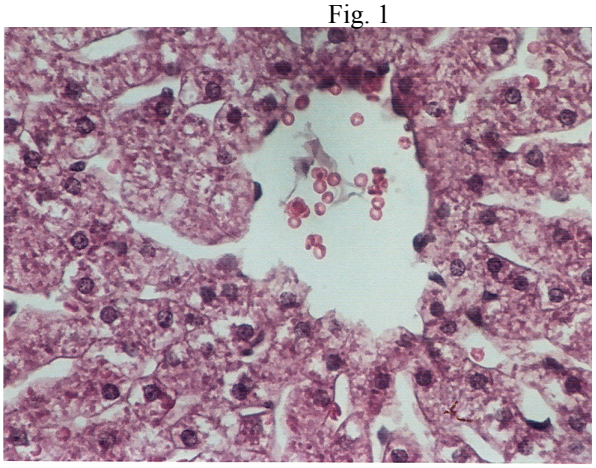

Fig.3

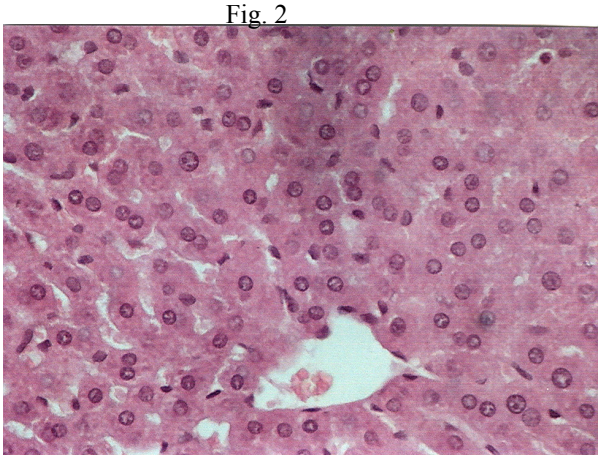

Fig.4

(H & E X 400)

4. Discussion

In the current study, D-Ga1N / LPS intoxication resulted in a significant increase in the serum level of diagnostic marker enzymes (AST, ALT and ALP) as compared to the control group. This is an indicative of cellular leakages and loss of functional integrity of cell membrane in liver. This observation is in agreement with [19] and [33]. The elevation of transaminases could be taken as an index of liver damage and this may be due to the fact that D-GalN / LPS administration disrupts plasma membrane permeability causing leakage of the enzymes from the cells. These findings is confirmed with the results of the histopathological investigation which revealed loss of architecture and cell necrosis with inflammatory collections in the central zone. In this study, oral pretreatment with PJ attenuated the D-GalN / LPS-induced elevation in the level of these diagnostic marker enzymes demonstrating the cytoprotectve activity of PJ which preserved the structural integrity to the hepatocellular membrane and liver cells architecture damage caused by D-GalN / LPS.

In the current study, liver damage induced by D-GalN/LPS was accompanied by a significant inhibition of liver DNA, RNA and protein contents. The metabolism of D-GalN may deplete several uracil

nucleotides including UDP-glucose, UDP-galactose and UTP which are trapped in the formation of uridine diphosphogalactosamine and it has thought that D-GalN induces liver injury by inhibiting the synthesis of RNA and protein through a decrease in hepatic UTP concentration which finally evokes the necrosis of liver cells [34,35]. Furthermore, bacterial endotoxin such as LPS is among the agents that cause immunological stimulation of Kupffer cells [36]. Activation of Kupffer cells contributes to liver injuries by releasing cytotoxic agents, inflammatory cytokines and ROS, this may lead to severe oxidative damage of the liver cells [37] and the cellular components like cell membrane, lipids, proteins and DNA **[38].** In addition, [39] also reported that D -GalN/LPS intoxication increases the neutrophil infiltration into the liver cells with increased release of ROS species from the activated neutrophils.

In the present work, administration of GalN/LPS caused also a significant increase in the MDA level together with a decease in the activity of the antioxidant enzymes CAT and SOD) and the non-enzymatic antioxidant GSH level in hepatic tissue reflecting an oxidative stress state. Our results are in agreement with those observed by [19,40] who reported a significant increase in lipid peroxides accompanied with decreases in the antioxidant parameters CAT and SOD due to D-GalN/LPS intoxication. This selective inhibition of antioxidant enzymes activities might be justified by the suggestion of [41] that D-GalN can selectively block hepatic transcription and indirectly blocks hepatic protein synthesis.

In this study, oral pre-treatment with PJ effectively protected the liver from the toxicity of D-Ga1N / LPS by decreasing the oxidation process proved by decreasing hepatic MDA together with increasing the serum activities of CAT and SOD as well as GSH level. Concomitantly, it partly prevented liver enzymes from elevation indicating the protection of the cell membrane from free radicals attack. The histopathological examination confirmed these results showing the improvement in the signs of necrosis and cellular infiltration together with the marked prevention of protein ; DNA and RNA loss in the hepatic tissue. These results are in accordance with the findings of [42], which demonstrated in vitro that besides scavenging free radicals and ROS, pomegranate also prevents DNA damage and with the results of [43] who confirmed the ability of pomegranate to protect DNA and preventing chromosomal damage in mice. In addition, [44] demonstrated that pomegranate extract afforded up to 60% protection against hepatic lipid peroxidation due to the maintenance of the GSH levels and activities of CAT, glutathione peroxidase, glutathione reductase, and glutathione-S-transferase. Furthermore, [45] found a direct correlation between antioxidant capacity and antimutagenic activity of pomegranate peels.

There is a possibility that orally administered PJ exerts a preventive effect on liver injury progression in D-Ga1N/LPS treated rats through its indirect antioxidant action to maintain antioxidant defense system in addition to its direct antioxidant action to scavenge ROS and to inhibit lipid peroxidation . The hepatoprotective property of PJ may be attributed to the presence of different bioactive components, mainly polyphenols, ellagitannins, condensed tannins, and anthocyanins which have antioxidant properties.

Thus, the present study confirms the hepatoprotective action of pomegranate against D-GalN/LPS induced hepatitis in rats which may accept a clinical importance because there is close resemblance between the multifocal necrosis produced by D-GalN/LPS and the lesion of viral hepatitis in humans. In coclusion; pomegranate could serve as a better remedy for liver disease and controlled clinical studies in viral hepatitis would be worthwhile.

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Corresponding author
Amal, A. Fyiad
Biochemistry Dept.
Genetic Engineering and Biotechnology Division,
National Research Centre, Cairo, Egypt.
amalfyiad@yahoo.com

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The Sun as a large Hydrogen Atom

Salah Eid

Faculty of Arts, Suez Canal University, Ismailia, Egypt
Salaheid050@gmail.com

Abstract: The solar planets orbit the Sun with velocities less than the velocity of an electron orbiting a proton in the fifth level of energy in hydrogen atom, while if a body B takes place at the hydrogen surface of the sun, this supposed body will orbit the Sun with the same velocity of this electron according to the gravitational law $\frac{GM_s}{R_s}$ with which we calculate the orbital velocity of any planet. The velocities of solar planets from Mercury to Pluto, therefore follow that of B, each according to its distance from the Sun's center. The Sun which is a hydrogen star therefore behaves as a large hydrogen atom with number of 'constant' levels of energy, the mentioned velocities being obtained from gravitational law means the disappearance of the borders between gravity and electromagnetism as gravity itself creates the electromagnetic bonds in the structure of the Sun where at its hydrogen surface the electron is at its fifth and last level of energy with its mentioned orbital velocity.
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Key words: hydrogen surface of the Sun, hydrogen atom, gravity, electromagnetism

1. Introduction

One evening , some months ago I decided to know the value of orbital velocity of a body nearer than Mercury to the Sun , just at its hydrogen surface, using the law of gravity in calculating this velocity I was very surprised when the value of it was that of an electron orbiting a proton at fifth level of energy! The accurate equivalence between the two velocities bears a deep meaning concerning the dream of scientists especially Einstein of unifying gravity with electromagnetism. For this reason I studied the subject seriously.

The Sun as a hydrogen star:

Like most of other stars, the Sun is made up mostly of hydrogen followed by helium [(1)] . From here came the theory that the source of Sun's energy arises from burning hydrogen into helium, this is not the place of discussing the correctness of this theory, what is important here is to emphasize on the Sun as a hydrogen star. For every million atoms of hydrogen in the sun there 98,000 of helium and less numbers of oxygen, carbon, neon, magnesium etc [(2)] . The surface of the Sun is composed of three layers, the photosphere, the chromosphere and the corona, they are respectively above each others .The chromosphere (800 km) deep is the most important layer of the sun or any other star [(3)] For us here this statement is so because it is the actual hydrogen surface of the Sun, the corona above it (800,000 km) or more [(4)] composed of electrons free from the attraction of the protons, thus at the corona layer thus comes the end of hydrogen in the Sun, and this means that electrons in the chromosphere must be at the fifth and last level of energy of hydrogen atoms just before getting free from the protons' attraction, the chromosphere appears as a thin red rim of light [(5)] , the red color confirms the idea that the electrons in the chromosphere are at the last or fifth level of energy in hydrogen atom before getting free from the attraction of the protons in the following corona layer, where they emit the longest wavelength in all the portions of hydrogen spectrum $(656.3\ nm)$ [(6)] concerning the red color.

The equivalence between two velocities:

The mentioned orbital velocity of the supposed B body at the hydrogen surface of the Sun is

$$\frac{GM_s}{R_s} = V^2 = 1.90x10^{-11}$$

Where G is the universal constant of gravity, M_s is the mass of the Sun in Kg , R_s is the radius of it in meters.

The mean orbital velocities of solar planets from Mercury to Pluto follow the velocity of this body, they are less than it as they are farthest than this body from the center of the Sun, but they all obey the same law of gravity, the velocities of the interior planets are

Body B $4.35x10^5$

Mercury $4.78x10^4$

Venus $3.50X10^4$
Earth $2.97x10^4$
Mars $2.41x10^4$ (7)

This means that the Sun is a large hydrogen atom, the planets with their velocities are extension of body B!

Now, the velocity of our body is the same velocity of an electron orbiting a proton at the fifth level of energy, where experimentally

$$\frac{13.6ev}{5^2} = 0.544 = 8.714x10^{-20} J = \frac{1}{2} m_e v^2$$

$$m_e v^2 = 1.74x10^{-19} J$$

$$v^2 = 1.91x10^{11} \quad \text{or} \quad v = 4.37x10^5$$

And this is the case in chromosphere layer before the beginning of corona layer above it. The equivalence is clear between the velocity of the body B orbiting the Sun at its hydrogen surface , and the velocity of the electrons orbiting protons in this surface. Is not this equivalence a fantastic fact?

From this equivalence we can see the relation between the radius of hydrogen atom and that of the Sun through the two expressions of gravitational and electromagnetic velocities as follows

$$\frac{GM_s}{R_s} = \frac{e^2}{4\pi m r \varepsilon_0}$$

Where $$\frac{r}{R_s} = \frac{e^2}{4\pi m \varepsilon_0 G M_s}$$

We can conclude any of the two radii from the other for any star, even it is neutron star where the radius between the electron and the proton is of nuclear range, the mass of the star is some multiple of the Sun and therefore the radius of the star is very short.

Conclusion:

The two main results from above discussion are

1- The Sun which is a hydrogen star behaves as a large hydrogen atom.
2- Gravity is equivalent to electromagnetism.

Corresponding author
Salah Eid
Faculty of Arts, Suez Canal University, Ismailia, Egypt
Salaheid050@gmail.com

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Community Participation in Community-based Prevention Programs; A Short Review of the Literature on Challenges to Breast Cancer Prevention Programs or Activities

Maryam Ahmadian[1]&Asnarulkhadi Abu Samah[2]

Department of Social and Development Sciences, Faculty of Human Ecology, Universiti Putra Malaysia 43400, UPM Serdang, Selangor, Malaysia
Corresponding Authors: marydian50@yahoo.com [1] & asnarul_n9@yahoo.com [2]

Abstract: This article reviews the potential of widely used psycho-social factors affecting community participation in community-based prevention programs among diverse communities. Besides, we specifically appraised the previous literature to look into the psycho-social, structural, and demographic factors which create latent challenges to community participation in breast cancer prevention programs. We believe communities with lack of behavioral and psycho-social change components are likely to have low motivations to participate in health programs against diseases such as breast cancer. Additionally, certain demographic characteristics and potential structural factors control a distinct participation in health programs. Clarification of participation in public health programs and its psycho-social, structural, and demographic attributes are keys to explicate why and how socio-cultural, behavioural, and multifaceted interventions should be main concern in the evaluation of community participation in health promotion programs. The idea here is rather to emphasize on community participation in breast cancer prevention activities for community development undertakings.
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Keywords: Community development; Community participation; Community-based prevention programs, Breast cancer

Introduction

Community development, as a strategy for a health promotion program, is an important attempt in the prevention of diseases. Furthermore, community-based prevention program is an approach to health promotion and disease prevention. Nowadays, the community-based health prevention program has brought about a new path to emphasize on investment in prevention and support care among any sub-population.

Community development strategies stress the principles of the Ottawa Charter for Health Promotion (1986) and emphasized on the community participation for seeking solutions for health problems. Health promotion encourages individuals and communities to take greater responsibility for their health (WHO, 1978). Nowadays, community-based health promotion programs have become an integral part of overall health promotion efforts (Shea and Basch, 1990). Community-based programs alter public health policies to change health behaviours and to reduce health risks of community members. Individual behaviour change is a basic priority for participatory activities which are planned by the government such as anti-smoking and nutrition campaigns, and displayed a lifestyle view of health promotion (Pinder, 1994).

With the rapid rise in health care costs all around the world, health services need to find effective ways to prevent diseases and promote health. At the same time, there is increasing evidence that community participation is a pivot for new public health programs and it can significantly improve communities' knowledge, attitudes and behaviours to contribute to health promotion. The problem is that the number of people attending the event does not denote participation. People are present, but they may not commit of what is going on (Rifkin, 2001). People have no participation in decision making level. However, Abu Samah et al., (2012) noted that community involvement in health tends to be assumed community participation.

The concept of health and health care, as defined by the World Health Organization (WHO) demands participation from communities. This concept impacts on the standard of housing, maternity and child care, nutrition and diet, potable water, and hygiene. At the WHO conference in Alma-Ata in 1978, health was interpreted beyond the absence of illness. Since that time, the concept of primary health care (PHC) has implied that a community takes responsibility for its own health. However, despite these endeavors, there are still many barricades and challenges which stand in the way of community participation in health programs.

The impact of challenges and barriers to community participation in community-based prevention programs are seldom studied among general population or sub-populations. The purpose is to understand even very simple experiences of general population to make program planners and managers consider community participation in health prevention programs and to facilitate the policy-making process, practically. Psycho-social, structural, and demographic attributes are crucial in the identifying challenges to public participation in health prevention programs.

One of the fastest growing public health concerns is breast cancer which needs community participation. The incidence of breast cancer increased over the past 20 years, and this fosters the worry about psycho-social and physical well-being of healthy women at risk of breast cancer. In line with that thought, very few studies have been done based on theoretical models regarding women's participation in breast cancer prevention groups such as support group (Gilbar & Neuman 2002; Cameron et al., 2005). In addition to this, at the structural level, social, economic and cultural barriers, and at the individual level, motivation can influence community participation in health care (Kapiriri et al., 2003).

This paper will outline the previous literature to look into the psycho-social, structural, and demographic factors to identify potential challenges to community participation in health programs, activities, and groups. A whole host of aspects came into play in this literature review, which we are only just beginning to appreciate community participation in public health prevention programs, particularly women's involvement in breast cancer programs or support groups based on voluntary work or getting health benefits.

Methodology

More than 80 articles published from the year of 1990 through 2012 were reviewed mostly by following electronic databases such as Science Direct, Pub Med, ISI Web of Sciences, and Google Scholar. The findings from applicable published studies were summarized to identify challenges and barriers to community participation in community-based health prevention programs. The inclusion decisive factor were "psychosocial factors", "structural factors", and "demographic factors " in combination with "community participation in health programs", "community participation in community-based prevention programs", and "women's participation in breast cancer prevention programs or groups". The factors influencing prevention programs were categorized in three main domains such as psychosocial issues, structural factors, and socio-demographic factors.

These studies confirmed that the decision to engage with a health prevention program is influenced by a variety of variables such as age, education, occupation, access to health services, attitude, and perceived barriers. In mapping out the factors, there are many factors which affect community participation in health prevention programs and many diseases which can be prevented by participation of lay people. But this study tends to fall under the divisions of psychosocial, structural, and demographic factors along with current health promotion programs (e.g. breast cancer, obesity, physical activity, family planning, etc.) which held by health care providers around the world. The underlying assumption is that these factors should be understood in terms of tangible challenges to women's participation in breast cancer prevention programs or activities.

Demographic Factors

Community participation in health programs is realized to be crucial in health and social development. Little is known about how often socio-demographic factors influence participation in community-based prevention programs. The relations between different age, gender, ethnicity, and socioeconomic characteristics controlled a mixed participation in health programs. However, the lack of serious commitment might be a barrier to the sustainability of community-based control strategies (Sindato et al., 2008).

These factors are too plentiful to be reviewed here deeply. For example, Nobles and Frankenberg (2009) discovered that socio-demographic characteristics of mothers are associated with the choice to participate in community activities regarding their children health in Indonesia. Similarly, Boyce (2001) has already noted that the numbers and types of community participation are influenced by geography (Cohen & Syme, 1985), socioeconomic status (Sills, 1968; Widmer, 1987), gender (Wells et al., 1990), and group heterogeneity (Litwin, 1986). Therefore, community-based strategies must be adaptable to the ecological local setting, cultural and social differences (Lloyd et al., 1992). In fact, a package of specific socio-demographic factors associated with preventive diseases will be essential for sustaining community participation in a public health setting.

Gender

With regard to age and gender, Boyce (2001) stated that attitudinal barriers to young women's participation demands women to be separated into different age groups to encourage participation. Boyce (2001) mentioned that gender also affects community participation and self help activities. For instance, men participated mostly in advocacy activities, while women participated in social support groups and self-help activities. Previous studies documented that

female, younger, white, better educated, and middle-class people tend to participate excessively in support groups related to health issues (Berglund et al., 1997; Deans et al., 1988; Krizek et al., 1999; Helgeson et al., 2000; Plass &Kock, 2001). In contrast, Sherman et al., (2008) did not find gender differences in public participation in cancer support groups.

Age

Age as a demographic factor has been a potential contributor to participation in health programs. As mentioned above, younger people are more likely to participate in health programs such as cancer support groups (Berglund et al., 1997; Deans et al., 1988; Krizek et al., 1999; Helgeson et al., 2000; Plass & Kock, 2001). There is little published information regarding the relationship between age and community participation in health programs, particularly in breast cancer prevention. In a recent study, there was a significant relationship between age and high level of volunteering in community–based breast cancer prevention programs in Iran (Ahmadian et al., 2011). However, in general, younger women tend to participate in family planning programs in Iran. This may relate to the higher education level or having a job among Iranian women who participated in family planning programs. In contrast to this, association between age and participation in cancer support group was not significant in a study in the United States (Sherman et al., 2008).

Education

In some way, there are various socio-demographic factors that hinder the community participation in health programs. According to Boyce (2001) community members with very low educational levels had minimal level of participation usually as clients and volunteers, and no interest in taking responsibility at project management positions. Despite the various benefits of local community participation in health prevention programs, it needs to be realized that their commitment to the possible gains from community participation is important.

Previous literature demonstrated that relationship between education and participation in cancer support group was significant and participants had slightly higher education than non-participants (Sherman et al., 2008). Stevens & Duttlinger (1998) also found that established participants in a breast cancer support group were mostly educated. Literature proved that there were trends for greater participation in breast cancer support group among those with higher education (Bauman et al., 1992; Meyer & Mark, 1995; Stevens & Duttlinger, 1998; Eakin & Strycker, 2001).

Similar to other western societies, a research in Iran showed that community participation levels in breast cancer prevention programs were influenced by women's education. Educated women were more enthusiastic to participate in programs regarding their health and well-being such as family planning and breast cancer programs (Ahmadian et al., 2011). These findings were supported by previous studies carried out by Ahmed (2003) and Sarker (2005) in Bangladesh that education grants women a voice against social injustice.

Marital status

With regard to community participation and the psycho-social well-being among retirees in the United States a study by Moen& Fields (2002) showed that community participation may be less important for the well-being of married, as opposed to unmarried because married people are less socially isolated than are those who are widowed, divorced, or never married. Authors also argued that married retirees would both be more suitable to participate in their communities and to benefit from that participation (Musick et al., 1999). They added that marriage itself is an integrating role, one with obvious emotional benefits to health (Pienta et al., 2000; Waite & Gallagher, 2000).

A recent study by Ahmadian et al., (2011) exposed that there is not a significant relationship between marital status and voluntary participation in breast cancer prevention activities among women attending outpatient clinics in Iran. Iranian women, particularly married ones may have no tradition of extensive involvement in community networks, especially in health programs. However, they support survival groups, after being healed from breast cancer, which shows that specific personal experience on medical issues will be an incentive to mobilize people against diseases such as breast cancer and to encourage them to voluntarily participate in health activities.

Occupation

There are some inconsistent results between the researches about the importance of occupation as a socio-demographic factor. It seems working people tend to participate in community based health programs which might drive from their communication with other people in the society or education. These people can be informed of their relevant medical programs such as those available work-place programs. Besides, they are less conservative than the other unemployed ones. For example, the employed women are able to consider the reality that their own health is equally important to the whole family health (Ahmadian et al., 2011). In a recent research which was conducted in Iran by Ahmadian (2011), women with full-time jobs have participated in health promotion programs such as breast cancer prevention programs more than those having part-time jobs, being unemployed and housewives. It can be concluded that participating

women with full-time jobs have less socioeconomic dependency (Ahmadian et al., 2011). In contrast, association between occupation and participation in cancer support group was not significant in a study in the United States (Sherman et al., 2008).

Income level

According to Boyce (2001) community members with the low-income levels had little participation usually as clients and volunteers, and showed no concern in taking responsibility at project management situations. Boyce (2001) also discovered that community members with low incomes had minimal levels of participation in health promotion projects in Canada. Similarly, Green and Kreuter (1991) indicated that economic status as an individual characteristic influences voluntary behavior in health programs, such as getting vaccinated and complying with a treatment schedule.

Likewise, income was not found to be a significant contributor in a recent study in Iran regarding women's participation in health promotion programs such breast cancer prevention (Ahmadian et al., 2011). Rich women including the old ones in Iran are keen on voluntary group programs, like charities or elderly care, but they do not appreciate the need for health seeking behaviours (Ahmadian, 2011). No matter how clear the benefits of a designed intervention may appear to those initiating it, the socioeconomic context may affect whether community involvement is made possible or delayed (Zakus and Lysack, 1998).

Psychosocial Factors

The effect of psychosocial factors on community participation or public participation in health and health inequalities has been characterized significantly in human and social capital research literature. Egan et al., (2008) cited from previous literature that psychosocial theories have persuaded policy-makers to develop public health strategies that take into account people's support networks, sense of control and empowerment, and the extent to which people participate in the local community.

We carried out a literature review of recent studies looking at how psychosocial factors such as attitude, beliefs, self-efficacy, social influence and perceive barriers, may relate to community participation in health programs, particularly preventive disease programs like breast cancer prevention community settings. We also found some specific psychosocial factors associated with community participation in health programs. However, more robust reviews should be done to make possible a better understanding of psychosocial factors and its effects on community participation in prevention programs within health care structures. Here, we began to emphasize the influences of psychosocial factors on people's behavior with regard to their participation in health programs. Nevertheless, understanding the psychosocial determinants of community participation in health programs could be a difficult endeavor in practice. As we are interested in community participation in health promotion programs, concerns must be drawn out for individual and structural factors which have been reviewed in the paper.

Attitude

The concept of community participation is not explicit to health because it underpins rural community development (Cheers, 1998), and social development (Midgley et al., 1986). In terms of health prevention programs, literature showed that community members' attitudes influence their participation in programs. When local community people change their attitude and behaviours, they would obtain a sense of program ownership and sustainability. For instance, in Uganda a health program develops community support through the use of participatory techniques to promote dynamic reflection on HIV/AIDS and to change local community attitudes. This program was successfully used to educate and mobilize entire communities to reduce their risk of becoming infected with HIV due to behavioural change at the community level (Welbourn, 1998).

Community involvement in the diagnosis and seeking solution of health problems is an old opinion of public health. But listening to the concerns of the community is important (Minkler, 1990). Beeker et al., (1998) also stated that public health practitioners should recognize environmental and community factors which influence health issues. The results of a research which was carried out in Iran showed that local people could acknowledge their own health needs and request more information from professionals to improve their own health based on their cultural attitude and historical roots (Behdjat et al., 2009).

Another study by Sharma (2007) in India showed that understanding participation in the community-based rehabilitation is referred to the attitude and behaviours of all actors involved in the community-based programs. Sindato et al., (2008) also noted that it is important to comprehend the people's attitudes before their involvement in a health program. In contrast, attitude regarding dengue fever prevention among Brazilian communities was not associated with effective dengue control actions (Claro et al., 2006).

With regard to breast cancer, women's participation in prevention programs at the community level was significantly related to their attitude (Ahmadian, 2011). We believe that more social scientist should come to assist local communities to

outline their own health needs, particularly about preventive diseases and to develop initiative approaches and solutions for meeting them. In a way, attitude towards participation in health programs may potentially explain some health outcomes.

Self-efficacy

Handy& Kassam (2004) indicated that individuals with low self-efficacy regarding health behaviour restrict their participation in rural NGOs in India. Smith-Morris (2006) also stated that non-participation in successful community-based diabetes program attributed to insufficient self-efficacy. Smith-Morris (2006) believed that program revision must be tied to certain psychosocial factors due to the importance of people's voluntary participation in community-based health programs. With regard to social capital and health programs, Kawachi et al., (2004) identified inconsistent evidence of relations between collective efficacy and social cohesion with health outcomes such as general health and child health. However, publication bias may influence our literature review.

Another study revealed that self-efficacy is not a salient factor for participation in breast cancer prevention programs among Iranian women (Ahmadian et al., 2011). The result of the study demonstrates a trend of community participation in health programs in less developed countries such as Iran. This trend includes lack of a strong sense of community among participants and low numbers of active participants since there is no actual formal program regarding breast cancer prevention in developing or less developed countries.

Belief

Previous study showed specific cultural belief and ethnic contexts had association with voluntary participation in health program (Boyd-Franklin, 1991; Guidry, et al., 1997; Mathews, Lannin, & Mitchell, 1994). Health belief is a vital part of community-based control program. Positive belief encourages people to control the diseases such as malaria individually and to increase their voluntary participation in control activities or programs (Grantham, 2009). Similarly, Zaim (1997) mentioned that in malaria control campaigns in southern Iran, health care professionals should take into account people's beliefs towards national malaria control programs in the region. He also mentioned that malaria control activities have been integrated into the primary health care system (PHC) in Iran. According to him, people's beliefs and behaviour towards national malaria control programs in southern Iran lead to control the disease and increase community participation in malaria control activities, particularly those measures aimed at reducing human-vector contact. Another study showed that belief is a prominent factor for participation in breast cancer prevention programs at the district level among Iranian women (Ahmadian et al., 2011).

Social Influence and Social Support

Social influence and social support within familial, marital relationship and social network can affect public participation in health programs. In fact, these factors mediate community involvement in health programs. With regard to women's participation in breast cancer support group, literature showed higher level of participation in support groups was associated with potential benefits of participation in support groups and consistent support over time (Stvense, 1998). Community participation or activity may reveal a general readiness for social engagement (Bauman et al., 1992; Taylor et al., 1986). Similarly, previous studies showed that social influence and social support are associated with participation in breast cancer prevention programs or cancer support groups (Ahmadian et al., 2011; Sherman et al., 2008). Previous literature has also shown that it is crucial to foster social support from partners and families both before and during interventions in order to facilitate program goals (Leonard et al., 2001). Taylor et al. (1986) similarly found that encouragement from spouses to attend a group was tied to greater participation.

Stevens & Duttlinger (1998) identified that demographic, medical, and psychosocial factors affect women's participation in breast cancer support groups. According to them, the most important barriers causing non-participation were anxiety, depression, stress, non-support, and aggression which were lower in established participants with higher participation. Furthermore, established participants were most educated, and most of their friends were diagnosed with cancer which supports the view that social influence is linked to better health and active participation. According to (Bauman, et al., 1992; Taylor et al., 1986), volunteer or community activity has been linked to the use of social influence and may reflect a broader readiness for social engagement.

Similarly, social networks influence health behavior, directly through normative pressure to change individual-level characteristics such as frequency of condom use, and indirectly through collective action to change community-level characteristics such as restrictive gender roles regarding HIV (Beeker et al., 1998). In practice, identifying social network factors affecting community participation in health program can be a difficult task. For example, Media attention to the disease such as breast cancer compared with other cancers may affect participation in the community support group (Sherman et al., 2008). Other researchers (Eakin & Strycker, 2001; Krizek et al., 1999) similarly stated that the level of awareness and

utilization of community support groups are higher among breast cancer patients than among those with other types of cancer.

Perceived Barriers

Theoretical perspectives rarely have been employed to investigate the use of community participation in community-based disease prevention programs. A recent study by Sherman et al., (2008) utilized the Health Belief Model (Rosenstock, 1974; Stretcher & Rosenstock, 1997) to identify the determinants of women's participation in a community-based cancer support group in Arkansas, USA. Authors suggested that the perceived barriers were associated with women's participation in cancer support group.

Martinez et al., (2001) examined the barriers to the physical activity of a faith-based community among churchgoers in a border region. They found some individual barriers to participation in the community-based physical activity program including lack of motivation, time, language, money, social support, family or household responsibilities, socio-cultural (fear) and environmental (traffic-related) barriers which reduce people's attendance in community-based health programs.

Likewise, Kapiriri et al., (2003) found that at the structural level, social, economic and cultural barriers influence local community participation in health in Uganda. In addition, Boyce (2001) also mentioned similar barriers in a study on community participation of disadvantaged groups such as poor women, street youth, and disabled persons in health promotion projects in Canada. In addition, Smith-Morris (2006) noted that non-participation in health programs such as diabetes program can happen due to barriers like lack of knowledge, income, social or family support.

Similarly to other authors' findings, Beeker et al., (1998) argued that public health practitioners have recognized that health is influenced by environmental and community factors. They added that community involvement in the diagnosis and solution of health problems is a long-standing opinion of public health. It encourages listening to the concerns and problems of community residents (Minkler, 1990). Green & Kreuter, (1991) also mentioned that despite early recognition of health behaviour in culture, geography, economic and political circumstances, health interventions should focus on individual characteristics, like motivation and skill, to change voluntary behaviours in health programs such as getting vaccinated. With regard to breast cancer, Sherman et al., (2008) cited that some practical barriers were associated with participation in support groups such as breast cancer groups.

As underlined by previous investigations (Eakin & Strycker, 2001; Plass & Koch, 2001), limited awareness of where to find a group was tied to lower participation. Moreover, as expected, those who lived in distant or rural areas reported less participation in support groups. Mostly, these individuals have fewer group services available in their local communities and appear reluctant to travel long distances to seek them. Geographical or transportation barriers have been cited as important obstacles by several investigators (Duncan & Cumbia, 1987; Thiel de Bocanegra, 1992; Llwelyn et al., 1999; Fukui et al., 2001).

Regarding women and breast cancer activity, a recent study showed that they overcame their barriers towards preventive behaviors such as mammography for higher level of participation in any community-based breast cancer prevention program in Iran (Ahmadian et al., 2011). However, they observed that those who participated in the programs lived nearby as well.

Thus far, we have discussed the specific barriers to public participation or community participation in health programs and the fact that community participation in health needs a pragmatic solution to identify these barriers prior to the success of community participation activities and process in health programs.

Structural Factors

This section introduces structural factor as one potentially useful determinant of community participation in health programs. Recent perspectives proves that community participation in health programs has appeared to be understood as relying more on structural factors in health care structures than on cultural factors in local communities. There is an increasing emphasis on political factors within and between health agencies, governments, and different levels of national health care systems. These perspectives put up new inquiries for community health programs and the strategy of community participation (Stone, 1992).

According to Sherman et al., (2008), structural factors influencing participation in health programs might incorporate practical problems such as transportation or distance (Bauman et al., 1992; Duncan & Cumbia, 1987; Fukui et al., 2001; Llewelyn et al., 1999; Thiel de Bocanegra, 1992), and include functional capacity (Duncan & Cumbia, 1987), financial obstacles, or competing family or care giving responsibilities (Fukui et al., 2001). Geographical or transportation barriers have been mentioned as important obstacles by several researchers (Duncan & Cumbia, 1987; Llwelyn et al., 1999; Fukui et al., 2001).

A structural perspective was utilized in another study on community participation of disadvantaged groups such as poor women, street youth, and disabled

persons in health promotion programs in Canada. This study showed the relationship between various dimensions of structure like social-cultural, organizational, political-legal-economic, and the community participation process. Participation was controlled by structural factors such as bureaucratic rules, perceived minority group relations, agency responsibilities, available resources, and organizational roles. The study came up with a conceptual model based on structural factors that is practical in explicating how key factors from federal and local levels can limit or ease the community participation process (Boyce, 2001).

With respect to patients participation in health program or groups, one should bear in mind that understanding patient preferences might also be an important structural questions regarding group composition and group format. Many groups are directed toward specific diseases (e.g., breast cancer) and these focused groups assist participants distinguish more readily with other members and hasten group cohesion (Leszcz et al., 2004; Cameron et al., 2005). Previous literature also cited by Sherman et al., (2007) in a study regarding cancer group psychotherapy interventions. In the same way, health care providers should clarify to people about the importance of common diseases and the reality of prevention programs in a small scale approach, as well as the benefits that participation in these programs can offer (Ahmadian and Abu Samah, 2012).

Limitations

This review explores the evidence of demographic, psychosocial, and structural factors and their associations with community participation in health programs, particularly breast cancer prevention or support programs in community setting. However, the review provides little proof on the factors inspiring community participation in health programs based on local community geography around the world. The overall lack of robust data on factors influencing community participation in health programs, this paper may reflect the deprived quality of some previous studies on these factors.

The literatures regarding socio-psychological attributes on community participation in health programs are relatively limited in theoretical depth. The present review cannot be addressed directly to previous literature review and much of this limitation may be linked to our approach to scoping the previous studies. Some of these limitations have also been cited in a systematic meta-review on psychological risk factors and health inequalities by Egan et al., (2008). Nevertheless, the benefit of factors influencing participation in health program can be concluded by highlighting the importance of specific factors in this review.

A review of previous literature showed that traditionally community participation has been assessed in quantitative forms, for example, by asking how many people have come to a meeting or how many people have joined in a community activity. The problem is that presence does not mean participation. People may be present, but have no commitment or understanding of what is going on (Rifkin, 2001). Therefore, it is a further limitation for studies related to community participation purpose. Another limitation is designing a framework for community participation to increase participation in health which might lead to unrealistic assumptions about participation in health programs.

Conclusion

Community development strategies emphasizes communities to take greater responsibility for their own health and changes in people's attitudes to improve their optimal health. However, a major contribution to this change is the attitudes of the professionals involved in health promotion programs.

It is clear that changes in people's behaviour and attitudes are a long-term process and should address questions about whether the specific demographic, psychosocial, and structural factors are effective to active community or public participation in health plans. Since people know what is going on, their positive attitude can reduce their suspicion about health promotion programs and simplify their accountability to the existing diseases prevention programs.

This review provides some information about demographic, psychosocial, and structural factors and community participation in health programs. We found some evidences of factors associated with breast cancer prevention programs or support groups to appraise the importance of breast cancer prevention in the public health setting. These factors may be useful determinants for public health intervention agendas in future.

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Relationship of some risk factors and symptoms in patients with acute coronary syndrome

[1]Hamid Sharif Nia (MScN, BScN) , [2]Ali Akbar Haghdoost(PhD), [3]Mitra Hekmat Afshar, [4]Rogheyeh Nazari, [5]Samieh Ghana(BSc), [6]Mohammad Ali Soleimani , [7]Nasim Bahrami and [8]Zahra Beheshti(MScN, BScN) *

[1] Faculty of Nursing and Midwifery of Amol, Mazandaran University of Medical Sciences, Sari, Iran. PhD Student of Nursing at Baqiyatallah University of Medical Sciences, Tehran, Iran.
Email: h.sharifnia@mubabol.ac.ir
[2] Assistant of professor Epidemiology and Biostatistics. Research Center for Modeling in Health Kerman University of Medical Sciences, Kerman, Iran.
Email: ahaghdoost@kmu.ac.ir
[3] Msc of critical care Nursing, Golestan University of medical sciences, Gorgan, Iran
Email: m.hekmatafshar@yahoo.com
[4] MSc, BSc in nursing. Mazandaran University of medical science, Sari, Iran
Email: Roojayanaz@yahoo.com
[5] Bachelor of midwifery Golestan University of medical science, Gorgan, Iran
[6] Nursing and midwifery faculty, Qazvin University of Medical Sciences, Qazvin, Iran & PhD student of nursing, Tehran University of Medical Sciences,Tehran, Iran. Email: soleimany.msn@gmail.com
[7] MSc, BSc in midwifery. Nursing and midwifery faculty, Qazvin University of Medical Sciences, Qazvin, Iran.
Email: Nbahrami87@gmail.com
[8] Faculty of Nursing and Midwifery of Amol, Mazandaran University of Medical Sciences, Sari, Iran.

***Corresponding author**: Zahra Beheshti, Email: Zahrabeheshti2003@yahoo.com Tel: +981212221919 Fax: +98 (0121) 2151220

Abstract: Acute coronary syndrome (ACS) is one of the major causes of death in the worldwide. Clinical manifestations are different. So it's necessary to have knowledge about the types of symptoms experienced by patients with ACS. This study was performed to assay the Relationship of some risk factors and symptoms in patients with acute coronary syndrome**.** This cross-sectional study, were studied 294 patients with acute coronary syndrome at least 24 hours after admission had survived. Data was collected by a questionnaire that included demographic data form and check list of some symptoms and history of risk factors. There was a significant relationship between STEMI with vomiting (OR=1.94) and anxiety (OR=1.83) and UA with vomiting (OR=0.42). Between sex with weakness (OR=2.29) and anxiety (OR=1.82), diabetes with dyspenea (OR=1.8), weakness (OR=1.02) and tinnitus (OR=2.06) and hyperlipidemia with weakness (OR=2.35) and tinnitus (OR=2.49) was available significant difference. The findings of this study indicate that the appearance of symptoms of acute coronary syndrome were different as for ECG changes and risk factors, and more focused on those symptoms that they are common with any other diseases. Since, many of the symptoms of acute coronary syndrome can be potentially dangerous and life threatening, accurate diagnosis and timely action is crucial for the patients.

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Key words: Acute Coronary Syndrome, Risk factors, Symptom

Introduction:

Acute coronary syndrome (ACS) refers to a group of heart disorders, identifies with various degrees of ischemic heart disease. The most common modes are unstable angina (UA), myocardial infarction with the ST segment elevation (STEMI) and without ST segment elevation (NSTEMI) (1). Although unstable angina is not defined yet, but it can be define as a stage between stable angina and myocardial infarction (2).

Recent studies have shown that around 840,000 patients diagnosed with acute coronary syndromes are admitted to hospitals in America (3). On the other hand, more than 13 million people in America suffering from coronary artery disease (CAD) are at a high risk of developing acute coronary syndrome (4). American Heart Association announced that acute coronary syndrome is one of the major causes of death in this country, So that in 1998, 459,841, or one of every five deaths was due to coronary artery disease. These statistics in 2001 reached to 1.1 million in Americans, which is estimate that about 40% of them will die of coronary syndrome (5). NSTEMI and UA estimate for about 2.5 million admissions annually worldwide, while ST elevation STEMI accounts for another 1 million(6) .

In the latest statistics of the Ministry of Health in 1380, almost 46% mortality in 18 provinces of Iran has been devoted to cardiovascular disease and myocardial infarction (7).

In 1970s, acute coronary syndrome and the relationship between coronary artery occlusion with no clinical manifestations were reported in studied. From then, different studies were done to evaluate the prognosis and risk factors in the patients and finally compared with acute coronary syndrome in those who had clinical manifestation, because their symptoms are related to the risk factors (8). Unfortunately, in developing countries in Asia has been little attention to ischemic heart disease (9), so that the main cause of pre-hospital death in patients with acute coronary syndrome is the delay in deciding for the choosing and starting of treatment and care (10).

To influence the onset of treatment, it's necessary to have information and knowledge about the types of symptoms experienced by patients with acute coronary syndrome (11). Because of the delay in providing hospital medical treatment, most patients also delayed by the lack of awareness of symptoms occurs, so nurses by educating about the symptoms can have an effective role in reducing therapy's delay (12).

Nikrvan quoted Ahmadi says because nurses are the closest person to the patients, so, any change in the patient's condition is noticed by them immediately. Given that one of the nurse's goals is to help diagnoses, they can acquire knowledge in the field of symptoms, to prevent the mortality of patients (7). With increasing nurses' knowledge about similarities and differences between acute coronary syndrome symptoms in patients with multiple risk factors, they can conduct instant and precise diagnosis and finally, effective triage can be established. So the extent of necrosis area and also mortality can be reduced (13). Although chest pain is the most important symptom for diagnosis of ACS, but it's associated with the non-typical and non-specific symptoms, such as quality, spreading, location and severity of chest pain. These symptoms have been reported different in various populations. Previous studies investigate, the differences in symptoms between men and women, while evidence suggests that symptoms differences can be seen in age, risk factors and race (14).

Considering the few studies have been conducted on this issue in developing countries (15), especially in Iran, this study investigates the Relationship of some risk factors and symptoms in patients with acute coronary syndrome.

Methods:

This cross-sectional study was conducted in Imam Reza hospital in Amol (Iran), which is in the north of Iran, has the population of one million, from May 2010 to July 2010.

The study is powered at 80% with a 2-sided 5% to achieve a statistically significance on a moderate standardized effect size of 0.4. 294 patients diagnosed with acute coronary syndrome, hospitalized in cardiac ward which had survived at least 24 hours, Purposive Sampling, enrolled to the study.

Patients were excluded if they had any history of Alcoholism, mental, psychological and verbal problems, decreased of consciousness, acute skeletal muscle (at least a week before the onset of symptoms), gastrointestinal diseases such as peptic ulcer, reflux and congestive heart failure.

The data collected by a researcher made questionnaire that included demographic data, history of risk factors such as sex, diabetes, hypertension and hyperlipidemia and also clinical coronary artery disease symptoms. Symptoms of coronary artery disease (CAD), such as chest pain, dyspnea and sweating were gathered from the patients by an expert interviewer. To determine the validity of the questionnaires, the content related validity was utilized and their validity was confirmed by 10 cardiologists in Amol University of Medical Sciences. Also, in order to determine the internal consistency reliability, Cronbach's alpha coefficient was used (r=0.91).

Definition:

Acute coronary syndrome: ACS has been identified with ST depression less than one mm, ST elevation more than two mm in leads V1-V4 or more than one mm in any other lead, inverted T wave in each lead or increase in blood levels of at least one biochemical marker (16).

STEMI: ST elevation more than two mm in leads V1-V4 or over one mm in any other lead was defined as STEMI.

NSTEMI: 1) The existence of more than 20 minutes to angina along with higher levels of troponin T or I. 2) ECG changes in ST segment depression and inverted T

wave, even a normal ECG was defined as NSTEMI (15).

Ethical Considerations:

No further diagnostic procedure or treatment imposed on the process of treating patients and all information obtained from the patients remained confidential and given no legal and real authority. The study was approved by the ethics committee of Babol University of Medical Science.

Statistics:

Data analyzed by using SPSS version 16 software with descriptive statistics, Chi-square and logistic regression. In the logistic regression model, the independent variables were treated as a binary variable. Results were expressed as crude and adjusted odds ratio and 95% confidence interval. The variables with *p* less than 0.1 in the crude analysis were entered in the adjusted model and in adjusted odds ratio all variables entered simultaneously by backward stepwise regression model. A probability level of $P<0.05$ was accepted as statistically significant.

Results:

From two hundred ninety four subjects participated in this study, 172 subjects (58.5%) with mean age 26/12(SD=67/59) years old and weights 19/15 (SD=75/73) Kg were men. 0ne hundred thirty three subjects (45.2%) had normal body mass, 245 subjects (83.3%) were Married, 180 of them (61.2%) were illiterate and 116 subjects (39.5) were smokers .

From the whole subjects with ACS, 121 subjects (41.1%) had UA (CI95%: 36 - 46), 141 (48%) had STEMI (CI95%: 42-53) and 32 (10.9 %) had NSTEMI (CI95%:6-13). Ninety two subjects (31.3%) of subjects had a history of diabetes, 121 (41.2%) had hypertension and 144 (49%) patients had hyperlipidemia. According to the results of this study, no significant relationship was found between NSTEMI and symptoms. But there was a significant relationship between symptoms such as chest pain (p=0.03), vomiting (p=0.006), hiccups (p=0.04), belch (p=0.03) and anxiety (p=0.007), with STEMI. Also, there was a significant relationship between symptoms such as sweating (p=0.01), nausea (p=0.01), vomiting (p=0.001), hiccups (p=0.01) and anxiety (p=0.01), with UA.

Table 1 shows crude and adjusted odds ratio for ischemic heart disease symptoms in acute coronary syndrome. Since, there was no significant relationship between NSTEMI and symptoms, researcher did not enter the symptoms with NSTEMI in logistic regression model.

On the other hand, according to the adjusted model, STEMI increases probability of vomiting and anxiety symptoms up to 96% and 83% respectively.

Also, UA reduces probability of vomiting symptom less than 58%. According to the chi-square test, although men had experienced chest pain more than women (p=0.04) but the symptoms of weakness (p <0.001), fatigue (p=0.02), hiccups (p=0.01) and anxiety (p=0.001) were reported in women more than the men.

Also, patients with diabetes, reported dyspnea (p=0.002), weakness (p<0.001), sweating (p=0.02), fatigue (p=0.001), hiccups (p=0.01), belch (p=0.01) and tinnitus symptoms (p=0.002) more than the patients without a history of diabetes.

Among the subjects, there was a significant relationship between a history of hypertension with symptoms of chest pain (p=0.04), dyspnea (p=0.04), sweating (p=0.01), nausea (p=0.01), vomiting (p=0.03) and hiccups (p=0.03). Finally, ACS Patients with hyperlipidemia experienced more symptoms of dyspnea (p=0.001), weakness (p<0.001), fatigue (p=0.003) and tinnitus (p<0.001).

Table 2 shows crude and adjusted odds ratio for ACS symptoms according to sex, diabetes, hypertension and hyperlipidemia risk factors in the logistic regression model.

According to the adjusted model, symptoms of weakness (129%) and anxiety (82%) are more likely in female gender.

These results showed subjects with diabetes, risk of dyspnea, weakness and tinnitus is up to 101%, 106% and 80% respectively. Also, hyperlipidemia increases the experience of symptoms such as weakness and tinnitus 135% and 149% respectively.

ACS patients with a history of hypertension in the crude model, showed the dyspnea 64%, vomiting 74% and hiccups symptoms 99% more than the other. But in adjusted logistic regression model, no symptoms remained in patients with a history of hypertension.

Discussion:

Ischemic heart disease is the main cause of disability and death in the most countries of the world. Despite the major improvements in diagnosis and treatment, one third of patients with ACS die. Half of these patients, within the first hour and before reaching to the hospital die. And two-thirds of those who survive do not ever fully recover and do not return to normal life (17).

According to the results of this study, although no significant relationship was seen between the symptoms of ischemic heart disease and NSTEMI, But in the study of Thuresson there was significant relationship between chest pain, nausea, vomiting and dizziness with NSTEACS (11). This result was not consistent with our research finding. NSTEACS (UA and NSTEMI patients) were included in the Thuresson study, which could explain this discrepancy. But in our research, those patients who had increased troponin T,

but no ECG changes and ST elevation, enrolled to the study. In the literature review no other study conducted on ischemic heart disease symptom and NSTEMI.

The present findings imply that patients with STEMI show more symptoms of vomiting and anxiety. This result is consistent with Thuresson research finding (11).

Nausea and vomiting may be established in a STEMI due to the activation of vagal reflex or stimulate left ventricular receptors that is part of the Bezold-Jarisch. These symptoms are seen in the inferior STEMI than the anterior. The more symptoms can be seen in inferior STEMI than the anterior. Although nausea and vomiting are the two common symptoms of STEMI, but when ACS pain is experienced in the epigastric region, it may simply mimic acute cholecystitis, gastritis or peptic ulcer symptoms (18).

On the other hand, the results of this study indicate that UA reduces the vomiting symptom, but may increases the symptoms such as sweating, pale cold skin, and sinus tachycardia (18). Unfortunately, there was no study found on ischemic heart disease symptoms in the patients affected by UA.

Coronary artery wall atherosclerosis progression which accompanies with accumulation of fat particles leads to many inflammatory reactions. On the other hand, bringing Macrophages to the damaged area, leads to the release of biochemical substances which increase endothelium damage and leads to platelet aggregation and clot formation.

Severity of ACS is associated with the involvement area and the degree of coronary artery occlusion with thrombosis. Complete occlusion of a coronary artery and cut off blood supply to the heart muscle lead to STEMI, and in the partial occlusion the UA/NSTEMI symptoms appear (19). According to our study women reported more symptoms such as weakness, fatigue, hiccups and anxiety than men, but men experienced just chest paint more than the women. In Devon study women had experienced more symptoms of dyspepsia, Palpitation, nausea, numbness fingertips, weakness, and cough than the men, but the men reported more dizziness (20).

Some studies reported that men had experienced more chest pain and dyspnea and women more sweating and dyspnea (21). Another study indicated that the appearance of vomiting, dyspnea, fatigue and anxiety in women is more than the men and also hiccups, sweating and fainting, are more common in the men (7).

Contradictory findings have reported in association with ACS symptoms with regard to sex, maybe along with many other diseases, which ultimately may cause a delay in decision making, diagnosis and treatment (20).

We found the patients with ACS who had a history of diabetes, experienced more dyspnea, weakness and tinnitus. Although in the study of Funk et al. there was no significant relationship between diabetes and ACS symptoms (15). But Culić have reported that diabetic patients have more experiences of dyspnea, weakness, cough and vomiting that may be due to neuropathy and Autonomic Dysfunction nerve fibers (14).

The risk of cardiovascular events in diabetic patients is 2-8 times more than Non-diabetic, and 75% of diabetic patients' mortality is due to CAD (18).

Diniz stated that diabetes is considered one of the main causes of tinnitus, which is accompanied with spiral ganglion neuron atrophy and eighth cranial nerve demyelination, so 70% of diabetic patients in his study reported tinnitus (22).

Also in our study, ACS patients with a history of hyperlipidemia experienced more weakness and tinnitus. The study of Culić have shown that weakness, nausea, hiccups, and tinnitus symptoms is more experienced in patients with AMI with a history of hyperlipidaemia (14).

Tinnitus is seen more in over 50 years old people, especially when other risk factors such as angina, hyperlipidaemia and diabetes are present (23).

The results suggest that the appearance of dyspnea, vomiting and hiccups in ACS patients with a history of hypertension is higher. According to the Culić study the symptoms of chest pain, belch, cough and weakness in AMI patients with a history of hypertension was higher (14). The results of this study did not confirm our result.

This difference may be due to this fact that all of the patients in colic study had AMI, but in our study a part of participants were patients who had unstable angina.

Conclusion:

The findings show that the appearance of symptoms of acute coronary syndrome according to the ECG changes and risk factors is different and more related to those symptoms that may be common in many other diseases.

Since, many of the symptoms of acute coronary syndrome can be potentially dangerous and life-threatening, accurate diagnosis and timely action is crucial for the patients.

Limitations of the Study:

We couldn’t assess another factor which affects ACS symptoms such as musculoskeletal disease, neurological and cognitive disorder and also lifestyle.

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Conflict of interest
There was no conflict of interest.

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Table 1: Ischemic heart disease-related symptoms in logistic regression model, patients with acute coronary syndrome

ACS	Logistic Regression	**C**rude model			**A**djusted model		
		OR	95%CI for OR	*P* value	OR	95%CI for OR	*P* value
	Symptoms						
	Chest pain	3.27	1.02-10.14	0.04			
	Vomiting	2.12	1.23-3.64	0.006	1.94	1.12-3.38	0.01
STEMI (yes*/no)	Hiccup	1.95	1.01-3.74	0.04			
	Belching	1.75	1.03-2.98	0.03			
	Anxiety	1.99	1.19-3.30	0.008	1.83	1.09-3.09	0.02
UA (yes*/no)	Sweating	0.50	0.30-0.84	0.009			
	Nausea	0.54	0.33-0.89	0.01			
	Vomiting	0.36	0.02-0.66	0.001	40.42	0.23-0.77	0.005
	Hiccup	0.04	0.19-84	0.01			
	Anxiety	0.52	0.31-0.89	0.01			

All variables with p<0.1 in crude analysis were entered in the adjusted model in step 1; then using backward stepwise method, only significant variables were selected in the final adjusted model. OR; Odd ratio, CI; Confidence Interval.

Table 2 :The relationship between acute coronary syndrome disease and risk factors such as sex, diabetes, hyperlipidaemia and hypertension

Logistic Regression		**C**rude model			**A**djusted model		
Risk Factors	Symptoms	OR	95%CI for OR	*P* value	OR	95%CI for OR	*P* value
Sex(male/female*)	Weakness	66 .2	4.33 -1.63	0.001 <	2.29	3.80 -1.37	0.001
	Fatigue	1.71	2.78 -1.05	0.02			
	Hiccup	2.18	4.15 -1.14	0.01			
	Anxiety	2.28	3.80 -1.37	0.001	1.82	3.11 -1.07	0.02
	Dyspenea	2.32	1.35 -2.32	0.002	1.8	3.18 -1.01	0.04
	Weakness	2.58	4.29 -1.55	<0.001	2.01	3.44 -1.17	0.01
	Sweating	1.95	3.49 -1.08	0.02			
Diabetes(yes*/no)	Fatigue	2.32	3.86 -1.39	0.001			
	Hiccup	2.19	4.19 -1.14	0.01			
	Blich	1.97	3.40 -1.14	0.01			
	Tinnitus	2.58	4.70 -1.41	0.002	2.06	3.86 -1.1	0.02
	Dyspenea	2.16	3.05 -1.34	0.002			
	Weakness	2.97	4.87 -1.81	0.001>	2.35	3.96 -1.4	0.001
Hyperlipidemia(yes*/no)	Fatigue	2.06	3.36 -1.27	0.003			
	Tinnitus	3.11	5.87 -1.64	0.001>	2.49	4.82 -1.28	0.007
	Dyspenea	1.64	2.67 -1.01	0.004			
Hypertension(yes*/no)	Vomiting	1.74	2.96 -1.02	0.004			
	Hiccup	1.99	3.87 -1.04	0.003			

*Reference

All variables with p<0.1 in crude analysis were entered in the adjusted model in step 1; then using backward stepwise method, only significant variables were selected in the final adjusted model.OR; Odd ratio, CI; Confidence Interval.

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Cohort Study on Hemolysis Associated with G6PD Deficiency in Jaundice Neonates

Seyed Hesamedin Nabavizadeh [1], Mehrdad Rezaie [2], Parisa Sabzali [3], Abdolhamid Barati [3], Mohammad Zoladl [1]

[1.] Social Determinants of Health Research Center, Yasuj University of Medical Sciences, Yasuj, Iran
[2.] (Corresponding author) Social Determinants of Health Research Center, Yasuj University of Medical Sciences, Yasuj, Iran and Department of Neonate Medicine, Shiraz University of Medical Sciences, Shiraz, Iran
[3.] Students Research Committee, Yasuj University of Medical Sciences, Yasuj, Iran
md_rezaie@yahoo.co.uk

Abstract: Glucose 6 phosphate dehydrogenase (G6PD) deficiency as an X-linked disorder is the most common human enzyme deficiency in the world. A Meta analysis regard G6PD deficiency showed its prevalence in Iran was between 2.1 to 7.6 percent. With regard to the fact that Iran is located in area with high prevalence of G6PD deficiency, and with respect to information that some other studies consider the role of hemolysis less important in the incidence of jaundice, therefore this cohort study was aimed to determine the relationship between hemolysis and G6PD enzyme deficiency by compare related data between 107 neonates suffering from jaundice having G6PD deficiency as experiment group, and the control group consisted of 127 neonates having normal G6PD enzyme activity. Result showed that the mean of bilirubin in the experiment group was 18.1 gram per deciliter, and 16.4 grams per deciliter in the control group (p=0.018). It can be concluded that there was no evidence of higher hemolysis among neonates suffering from jaundice having G6PD enzyme deficiency compared to neonates suffering from jaundice having normal G6PD enzyme activity. It also can be concluded that hemolysis is not an important factor in the incidence of jaundice in children having G6PD enzyme deficiency.
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Keywords: Hemolysis; neonate; Hyperbilirubinemia; G6PD deficiency

1. Introduction

Glucose 6-phosphate dehydrogenase is an enzyme found in normal red blood cells. Its primary metabolic role in normal concentration is to protect red cells against oxidative damage (George and Akani, 2011). This enzyme is a crucial enzyme in the regenerative mechanism of the aerobic cells too. Although patients with the enzyme deficiency in all tissues have G6PD deficiency activity, but it seems that except in red blood cells, it has no major dysfunction symptoms in other body tissues in a way that they are not marked due to the dysfunction of this enzyme (Behrman, Kliegman, and Jenson, 2008).

Glucose-6-phosphate dehydrogenase (G6PD) deficiency as an X-linked disorder which affecting mostly African, Mediterranean and far-eastern populations is the most common human enzyme deficiency in the world; it affects an estimated 400 million people (Iranpour, Hashemipour, Talaei, Soroshnia, and Amini, 2008). This enzyme deficiency is widely observed in tropical and subtropical regions consist of Africa, South Europe, The Middle East, South Asia, and Oceania (Cappellini and Fiorelli, 2008).

Although according to WHO, Iran is in a moderately high incidence area for G6PD deficiency (Mohammadzadeh, Jafarzadeh, ShahFarhat, Keramati, Badiee, Esmaily, and Amiri, 2009) and there is a 10-14.9% prevalence of G6PD deficiency in this country (Nabavizadeh and Anushiravani, 2007) but a meta analysis regard G6PD deficiency showed its prevalence in Iran was between 2.1 to 7.6 percent (Nkhoma, Poole, Vannappagari, Hall, and Beutler, 2009).

The difference in incidence is related to the different areas of residence and the groups themselves which are under study. The majority of people having G6PD enzyme deficiency are unmarked throughout their lives, but these people are at risk of producing neonate jaundice and increasing the risk of acute hemolysis due to contacting oxidants (Cappellini and Fiorelli, 2008).

The clinical manifestations of G6PD deficiency vary from no symptoms to acute haemolytic anaemia or severe chronic haemolytic anemia (Rahimi, Raygani, Siabani, Mozafari, Nagel, and Muniz, 2008).

Neonatal jaundice caused by G6PD enzyme deficiency is rarely observed on first day after birth. It is usually observed on day two or three. In most cases, neonates do not suffer from severe anemia; however, the presence of factors such as prematurity, infection, and environmental oxidants, neonatal jaundice due to G6PD enzyme deficiency may be severe, which may lead to brain damage or even death. Although jaundice due to G6PD enzyme

deficiency after neonatal stages may be due to Hemolysis, and as a result, the increase of bilirubin, but according to various studies, this is not true among neonates, and the incidence of jaundice at this stage is related to other factors such as liver dysfunction (Cloherty, Eichenwald, and Stark, 2008). With regard to the fact that Iran is located in an area with high prevalence of G6PD enzyme deficiency, and also with respect to the information that some other studies consider the role of hemolysis less important in the incidence of jaundice, therefore this study was aimed to determine the relationship between hemolysis and G6PD enzyme deficiency among neonates suffering from jaundice admitted at the Imam Sajjad Hospital of Yasuj, Iran.

2. Material and Methods

The samples of this cohort study were selected among neonates suffering from jaundice who admitted at the Imam Sajjad Hospital of Yasuj, Iran. The sample size estimated approximately 105 per each group by the formula with Z 0.95(1.96), G6PD deficiency ratio of seven percent, and d=0.05, and also considering the possibility of a 10 percent sample loss.

In research process, 107 neonates suffering from jaundice having G6PD enzyme deficiency were selected as experiment group, and the control group consisted of 127 neonates having normal G6PD enzyme activity; so 234 admitted neonates suffering from jaundice in the Neonatal Unit of Imam Sajjad Hospital of Yasuj were studied.

Data regarding age, place of parent's residence, laboratory findings were gathered from both groups by a questionnaire designed for this purpose. The collected data were analyzed by the SPSS software using inferential and descriptive statistics.

3. Results

In the present cohort study conducted on neonates suffering from jaundice neonates who admitted at the Imam Sajjad Hospital from 2008-first quarter of 2009, a total number of 234 neonates were studied in two groups. These two groups consisted of an experiment group of 107 neonates (%45.7) which had G6PD enzyme deficiency and a control group, 127 neonates (%54.3), having a normal range of G6PD enzyme activity.

The mean age of the neonates was 4.6±4.2 days, which the mean age of hospitalization of the experiment and control groups was 4.7 and 4.5 days respectively showing no significant difference.

The average of overall hospitalization period for both experiment and control groups were 4.2 and 4.4 days respectively. Despite the existence of some differences, no significant statistical differences were observed.

The mean of hemoglobin of the infants at the time referring to the hospital were 15.1 gram per deciliter in the experiment group, and 15 gram per deciliter in the control group. These amounts of hemoglobin showed no significant statistical difference.

The average amount of bilirubin in the experiment group was 18.1 gram per deciliter, and 16.4 grams per deciliter in the control group (p=0.018).The higher rate in the experiment group led to a significant statistical difference (Table 1).

Table 1. Bilirubin amount (milligram per deciliter) in admitted jaundice neonates based on status of deficiency of glucose 6 phosphate dehydrogenize

Group / Bilirubin	Case	Control	Total
Mean	18.1	16.4	17.2
SD	5.2	5.2	5.2
	P= 0.018		SIG

In the meantime, the highest amount of Bilirubin in both the experiment and control grouip was 30 and 39 respectively.

Considering the average amount of reticulocyte, it was 2.1 and 1.7 for the experiment and control group respectively. Although p=0.086, and also considering the high amount of reticulocyte in the experiment group, no significant difference was observed.

Considering hemoglobinuria, from the 212 answers from the questionnaires, hemoglobin was found in the urine of 19 neonates (%9), its details showed in table 2. The calculated relative risk associated with the occurrence of hemoglobinuria in infants with G6PD enzyme deficiency was 1.24.

Table 2. hemoglobinuria in admitted jaundice neonates

Hemoglobinuria / Group	Number	Percent
Case	11	10.3
Control	8	6.45
Total	19	8.2
SIG	P=0.47	

All the neonates in both groups had negative compose. Regarding mothers' blood group, 49 (%46.2) were O, 37 (%34.9) were A, 14 (%13.2) were B, and 6 (%5.7) were AB. Moreover, 97 (%91.5) were +Rh, and (%8.5) were –Rh.

From the 124 correct answers of the control group, the amount of blood groups of O, A, B, and AB were 60, 33, 29, and 2 respectively. 116 neonates were +Rh, and 8 were –Rh.

The amount of P regarding the mothers' blood group was %66. The number of blood group O, A, B, and AB of the neonates were 49, 31, 21, and 5 respectively. These amounts for the control group were 46, 38, 35, and 3 respectively. 104 neonates of the experiment group were +Rh and 2 were –Rh, but for the control group was 117 and 5 respectively, which showed no significant difference regarding blood groups and the Rh in both groups.

4. Discussions

In the present study, no statistical significance was observed between the amount of Reticulocyte, hemoglobin, and Humoglubinory between case and control group ($p>0.05$). So, it can be concluded that there was no evidence of higher hemolysis among neonates suffering from jaundice having G6PD enzyme deficiency compared to neonates suffering from jaundice having normal G6PD enzyme activity. It also can be concluded that hemolysis is not an important factor in the incidence of jaundice in children having G6PD enzyme deficiency.

A study regard evaluation of Glucose 6 phosphate dehydrogenase deficiency without hemolysis in icteric newborns showed that despite reporting hemolysis among a number of patients having G6PD enzyme deficiency, in the majority of the cases with enzyme deficiency (%58.3), no sign of hemolysis was observed(Eghbalian and Monsef, 2007). This is in accordance with the results of the present study. It should be noted that the number of hemolysis among neonates with G6PD enzyme deficiency in the Eghbalian study is higher than other studies, which may be due to the small fraction of neonates with G6PD enzyme deficiency in his study.

In a study conducted in Nigeria, 40 percent of neonates suffering from jaundice had G6PD enzyme deficiency, which most cases had no sign of hemolysis (Ahmad, Yulubu, and Hendricks, 1999).Also, In study in India, it was observed that 12 percent of the neonates suffering from jaundice had G6PD enzyme deficiency, and among these, despite that 48.7 percent suffered from severe jaundice, no case of hemolysis was observed (Madam, Sundaram, and Bhargava, 2001).

In another study conducted at Saudi Arabia, 18.4 percent of the neonates suffering from jaundice had G6PD enzyme deficiency, but no case of hemolysis was observed (Yaish, Niazi, al Shaalan, Khan, and Ahmed, 1991).

In all the studies mentioned above, and also a study conducted in Malaysia (Jalloh, Van Rostenberghe, Yusoff, Ghazali, NikIsmail, Matsuo, Wahab, and Nishio, 2005), and a study conducted in Mazandaran province, Iran (Ahmadi and Ghazizadeh, 2008) with various severities reported, no sign of interference of hemolysis as a main factor of Hyperbilirubinemia was observed among neonates having G6PD enzyme deficiency, which is consistent with the results of the present study.

A report from Birmingham reveals a fatal incidence of severe hemolysis in a neonate suffering from jaundice having G6PD enzyme deficiency (Aaron, 2007). Also another study describes a case of hemolysis and Hyperbilirubinemia among a triplet having G6PD enzyme deficiency (Shah and Yeo, 2007). These cases were exceptional individual cases which cannot undermine the results of the present and previous studies.

It is worth to note that with respect to the results of the present study which has a considerable sample size (a similar sample size of the Saudi Arabia study), it can be said that the interference of hemolysis on neonates suffering from jaundice having G6PD enzyme deficiency is negligible and even be ignored.

In general, based on the present study, there is a significant difference in the severity of jaundice (amount of bilirubin in the blood) between both the experiment and the control group, in such a way that the severity of jaundice in children having G6PD enzyme deficiency is approximately two units more than neonates having normal G6PD enzyme activity. This result is in accordance with almost all of the studies mentioned above.

In this study no meaningful difference regarding the age of admitted neonates and the period of hospitalization were observed. This could explain the short hospitalization duration of the neonates suffering from jaundice having G6PD enzyme deficiency who rapidly admitted to the hospital.

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Corresponding Author:
Dr. Mehrdad Rezaie
Social Determinants of Health Research Center
Yasuj University of Medical Sciences
Yasuj, Iran
Department of Neonate Medicine

Shiraz University of Medical Sciences
Shiraz, Iran
E-mail: md_rezaie@yahoo.co.uk

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Pathogenetic Role of Matrix Metalloproteinase-2 and Matrixmetalloproteinase-9 in Behcet's Disease

Sahar S Ganeb[1], Howyda M Kamal[2] and Ayser A Fayed[3]

Rheumatology, Rehabilitation & Physical Medicine[1], Clinical & Chemical Pathology[2] and Ophthalmology[2] Departments, Benha University, Faculty of Medicine, Egypt
sahar_ganeb@yahoo.com

Abstract: Objective: To assess serum levels of Matrix metalloproteinases-2 (MMPs-2) and MMP-9 in Behcet's disease (BD) patients to investigate the possible association between MMP-2 and MMP-9 serum levels with clinical manifestations and disease activity. Methodology: Thirty BD patients and 30 age and sex matched healthy controls were included. Thorough clinical examination with stress on dermatological, locomotor, neurological and ophthalmologic manifestations. Assessment of disease activity was done. We compared the activity scores of patients with their serum levels of MMP2 and MMP-9. Assessment of ESR, CRP, MMP-2 and MMP-9 serum levels by ELISA were performed. Results: A statistical significant increase ($P<0.001$) in MMP-9 levels was found in BD patients in comparison with the control group, while there was non-statistical significant difference ($P>0.05$) in MMP-2 levels in BD patients in comparison with the control group. Within the BD patients' group, there were elevations of MMP-2 and MMP-9 serum levels in BD patients with vascular lesions, CNS lesions and disease activity ($P<0.05$). There were statistical significant positive correlations between MMP-2 and MMP-9 serum levels with disease activity score ($r= 0.425$, $P<0.05$), ($r= 0.413$, $P<0.05$) respectively and vascular lesions ($r = 0.394$, $P<0.05$), ($r = 0.458$, $P<0.05$) respectively. Conclusion: Increased serum levels of MMP-2 and MMP-9 in BD patients can be considered as a pathogenetic marker of BD disease activity. These higher levels correlated with systemic involvement and were associated with various clinical manifestations.

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Keywords: Behcet's disease, Matrix metalloproteinases, disease activity of Behcet's disease.

1. Introduction

Behcet's Disease is a chronic inflammatory multisystem disorder of unknown disorder characterized by recurrent orogenital ulcers, and skin lesions, ocular involvement which is frequent and sever, often bilateral, rapidly compromising the visual function. Eye lesion have been found including anterior uveitis, posterior segment involvement with vasculitis, vitritis, retinal oedema, and venous occlusion. Eye involvement my also be associated with neurological manifestations like optic neuropathy. The disease frequently occurs in Mediterranean countries, the Middle East, and eastern Asia, there are no pathognomonic laboratory tests or histologic findings specific to BD, thus, the diagnosis is based on clinical criteria, and various criteria have been proposed. The most commonly used criteria are those of the International Study Group for BD, and these require recurrent oral ulceration plus at least two of the following: recurrent genital ulcerations, eye lesions (such as uveitis), skin lesions (such as erythema nodosum or folliculitis), and a positive skin pathergy test (1,2).

MMPs are a large family of proteolytic enzymes involved in an array of physiological and pathological processes from development, morphogenesis, reproduction, wound healing, and aging to inflammation, angiogenesis, neurological disorders, and cancer cell invasion and metastasis (3).

MMPs differ structurally and that each MMP has the ability to degrade a particular subset of matrix proteins. The protein products are, however, classified by shared functional and structural characteristics (4). Based on the substrate specificity, the family of MMP enzymes is subdivided into subgroups such as stromelysins (MMP-3, -10 and -11), collagenases (MMP-1, -8 and -13), gelatinases (MMP-2, and -9) and membrane-type MMPs (MMP-14, -17, -22, -24, -25) (5).

Among these, gelatinases comprised of gelatinase A (MMP-2) and gelatinase B (MMP-9), they have unique ability to degrade the type-IV collagen, a major component of the basement membrane (6). The gelatinases are secreted as zymogens and cleave to the active form and their function is tightly regulated by several different mechanisms (7).

Together, the MMPs are able to process or degrade all the known protein components of the extracellular matrix (ECM) (4,8). They are a family of enzymes that regulate the ECM environment and whose activity has been implicated in normal and pathological processes (9,10). Each ECM element is cleaved by a specific MMP or MMP group (11). Pro-

inflammatory cytokines such as interleukin-1 (IL-1) and tissue necrosis factor-α (TNF-α) have been shown to up-regulate MMP-9 (12,13). MMP-9 is considered to be a key determinant of extracellular matrix degradation, having collagen as the main substrate (14).

MMP-9 appears more likely than MMP-2 to be involved in the pathophysiology of Giant cell arteritis. MMP-9 not only participates in the degradation of elastic tissue but also is associated with intimal hyperplasia, subsequent luminal narrowing, and neoangiogenesis (15).

Aim of the work:

The present study aimed to assess the serum levels of MMP-2 and MMP-9 in Behcet's disease patients, to investigate the possible pathogenetic association between MMP-2 and MMP-9 serum levels with clinical manifestations and disease activity.

2. Patients and Methods

Thirty patients, diagnosed as BD according to the international study group criteria (ISGC, 1990) (16), and thirty age and sex matched healthy volunteers serving as a control group were enrolled in this study.

All participants gave informed consent and the local Ethical Committee approved the study.

Exclusion criteria: Patients who had other illnesses that might affect the results of the study, such as other autoimmune diseases, herpes simplex, renal, hepatic diseases or diabetic patients were excluded from the study.

All BD patients were subjected to the following: detailed history taking, thorough clinical examination with stress on dermatological, locomotor, neurological and ophthalmological disorderd , assessment of disease activity was done at time of blood sampling according to the Leeds activity score system (17). Patients with BD were categorized as active (total activity score ≥5) or inactive (total activity score <5).

Laboratory Investigations:

Seven milliliters of venous blood was drawn. One ml on EDTA for complete blood picture by sysmex kx 21. Two mls on Na citrate (with ratio1:4) for ESR estimation by Westergren method recorded in mm/hr.

The remaining blood sample was put in a plain tube, left to clot for 20 mins., then centrifuged at 3000 rpm for 30 mins. The serum was used in the measurement of C-reactive protein (CRP) by the turbidity assay as specified by the manufacturer (Orion diagnostic, cat. No.67977), with an established normal range of (0 - 0.8 mg/dl) and the rest of serum samples were stored at -20°C until measuring MMP-2 and MMP-9 levels by ELISA.

Measurements of MMP-2 and MMP-9:

The levels of MMP-2 was measured by human MMP-2 ELISA cat.No: BBT0459R manufactured by (Biovendor laboratorni medicina a.s). Sensitivity <10 pg/ml. Concentrations of unknown samples were determined from a curve obtained by the standards.

MMP-9 was measured by human MMP-9 ELISA cat. No. RBMS2016/2R manufactured by (Biovendor laboratorni medicina a.s). The detection limit was ≤ 0.05 ng/ml, with inter-assay and intra-assay coefficient of variation (CVs) were 10.2% and 7.3% respectively.

Statistical analysis:

The collected data were presented and analyzed using SPSS version 17 soft ware. Suitable statistical techniques were calculated as number and percent or mean and standard deviation. Student "t" test, was used as a test of significance and 95% CI. The relationship between the variables was evaluated by Spearman's correlation.

3. Results

This study included 30 patients, 19 males (63.3%) and 11 females (36.6%). Their ages ranged from 21-51 years with a mean of 37.52±7.74 years. Thirty age and sex matched healthy volunteers serving as a control group- 18 males (60%) and 12 females (40%), aged from 21-53 years with a mean of 33.29±9.82 years were included in this study. Patients' disease duration ranged from 2 to 11 years with a mean of 5.73±3.5 years.

Patients were taking corticosteroids and/or immunosuppressive agents in variable doses.

Table (1) Shows clinical manifestations of BD patients: Skin lesions: erythema nodosum, sterile pustules or papules in 13 patients (43.3%). Arthritis in 14 patients (46.6%). Ocular disorders in 13 (43.3%): uveitis in 11 patients (36,7%), optic neuritis in one eye (3.3 %) and retinal vasculitis in one patient (3.3%) (figure 1) displayed a picture of optic neuritis (a) and a fluorescein angiography of a case of retinal vasculitis (b) . Vascular lesions in 5 patients (16.6%): venous thrombosis in 4 patients (13.3%) and arterial thrombosis in one patient (3.3%) were encounterd, and 50% of patients had disease activity.

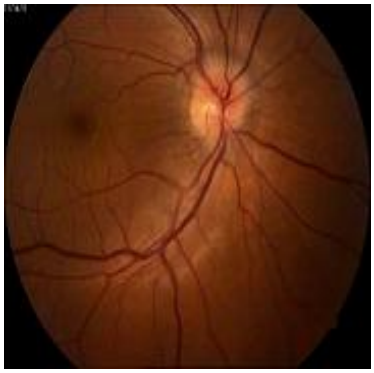
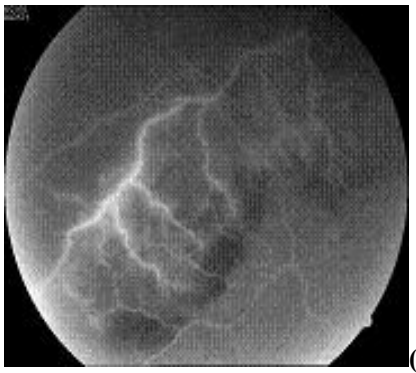
(a)   (b)

Figure [1] : a picture of optic neuritis (a) ,a fundus fluorescein angiography of a case of retinal vasculitis (b)

Table (1): Clinical manifestations of BD patients (no=30).

Clinical manifestations	No of patients	Percentage
Oral aphthous ulcers	18	60%
Genital ulcers	8	26.6%
Skin lesions	13	43.3%
Arthritis	14	46.6%
Ocular manifestations	13	43.3%
Vascular lesions	5	16.66%
CNS manifestations	2	6.6%
Disease activity	15	50%
+ve skin Pathergy test	8	26.6%

Table (2): Shows that there was no statistical significant difference (P>0.05) in MMP-2 levels in BD patients in comparison with the control group, while the levels of MMP-9 were significantly higher (P<0.001) in BD patients in comparison with control group.

Table (2): ***ESR, CRP and serum MMP-2 and MMP-9 levels in patients and controls.***

Parameters	Patients mean±SD Number=30	Controls mean±SD number=30	t	P	95% CI
ESR	39.59±15.63	14.57±5.52	8.3	<0.001*	18.9-31.1
CRP	9.9±5.3	0.4±0.2	9.8	<0.001*	7.6 - 11.4
MMP-2 ng/ml	7.1±5.3	5.14±3.51	1.69	>0.05	0.4 -4.3
MMP-9 ng/ml	36.48±16.43	18.74±12.12	4.76	<0.001*	10.3-25.2

P<0.05*= significant **P>0.05**= nonsignificant

The association between individual clinical manifestations and serum MMP-2 levels revealed significant elevation of MMP-2 serum levels in BD patients with skin lesions, vascular lesions, CNS lesions and disease activity (P<0.05), while there were no association between serum MMP-2 levels and oral ulcers, genital ulcers, arthritis, ocular lesions or a positive skin Pathergy test (P> 0.05), (table 3).

Table (3): Serum MMP-2 in patients with different clinical Manifestations compared to those without in BD patients (no=30).

	Oral ulcers (no18)	Genital ulcers (no=8)	Skin lesions (no=13)	Arthritis (no=14)	Occular lesions (no=13)	Vascular lesions (no=5)	CNS lesions (no=2)	Disease activity (no=15)	+ve Pathergy (no=8)
With	6.92±2.8	7.34±5.1	7.34±2.6	6.28±4.1	6.85±4.7	12.19±2.7	10.22±4.1	7.71±2.9	6.78±4.7
without	6.83±2.1	6.31±2.7	5.48±1.4	5.91±3.2	6.37±3.9	5.21±1.9	5.43±2.3	5.33±2.1	6.14±4.1
t	0.094	0.72	2.52	0.28	0.3	7.01	2.74	2.57	0.36
P	0.92	0.47	0.019*	0.78	0.76	0.0000*	0.01*	0.016*	0.72

P<0.05*= significant P>0.05= non significant

Table (4): Shows that MMP-9 serum levels were significantly elevated in BD patients with skin lesions, ocular lesions, vascular lesions, CNS lesions and disease activity (P<0.05), while there were no increase in MMP-9 serum levels with oral ulcers, genital ulcers, arthritis or a positive skin Pathergy test (P> 0.05).

Table (4): Serum MMP-9 in patients with different clinical Manifestations compared to those without in BD patients (no=30).

	Oral ulcers (no=18)	Genital ulcers (no=8)	Skin lesions (no=13)	Arthritis (no=14)	Occular lesions (no=13)	Vascular lesions (no=5)	CNS lesions (no=2)	Disease activity (no=15)	+ve Pathergy (no=8)
With without t t P	38.93±10.1 37.85±11.2 0.27 0.79	39.52±19.7 36.67±14.5 0.43 0.66	38.99±7.01 34.01±5.01 2.17 0.83	38.39±15.8 35.16±15.3 0.56 0.57	39.97±10.3 32.13±9.82 2.12 0.042*	45.21±13.4 31.78±10.4 2.52 0.018*	48.64±15.3 30.31±11.8 2.1 0.045*	46.93±31.8 26.76±16.5 2.18 0.037*	38.16±13.2 34.84±8.40 0.82 0.42

P<0.05*= significant **P>0.05**= non significant

The present work demonstrates that in the correlation between serum MMP-2 levels and different clinical and laboratory parameters among BD patients, there was statistically significant positive correlation with disease activity score (r = 0.3425, P<0.05) figure (2) and vascular lesions (r = 0.394, P<0.05).

Correlating between MMP-9 serum levels and some variables among BD patients showed statistically significant positive correlations with disease activity score (r = 0.413, P<0.05) (figure 3) and vascular lesions (r = 0.458, P<0.05).

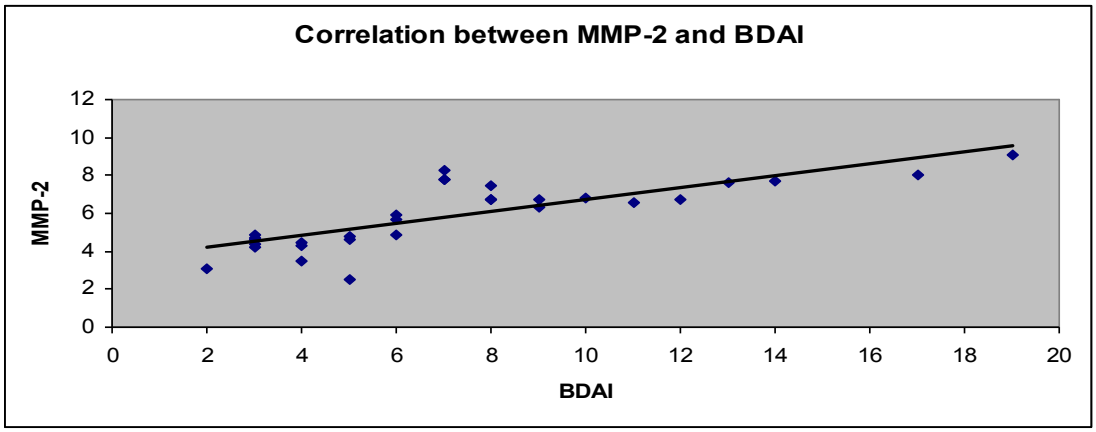


Figure [2]

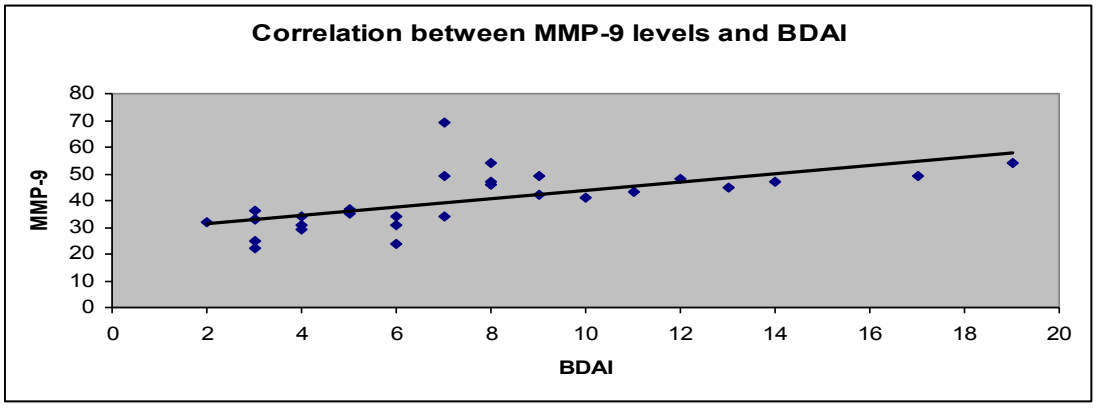


Figure [3]

4. Discussion

Behçet's disease (BD) is a chronic systemic inflammatory disorder affecting multiple organs with a generalized vasculitis (18). It is also described as the triad of recurrent oral and genital ulcers, iritis with hypopyon, retinal vasculitis and posterior uveitis , primarily affecting young adults aged 25–35 years (1,19,20).

Aksoy et al. (2011) (21), reported that the numbers of BD patients suffering from individual clinical manifestations of the disease to varying degrees were as follows: 56% oral ulceration, 40% genital ulceration, 32% ocular involvement, 40% vascular involvement, 40% arthritis/arthralgia and 48% cutaneous lesions, such as erythema nodosum and papulopustular eruption. These results were comparable to our study.

Pandrea et al. (2007) (22), reported that vascular lesions affect 7–29% of BD patients at least once during the clinical course of the disease.

Almost 90% of their patients were diagnosed with superficial thrombophlebitis, and up to 35% developed major thromboembolic complications involving the superior and/or inferior vena cava, arteries were less frequently involved (23).

In this study, the serum levels of MMP-2 showed non statistical significant difference ($P>0.05$) in BD patients in comparison with the control group. On the other hand, the serum levels of MMP-9 were significantly higher ($P<0.001$) in BD patients in comparison with the control group. These results were in accordance with those reported by Pay et al., (2007) (24).

In the current study the association between individual clinical manifestations and serum MMP-2 levels revealed elevation of MMP-2 serum levels in BD patients with skin lesions, vascular lesions, CNS lesions and disease activity ($P<0.05$).

Pay et al., (2007) (24), in their study on 58 BD patients found that the serum levels of both MMP-2 and MMP-9 in patients with systemic involvement, vascular involvement, thrombotic involvement and aneurysm formation were higher than those of healthy controls. Also, they found that the serum level of MMP-9 in patients with ocular disease was higher than those of healthy controls. Therefore, they proposed that MMP-2 and MMP-9 may play a pathogenic role in vasculo-Behçet's disease complicated with aneurysm formation. These results are in accordance with our findings where MMP-9 serum levels were significantly elevated in BD patients with, ocular, vascular and CNS lesions and with disease activity ($P<0.05$).

Also, Pay et al., (2007) (24) stated that serum levels of MMP-2 and MMP-9 were not found different compared to those with mucocutaneous involvement. This coincides with our results as there were no association between serum MMP-2 and MMP-9 levels and oral ulcers or genital ulcers. On contrary to their study our results showed significant elevation of MMP-2 and serum levels in BD patients with skin lesions.

Lorelli et al. (2002) (25), demonstrated that the plasma MMP-9 levels were higher in the patients with abdominal aortic aneurysm compared with healthy patients. The causes of aneurysms were either atherosclerosis and/or degeneration (26). This supports our finding, as there were significant increase MMP-9 serum levels in patients with vascular lesions.

Vascular involvement in Behçet syndrome is most likely to involve thrombosis in the venous system, but arterial lesions are associated with greater risks (20,23). The pathogenesis is considered to be vasculitis resulting in obliterative endarteritis of the vasa vasorum supplying the medium and large vessels, the overall mortality in Behçet syndrome is 3%–4%, and the most common cause of death is aneurysmal rupture (19,27).

Studies on a possible association between the occurrence of thrombosis and thrombophilia in patients with BD are controversial, Venous and arterial thrombosis occur in patients with this disease and are associated with significant morbidity and mortality, (28). Because of their ability to destroy elastin, MMP-2 and -9 have been hypothesized to play a primary role in the internal elastic lamina degradation (15).

Johnson and Galis (2004) (29), demonstrated differential regulation between these MMPs in vivo with regard to smooth muscle cells (SMCs) migration and cell-mediated collagen organization and they reported: whereas MMP-2 and MMP-9 may have similar matrix-degrading abilities, only MMP-9 appears to play an additional role in SMC attachment to the matrix, this may help in tissue remodeling.

This study revealed that there were no association between MMP-9 serum levels and positive skin Pathergy test ($P> 0.05$), this result agreed with the study of Aksoy et al., (2011) (21) who found no association between MMP-9 serum levels and BD group with the presence of a positive pathergy test.

Senzaki et al.,(2001) (30) demonstrated that levels of MMPs including MMP-2 and MMP-9 were significantly higher in patients with Kawasaki disease as compared to controls, and with the levels of MMP-9 in Kawasaki disease patients with coronary artery lesion being higher than those without coronary artery lesion. Takeshita et al., (2001) (31) revealed that MMP-9 was generated from circulating leukocytes. Matsuyama et al., (2003) (32) reported that the levels

of MMP-2 and MMP-9 were higher in patients with Takayasu arteritis than in controls, but only MMP-9 was found to be correlated with disease activity score.

The present work demonstrates that in the correlation between both serum MMP-2 and MMP-9 levels and different clinical and laboratory parameters among BD patients, there were statistical significant positive correlations with disease activity score and vascular lesions.

In accordance with the current literature, Pay et al., (2007) (24) found that MMP-2 and MMP-9 had statistical significant correlations with BD activity score and they concluded that serum MMP-2 and MMP-9 levels can be used as an activity indicator for vasculo-Behçet's or active Behçet's patients, respectively.

On the contrary, Aksoy et al. (2011) (21) revealed that plasma levels of MMP-9 did not display a positive correlation with BD activity index scores.

Conclusion: Increased serum levels of MMP-2 and MMP-9 in BD patients can be considered as a pathogenetic marker of disease activity. These levels correlated with systemic and vascular involvement, so their assessment would most likely help to improve the clinical outcome of patients affected with this disease.

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Corresponding author
Sahar S Ganeb
Rheumatology, Rehabilitation & Physical Medicine
Faculty of Medicine, Benha University,,Egypt
sahar_ganeb@yahoo.com

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Prevalence of Left Ventricular Diastolic Dysfunction among Hypertensive Adults in Klang Valley, Malaysia

Ching Siew Mooi[1], Chia Yook Chin[2], Wan Azman Wan Ahmad[3], Mehrdad Jalalian[4]

[1.] Department of Family Medicine, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, 43400 Serdang, Selangor D.E., Malaysia
[2.] Department of Primary Care Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia, Affiliation: Curtin University, Australia
[3.] Department of Medicine, Faculty of Medicine, University of Malaya, 50603 Kuala Lumpur, Malaysia
[4.] Editor In-Chief, Electronic Physician Journal, Mashhad, Iran
chingsmlcl2004@yahoo.com

Abstract: Heart failure in many patients is due to left Ventricular Diastolic Dysfunction (LVDD), but little is known about its prevalence among hypertensive adults, especially in the primary care setting. , This quantitative study aims to evaluate the prevalence and factors associated with LVDD. A cross-sectional study was conducted among 359 hypertensive patients who underwent echocardiography to define their cardiac structure and function. The peak ratio of early to late diastolic filling velocity was used to assess the LVDD. The Framingham Coronary Heart Disease risk score was derived from the most recent blood test available in the previous year. SPSS version 19 was used to analyze the data. Echocardiographic LVDD was found in 68% of the participants. Of the 243 hypertensive subjects who had LVDD, 69.5% did not have any left ventricular hypertrophy (LVH) while 30.5% had LVH. Age (odds ratio (OR) 1.11, 95% confidence interval (CI) 1.07-1.15), fasting blood glucose (OR 1.18, 95% CI 1.02-1.37), poor blood pressure control (OR 1.93, 95% CI 1.12-3.32), central obesity (OR 2.06, 95% CI 1.17-3.64), and LVH (OR 2.76, 95% CI 1.29- 5.90) were found to have a significant positive relation with LVDD. Poor hypertension control, diabetes, older age, central obesity, and LVH are the predictors for the development of diastolic dysfunction.

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1. Introduction

Hypertension is one of the main causes of preserved Left Ventricular Ejection Fraction (LVEF) heart failure where diastolic dysfunction is present instead. Heart failure with normal left ventricular ejection fraction (HFNEF) or preserved LVEF heart failure also known as diastolic heart failure consists of a clinical syndrome characterized by the symptoms and signs of heart failure, a preserved ejection fraction (EF), and left ventricular diastolic dysfunction (LVDD) (1). The incidence of HFNEF is common in older females with diabetes and patients with uncontrolled blood pressure (2, 3, and 4). As such, it could become the most common type of heart failure in the community as the aging population increases substantially worldwide (5-8). In addition, studies have reported that the prognosis of HFNEF is ominous and comparable to heart failure with reduced ejection fraction (9-12). However, the importance of this public health problem has been under-recognised as it is undiagnosed in most of the patients, resulting in a lack of optimal treatment in the community.

Early diagnosis of LVDD is essential as studies have shown that pre-clinical LVDD is the first observable manifestation of heart failure (5, 13). LVDD has also been found to be associated with marked increases in all-cause mortality and morbidity in many studies (8, 14, and 15). However, little is known about the characteristics that predispose individuals to anabnormal diastolic function among hypertensive individuals in the primary care setting. Such information is essential for improving adherence to clinical practice guidelines since treatment at an early asymptomatic stage may delay or prevent progression to symptomatic heart failure and its consequences. Thus, this study aims to determine the prevalence and predictors of diastolic dysfunction among hypertensive individuals in the primary care setting.

2. Material and Methods

This cross-sectional study was conducted in a hospital-based outpatient clinic in Klang Valley, Malaysia. The inclusion criteria were patients with hypertension, as determined when their case record fulfilled the following criteria:

- Either documented diagnosis of hypertension according to World Health

Organization (WHO)-International Hypertension Society (ISH) criteria, or:

- Those whose current treatment consisted of lifestyle modification or anti-hypertensive agents.

Patients with hypertension who were 18 to 70 years old were recruited from 1st of June 2009 to 30th of September 2009. We choose an arbitrary upper age limit of 70 years in this study in order to avoid the widely known effect of aging on the mitral inflow patterns on echocardiogram (3).

Demographic data and smoking status of the patients were obtained during face-to-face interviews. Co-morbidities, including diabetes, ischaemic heart disease (IHD), and stroke, were also recorded. Lipid profile and fasting blood glucose were obtained from patient records in 2009. The Framingham CVD risk scores (FRS) were calculated based on age, total and high-density lipoprotein cholesterol, systolic blood pressure, treatment for hypertension, smoking, and diabetes mellitus status.

All patients underwent echocardiography tests to clarify the cardiac structure and function. Classical M-mode with a two-dimensional Doppler echocardiography video recorder was used, together with a Siemens model equipped with 2.5 MHz transducer. Subjects were examined in the left lateral decubitus position with left parasternal, and the apical chamber views were taken as indicated during the test(16).The peak early (E) and late (A) diastolic velocities were measured from the transmitral flow signal. LVDD was diagnosed by looking at the E/A ratio reading; "E wave velocity" stands for the highest velocity during the early rapid filling diastolic phase and "A wave velocity" refers to the highest velocity during the late filling (atrial systole) phase. The E/A ratio is derived from the ratio of the peak early ventricular filling velocity to the peak atrial filling velocity. In a normal situation, the E/A ratio falls between 1 and 2; in LVDD, the E/A ratio is < 0.75 (17), in which there is a decrease in early transmitral LV filling and an increased proportion of filling during atrial contraction. HFNEF was diagnosed when the patient had clinical symptoms suggestive of heart failure, together with the presence of LVDD in a preserved ejection fraction. Echocardiography left ventricular hypertrophy (LVH) is defined as the left ventricular posterior wall thickness together with the inter-ventricular septal thickness ⩾11mm (18).

Patients' height and weight were determined using a digital scale. Body mass index (BMI) was calculated as weight in kilograms per square of the height in meter (kg/m^2). Abdominal obesity was obtained using a measuring tape according to standard procedure. Using the Asian Pacific's obesity guideline, obesity and central obesity were defined as having a BMI of more than 27.5 kg/m^2 and waist circumference ≥ 90cm in men and ≥ 80 cm in woman respectively (19). Blood pressure was taken using a mercury sphygmomanometer. The average of three blood-pressure readings was used to determine the control of blood pressure. The target blood pressure (BP) was defined as <140/90 mmHg among hypertensive patients and <130/80 mmHg among hypertensive patients with diabetes (20, 21). SPSS statistical software version 19 (SPSS IBM New York, United States) was used. Continuous data are described as mean and standard deviation or median and interquartile range (25-75th percentiles) if the distribution is skewed. Categorical data are reported as proportions (percentage). The Chi-square test was used to examine the associations among the different variables of the study. Multivariate logistic analysis was used to look for the predictors of the diastolic dysfunction. All analyses were done with 95% confidence intervals (CI), and the level of significance was determined at $p<0.05$. Ethical approval was obtained from the Medical Ethics Committee of the Faculty of Medicine University Malaya.

3. Results

A total of 359 patients with hypertension were enrolled in the study. However only 356 respondents were enrolled in the analyses as three of the echocardiography results were not reliable for LVDD diagnosis. The median age of patients was 59.4 ±10 years, with 52.1% being aged >60 years. The median duration of BP was 8 ± 12 years, and patients' mean systolic blood pressure was136.3 ± 13.9 mmHg while mean diastolic blood pressure was 81.5 ± 7.7 mmHg. The mean BMI was 26.8 ± 4.7 kg/m2.

Table 1. The demographic and clinical Characteristics of the study populations (n= 356)

Variables	Value
Age, years	59.4 ± 10
Females, n (%)	208 (58%)
Race, Malay: Chinese: Indian, n (%)	99,180,76 (27.6%,50.1%,21.2)
Co-morbidities, n (%)	44 (12.3%)
Diabetics' hypertensive, n (%)	147 (40.9)
Duration of blood pressure (years)	8 ± 12 years
Mean blood pressure (mmHg)	136.3 ± 13.9/ 81.5 ± 7.7
ACEI/ARB use, n (%)	203 (56.5)
Statin use, n (%)	258 (71.9)

Participants were predominantly female (58%), Chinese (50.1%) and individuals who had completed secondary education and above (86.1%). Females were found to have a better blood pressure control rate than males (41.5% versus 40.9%) and a lower percentage of LVH (19.5% versus 29.5%).Vascular co-morbidities were recorded among 44 (12.3%) individuals. Stroke was more common than cardiovascular events (7.5% versus 6.7%). The majority of patients were treated with ACEI or ARBs (56.5%). Demographic and clinical characteristics of all patients who met the study inclusion criteria are shown in Table 1.

Table 2. Patients' Clinical Characteristics Based on LV Diastolic Function

Characteristics	**Overall (n=356)**	**Presence of LVDD (n=243)**	**Absence of LVDD (n=113)**	**P-value**
Age, years	59.3 ± 7.5	60.7 ± 6.4	56.5 ± 8.7	<0.001
Target BP achieved (n, %)	147 (41.3)	153 (63.0)	56 (49.6)	0.017
Left ventricular hypertrophy (n, %)	85 (23.9)	74 (30.5)	11 (9.7)	0.001
Central obesity (n, %)	213 (59.8)	157 (64.6)	56 (49.3)	0.007
Fasting plasma glucose (n, %)	6.6 ± 2.1	6.8 ± 2.4	6.0 ± 1.4	0.006
Female gender (n, %)	207 (58.1)	138 (56.8)	69 (61.1)	0.447
Ischaemic heart disease (n, %)	22 (6.2)	19 (7.8)	3 (2.7)	0.073
Framingham risk score points (n, %)	20.7(8.2)	22.6 (7.7)	16.7(7.6)	0.001
Duration of BP, months	125 ± 88	133 ± 90	107 ± 82	0.148
Smoking (n, %)	25(7.0)	14(5.8)	11(9.7)	0.919
Consume alcohol (n, %)	98 (27.5)	65(26.7)	33(29.2)	0.629
ARB/ ACEI agents	200(56.2)	141(58.0)	59(52.2)	0.304
BMI ± SD, kg/m2	26.8 ± 4.7	27.1 ± 4.6	26.1 ± 4.9	0.064
Home BP monitoring (n, %)	177(49.7)	126(51.9)	51(46.4)	0.090

ARB: Angiotensin Receptor Blockers
ACEI: Angiotensin Converting Enzyme Inhibitors
BP: Blood pressure
*statistically is significant as the p-value is <0.005

Table 3. Factors associated with left ventricular diastolic dysfunction

Characteristics	**Univariate model**		**Multivariate model**	
	OR (95% CI)	P Value	OR* (95% CI**)	P Value***
Age	1.08(1.05-1.11)	<0.001	1.11(1.07-1.15)	<0.001
Poor BP control	1.73(1.10-2.72)	0.017	1.93(1.12-3.32)	0.018
Left ventricular hypertrophy	4.06(2.06-8.01)	<0.001	2.76(1.29-5.90)	0.009
Central obesity	1.86(1.18-2.92)	0.007	2.06(1.17-3.64)	0.012
Fasting plasma glucose	1.22(1.06-1.40)	0.006	1.18(1.02-1.37)	0.025
Female gender	1.19(0.76-1.88)	0.447	0.85(0.49-1.49)	0.574
Ischaemic heart disease	0.32(0.09-1.11)	0.073	1.76(0.46-6.82)	0.411
Framingham risk score points	1.09(1.06-1.12)	<0.001	1.02(0.97-1.06)	0.461

* OR: Odds Ratio
** CI: Confidence Interval
***statistically is significant as the p-value is <0.005
* Adjusted for age, blood pressure control left ventricular hypertrophy, central obesity, fasting blood sugar, female gender, ischemic heart disease and Framingham risk scores.

The prevalence of echocardiography LVDD and HFNED was 68% and 10.3%, respectively. Of the 243 hypertensive subjects who had LVDD, 30.5% had LVH. Subjects with LVDD were found to be older and have poorer blood pressure control, left ventricular hypertrophy, central obesity, fasting plasma glucose, and higher Framingham risk scores. The clinical characteristics and the left ventricular diastolic dysfunction statuses on the echocardiogram of all patients are shown in Table 2.

Table 3 shows the odds of having LVDD based on multiple logistic regressions after adjusting for established LVDD risk factors. Patients who are older (odds ratio (OR) 1.11, 95% confidence interval (CI) 1.07, 1.15) with poorer blood pressure control (OR 1.93, 95% CI 1.12, 3.32), higher fasting plasma glucose (OR 1.18, 95% CI 1.02, 1.37), central obesity (OR 2.06, 95%CI 1.17, 3.64), and underlying LVH (OR 2.76, 95% CI 1.29, 5.90) were significantly associated with the development of LVDD. However, no correlation existed between both mean FRS and LVDD.

4. Discussions

In our study, the overall prevalence of LVDD among hypertensive patients, as estimated from echocardiography measurement, was as high as 68%. The prevalence of diastolic heart failure was 10.3%. The reported prevalence of LVDD in hypertensive patients varies from 46% to 85% (22-26). The prevalence is varies widely as the characteristics of the studied population, choice of imaging modalities, and criteria used to diagnose LVDD varied in the previous studies. This result is in keeping with other studies in Europe and Africa. Although the majority of patients with the LVDD in this study were asymptomatic, we still need to be vigilant as study reported that nearly a fifth of diastolic dysfunction was associated with the subsequent development of heart failure (27) in which suggesting a high progression of LVDD to overt heart failure. Besides that, hypertension (HPT) is currently one of the most common public health problems in Malaysia (28) as the lifespan has increased in the population (29, 30). With the lifespan expected to continue to increase, the prevalence of hypertension is also expected to increase (20). Hypertension is often associated with increased risk of cardiovascular disease, which subsequently leads to the development of heart failure. These results should also prompt us to use more ACEI/ARB, if not already used, as these agents have been shown to slow down the progression to diastolic heart failure (31, 32). Furthermore, doctors need to adhere more to the national practice guidelines and provide optimal treatment of BP to delay the new onset of diastolic heart failure.

Our study found that age is one of the factors associated with the development of LVDD, which concurs with many other studies (2, 3, and 33). This can be explained by the fact that hypertension actually amplifies the vascular changes in the aging heart and worsens the decrease in left ventricular compliance, resulting in physical deconditioning (34). Our study indicated that LVH is a predictor of the presence of LVDD. One possible explanation of why LVH causes LVDD could be because of the enhanced sensitivity to volume overload from the increase in left ventricular remodeling and dilatation of with volume-dependent elevation of the filling pressures (4). Similarly, uncontrolled chronic hypertension seems to be the main culprit of LVH, which subsequently causes the development of LVDD and heart failure (35, 36, and 37).

Surprisingly, central obesity was shown to have a relationship with the LVDD instead of BMI as reported by other studies (38). Asians are known to have higher abdominal obesity despite having the same BMI (36). In other words, central obesity is more accurate and better at representing the CV risk factors, particularly in the Asian population (37). Furthermore, insulin resistance has been reported to be an independent predictor for LVDD (35, 39), which explains this finding.

With regard to other factors, in our study, we observed that LVDD was strongly predicted by the diabetic hypertensive disorder. According to the literature, glucose intolerance and type two diabetes mellitus negatively impact the midwall systolic mechanism and diastolic filling, as mentioned in the Strong Heart Study and HyperGEN study (40, 41, and 42). Thus, this may explain LVDD is the early manifestations of diabetic cardiomyopathy (43). In addition, the prevalence of T2DM is reported to be much higher in hypertensive patients than the common population (44, 45) Thus, not surprisingly the risk of death from the cardiovascular event is absolutely higher among hypertensive patients with underlying diabetes, as shown in the literature (46).

Previous studies have shown that Framingham risk score has a relationship with left atrial volume, which is a surrogate in expressing the severity of diastolic dysfunction (47). However, our study failed to show such a relationship between Framingham risk score and LVDD. This is probably because the FRS may underestimate the CVD risk in those high-risk groups such as Asian population and diabetes patients (48, 49, and 50). Indeed, 40.9% of the patients in our study had underlying diabetes,

which may explain the negative association between LVDD and FRS in our study.

In terms of gender, females were found to have a better blood pressure control rate than males (41.5% versus 40.9%) and a lower percentage of LVH (19.5% versus 29.5%). This could explain the negative relationship between female gender and the development of LVDD; however, further study is needed to examine this aspect.

Cardiac catheterization remains the gold standard for diagnosing diastolic dysfunction. However, the use is restricted as this is an invasive procedure and not practical in routine daily practice. Tissue Doppler imaging sounds to be a better tool compared to the conventional standard echocardiography given that its measurement of the transmittal flow is independent of other confounding factors. However, this tool is not available in the primary care setting.

In summary, the prevalence of LVDD is high among the hypertensive population in the primary care setting. Every effort needs to be put in for early detection. Older age, poorer blood pressure control, presence of left ventricular hypertrophy, central obesity, and fasting plasma glucose levels were significantly associated with a higher risk of LVDD.

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Corresponding Author:
Dr. Ching Siew Mooi,
Department of Family Medicine,
Faculty of Medicine and Health Sciences,
Universiti Putra Malaysia,
43400 Serdang, Selangor D.E., Malaysia
Email: chingsmlcl2004@yahoo.com

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A Factor Component Analysis of the Sources of Income Inequality in the Limpopo River Basin of South Africa

**Abayomi Samuel Oyekale and Sibongile Sylvia Vutela-Tekana

Department of Agricultural Economics and Extension, North-West University, Mafikeng Campus, Mmabatho, 2735, South Africa. asoyekale@yahoo.com. ** Corresponding author

Abstract: Income inequality is detrimental to economic development because of its direct linkage to crimes, political unrest and corruption. This study analyzed the contributions of different sources of income to inequality in the South Africa's Limpopo River Basin. The data used were for 704 households that provided information on sources of their income. The decomposition method proposed by Stark *et al* (1996) was used. The results show that incomes from crops, livestock and non-farm assets constitute the highest proportions of rural households' income. Inequality is generally high in all the districts with Rustenburg and Witrivier having the highest Gini coefficients. Out of the income sources, crop and livestock sources increased inequality. It was recommended that efforts to redress inequality should include promotion of non-farm enterprises and ensuring conducive environment for people to work in any part of the country without fear of molestation irrespective of race, among others.
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Keywords: Inequality, income sources, factor component, Limpopo River Basin

1. Introduction

Development economists have over the past few decades debated and seriously advocated for rapid reduction in poverty and inequality. Despite some commitments shown by many developing countries towards achieving these, in many cases, there is lack of strong political will and sincere commitments. In many countries, increase in income inequality raises serious humanitarian concerns and some fears of political stability. It is now clear that without equity in access to physical and financial resources, the policy environment that is required for rapid economic growth cannot be provided where wide inequality persists (Clarke *et al*, 2003; Oyekale *et al*, 2006).

Since the 1990s, development policy makers have been concerned about pertinent issues that are related to how much of the dividends of economic growth reach the poor (Kakwani *et al*, 2004). It has also become evident that economic reforms that are required for rapid poverty alleviation are those that can deliver more of the benefits of growth into the hands of the poor. This had been tagged *pro-poor growth* and emphasis had been placed on increase in the average income of the poor and concurrent reduction in inequality. Therefore, the theoretical and empirical attentions that are given by development economists to the distribution of income and wealth are worthwhile, because high level of income inequality produces an unfavorable environment for economic growth and human development.

The Millennium Development Goals (MDGs) provide a timely blueprint and urgent reminder to policy makers about the need to prioritize their development agendas in a manner that achieves better living conditions for the marginalized poor population. In many instances, however, bridging the gaps between the few extremely rich and the extremely poor majority remains a daunting challenge. This is because of peculiar characteristics of the poor that facilitated their being trapped in poverty. For instance, most of the times, the poor lacks basic education, access to financial resources, access to land and ability to utilize emerging opportunities within the economy for the utmost benefit of lifting households' incomes above the national averages. Therefore, some targeted reforms do not often bring rapid results in the form of reduction in poverty and inequality.

There is now consensus among policy makers that poverty cannot be reduced if the level of inequality is high (Addison and Cornia, 2001). This finding has completely rebuffed earlier theories of development that emphasized inequality as a pre-condition for economic growth and poverty reduction (Aigbokhan, 2008). Conceptually, inequality implies dispersion of a distribution, whether one is considering income, consumption, or some other welfare indicators or attributes. Although distinct as concepts, income inequality is often studied as part of the broad analyses covering poverty. However, inequality is a broader concept than poverty because it is defined over the whole distribution (Litchfield, 1999; Cowell, 1999).

Decomposition of income inequality is desirable because it enables us to examine the

contribution to inequality of particular group or households with specific characteristics. It can also be used to assess the influence of different income components on overall inequality. Studying inequality is also important because its interaction with other economic problems often results in discontent, violence and corruption. Therefore, as part of microeconomic objectives, governments often give equitable distribution of income a priority (Oyekale, 2006).

In South Africa, the poverty situation is quite pathetic despite existence of social infrastructure that is comparable to what obtains in many developed countries. The country is sharply divided into two groups of the affluent and the destitute. It had been estimated that while more than halve of South African population is poor, majority of the poor live in rural areas Similarly, South Africa's income inequality in 1993 was the fourth worse out of 105 countries (Madzwamuse, 2010).

Specifically, high levels of inequality in South Africa can be traced to history of colonialism and apartheid. Majority of the black population were dispossessed of their land and denied access to vital development resources and adequate services such as health care, housing and education. After national independence and return to democratic government in 1994, policies and economic reforms to redress inequality in access to resources have been put in place. This is to ensure respect of fundamental human rights and provision of a development approach that focuses on human justice, resource equity and economic sustainability (Madzwamuse, 2010). However, not much success had been achieved because the bulk of the nation's resources are still concentrated in the hands few affluent segment of the population.

The situation in the Limpopo River Basin is more devastating due to small land holdings of majority of the farmers. In South Africa at large, commercial farming occupies 85 percent of the countryside farming activities. Although contributing a lot to South Africa's Gross Domestic Product (GDP), the Limpopo River Basin is particularly susceptible to adverse climatic situations like drought, flood and hailstorms. Increasing population pressure is putting serious pressure on the natural resources, with persistent degradation resulting from agricultural intensification. Therefore, currently available natural resources cannot provide rural people guaranteed livelihoods to escape from poverty. This paper therefore seeks to determine the sources of income inequality in the Limpopo River Basin. The remaining sections of the paper present the adopted methodology, discussions of the results from data analysis and the policy issues that emanated from the findings.

2. Materials and Methods

The data and sampling methods

This study used the data that were collected by the International Food Policy Research Institute (IFPRI) and the Center for Environmental Economics & Policy in Africa (CEEPA). Permission to download the data was granted by IFPRI. The survey was based on 794 households that completed the questionnaires out of 800 that were initially targeted. However, due to lack of data on income sources, only 704 households were used for this study. The multi-stage sampling method was used to select 20 districts in the South Africa's Limpopo River Basin. The selected districts reflect key Water Management Areas (WMAs) and agricultural production activities. At the first stage, total number of sample districts was identified. At the second step, 20 districts were selected out of the 5 WMAs. The third step involved determining the distribution of the 20 districts across the 4 provinces in the basin. The Gauteng (2), Limpopo (9), Mpumalanga (6) and North West (3) were selected. The fourth step involved random sampling of farm households that undertook some farming activities during the April 2004 to May 2005 farming season. The survey was carried out between August and November 2005.

Analytical methods

We followed the approach of Lerman and Yitzhaki (1985) which was adopted by Azam and Shariff (2009). The specification begins by expressing Gini-coefficient for total income inequality G as follows:

$$G = \sum_{k=1}^{K} S_k G_k R_k \qquad 1$$

where *Sk* represents the share of component *k* in total income, *Gk* is the source Gini corresponding to the distribution of income from source *k*, and *Rk* is the Gini correlation between income from source *k* and total income.

$$R_k = cov\{Y_k, F(Y)\}/cov\{Y_k, F(Y_k)\} \quad ..2$$

where $F(Y)$ and $F(Y_k)$ are the cumulative distributions of total income and income from source k respectively. Stark *et al* (1996) submitted that equation 1 can be decomposed into three components which show how important the income source is with respect to total income (S_k), how equally or unequally distributed the income source is (G_k) and how the income source and the distribution of total income are correlated (R_k). Lerman and Yitzhaki (1985) showed that using this approach, it is possible to determine effect of small changes in a specific income source on inequality, holding income from all

other sources constant. If there is a small change in income from source k that is equal to eY_k, where e is close to 1 and Y_k represents income from source k, we can show that the partial derivative of the Gini coefficient with respect to a percent change (e) in source k is equal to

$$\frac{\partial G}{\partial e_k} = S_k(R_k G_k - G) \quad ..3$$

where G is the Gini coefficient of total income inequality prior to the income change. The percent change in inequality resulting from a small percent change in income from source k equals the original contribution of source k to income inequality minus source k's share of total income:

$$\frac{\frac{\partial G}{\partial e_k}}{G} = \frac{S_k R_k G_k}{G} - S_k \quad ..4$$

3. Results

Average income from different sources

Table 1 shows the average income from different sources as reported by selected farmers in the Limpopo River Basin of South Africa. It shows that Cullinan and Tzaneen (Letaba) districts have highest average annual nonfarm labour incomes with R 27425.00 and R 26618.60 respectively. However, Brankhortspruit and Brits have the lowest average annual non-farm labour incomes with R 3408.70 and R 3976.92 respectively. While many of the districts recorded no income for gifts, average annual incomes obtained through remittances are too small. The bulk of the incomes were derived from crops, livestock and non-farm assets. The largest annual average incomes from crops were recorded for Rustenburg and Lephalale (Ellistras) with R 425053.31 and R 147144.15 respectively. Lowest values were recorded for Brankhortspruit and Brits with R 1865.22 and R 2493.46 respectively. Under livestock average incomes, the highest were for Witrivier and Warmbad with R 496589.58 and R 82986.54, respectively. Incomes from non-farm assets are highest in Rustenburg and Witrivier with R 461235.97 and R 198022.92, respectively. Also, average total income is highest in Rustenburg and Witrivier with R 926772.45 and R 805008.67, respectively.

Table 1: Average incomes from different sources in selected districts of South Africa's Limpopo River Basin

District	Non farm labour	Gift	Remitances	Crop	Livestock	Pension	Savings	Farm asset	Non farm asset	Total income
Brankhortspruit	3408.70	295.65	217.39	1865.22	8413.04	4271.30	1153.48	260.87	16138.70	36024.35
Brits	3976.92	0.00	785.77	2493.46	21514.27	5612.31	0.00	0.00	33198.04	67580.77
Carolina	7080.00	0.00	120.00	26780.88	7322.80	4232.80	31.20	0.00	10789.60	56357.28
Cullinan	27425.00	0.00	0.00	11175.00	0.00	2340.00	0.00	0.00	45595.00	86535.00
Krugersdorp	14280.00	30.00	1248.00	49511.50	7545.00	7848.00	0.00	0.00	76978.50	157441.00
Lephalale (Ellistras)	10783.28	32.79	769.18	147144.15	5220.90	2599.18	306.89	0.00	46147.72	213004.08
Lydenburg	7495.29	0.00	664.71	37791.09	3290.47	4849.41	0.00	0.00	48783.29	102874.26
Makpopane	16336.00	20.00	108.20	20977.40	3604.84	577.20	96.00	0.00	41769.00	83488.64
Marico	6087.80	0.00	121.95	55229.88	35687.15	2961.95	0.00	0.00	69497.00	169585.73
Messina	4662.86	0.00	2057.14	102901.97	8858.86	1837.66	0.00	0.00	30163.69	150482.17
Middelburg	24430.87	239.13	272.61	12903.04	27048.39	4265.65	1173.91	43.48	117207.09	187584.17
Nebo	27677.78	0.00	453.89	18772.22	30551.72	5947.50	833.33	0.00	13481.67	97718.11
Nkomazi	14882.76	0.00	995.86	5217.93	7328.93	5257.24	0.00	0.00	33450.31	67133.03
Rustenburg	13996.55	34.48	0.00	425053.31	23871.79	2163.10	417.24	0.00	461235.97	926772.45
Soutpansberg	12673.49	5.56	782.70	5173.90	4843.19	2745.71	555.56	0.00	26408.79	53188.90
Thabazimbi	16340.67	4.50	65.33	11805.67	1575.00	4475.33	0.00	0.00	34617.67	68884.17
Thohoyandou	16266.67	0.00	5017.33	40347.07	2093.89	4601.56	422.00	0.00	45692.69	114441.20
Tzaneen (Letaba)	26618.60	0.00	2265.81	50261.47	11202.09	3482.79	190.70	2674.42	96191.93	192887.81
Warmbad	7573.08	80.77	2968.08	4983.27	82986.54	4296.54	230.77	923.08	18223.08	122265.19
Witrivier	9746.88	2.08	90.00	94931.38	496589.58	4430.00	1195.83	0.00	198022.92	805008.67

Table 2: Rural Gini-coefficients across the districts in Limpopo River Basin of South Africa

District	Estimated S-Gini	Population Share	Income Share	Absolute Contribution	Relative Contribution
Brankhortspruit	0.4625	0.0327	0.0059	0.0001	0.0001
Brits	0.5983	0.0369	0.0125	0.0003	0.0003
Carolina	0.7250	0.0355	0.0100	0.0003	0.0003
Cullinan	0.5766	0.0057	0.0025	0.0000	0.0000
Krugersdorp	0.6783	0.0142	0.0112	0.0001	0.0001
Lephalale (Ellistras)	0.7930	0.0866	0.0925	0.0064	0.0075
Lydenburg	0.7385	0.0483	0.0249	0.0009	0.0011
Makpopane	0.5433	0.0710	0.0297	0.0011	0.0014
Marico	0.7238	0.0582	0.0495	0.0021	0.0025
Messina	0.7597	0.0497	0.0375	0.0014	0.0017
Middelburg	0.7446	0.0653	0.0614	0.0030	0.0035
Nebo	0.6578	0.0511	0.0250	0.0008	0.0010
Nkomazi	0.6260	0.0412	0.0139	0.0004	0.0004
Rustenburg	0.9447	0.0412	0.1914	0.0074	0.0088
Soutpansberg	0.6350	0.0895	0.0239	0.0014	0.0016
Thabazimbi	0.5432	0.0426	0.0147	0.0003	0.0004
Thohoyandou	0.7473	0.0639	0.0367	0.0018	0.0021
Tzaneen (Letaba)	0.7272	0.0611	0.0591	0.0026	0.0031
Warmbad	0.8056	0.0369	0.0226	0.0007	0.0008
Witrivier	0.9370	0.0682	0.2751	0.0176	0.0208
Within Group	---	---	---	0.0486	0.0576
Between Group	---	---	---	0.4860	0.5757
Overlap	---	---	---	0.3097	0.3668

Table 2 shows the results of income inequality decomposition across the districts in the South Africa's Limpopo River Basin. It reveals that Witrivier and Rustenburg had the highest income shares with 27.51 percent and 19.14 percent, respectively. The districts with lowest shares of total incomes are Cullinan and Brankhortspruit with 0.25 percent and 0.59 percent respectively. Similarly, the table shows the Gini coefficient of incomes across the districts. It reveals that inequality is lowest among farmers in Brankhortspruit and Thabazimbi with 0.4625 and 0.5432 respectively. The districts with highest income inequality are Rustenburg and Witrivier with 0.9447 and 0.9370 respectively. The results generally show that income inequality is generally high across the districts. The results also show that out of the Gini coefficient of 0.893 that was computed for the combined data, Witrivier accounted for the highest relative contribution of 2.08 percent among the districts. However, between group inequality accounts for 57.57 percent, while within group inequality accounts for 5.76 percent. The interaction of within group and between group inequality accounts for 36.68 percent of total inequality.

Table 3 shows the results of inequality decomposition based on the different sources of incomes that were reported by the farmers. It reveals that while the share of non-farm labour income in the total income is 6.79 percent, its relative marginal effect is with negative sign. It implies that holding every other thing constant, a one percent increase in non-farm labour income will reduce inequality by one percent. Therefore, promotion of economic activities that can lead to more income generating opportunities from non farm activities in the rural areas will deliver more incomes into the hand of the poor.

Incomes received as gifts accounts for very low percentage (0.01) of total income. However, its relative marginal effect reveals that if income in this category is increased by one percent, holding every other income constant, inequality will decline by 0.02 percent. This implies that efforts by the rural communities to exist in more cohesion by facilitating sharing will lead to reduction in inequality. This is expected because in many cases, it is the poor that are

unable to meet their needs and then seek for financial assistances.

Incomes received as remittances account for 0.50 percent of the total income. Its relative marginal coefficient reveals that holding incomes from other sources constant, a one percent increase in the incomes from that source will reduce inequality by 0.26 percent. Incomes realized from crops account for 29.61 percent of the total income. However, holding incomes from other sources constant, a one percent increase in the incomes from crops will increase inequality by 1.82 percent. Similar finding is reported for livestock, which accounts for 23.90 percent of total incomes, but would increase inequality by 2.0 percent if there is a one percent increase in the incomes to that source, holding every other income constant.

Income from pension accounts for 1.84 percent of the total income but have a relative marginal effect of -0.0130. This implies that holding every other income source constant, a one percent increase in the income from pension will reduce inequality by 1.30 percent. The incomes that were generated from savings account for 0.19 percent of total income. However, holding incomes from other sources constant, a one percent increase in the saving income will reduce inequality by 0.08 percent. Farm asset income accounts for 0.10 percent of the total income. A one percent increase in income realized from that source holding every other income source constant will reduce total inequality by 0.01 percent. Non farm asset income accounts for the highest proportion of total income (37.04 percent). Also, a one percent increase in this income source, holding incomes from other sources constant will reduce total inequality by 1.16 percent.

Table 3: Gini decomposition by sources of income in the Limpopo River Basin of South Africa

Source	Coeff. of Concentration	Share	Relative Contribution	Absolute Contribution	Relative Marginal Effect
Non farm labor	0.7195	0.0679	0.0579	0.0489	-0.0100
Gift	-0.2653	0.0002	-0.0001	0.0000	-0.0002
Remittances	0.4086	0.0050	0.0024	0.0021	-0.0026
Crop	0.8962	0.2961	0.3143	0.2653	0.0182
Livestock	0.9150	0.2390	0.2590	0.2187	0.0200
Pension	0.2511	0.0184	0.0055	0.0046	-0.0130
Savings	0.5077	0.0019	0.0012	0.0010	-0.0008
Farm asset	0.7532	0.0010	0.0009	0.0008	-0.0001
Non farm asset	0.8179	0.3704	0.3588	0.3029	-0.0116

4. Recommendations

The results have shown high levels of income inequality among farmers in the Limpopo River Basin. Although within group inequality accounts for lesser proportion of total inequality across the selected districts, it also very obvious that income inequality in districts like Rustenburg and Witrivier are intolerably high. Therefore, government needs to redress the contributing factors to inequality by profiling detailed resource endowments and access by poor households in each district and address the driving forces of inequality in a more critical manner.

The results have shown that promotion of economic activities that can lead to more income generating opportunities from non farm activities in the rural areas will deliver more incomes into the hand of the poor. There is therefore the need for government and private sector interventions in creating more opportunities for non-farm business operations in the rural areas. Such efforts can be channeled through skill development for small scale business operations and provision of small loans. It should be emphasized that as more retail businesses develop and grow, rural income inequality will decline.

Gift income receipts reduce income inequality. Facilitation of social capital and networks in rural area will therefore be an important factor in reducing inequality. Also, because remittances reduce inequality, creation of conducive environment for people to move to other parts of the country to establish business or work without fear of molestation or sectionalism will go a long way in reducing inequality. Although incomes from crop and livestock account for significant proportion of total income, they are inequality increasing. There is the need for government interventions in identifying the pressing needs of poor small scale farmers to boost their farm income. Such efforts should address inadequate access to land and other production input.

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Address of Corresponding Author
Department of Agricultural Economics and Extension, North-West University, Mafikeng Campus, Mmabatho, 2735, South Africa.

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CLIMATE CHANGE AND COCOA PRODUCTION EFFICIENCY LOSSES IN ONDO EAST LOCAL GOVERNMENT, NIGERIA

A. S. Oyekale

Department of Agricultural Economics and Extension, North-West University Mafikeng Campus, Mmabatho, 2735 South Africa. abayomi.oyekale@nwu.ac.za

Abstract: Effect of climate change on cocoa agriculture cannot be underestimated. This study assessed efficiency differentials in cocoa production under with and without climate change scenarios. The data were collected using multi-stage sampling method. Data were analyzed with simple descriptive statistics and stochastic frontier approach. The results show that cocoa farmers are ageing (μ = 54 years) and many owe small farms (μ = 9.15 ha). Also, production input elasticities when under normal climate are all positive, while those for chemical and spraying hour are negative when there is climate change. Return to scale under climate change is higher (2.097075) than without climate change (1.825603), although lower output under the former still implies low productivity. Average production efficiency with climate change is 65.14 percent while it is 83.75 percent without climate change. The study recommended development of viable and cost effective chemicals to curtail increasing incidence of pests and diseases as a result of climate change, among others.
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Keywords: cocoa, climate change, technical efficiency, stochastic frontier

1. Introduction

Cocoa (*Theobroma cacao*) is a low altitude crop that grows from sea level up to an altitude of 700m. It was introduced to Nigeria from the American Continent in 1874. Its rainfall requirement ranges between 1000 to 3000 mm per annum, in absence of which irrigation will be required. Cocoa production is very sensitive to moisture stress and excess soil water portends serious constraint to its optimum performance (Obatolu *et al*, 2003).

Commercial production of cocoa in Nigeria began in the then Western Nigeria between 1889 and 1890. In 1965, cocoa cultivation had gained prominence to the extent that Nigeria became the second largest producer in the world. This production euphoria was however thwarted by discovery of petroleum, after which the agricultural sector was partly neglected.

Among the reasons that have been given for decline in cocoa production are farmers' small land holdings, transportation problem, scarcity of human labour, low capital investment and variability in climatic factors (Adegeye, 1996). Also, while climate change poses serious challenges to Nigerian agriculture, some of the proposed options to address it in the Kyoto Protocol imply some future economic downturn. This is because oil generates more than 90 percent of the country's foreign exchange revenues. Avoidance of impending economic doom requires that the country should diversify her sources of income, and cocoa agriculture easily comes to fore.

Farmers' interest in cocoa production was resuscitated after government implemented the Structural Adjustment Program (SAP) in 1986. The major components of SAP included market-determined exchange rate and interest rates, liberalized financial sector, trade liberalization and commercialization/privatization of a number of public enterprises. With the scrape of the Commodity Marketing Boards that brought a lot of pricing inefficiency in cocoa marketing due to price-giver role it played, cocoa farmers were motivated to grow the crop as well as rehabilitating their old farms.

It should be emphasized that cocoa remains the second largest foreign exchange earner after petroleum (Adegeye, 1996; Izuchukwu). Apart from providing foreign exchange to the exporting countries, cocoa is a means of conserving foreign exchange. This is achieved by locally producing cocoa based products such as cocoa-butter, cocoa cake, cocoa powder and cocoa wine, among others.

However, Nigeria's lost glory in cocoa exports is yet to be restored because the country has slumped to the fifth position in global production, while accounting for just about 5 percent of world's total outputs. Government has taken some initiatives to revive cocoa production through some rebirth processes. However, among the most pressing limiting factors is climate change. This is because every stage of cocoa production requires adequate weather conditions (Nabuurs *et al*., 2007). Also, cocoa is highly susceptible to drought, and the pattern of its cultivation is related to rainfall distribution (Anim-Kwapong and Frimpong, 2005).

Black pod disease accounts for quite a lot of cocoa production losses by attacking the ripened or

very young pods (Opoku *et al* 1999). The disease is closely related to the pattern of rainfall distribution. It is more prevalent in damp situations with utmost pod infection in years when the short dry period from July to August is very wet. Ondo state, being the highest cocoa producing state in Nigeria has records of fluctuations in some climatic parameters, especially rainfall, temperature and sunshine hours. Madu (no date) found that among the states in South West Nigeria, Ondo records the highest climate change vulnerability based on indices aggregated from several indicators. Also, Nigerian Meteorological Agency (NIMET) (2011) noted that in August 2010, some places in the South West including Ondo state recorded rainfall values that were 200-300 percent higher than normal.

This paper seeks to answer the question: what is the efficiency loss in cocoa production that results from climate change? This is fundamental because climate change is a production shock that subjects farmers to operate below the production frontier. Adaptation is only able to reduce production losses resulting from climate change. In economic sense, the farmer will be technically inefficient. This has some welfare implications for the farmers since their incomes are adversely affected. In the remaining parts of the paper, the materials and methods, the results and discussions and the recommendations have been presented.

2. Materials and Methods

The study area

The study was carried out in Ondo East Local Government Area (LGA), which is one of the 18 Local Government Areas in Ondo state. The 2006 National Population Census put the population of the LGA at 76,092 people (National Bureau of Statistics, 2009). The LGA is characterized by tropical climate with rainy season from April to October, while the dry season is from November to March. The state is predominantly agrarian with about 70 percent of the labour force engaged in agriculture. Cocoa is the primary cash crop with regular intercrops with food crops such as yam, cocoyam, cassava, plantain etc.

Sources of Data

Primary data that were collected through personal interviews and administration of questionnaires were used. The multi-stage sampling method was used. At the first stage, twelve villages were selected randomly from the list of available villages in the LGA. At the second stage, households were randomly sampled in the selected villages based on their total estimated number of households. Although 120 questionnaires were administered, only 99 contained complete and useful information to be used for the analysis.

Analytical method

Economic literature suggests several alternative approaches to measuring productive efficiency. These methods can be grouped into non-parametric and parametric frontiers. Nonparametric approach uses linear programming and does not impose any functional form on the production frontiers. The most popular non-parametric approach is the Data Envelopment Analysis (DEA). The parametric approach imposes a functional form on the production function, and makes some assumptions about the data. The most common functional forms include the Cobb–Douglas, Constant Elasticity of Substitution and Translog Production Functions (Coelli *et al*, 2004).

The other distinction is between deterministic and stochastic frontiers. Deterministic frontiers assume that all the deviations from the frontier are as a result of firm's inefficiency, while stochastic frontiers assume that part of the deviations from the frontier is due to random events (reflecting measurement errors and statistical noise) and part is due to firm specific inefficiency. The stochastic frontier production function model has the advantage of allowing simultaneous estimation of individual technical efficiency, as well as the determinants (Coelli *et al*., 1994).

The stochastic frontier production function that was used in this study can be illustrated with a farm using n inputs $(X_1, X_2, \ldots, X_n)$ to produce output Q_i. Efficient transformation of inputs into output is characterized by the production function $f(X_i)$, which shows the maximum output obtainable from various input vectors. The stochastic frontier production function assumes the presence of technical inefficiency of production. Hence, the function is defined as:

$$Q_i = f(X_i, \beta)\exp(v_i - u_i) \quad .1$$

where v_i is a random error which is associated with random factors not under the control of the farmers. The model is such that the possible production Q_i is bounded above by the stochastic quantity, $f(X_i, \beta)\exp(v_i)$. The random error (v_i) is assumed to be normally distributed $N(0, \sigma_v^2)$ random variable that is independent of u_i.

Technical efficiency of an individual farmer is defined in terms of the ratio of the observed output to the corresponding frontier output, given the available technology. We specified the farmers' production function by defining the Cobb-Douglas function as:

$$Log\,Q_i = \alpha_i + \beta_i \sum_{i=1}^{6} Log X_i + (v_i - u_i) \quad .2$$

Q_i represents cocoa output of i-th farmer measured in kg, X_1 represents hired labour (man days), X_2 represents family labour (man days), X_3 represents the land area (hectares), X_4 is chemical input (litre), X_5 is spraying hours, X_6 is cocoa bean processing time (hours), β_i are coefficients to be estimated. The u_i is the technical inefficiency effect, which can be defined as:

$$u_i = \theta + \rho_i \sum_{i=1}^{n} Z_i + k_i \quad .3$$

Where Z_i are spraying interval, death of cocoa trees, capsid infection, not drying cocoa beans, repeat spraying, sex, age, marital status, education, income sources, experience, irrigate, market access, losses from capsid, losses from black pod disease, quality reduced.

3. Results

Socio-economic characteristics of the farmers

Table 1: Distributions of farmers' socioeconomic characteristics

Variable	Frequency	%
Sex		
Male	90	90.9
Female	9	9.1
Marital status		
Married	86	86.9
Single	13	13.1
Education		
None	15	15.1
Primary	31	31.1
Secondary	18	18.2
Adult	6	6.1
Tertiary	29	29.3

Source: Field survey Data, 2008.

Table 1 shows that cocoa farmers were predominantly males, making up of about 90.9% of the total respondents. Also, majority of the sampled population were married (86.9%), while the remaining 13.1% were single. About 15.1% of the respondents had no formal education, while just 6.1% had adult education. Primary education was attained by 31.3%, while 18.2% had secondary education. This shows that majority of the respondents were literate with about 94.9% having some form of formal education.

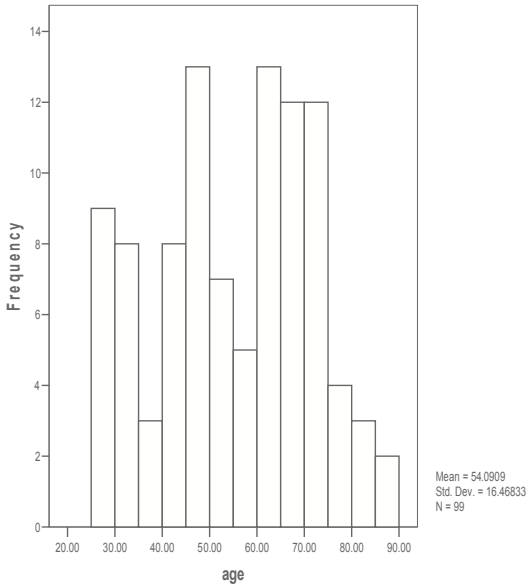

Figure 1: Distribution of cocoa farmers' age

Figure 1 shows the distribution of farmers' age. It reveals that although the average age is 54 years, highest concentration falls within 60-75 years of age. This shows that majority of them are aged. Also, the oldest farmer was 89 years old, while the youngest was 25 years old. The results are pointing to the fact that cocoa farmers' population in Ondo state is ageing.

Figure 2 also shows the distribution of land areas cultivated to cocoa. It reveals that average farm size is about 9 hectares with majority of the farmers having less than 10 hectares of land.

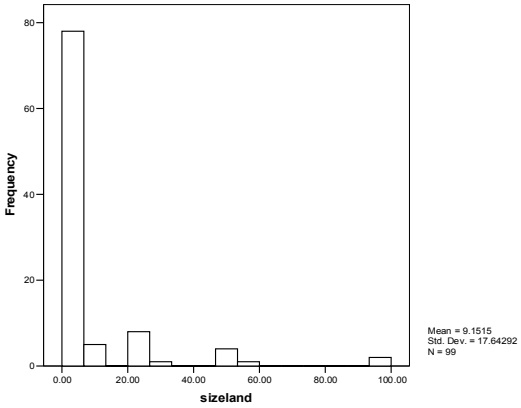

Figure 2: Distribution of farmers' cocoa land areas

Forms of climate change noticed and their importance for cocoa production

Table 2 presents the different forms of climatic change that had been noticed by the farmers. It shows that monthly rainfall that was lower than

normal average was observed by 58.6% of the farmers, while high monthly rainfall was observed by 21.2%. Similarly, 11.1% of the respondents noticed unfavorable sunlight, while 4.0% noticed high temperature.

Table 2: Distributions of respondents by noticed climate changes

Climate change	Frequency	Percentage
High rainfall	21	21.2
Low rainfall	58	58.6
High temperature	4	4.0
Unfavorable sunlight	11	11.1
More than one response	5	5.0
Total	99	100.0

Source: Field Survey 2008

Table 3 also shows the perception of the farmers about importance of some climatic variables in cocoa production. It reveals that 97 percent of the farmers indicated that rainfall is most important for cocoa growth and development of the pods.

Table 3: Distributions of respondent by degree of importance of climate variables in cocoa production

Climate variables	Frequency	Percentage
Rainfall	96	97.0
Temperature	1	1.0
Others	2	2.0
Total	99	100.0

Source: Field Survey 2008

Climate Change and production efficiency losses

Table 4 shows the Maximum Likelihood Estimates (MLE) of the production function that was estimated. The analyses were conducted for the present situation whereby farmers complained about climate change. Farmers were also asked to estimate their cocoa production losses that are due to farm infections by several diseases that are directly associated with climate change. The addition of farmers' production losses to what they eventually got gives us an idea of what their outputs would have been, if the climate was adequate. This was referred to as "normal climate". The models produced a good fits of the data because the likelihood ratio and the Wald Chi square values are statistically significant ($p<0.01$).

The table shows that under normal climate, the parameter of hired labour is not statistically significant ($P>0.10$), whereas it is significant under abnormal climate ($p<0.01$). The elasticity differential for hired labour is also positive (3.77%). This implies that efforts to increase hired labour by 1% will increase output more under the problematic climate scenario that if things were normal. The parameters of family labour for the two results are statistically significant ($p<0.01$). Under normal climate, increasing family labor by 1% will result in 0.76% increase in cocoa output. However, with climate change, increasing family labour by 1%will lead to 1.38% increase in output. This shows that with climate change, cocoa outputs can increase with the use of more family labour. This can be explained from the fact that the owns the farm and would do everything possible to do an effective and lasting job.

However, although both are statistically significant ($p<0.01$), the elasticity coefficient of land under normal climate (0.5691221) is higher than that with climate change (0.5676654). This shows that land productivity declines with climate change. This is expected because proper interaction of normal climatic parameters with land is needed for output optimization.

The elasticity of chemical input under normal climate is not statistically significant ($p>0.10$), whereas it is significant under climate change ($p<0.01$). The results however show that whereas the parameter is with positive sign without climate change (0.1147638), it has negative sign under climate change (-.1005723). The implication is that without climate change, increase in chemical input by 1% will increase output by 0.11%. Similar increment will lead to reduction in cocoa out by 0.10 percent when climate has changed. Therefore, the result points to the fact that chemicals had been overused under climate change. This is expected because farmers indicated that due to high infection of their farms with black pod disease, they were compelled to spend more money on chemicals.

The elasticity of spraying hour (.0366154) is not statistically significant ($p>0.10$), while that under climate change (.0280924) is significant ($p<0.01$). The results also show that increasing spraying hour by 1% will lead to about 0.03% increase in cocoa output under climate change. Elasticity of processing hour under climate change is statistically significant ($p<0.01$) but with negative sign. Under normal climate, processing hour elasticity is not statistically significant ($p>0.10$) but with positive sign. The results are showing that the bulk of the problem with cocoa output under climate change does not lie in processing, but farm-based challenges in the form of cocoa pods that are being destroyed by pests and diseases.

Table 4: Maximum Likelihood Estimate (MLE) Parameters for Cocoa Production Function and Determinants of Inefficiency

Variables	Parameters	Standard error	t-value	Parameters	Standard error	t-value
	Normal climate			Climate change		
Hired labour	.2550139	.1932953	1.32	.2927343	9.39e-06	31160.54
Family labour	.7580476	.2150934	3.52	1.376696	4.87e-06	2.8e+05
Land area	.5691221	.101428	5.61	.5676654	5.03e-06	1.1e+05
Chemicals	.1147638	.13892	0.83	-.1005723	4.70e-06	-2.1e+04
Respraying Hours	.0366154	.1588064	0.23	.0280924	4.89e-06	5742.30
Processing Hour	.0920401	.1211774	0.76	-.0675404	3.29e-06	-2.1e+04
Constant	2.893974	.1263398	22.91	3.16226	4.76e-06	6.7e+05
lnsig2v	-2.397072	.2068219	-11.59	-38.93253	437.1132	-0.09
Wald Chi Square	96.90***			6.25e+11		
LR	-34.111608				-16.311394	
Returns to scale	1.825603			2.097075		
Inefficiency model						
Spraying interval	.0371973	.1243895	0.30	-.0163461	.0561049	-0.29
Death of cocoa tress	-1.517047	1.282399	-1.18	-.5203255	.4015736	-1.30
Capsid infection	-2.289707	1.289083	-1.78	-1.192085	.4508137	-2.64
Not processing pods	-.070646	1.58159	-0.04	1.22256	.6473814	1.89
Repeat spraying	2.118531	1.429468	1.48	.3112483	.4348759	0.72
Sex	-1.276063	1.131265	-1.13	-.768567	.7126002	-1.08
Age	.071356	.0508786	1.40	.0316	.0235493	1.34
Marital status	.4740244	1.743386	0.27	.0282211	.5978307	0.05
Education	-1.562247	1.324355	-1.18	-.5957948	.628162	-0.95
Income sources	-1.233598	1.126063	-1.10	-1.047092	.5344456	-1.96
Experience	.0374346	.0425419	0.88	.0119882	.0231066	0.52
Irrigate	-1.503439	1.226152	-1.23	-.987425	.5657554	-1.75
Market access	3.106482	2.403053	1.29	1.761802	.6750647	2.61
Losses from casid	8.97e-07	.0000132	0.07	1.16e-06	3.09e-06	0.38
Losses from other diseases	-8.50e-06	.0000149	-0.57	-4.97e-06	8.80e-06	-0.56
Quality reduced	-.0769583	1.406055	-0.05	-1.079921	.6145109	-1.76
Constant	-8.61158	4.234511	-2.03	-1.460272	1.423423	-1.03
Sigma	.3016355	.0311924		3.51e-09	7.68e-07	

The results further show that returns to scale under normal climate is 1.825603, while it is 2.097075 with climate change. This show that if all inputs are increased by 1% under normal climate, cocoa output will increase by 1.83%. However, it will increase by 2.1 percent with climate change. The implications of these findings should be critically examined because farmers' outputs are generally low under climate change. Therefore, though returns to scale is higher, in absolute form, returns to cocoa investment under climate change is still low.

Determinants of cocoa production inefficiency

The determinants of inefficiency in cocoa production are shown in lower segment of table 4. The results reveal that many of variables under normal climate are not statistically significant ($p>0.10$). However, form those that are statistically significant, those farmers that indicated capsid infection have significantly lower inefficiency ($p<0.10$) with and without climate change. This not expected but the use of chemicals could have neutralized the expected impact of capsid infection on inefficiency. Also, those that were not processing (drying) their cocoa pods due to inadequate climate have significantly higher inefficiency ($p<0.10$). This is expected because selling the cocoa pods while wet will make the produce buyers to underestimate what it would have weighed if dried. Therefore, output of such farmer will be underestimated.

Farmers with other sources of income have significantly lower inefficiency ($p<0.05$) when there

is climate change. This is expected because their time will be allocated to other sources of income if the climate is not adequate to carry out farm operations on their cocoa farms. Also, having other sources of income may imply being able to pay for necessary inputs that are required on cocoa farms. The farmers that irrigated their farms under climate change have significantly lower inefficiency ($p<0.10$). Irrigation would reduce death of cocoa trees, especially the young trees. However, market access significantly increases inefficiency with climate change ($p<0.10$). This shows that cocoa farmers are not able to explore opportunities afforded by market access because majority of them are already indebted to produce buyers that must buy their cocoa beans at regulated prices.

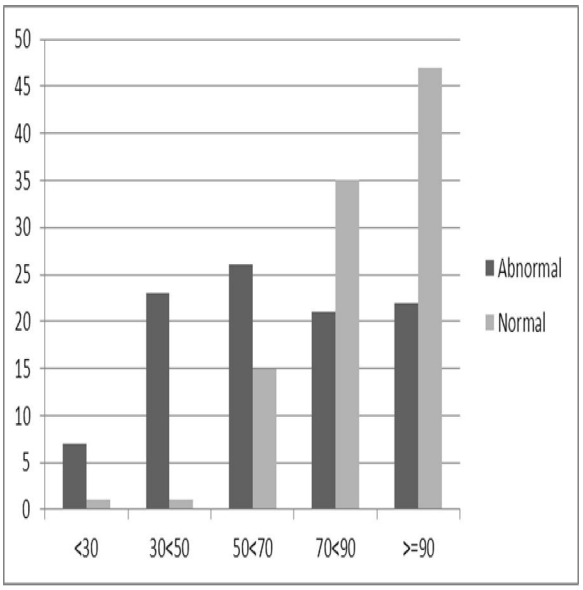

Figure 3: Distribution of technical efficiency in cocoa production under normal and abnormal climate

Figure 3 shows the distribution of cocoa production efficiency with and without climate change. It shows that while majority of the farmers have efficiency levels that are above 70 percent without climate change, the distribution is more towards less than 70 percent with climate change. Average efficiency with climate change is 65.14 percent, while it is 83.75 percent without climate change. This implies an efficiency loss of 18.61 percent.

4. Recommendations

Climate change poses serious challenge to agricultural production, and cocoa is among the crops that are extremely vulnerable. The findings of this study have highlighted some policy issues which are discussed below. First, there is need to inject younger blood into agricultural population in Nigeria. This can be done by providing incentives in the form of input provision and opening up of some forest reserves for cocoa production.

It was also found that adapting to climate change to sustain cocoa production requires more of family and hired labour. Given recent migration of youths from rural areas to urban areas in search for greener pasture, it is not sure whether such requirements can be met. Already, the use of child labour to carry our some menial operations in cocoa production had been frowned at by the International Labour Organization (ILO). The implication is that with shortage of labour in rural areas, the effects of climate change may not be easily rectified.

Also, chemical input had been over used by farmers in order to meet curtail the impact of diseases and pests on cocoa farms. Therefore, there is need for more research into developing more vibrant chemicals that will be more effective. Research should also focus on developing low cost dryers because it was found that some farmers were selling wet cocoa beans due to difficulties in having sufficient sunlight for drying the cocoa beans. Cocoa farmers should also be trained in alternative skills that can generate income. Therefore, introduction of cocoa farmers to other farm-based operations that can generate income will go a long way in curtailing the impacts of climate change.

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Field Studies on *Caligus Disease* among Cultured *Mugil Cephalus* in Brackish Water Fish Farms

[1]Noor El- Deen, A. E; [1]Abdel Hady, O.K; [2]Shalaby, S. I and [1]Mona S. Zaki

[1]Department of Hydrobiology, Vet. Div, NRC
[2]Department of Reproduction, Vet. Div, NRC
dr_ahmednoor2002@yahoo.com

Abstract: This study deals with clinical, post-mortem examination and parasitological identification of *Caligus sp* affecting infested *Mugil cephalus*, at Kafr El Sheikh Governorate fish farms. Histopathological examination of gills was investigated and recorded. Some treatment trials with freshwater, Metriphonate and freshly prepared Potassium permanganate were done. Freshwater with a 20-min immersion was successfully killed all copepods. While, short-term treatment for 30-min and 50-min immersion treatments with Metriphonate (20mg/l) and freshly prepared Potassium permanganate (10 mg/l) respectively, against *Caligus sp* infested *Mugil cephalus* were less effective than fresh water. These results concluded that, fresh water considered an effective in elimination of *Caligus sp* in *Mugil cephalus*.
[Noor El- Deen, A. E; Abdel Hady, O.K; Shalaby, S. I and Mona S. Zaki. **Field Studies on *Caligus Disease* among Cultured *Mugil Cephalus* in Brackish Water Fish Farms.** *Life Sci J* 2012;9(3):733-737] (ISSN:1097-8135). http://www.lifesciencesite.com. 103

Keywords: *Caligus disease*, *Mugil cephalus*, Trichlorfon, Potassium permanganate, freshwater.

1. Introduction

Parasites have recently been highlight at serious pathogenic problems in cultured mullet fish in marine and brackish water **(1)**. Among the parasites, Copepode family is commonly found on fishes cultured in brackish water **(2)**. Most of the Caligus species are exclusively marine and some species attack brackish water fishes **(1).** In Egypt, such parasites were isolated from mullet fish *Mugil cephalus* (3**)**. Attachment sites of these copepods were the body surface and gill cavities of the fish **(4)**. However, don't actually kill *Mugil cephalus*, unless the parasites occur in very large numbers, but the growth rate and market value of the *Mugil cephalus* may be reduced (**5** and **6)**. Fish with large numbers of parasites were emaciated; the body colour was darkened, Extensive abrasions with loosing and sloughing of the skin. The respiratory disturbance is accompanying with massive mucous secretions and severe destruction of the gills as well as causing severe losses and mortalities up to 50% (**7** and **3).** Losses associated with disease are the result of direct mortality due to secondary infections **(8** and **9).** *Caligus sp* respond differently to treatment. Fresh water controls *Caligus sp* (**10**) perhaps because of their osmoregulatory tolerances, which appears to be restricted to marine and brackish conditions **(11).** This study describes a simple and effective treatment against this pathogenic parasite.

2. Material and Methods

Fish:

A total number of 56 *Mugil cephalus* (ranged from 25-30 cm total length) infested with a parasitic copepod *Caligus* sp were collected from brackish water fish farms corresponding to Brullus lake and transported in tanks partially filled with brackish water pumped from the same ponds, were aerated and transported to Lab. of hydrobiology Dept. NRC. Before testing, two storage tanks were filled with water, the first with the same water of ponds and the second with freshwater. Eight aquaria (40 x 60 x 30 cm) were filled with 100 liter of water and aerated; seven aquaria were filled with brackish water had a pH of 8.2 and a salinity of 10 %o, and one was filled with freshwater had a pH of 7.2 obtained from the storage tanks. Freshly prepared Potassium permanganate (Akmavet) and Metriphonate (trichlorfon 97% active ingredient); (Adwia) were added to the tanks according to the experimental protocol (Table 1). There were seven treatment aquaria and one control. All *Mugil cephalus*, individually examined to record infestations and observed for clinical pictures. *Mugil cephalus* were exposed to the various treatments for either 5 -30 min (short-duration bath) or 12 h (long-duration bath) and immediately examined after exposure times.

Water:

Seven water samples collected from the same ponds *Mugil cephalus* and one from storage fresh water tank; in July at summer season 2011 for estimation of pH and salinity. A clean water sampling bottles each of about 1litre volume, equipped with stoppers. The water samples were taken a hand's breadth below the pond water surface. The bottles were labeled with locality, date and time of collection. The water samples were collected from 10 pounds of

Mugil cephalus and the average of their analysis were taken.

Clinical examination:

The collected *Mugil cephalus* were examined clinically according to the methods described by **(12)** paying an attention to the *Mugil cephalus* behaviors in the ponds, changes in colour, respiratory manifestations with a special attention to the gills.

Parasitological examination:

The microscopic parasites were collected by a fine brush, special needle or eye dropper, washed for several times in fresh water until the specimens had died and left in refrigerator at 4°C to completely relaxed. The crustaceans examined directly under light microscope. The isolated Copepods species were identified according to **(13,14,15 and 16).**

Physico – chemical analysis of water:

A total nine water samples, were collected from the different fish ponds; and one from stored freshwater tank. Determination of pH value of water samples collected from examined ponds and samples of storage freshwater tanks was measured by means of a digital PH meter (Ph. CP. HANNA instruments. Italy) and salinity (ppt) was estimated by DR 2010 (at wave length 530, programs 88).

Histopathological examination:

The affected skin , gills and gill arches of *Mugil cephalus* were fixed in 10% phosphate buffered formalin, then dehydrated in ascending grades of alcohol and cleaned in xylol, then embedded in paraffin wax and cut into thin sections (5um) and floated on warm water (just below the melting point of the paraffin) the sections are lefted from the water bath on microscope slides, coated with a minimal amount of Myer's albumin then allowed to dry thoroughly and then stained with (H&E) stain according to **(17).**

Treatment trials:

1- Freshwater. It was used with a 20-min freshwater dip treatment.
2- Potassium permanganate (KMnO4). It was used freshly prepared in a concentration of 5 mg/l for (10-min) immersion treatment.
3-Metriphonate (Trichlorfon) is an organophosphate insecticide and can be used at 0.25 to 20 mg/l, as a continuous bath for seven to ten days for (20-min) immersion treatment.

3. Results

Clinical examination:

The clinical signs of affected *Mugil cephalus* were loss of appetite, become debilitated with extensive mucous, rubbing against ponds plastic silk, nervous and respiratory manifestations. In addition, extensive focal brown spotted dotes on the skin, fins and red appearance on mouth can be seen (Fig.1 and 2).

Parasitological examination:

The microscopical examination revealed large numbers of copepods which was attached firmly to skin, fins and gill archer. The length of copepods were ranges from 5 to 6 mm long, the body was flattened, elongated or spherical with brown spotted colouration and have two characterized long bar-shaped egg pouches or strings. The crustacean parasite which was collected from some infected *Mugil cephalus* were identified as *Caligus sp* (Fig. 3).

Histopathological examination:

The parasites induced many branchial lesions showed severe inflammatory reactions to advanced degenerative and hyperplastic changes. The inflammatory changes were mainly observed in gill arch and congestion of lamellar blood vessels and adhesion of most gill filaments and lamellae (Fig. 4 and 5).

Treatment trials:

Treatment trials of naturally infested *Mugil cephalus* with *Calgius sp*, were applied using freshwater, freshly prepared Potassium permanganate and Metriphonate. It was noted that, the best suitable and effective treatments were freshwater followed by freshly prepared Potassium permanganate as it cause a great damage to the parasite at concentration of 10 mg/liter for 5 min and Metriphonate at a concentration of 20 mg /liter for 20 min.(Table, 1).

Table 1: Showing condition of the fish and copepods after different treatments and time exposures to remove *Caligus sp* from *Mugil cephalus* .

Treatment	Time	Treatment Concentration	Condition of *Mugil cephalus*	Condition of Copepods	Number of free copepods	Number of attached copepods
Fresh water		12 h	Good	Dead	10	0
Freshly prepared Pot.permanganate	20 min	3 mg/L	Good	Inactive	3	5
Freshly prepared Pot.permanganate	10 min	5 mg/L	Good	Dead	8	0
Freshly prepared Pot.permanganate	5 min	10 mg/L	Stressed	Dead	7	0
Metriphonate	30 min	10 mg/L	Good	Inactive	5	2
Metriphonate	20 min	20 mg/L	Good	Dead	9	0
Metriphonate	10 min	30 mg/L	Stressed	Dead	8	0
Brackish water control	12 h	10 %o	Good	Active	28	20

Figure (1): Showing *Mugil cephalus* red mouth X 40.

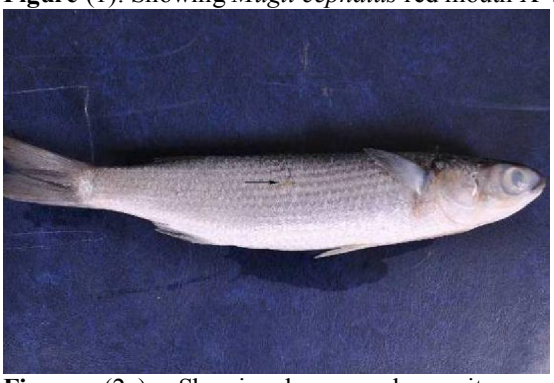

Figure (2) : Showing brown red parasite on skin (arrow) of *Mugil cephalus* .x 40

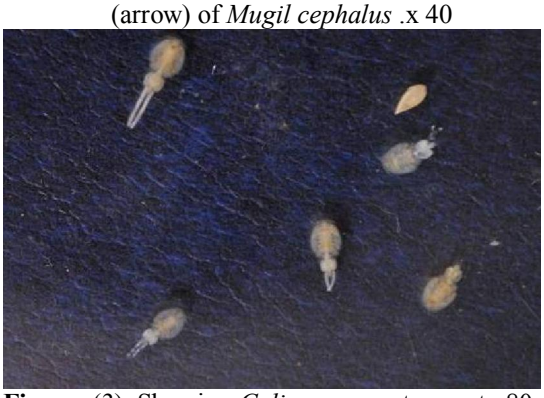

Figure (3): Showing *Caligus sp*. wet mount.x80

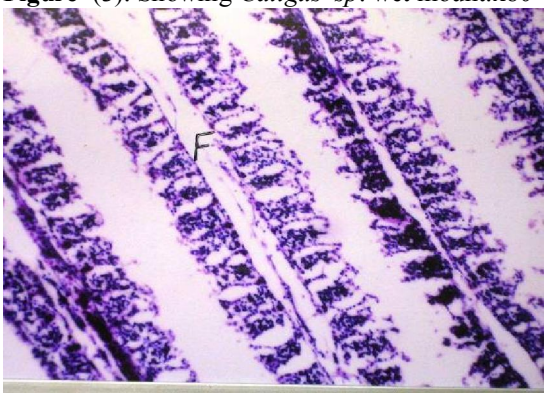

Figure (4): Gill of *Mugil cephalus* showing hyperplasia in the lamellae of the filaments (F) . H&E X 40.

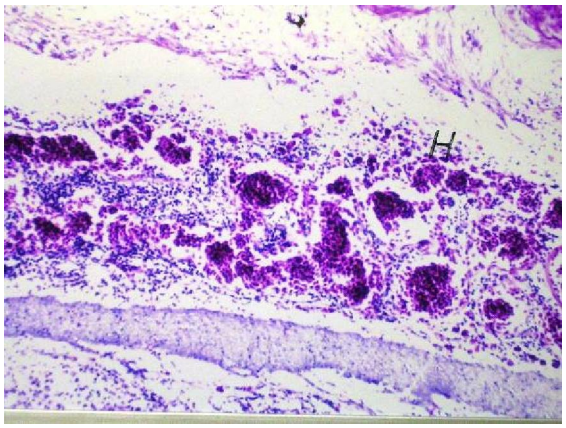

Figure (5): Gill of *Mugil cephalus* showing haemorrhage in the arch (H). H&E X 40.

4. Discussion

The present study deals with *Caligus disease* among cultured *Mugil cephalus* in brackish water at Kafr El Sheikh Governorate fish farms, Egypt. *Mugil cephalus* was in the aggregation as groups at the water inlet with severe respiratory distress and swam rapidly in circles, gulping of air at the water surface. These signs may be attributed to massive mucous secretions due to the irritation from contact of parasites and egg strings with the gill filaments & gill damage. Such results were nearly similar to that found by (**3, 18 and 19**). The results showed focal hemorrhage, abrasions on the skin and mortality was observed. These may be attributed to the parasites penetrate skin for fed and facilitate the invasion of the opportunistic micro-organisms. The present results agree with **(20, 5, 21 and 19).** The gross external examination of *Mugil cephalus* showed paleness of gills with numerous focal brown dots on the gill arches mainly unilateral and in some cases was bilateral. Alternative dark congested pale ischemic areas, marbling appearance of gills. These results are nearly similar to that reported by **(22)** and it may be attributed to destruction of the efferent vessels. The mechanism by which the destruction of the efferent vessels may happen by copepod crustaceans, where the blood pressure is low and no extensive haemorrhages are caused and the very short clotting time of blood brings about rapid occlusions of the vessel then thrombus is formed resulting in ischemia, which in turn leads to necrosis. Such explanation was recorded by **(23).**

In this study, histopathological examinations of the infested gills revealed congestion of lamellar and bronchial blood vessels with severe inflammatory cell infiltration. In advanced stages, adhesion of most secondary lamellae in some gills was observed. These results may be attributed to the mechanical injury

induced by the parasite that ingest infiltrated cells and epithelial cells that proliferate due to stimulation of attachment leads to slow haemorrhage, rapid blood clot, thrombus, ischemia and finally necrosis which manifests the picture of marbling appearance. The present findings nearly agree with results reported by **(24** and **5)**.

Regarding the prevalence of *Caligus disease* in relation to different some water parameters. The results revealed that, *Caligus* infestation found in *Mugil cephalus* cultured in brackish water , it may be attributed to the parasite cannot tolerate substantial osmatic changes in fresh water. The present findings nearly agree with the results found by **(25 and 26).**

Concerning the treatment using fresh water, it was revealed that the effectiveness of the fresh water treatment may be attributed to osmotic concentration. The present findings nearly agree with the results found by **(11).** Freshly prepared Potassium permanganate 5 mg per liter treated *Caligus sp* without harming in aquaria water. This may be attributed to strong oxidizing properties of Potassium permanganate, the present findings nearly agree with results found by **(27** and **28)** who recorded that Potassium permanganate kill skin and gill pathogens via its strong oxidizing properties. Moreover, the present study revealed that the effective concentration of Metriphonate was 20 mg per liter for 20 min. It was suggested that the effectiveness Metriphonate was due to its acetylcholine inhibiting effect on Caligus sp. These findings nearly agree with (**29,30, 31** and **32).** They reported that Metriphonate cause asphyxia and toxicity of the parasite. The results with (**33)** recorded that the standard *Caligus sp* treatment *in Salmon* appears to be Trichlorfon (300 mg/L, 15-60 min, 3-12°C).The results with pike may be due to the difference of the species , land host and /or they may be also due to difference of environmental conditions and /or breeding system.

In the present study, these results concluded that, fresh water considered the best and effective to eliminate *Caligus sp.* which affect *Mugil cephalus* in brackish water fish farms.

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Cranial Magnetic Resonance Imaging (MRI) Changes in Severely Malnourished Children before and after Treatment.

Ashraf M. El-Sherif[1] , Gihan M. Babrs[2] and Ahlam M. Ismail[3]

Departments of Radiology[1] and Pediatrics[2]- Faculty of Medicine, Minia University
Department of Pediatrics[3], Faculty of Medicine, South Valley University, Egypt
gihanbabrs@hotmail.com

Abstract: Protein energy malnutrition is an important problem in developing countries. Neurological changes is associated with sever malnutrition. **Aim of Work**: Our study seeks to document the morphological changes in the brain of infants suffering from severe malnutrition of both edematous and non-edematous types and to follow up these changes and its outcome after nutritional rehabilitation according to the WHO regimen using cerebral MRI imaging. **Patients and Methods:** Seventeen children suffering from severe malnutrition were included in this study. Patients included 7 males and 10 females and their ages ranged from 2 to 24 months who had attended Minia University outpatient clinic. All the children were evaluated and treated in hospital according to WHO standardized protocol for management of severe malnutrition. Patients were referred to Radiology Department for MRI of their brains on admission and again after 90 days of treatment. **Results:** Cerebral atrophy and ventricular dilatation are common findings in the brains of children suffering from moderate and severe PEM. Children with both edematous and non-edematous types of PEM are almost equally affected. However, the changes are reversible in most cases when nutritional rehabilitation is undertaken. Brain myelination process doesn't show significant delay in these patients and the brain stem and cerebellum were normal in all of them. **Conclusion:** Severely malnourished children should be evaluated by Z score and treated by WHO recommendation. Cranial MRI findings in these patients include brain atrophy and ventricular dilatation but these changes are reversible so, early treatment is very important and can help to prevent permanent neurological derangements.

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Key Words: Protein energy malnutrition – Cerebral imaging - Brain atrophy - MRI – Pediatrics .

1. Introduction

Still, up till now in the 21 century, Protein energy malnutrition (PEM) is an important public health problem in the developing countries. (PEM), a natural ramification of poverty, continues to be a perennial source of concern to a large segment of the world population. The developing nervous system of a child is especially vulnerable to deprivations in nurture. Peripheral nerve and muscle derangements are clinically evident by weakness, hypotonia and hyporeflexia in accordance with severity and duration of PEM. (1)

The brain of the child is one of the most vulnerable organs affected during growth with potential morphological changes, which can be detectable with neuroimaging technology. (2) Several neuropathological studies of the brain have shown that PEM may have adverse impact on the number of neurons and synapses, degree of myelination, and total cerebral lipid content of the developing brain .(3)

Previous cranial imaging studies of the brain in patients with protein energy malnutrition (PEM) showed that cerebral atrophy and ventricular dilatation are common findings (4) but, fortunately these cranial changes are reversible after achieving nutritional rehabilitation(5).

The present study seeks to document the morphological changes in the brain of infants suffering from acute severe malnutrition of both edematous and non edematous types and to follow up these changes and its outcome after nutritional rehabilitation by the standardized WHO regimen using cranial MR imaging.

2.Patients and Methods

This prospective study included seventeen children with age range (2 to 24 months), who had attended Minia University outpatient clinic over the period from May 2010 to May 2011 suffering from severe protein energy malnutrition and failure to thrive. Children were clinically examined and their measurements including weight, height and mid upper arm circumference (MUAC) were taken and plotted using the National Centre for Health Statistics reference values NCHS/WHO. The children were considered to have severe acute malnutrition if they had any of the following:

1- Height for age -2 SD from the median (z-scores) of normal child.

2- MUAC measurement under 11.0 cm.
3- Edema in both feet and legs.

All the children were evaluated and examined for presence of any complications as gastro-enteritis (GE), pneumonia and sepsis. Laboratory investigations including complete blood count, serum creatinine and urea, stool analysis, and electrolytes and serum albumin had been carried out.

Our patients were treated in hospital according to WHO standardized protocol for management of severe malnutrition which includes the following essential ten steps [6]:

1- Treat/prevent hypoglycemia
2- Treat /prevent hypothermia
3- Treat/prevent dehydration
4- Correct electrolyte imbalance
5- Treat/prevent infection
6- Correct micronutrient deficiencies
7- Start cautious feeding
8- Achieve catch up growth
9- Provide sensory stimulation and emotional support
10- Prepare for follow up after recovery

Patients were considered for hospital discharge when their weight for height reached 90% of median.[6]

All 17 patients were referred to Radiology Department for MR imaging of their brains on admission and again after 90 days of treatment. T1 and T2 weighted sagittal and axial MRI images were acquired as well as fluid attenuation inversion recovery (FLAIR) sequences using General Electric 0.2 T open MRI system.

3. Results

Seventeen children suffering from severe malnutrition were included in this study. Patients included 7 males and 10 females and their age ranged from 2-24 months.

The children were classified clinically on admission into 2 groups according to presence or absence of edema into 9 edematous and 8 non-edematous **(Table 1).** There was no significant correlation between the presence of edema and age, sex, weight, length and /or head circumference. But there was significant correlation between the presence of edema and the child's weight for height Z score number with *P* value 0.01.

Table (2) shows that edema was more common in children presented with gastroenteritis (*P*-value = 0.01) than in children presented with complications other than gastroenteritis like pneumonia, sepsis and/ or convulsion. Results of laboratory investigations showed no significant difference between edematous & non-edematous groups except for the presence of RBCs in the stool analysis of children with gastroenteritis (*P* = 0.002) (**Table 3**).

All children were treated in our hospital according to WHO standardized protocol for management of severe malnutrition.

Table (1) Demographic characteristics of 17 severely malnourished children on admission

	Edematous malnutrition (NO = 9)	Non edematous malnutrition (NO = 8)	*P* -Value
Age (months) mean ± SD	9.2±7.04	6.4±3.7	0.3
Sex: **Male No (%)** **Female No (%)**	 3 (33.3%) 6(66.7%)	 4 (50%) 4 (50%)	0.6
Weight(kg) mean ±SD	5.2±2.6	3.7±1.01	0.1
length (cm) mean ±SD	57.3±7.5	64.4±11.5	0.1
Head circumference (cm) mean±SD	41.4±3.7	38.8±3.5	0.1
Weight for height Z score No (%) **<-2 SD** **<-3 SD** **<-4 SD**	 6 (66.7%) 3 (33.3%) 0 (0)	 0 (0) 6 (75%) 2 (25%)	0.01

Table (2) Associated complications in severely malnourished children

	Edematous malnutrition (NO = 9)	Non edematous malnutrition (NO = 8)	*P*- Value
Pneumonia (%)	0	2 (25%)	-
Gastroenteritis (%)	7 (77.8%)	2 (25%)	0.01
Sepsis (%)	1 (11.1%)	2 (25%)	0.2
Convulsions*	1 (11.1%)*	0	-

*Convulsions in this patient was related to hypocalcaemia

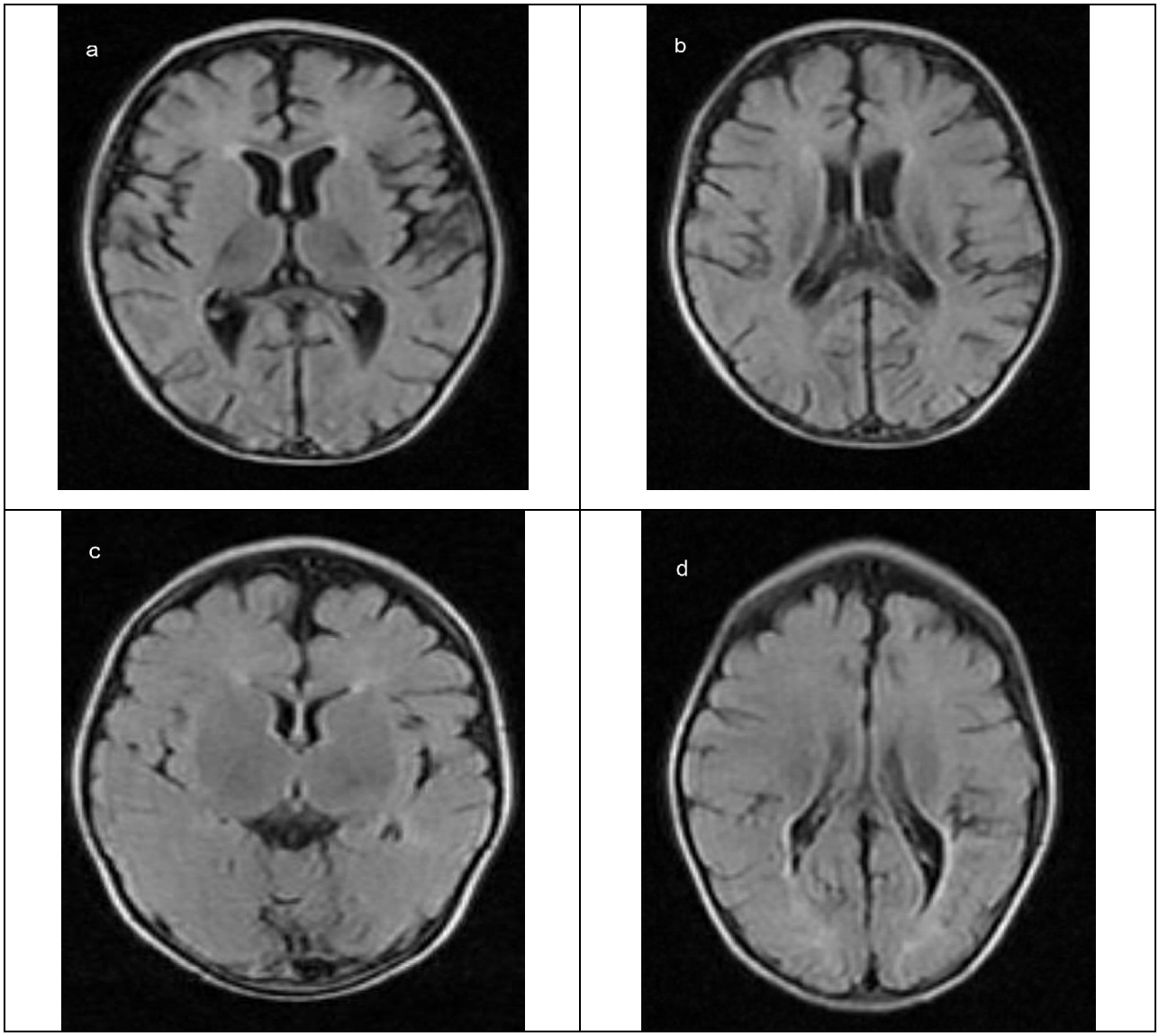

Fig. 1 Axial FLAIR MR images at the level of the basal ganglia. **a** and **b** 10 months old patient on admission showing mild brain atrophy in the form of dilated ventricles and prominent cortical sulci. **c** and **d** follow-up MRI of the same patient at day 90 after treatment showing resolution of the signs of cerebral atrophy with normal ventricular size and cortical sulci.

Table (3): Laboratory findings in severely malnourished children.

	Edematous malnutrition (NO = 9)	**Non edematous malnutrition (NO = 8)**	***P*- Value**
Hb (gm/dl)	9.4±1.5	10.5±1.03	0.4
WBCs ($\times 10^3$)	9.08±3.9	11.8±4.8	0.2
Platelets ($\times 10^3$)	364.8±163.5	502.1±221.2	0.1
Stool: pus cells	2.8±0.7	2±1.6	0.1
RBCs	5.1±4.9	35.6±18.5	0.002
Urine: pus cells	3.2±2.6	3.5±2.01	0.7
RBCs	2.2±1.6	2.2±1.9	0.9

Table (4): Cerebral MRI findings in severely malnourished children.

	Edematous malnutrition (No = 9)	Non edematous malnutrition (No = 8)	*P*- Value
Dilated ventricles	5 (55.6%)	5 (62.5%)	0.3
Cerebral atrophy	7 (77.8%)	7 (87.5%)	0.3
PVWM changes	1 (11.1%)	3 (37.5%)	0.1

Table (5). The distribution of patients according to the degree of cerebral atrophy as seen by MRI on admission and on follow-up

Degree of cerebral atrophy	14 patients with cerebral atrophy		*P*- Value
	On admission	On day 90	
Mild	2 (14.3%)	0	0.2
Mild to Moderate	7(50%)	0	
Moderate	5(35.7%)	2 (14.3%)	

All 17 children included in this study had MRI scans of their brains on admission and on follow-up at 90 days later. Fourteen patients (82%) had positive MR findings on admission consistent with cerebral atrophy while 3 (18%) patients had normal MRI scans. The abnormalities included all or some of the following: widened cortical sulci and sylvian fissures, widened interhemispheric fissures and cerebellar folia, dilated ventricles and enlarged basal cisterns. In addition, 4 patients (28%) had periventricular white matter (PVWM) changes (**Table 4**). There was no significant difference between both edematous and non edematous groups regarding the MRI findings. The degree of cerebral atrophy was determined as mild, mild to moderate or moderate. In two children the changes were mild, in seven they were mild to moderate and in five they were moderate (**Table-5**). Ventricular dilatation, particularly the frontal horns of the lateral ventricles was found in 10 (71%) of 14 patients with cerebral atrophy. On follow-up MRI scans performed 90 days later, the degree of cerebral atrophy had improved in 2 patients and completely resolved in 12 (86%) patients (Figure 1). Grey and white matters of the cerebral hemispheres were equally affected while the brain stem and the cerebellum did not show any abnormality in any of our patients. The myelination distribution appeared to be appropriate for age with no myelination delay detected in any of the examined children.

4. Discussion

In our study we demonstrated that there was significant correlation between the presence of edema and the child's weight for height Z score number with P value 0.01, that was coming in harmony with the results of Bhoomika et al., 2008 that concluded that using Z score system is the best way to determine degree of malnutrition(7).

Also we found that, edema was more common in children presented with gastroenteritis (P = 0.01) than in children presented with other complications .The results of laboratory investigations showed no significant difference between edematous and non-edematous groups except for the presence of RBCs in the stool analysis of children with gastroenteritis (P = 0.002) and that come in agreement with the results got by Pawellek et al., 2008,who found that PEM is most frequently associated with acute infections, especially gastroenteritis and chronic diarrhea (8) .

Several neuropathological studies of the brain have shown that PEM may have adverse impact on the number of neurons and synapses, degree of myelination, and total cerebral lipid content of the developing brain(3).

In the present study we found that cerebral atrophy and dilated ventricles were the commonest MRI findings seen in the children suffering from severe malnutrition and that comes in concordance with the study carried by Atalabi et al., 2010 (2).

The majority of children with acute protein energy malnutrition in this study (82%) showed MRI features of cerebral atrophy. Researchers in earlier studies demonstrated similar results using computed tomography (CT) (5) and MRI (2). In order to investigate whether these cerebral atrophic changes are reversible or not, researchers have monitored these changes during nutritional rehabilitation and reported complete resolution by day 90 in the majority of their patients (7). Likewise, in this study the results on follow-up brain scans came to confirm significant improvement after nutritional rehabilitation and documented complete resolution of the findings in 12 of 14 patients with initial cerebral atrophy of different degrees. The pathophysiologic changes leading to cerebral atrophy in children with protein energy malnutrition, however, remained a subject of research and postulations. Gunston et al., suggested that loss of myelin lipid accounts for the cerebral shrinkage seen in their patients and restoration of lipid to the myelin membrane with refeeding accounts for the reversal of cerebral shrinkage (7). However, in their study as well as in

our study there was no significant nutritional impact on the myelination process and it was regarded as normal for the patient's age. In another patient population with anorexia nervosa, Heinz et al have reported reversible cerebral atrophy and proposed that fluid moves out of intravascular spaces as a result of decreased colloid osmotic pressure and floods the subarachnoid spaces, dilates the ventricles, and widens the cisternal spaces and sulci. When nutrition is improved the plasma proteins rise, and the extracellular fluid moves back into the intravascular space (9,10). Although it was once postulated that loss of myelin lipid accounts for the cerebral shrinkage (7), our study demonstrated that the myelination process was determined as appropriate for age. Likewise, Hazin et al., (11) reported myelination delay in only 2 of their series of 20 children with severe PEM

Conclusion

Cerebral atrophy and ventricular dilatation are common findings in the brains of children suffering from moderate and severe PEM. Children with both edematous and non-edematous types of PEM are almost equally affected. However, the changes are reversible in most cases when nutritional rehabilitation is undertaken. Brain myelination process doesn't show significant delay in these patients and the brain stem and cerebellum are normal in all of them.

Corresponding author
Gihan M. Babrs
Department of Pediatrics, Faculty of Medicine,
Minia University
gihanbabrs@hotmail.com

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On some lower bounds and approximation formulas for $n!$

Mustafa A. OBAID

King Abdulaziz University, Faculty of Science, Mathematics Department,
P. O. Box 80111, Jeddah 21589, Saudi Arabia.
drmobaid@yahoo.com

Abstract: In this paper, we present the following new inequality of $n!$ $n! > \sqrt{2\pi}\ n^{n+1/2} e^{-n+\sum_{r=0}^{\infty}\left\{(2n+2r+1)\tanh^{-1}\left(\frac{1}{2n+2r+1}\right)-1\right\}}$ $n \in \mathbb{N}$. Also, we deduce that the approximation formula $n! \sim \sqrt{2\pi}\ n^{n+1/2}\ e^{-n+\sum_{k=1}^{m}\frac{2^{-2k}}{2k+1}\varsigma(2k,n+1/2)}$ has rate of convergence equal to n^{-2m-1} for $m = 1, 2, 3, \cdots$. Thus, we can choose the approximation formula that we want it convergence to $n!$ by a known rate.
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MSC 2010 classification : 33B15, 26D07, 41A60.

1 Introduction.

There are many different upper and lower bounds for $n!$ presented by several authors [4, 3, 21, 20, 17, 8, 9]. Most bounds are of the form

$$\sqrt{2n\pi}\left(\frac{n}{e}\right)^n e^{a_n} < n! < \sqrt{2n\pi}\left(\frac{n}{e}\right)^n e^{b_n}, \quad (1)$$

Where a_n and b_n tend to zero through positive values. P. R. Beesack [2] presented the following important result:

Theorem 1.

$$\sqrt{2\pi n}\left(\frac{n}{e}\right)^n e^{a_n} < n! < \sqrt{2\pi n}\left(\frac{n}{e}\right)^n e^{b_n}$$

$n \geq 1$, (2)

where the two sequences a_n, $b_n \to 0$ as $n \to \infty$ and satisfy

$$a_n - a_{n+1} < \sum_{k=1}^{\infty}\frac{1}{2k+1}\frac{1}{(2n+1)^{2k}} < b_n - b_{n+1}. \quad (3)$$

For the q-factorial which is defined by [5]

$$[n]_q! = n_q[n-1]_q \cdots [2]_q[1]_q,$$

where $[x]_q = \frac{1-q^x}{1-q}$ is the q-number of x, Mansour and et al [6] presented the following q-analog of the Beesack's result (2):

Theorem 2. The q- factorial $[n]_q!$ satisfies the double inequality

$$(q,q)_\infty(1-q)^{-n}e^{f_q(n+1)} < [n]_q!\,(q;q)_\infty(1-q)^{-n}\ e^{g_q(n+1)},\ n \geq 1; 0 < q < 1 \quad (4)$$

where $f_q(n)$ and $g_q(n)$ are two sequences tend to zero through positive values and satisfy

$$f_q(n) - f_q(n+1) - \log(1-q^n) < g_q(n) - g_q(n+1), \quad n \geq 1. \quad (5)$$

Recently, Mansour and et al [7] presented a new proof of Beesack's result (2) and deduced the following upper bounds of $n!$:

Theorem 3.

$$n! < \sqrt{2\pi n}\,(n/e)^n\ e^{M_n^{[m]}} \quad n \in \mathbb{N} \quad (6)$$

$$M_n^{[m]} = \frac{1}{2m+3}\left[\frac{1}{4n} + \sum_{k=1}^{m}\frac{2m-2k+2}{2k+1}\,2^{-2k}\varsigma\,(2k, n+\frac{1}{2})\right]$$

$$m = 1,2,3,\cdots,$$

where $\varsigma(x)$ is the Riemann Zeta function.

In this paper, we will use the technique of [7] to introduce a family of lower bounds of $n!$. Hence, we will deduce some new approximation formulas for large $n!$ and we will study their rates of convergence.

2 A New family of lower bounds of $n!$

To find some lower bounds of the series $\sum_{k=1}^{\infty}\frac{1}{2k+1}\frac{1}{(2n+1)^{2k}}$ we observe firstly that

$$\sum_{k=1}^{\infty}\frac{1}{2k+1}\frac{1}{(2n+1)^{2k}} > \sum_{k=1}^{m}\frac{1}{(2k+1)(2n+1)^{2k}}, \quad m = 1,2,3,\cdots$$

So, we can consider the recurrence relation

$$L_{n,m} - L_{n+1,m} = \sum_{k=1}^{m}\frac{1}{(2k+1)(2n+1)^{2k}}, \quad m = 1,2,3,\cdots \quad (7)$$

which has the following solution form

$$L_{n,m} = L_{0,m} - \sum_{i=1}^{n-1}\left(\sum_{k=1}^{m}\frac{1}{(2k+1)(2i+1)^{2k}}\right)$$
$$= L_{0,m} - \sum_{k=1}^{m}\frac{1}{2k+1}\left(\sum_{i=1}^{n-1}\frac{1}{(2i+1)^{2k}}\right).$$

By using the relation [18]

$$\sum_{i=1}^{n-1}\frac{1}{(2i+1)^{2k}} = -1 - (2^{-2k}-1)\varsigma(2k) - 2^{-2k}\varsigma(2k, n+1/2)$$
$$= -1 - \frac{(-1)^{k-1}(1-2^{2k})}{2(2k)!}B_{2k}\pi^{2k} + 2^{-2k}\varsigma(2k, n+1/2)$$

where $\varsigma(x)$ is the Riemann Zeta function and $B_r's$ are Bernoulli's numbers, we get

$$L_{n,m} = L_{0,m} + \sum_{k=1}^{m}\frac{1}{2k+1}\left(1 + \frac{(-1)^{k-1}(1-2^{2k})}{2(2k)!}B_{2k}\pi^{2k} + 2^{-2k}\varsigma(2k, n+1/2)\right).$$

Also

$$\sum_{i=1}^{\infty}\frac{1}{(2i+1)^{2k}} = \frac{(-1)^{k-1}(2^{2k}-1)}{2(2k)!}B_{2k}\pi^{2k} - 1.$$

Hence, we can choose

$$L_{0,m} = \sum_{k=1}^{m}\frac{1}{2k+1}(\varsigma(2k)(1-2^{-2k}) - 1), \quad (8)$$

which satisfies

$$\lim_{n\to\infty} L_{n,m} = 0, \quad m = 1,2,3,\cdots.$$

Then we obtain the following result:

Theorem 4.

$$n! > \sqrt{2\pi}\; n^{n+\frac{1}{2}}\, e^{-n+\sum_{k=1}^{m}\frac{2^{-2k}}{2k+1}\varsigma\left(2k, n+\frac{1}{2}\right)} \quad n, m \in \mathbb{N} \quad (9)$$

where $\varsigma(x)$ is the Riemann Zeta function.

In the following result, we will prove that the increasing of the value of m in the lower bound $L_{n,m}$ will improve its value.

Lemma 2.1.

$L_{n,m+1} > L_{n,m} \qquad m, n = 1,2,3,\cdots.. \quad (10)$

Proof.

From [9] we get

$$L_{n,m+1} = \sum_{k=1}^{m+1}\frac{2^{-2k}}{2k+1}\varsigma(2k, \frac{n+1}{2})$$
$$= L_{n,m} + \frac{2^{-2m-2}}{2m+3}\varsigma(2m+2, n+1/2).$$

But $\varsigma\left(2m+2, n+\frac{1}{2}\right) > 0$, then

$$L_{n,m+1} - L_{n,m} > 0.$$

Theorem 5.

$$n! > \frac{\sqrt{2\pi}\, n^{n+1/2}}{e^{-n+\sum_{r=0}^{\infty}\left\{(2n+2r+1)\tanh^{-1}\left(\frac{1}{2n+2r+1}\right)-1\right\}}} \quad n \in \mathbb{N} \quad (11)$$

Proof. Using (9) at m tends to ∞, we obtain

$$L_{n,\infty} = \sum_{k=1}^{\infty}\frac{2^{-2k}}{2k+1}\varsigma(2k, n+1/2).$$

But

$$\varsigma(2k, n+1/2) = \sum_{r=0}^{\infty}\frac{1}{(n+1/2\ \ +r)^{2k}},$$

then

$$L_{n,\infty} = \sum_{r=0}^{\infty}\sum_{k=1}^{\infty}\frac{1}{(2n+2r+1^{2k}(2k+1)}.$$

Using the relation

$$\tanh^{-1}x = \sum_{t=0}^{\infty}\frac{x^{2t+1}}{2t+1}; \qquad |x| < 1,$$

then we get

$$L_{n,\infty} = \sum_{r=0}^{\infty}\left\{\begin{matrix}(2n+2r+1)\tanh^{-1}\\((2n+2r+1)^{-1}) - 1\end{matrix}\right\}.$$

3 Convergence rate of the approximation formula

$$\boldsymbol{n! \sim \sqrt{2\pi}\, n^{n+1/2} e^{-n+\sum_{k=1}^{m}\frac{2^{-2k}}{2k+1}\varsigma(2k, n=1/2)}}.$$

C. Mortici [10]—[16] presented a new method to measure the convergence rate of some asymptotic expansions. Also, he use this method to accelerate and construct some approximation formulas. The following lemma contains the Mortici result.

Lemma 3.1.

If $(\varphi_n)_{n\geq 1}$ is convergent to zero and there exists the limit

$$\lim_{n\to\infty} n^k(\varphi_n - \varphi_{n+1}) = l \in \mathbb{R} \quad (12)$$

with $k > 1$, then there exists the limit:

$$\lim_{n\to\infty} n^{k-1}\varphi_n = \frac{l}{k-1}$$

To measure the convergence rate of the formula $\sqrt{2\pi n}\,(n/e)^n e^{L_{n,m}}$, define the sequence $(\varphi_n)_{n\geq 1}$ by the relation

$n! = \sqrt{2\pi n}\,(n/e)^n e^{L_{n,m}+\varphi_n}; \quad n = 1,2,3,\cdots$ (13)

The value of the approximation formula will be better whenever $(\varphi_n)_{n\geq 1}$ convergence to zero faster. Using the relation (13) we get

$\varphi_n = \ln n! - \ln\sqrt{2\pi} - (n+1/2)\ln n + n - L_{n,m}$

And hence

$\varphi_n - \varphi_{n+1} = (n+1/2)\ln(1+1/n) - 1 + L_{n+1,m} - L_{n,m}$.

By using the expansion [1]

$(n+1/2)\ln\left(1+\frac{1}{n}\right) - 1 = \sum_{k=1}^{\infty}\frac{1}{2k+1}\frac{1}{(2n+1)^{2k}}$ (14)

and the relation [7], we have

$$\varphi_n - \varphi_{n+1} = \sum_{k=m+1}^{\infty} \frac{1}{(2k+1)(2n+1)^{2k}}.$$

Then

$$\lim_{n\to\infty} n^{2(m+1)}(\varphi_n - \varphi_{n+1}) = \frac{1}{(2m+3)2^{2(m+1)}}; \quad n, m = 1,2,3,\cdots \quad (15)$$

Now we get the following result according Mortici result:

Theorem 6. The rate of convergence of the sequence φ_n is equal to n^{-2m-1}, since

$$\lim_{n\to\infty} n^{2m+1}\varphi_n = \frac{1}{(2m+1)(2m+3)2^{2(m+1)}}.$$

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Evaluation of Polymerase Chain Reaction and Culture for the Diagnosis of Corneal Ulcer

Rania A. Khattab[1], Salwa A. Rasmy[1], Yasser M. Ragab[1], Dalia G. Said[2], Maha M. Abdelfatah[2], Mohamed A[3]. Shemis, Dalia M. Ezzat[3] and Fatma Elzahraa S. Abdel Rahman[3]

[1]Microbiology and Immunology Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt
[2]Research Institute of Ophthalmology, Corneal unit and Microbiology Department, Cairo, Egypt
[3]Theodor Bilharz Research Institute, Giza, Egypt
Khattab500@yahoo.com lady_ok@yahoo.com

Abstract: Purpose: To compare polymerase chain reaction (PCR) to microbial culture for the detection and identification of bacterial and fungal microorganisms in microbial keratitis. **Methods:** Corneal scrapings from 150 patients clinically diagnosed as microbial keratitis, who attended the Research Institute of Ophthalmology cornea clinic were cultured, analysed by PCR and the results were compared. **Results:** Of the 150 patient samples, 104 (69.3%) were culture-positive (76 for bacteria, 19 for fungi and 9 were mixed culture); and 46 (30.7%) were culture-negative. Of these 150 patient samples, 130 (86.7%) were positive by PCR (74 bacterial, 18 fungal and 38 mixed infection); and 20 (13%) were PCR-negative. Of the 76 culture-positive for bacteria, 73 (96%) were positive by PCR; 17 (89.5%) out of 19 samples culture-positive for fungi were positive by PCR and 8 (89%) out of 9 samples culture-positive for mixed infection were PCR-positive. Of the 46 culture-negative samples, 32 (69.5%) yielded pathogen deoxyribonucleic acid (DNA) products and 14 were PCR-negative. The sensitivity of PCR in detecting bacterial, fungal, mixed culture and no growth keratitis was 94%, 86%, 88%, 79% respectively while the specificity was 90%, 82%, 95% and 83% respectively. **Conclusion:** PCR detects microbial DNA in the majority of bacterial and fungal corneal ulcers, and identifies microorganisms in a high proportion of culture-negative cases. PCR may be used as an adjunct to culture to identify microorganisms in microbial keratitis. Although being expensive, PCR remains a promising tool for faster and highly sensitive diagnosis of microbial keratitis.
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Key words: diagnosis- microbial keratitis- PCR- culture.

1. Introduction

Standard microbiological techniques for diagnosing microbial keratitis rely on culturing the organisms in nutrient media. The frequency of apparent diagnostic failure (that is, no organism is isolated though an infection is clinically evident) ranges from 20% (1) to 60% (2). An additional problem is that such techniques require days to weeks for complete results, which can significantly delay appropriate treatment.

The polymerase chain reaction (PCR) is a highly sensitive and rapid technique for amplifying analytic quantities of deoxyribonucleic acid (DNA) from infinitesimal starting quantities. When applied to the detection of pathogen DNA, the technique can be used to rapidly identify the presence of specific organisms (3). The potential utility of polymerase chain reaction (PCR) based techniques for improving the diagnosis of ocular infection is well recognized (3,4), and the use of PCR for this purpose is expanding (5,6). The aim of the current study is to compare culture and microbial PCR results in a series of patients presenting with corneal ulcer and to study the sensitivity and specificity of each method in diagnosing microbial keratitis.

2. Materials

Patients

Our study included 150 (77 males and 73 females) patients with clinical evidence of microbial keratitis who attended the outpatient clinic corneal unit department of the Research Institute of Ophthalmology, Cairo, Egypt. Their ages ranged from 2 to 83 years (mean 43 years). A total of 50 patients were used as controls (30 males and 20 females), their ages ranged from 25 to 50 years (mean 35 years). Control patients had normal ocular examination with no tear film dysfunction. A detailed history was taken and a thorough slit-lamp examination was done for all patients. In patients with microbial keratitis, the size, depth and margins of the infiltrate were noted. Any epithelial defect was photographed and measured. Corneal scrapings were taken from the base and edge of the ulcers with a sterile blade, after installing local anaesthetic solution (4% xylocaine) in the eye.

Methods

Culture

The material obtained by scraping from the leading edge and the base of each ulcer was inoculated directly onto sheep blood agar, chocolate agar, and Sabouraud dextrose agar (SDA) for bacterial and fungal culture. Culture growth was read within a maximum of 5 days for bacterial growth and two weeks for fungal growth. If positive, the colony was further analysed by standard biochemical tests until a specific species was identified.

To evaluate the diagnostic value of each assay, statistical analysis was done for calculating the sensitivity, specificity, positive predictive value

(PPV) and negative predictive value (NPV) using Medcalc program.

Polymerase chain reaction

Sample collection and DNA Extraction

After culture samples had been obtained, a sterile swab was used to obtain a corneal scrape from the base and leading edge of the corneal ulcer for PCR assay. The swab was placed into a sterile micro centrifuge tube, capped and immediately transferred to -70°c for storage until processing. DNA from all samples was extracted within one month of receipt.

Briefly, DNA was extracted from each swab using QIA amp DNA Micro extraction kit from Qiagen according to manufacturer's instructions. QIA shredder from Qiagen was also used to harvest the lysate.

DNA amplification:

The primers used in this study, their sequence, product size and references are shown in table (1). Specificity of the primers was tested using DNA of various strains available in our microbiology and immunology laboratory. All the strains used as positive controls were laboratory isolates like *Staphylococcus aureus, Staphylococcus epidermidis, Pseudomonas aeruginosa, Candida* spp., *Aspergillus* spp. and *Fusarium* spp..

I-Conditions for universal bacteria:

16srRNA primers (27-6 and 28-6) dissolved in 165 µl dist.H_2O to reach final conc. 50pmole/µl. (Figure 1).

PCR reaction mix (50µl):

DNA 1µl, taq 0.25µl, primers (pF 0.5 +pR 0.5), 5x buffer (GoTaq Reaction buffer) 10µl, dNTPs 4µl (2mM) and complete with dist. H_2O 33.75µl

PCR program:

Initial denaturation 96□C for 3min., denaturation 95□C for 15sec., both extension and annealing in one step 55□C for 30sec., 40 replication cycles , final extension 55□C for 10min., stop reaction at 4□ for 10min.

II- Conditions for the bacterial species analysed: (*Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas* spp.)

PCR reaction mix (50µl) for bacterial genotyping:

DNA 4µl, taq 0.5µl, 5x flexi buffer 10µl, dNTPs 1µl (10mM), Mg 7.5µl for *S.aureus* and 6.5µl for *S.epi* and *pseudomonas*, primers (0.3For +0.3Rev) complete with dist. H_2O

1-*Staphylococcus aureus*:

By using forward primer (9-6) and reverse primer (10-6), (Figure 2). (9-6 dissolved in 226µl and 10-6 dissolved in 240µl dist. H_2O)

PCR program:

Initial denaturation 95□ for 5min., denaturation at 95□c for 10 sec., annealing 60□c for 10sec., extension 72□c for 22 sec.45 cycles of replication and final extension 72□c for 5min.

2-*Staphylococcus epidermidis*:

By using GYRb FoR-9 and GyRb Rev-9, (Figure 3). (GYRb For-9 dissolved in 188µl and GYRb Rev-9 dissolved 185µl dist. H_2O).

PCR program:

Initial denaturation 95□ for 5 min., denaturation step at 94□c for 30 sec., annealing 55□c for 30 sec., extension at 72□c for 1min.,40 cycles of replication and final extension at 72□c for 2 min.

3-Pseudomonas* spp.*:

By using 21-6 and 22-6 primers**, figure (4).** (21-6 dissolved in 185µl and 22-6 dissolved in 205µl dist. H_2O)

PCR program:

Initial denaturation 95□c for 2min., denaturation 94□c for 20sec., annealing at 51□c for 20sec., extension at 72□c for 40sec., 40 cycles of replication and final extension at 72□c for 1 min.

III- Conditions for universal fungus:

ITS primers(ITS 1-9 and ITS 4-9) dissolved in 200µl dist. H_2O for final conc. 50pmole/µl, (Figure 5).

PCR reaction mix. (50µl):

DNA 4µl, taq 0.25µl, primers (pF 0.25 + pR 0.25), 5x buffer (Go Taq Reaction buffer) 10µl, dNTps 8µl (2 mM) complete with dist. H_2O 27.25 µl

PCR program:

Initial denaturation 95□c for 5min., denaturation at 95□c for 30sec., annealing 58□c for 30sec., extension at 72□c for 1min., 35 replication cycles ,final extension at 72□c for 10min., and stop the reaction at 4□c for 5min.

IV- Conditions for the fungal species analysed:

(*Aspergillus* spp*, Fusarium* spp.and *Candida* spp.)**

PCR reaction mix (25µl) for fungal genotyping:

DNA 1µl, taq (5U/µl) 0.2 µl, primers (0.125µl For. + 0.125µl Rev) (50pmole), dNTps 4µl (2 mM), 5x buffer (Go Taq reaction buffer) 5µl complete with dist. H_2O.

PCR program:

Initial denaturation 95□C for 5min., denaturation 95□C for 30sec., annealing 66□C for *Aspergillus* spp.,57□C for *Fusarium* spp. and 61□C for *Candida* spp. for 30sec., extention 72□C for 20sec.,40 repeating replication cycles, final extension 72□C for 7min**.**

1- *Aspergillus* spp. primers ASFu For-9 and Asfu Rev-9, product size 520 bp(Figure 6). (Dissolving of AsfuFor-9 by add 300µl dist.H_2O, and 255 µl to AsfuRev-9).

2-*Fusarium* spp. by FusoFor and FusoRev, product size 565 bp (Figure 7).

(Dissolving FusoFor by add 230µl injection water, and 220 µl to FusoRev)

3- *Candida* spp. by CAFOR2 -9 and CAREV3 -9,product size 402 bp (Figure 8). (Add 210 µl dist. H_2O to CaFor2-9 and 260 to CaRev3-9).

- PCR product was run on 1.5% agarose gel for medium product size (>400 bp) and 2% agarose for small products size (<250 bp), samples run with 100pb ladder
- Electrophoresis voltage range from 100:200 v, depend on the size of the gel

Small gels (50 ml) run on 100:120 V, large gels (100ml) run on ≈150 v. Microkit from Qiagen, 100bp

ladder from fermentas, Taq (5 u/µl) with its buffers and also dNTP mix from promega and primers from Bio NEER.

Table (1): Sequences of primer sets used

Microorganism	Primer Sequence	Product Size (bp)	References
Universal primer for bacteria	F 27 – 6: GGA GGA AGG TGG GGA TGA CG R 28 – 6: ATG GTG TGA CGG GCG GTG TG	241 bp	Samadi *et al*. (7)
Universal primer for fungi	F ITS 1 -9: TCC GTA GGT GAA CCT GCG G G R ITS 4 -9: TCC TCC GCT TAT TGA TAT GC	601 bp	Lindsley *et al.* (8)
S. aureus	F 9-6: CAA TGC CAC AAA CTC G R 10-6: GCT TCA GCG TAG TCT A	477 bp	Sakai *et al*. (9)
S. epidermidis	GYRB FOR. -9: CAG CAT TAG ACG TTT CAA G GYRB REv. -9: CCA ATA CCC GTA CCA AAT GC	251 bp	Yamada *et al*. (10)
Pseudomonas spp.	F 21-6: GAC GGG TGA GTA ATG C CTA R 22-6: CAC TGG TGT TCC TTC CTATA	618 bp	Theodore *et al.* (11)
Aspergillus spp.	ASFUFOR -9: CCA ATG CCC TTC GGG GCT CCT ASFUREV -9: CCT GGT TCC CCC CAC AG	520 bp	Emma *et al.* (12)
Fusarium spp.	FUSOFOR -9: CCA ATG CCC TCC GGG GCT AAC FUSOREV -9: GCA TAG GCC TGC CTG GCG	565 bp	Emma *et al.* (12)
Candida spp.	CAFOR2 -9: GGG AGG TAG TGA CAA TAA ATA AC CAREV3 -9: CGT CCC TAT TAA TCA TTA CGA T	402 bp	Emma *et al.* (12)

F= forward R= reverse

Table (1) shows the sequence of the primers used in this study, product size and references.

Universal bacteria

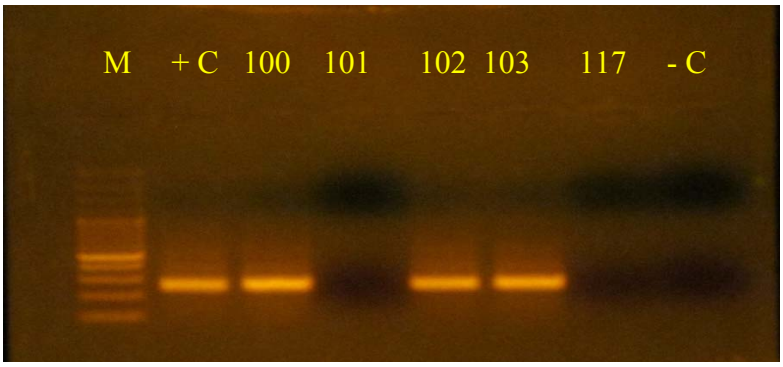

Figure (1): Agarose gel visualized in an ultraviolet transilluminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 2: positive control. Lane 8: negative control. Lanes 4, 7: negative samples. Positive PCR results are seen in lanes 3, 5, and 6. Product size 241 bp.

+C = positive control, -C = negative control, M= marker.

Staphylococcus aureus

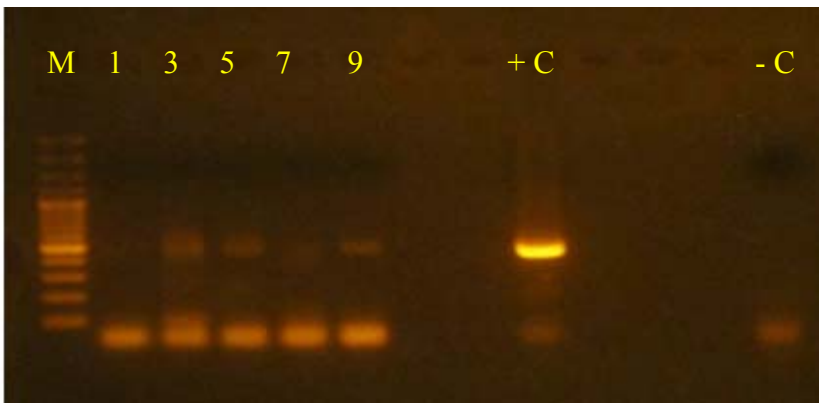

Figure (2): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 7: positive control. Lane 8: negative control. Lane 2: negative sample. Positive PCR results as seen in lanes 3, 4, 5, and 6. Product size 477 bp.

Staphylococcus epidermidis

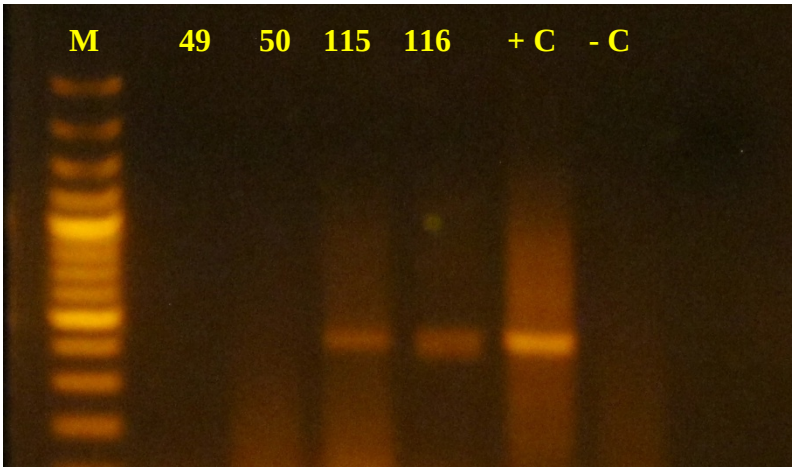

Figure (3): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 6: positive control. Lane 7: negative control. Lanes 4 and 5: positive samples. Lane 2 and 3: negative samples. Product size 251 bp.

Pseudomonas

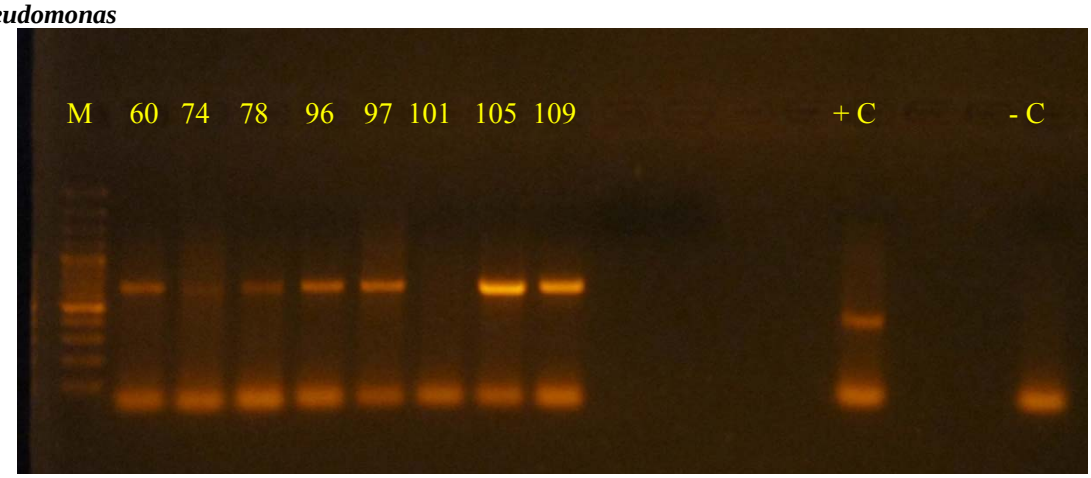

Figure (4): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 10: positive control. Lane 11: negative control. Lane 7: negative sample. Positive PCR results are seen in lanes 2, 3, 4, 5, 6, 8 and 9. Product size 618 bp.

Universal fungi

.

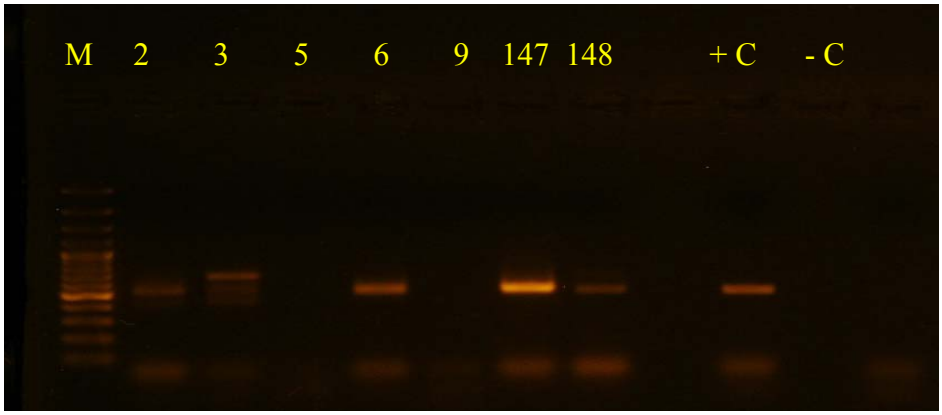

Figure (5): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 9: positive control. Lane 10: negative control. Lanes 4 and 6: negative sample. Positive PCR results are seen in lanes 2, 3, 5, 7 and 8. Product size 601 bp.

Aspergillus

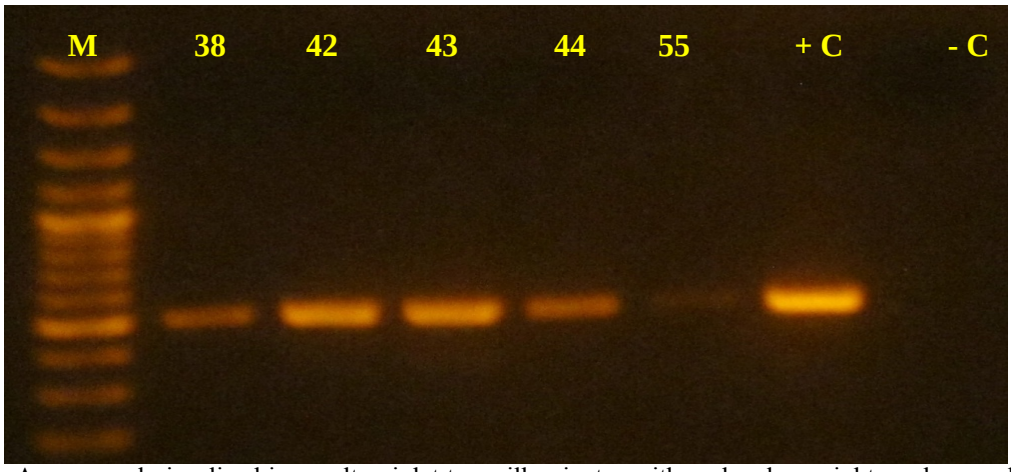

Figure (6): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 7: positive control. Lanes 8: negative control. Lanes 2, 3, 4 and 5: positive samples. lane 6: negative sample. Product size 520 bp.

Fusarium

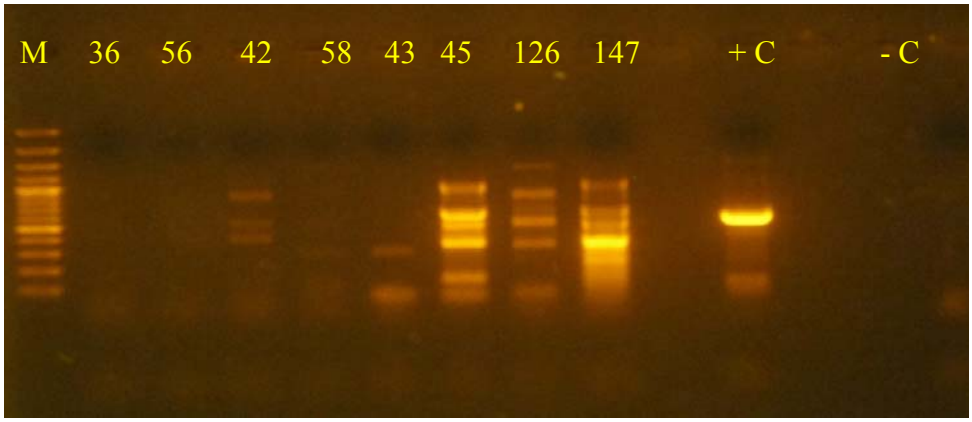

Figure (7): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1 molecular weight marker. Lane 10: positive control. Lane 11: negative control. Lanes 2 and 3: negative samples. Positive PCR results are seen in lanes 4, 5, 6, 7, 8 and 9. Product size 565 bp.

Candida

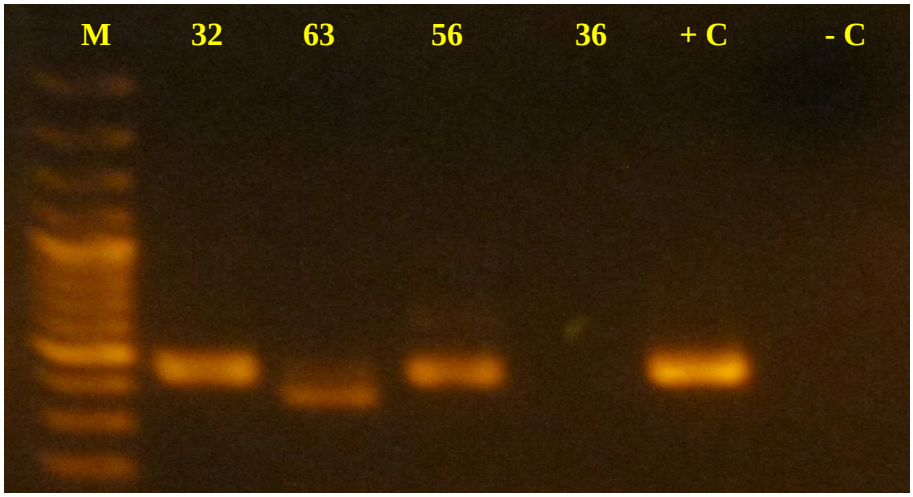

Figure (8): Agarose gel visualized in an ultraviolet trans illuminator with molecular weight markers and amplified DNA fragments. Lane 1: molecular weight marker. Lane 6: positive control. Lane 7: negative control. Lanes 2, 3 and 4: positive samples. lane 5: negative sample. Product size 402 bp.

3. Results

Culture results: Of the 150 patient samples, 104 (69.3%) were culture-positive (76 bacterial, 19 fungal and 9 mixed) and 46 (30.7%) were culture-negative as shown in figure (9). Gram positive cocci (71%) were predominantly isolated from the total number of bacterial cultures and *Aspergillus* spp. (48.5%) for fungal cultures was the most common fungal isolate.

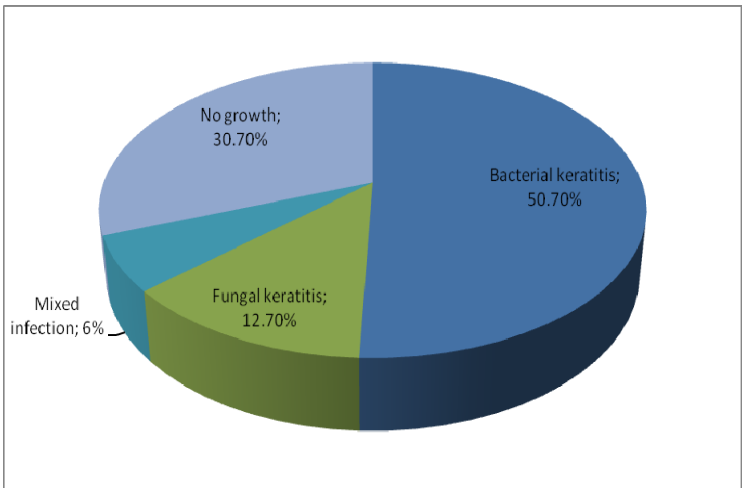

Figure (9): Culture results from 150 cases of microbial keratitis.

Polymerase Chain Reaction results:

Of the 150 samples derived from corneal ulcers, 130 (86.7%) were PCR-positive (74 bacterial, 18 fungal and 38 mixed infection) and 20 (13%) PCR-negative. Of the 76 samples culture-positive for bacteria, 73 (96%) were PCR-positive for bacteria. Of the 19 samples culture-positive for fungi, 17 (89.5%) were PCR-positive for fungi and 8 (89%) out of 9 samples culture-positive for mixed infection were PCR-positive. On the other hand, of the 46 culture-negative samples, 32 (69.5%) were PCR-positive, (one bacteria, one fungal and 30 mixed) and 14 PCR-negative. The sensitivity and specificity of PCR for the detection of microbial keratitis against the gold standard culture technique are showed in table 2 and figure 10.

Of the 74 samples positive by bacterial PCR, 73 (98.6%) were culture positive and 1 culture-negative. Seventeen (94%) out of the 18 fungal PCR positive samples were culture-positive for fungi and 1 culture-negative. Out of the 38 PCR-positive mixed infection results, 8 (21%) were also mixed culture -positive and 30 were culture-negative. Of the 20 PCR-negative samples 14 (68%) were also culture-negative, 3 culture-positive for bacteria, 2 culture-positive for fungi and 1 mixed infection culture-positive.

PCR yielded the same organism as culture in 53 samples for bacterial isolates and in 23 samples for fungal cultures (mixed infection was included). Of the 26 ulcers culture-positive for *S.aureus*, 22 (84.6%) matched PCR results, for *S. epidermidis* 23 (76.7%) out of 30 culture-positive samples were PCR-positive and for *Ps. aeruginosa* of the 10 culture-positive samples 8 (80%) were PCR-positive. Of the 4 ulcers culture-positive for *Fusarium* spp., 3 (75%) matched PCR results while for *Candida* spp., 6 (85.7%) out of the 7 culture-positive were PCR-positive and for *Aspergillus* spp. 14 (93.3%) out of the 15 culture-positive were PCR-positive. The sensitivity and specificity of PCR for the detection of bacterial and fungal pathogens are presented in table 3 and figure 11.

Table 2: Correlation between polymerase Chain Reaction and culture - based diagnosis of bacteria and fungi from corneal scrapes obtained from 150 cases with infective keratitis.

Culture results	No of cases	PCR Positive	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Positive predictive value (%) (95% CI)	Negative predictive value (%) (95% CI)
Bacterial keratitis	76	73	94%	90%	85%	40%
			77% to 97%	62% to 96%	57% to 90%	25% to 44%
Fungal Keratitis	19	17	86%	82%	75%	43%
			55% to 91%	65% to 86%	58% to 79%	27% to 49%
Mixed infection keratitis	9	8	88%	95%	38%	87%
			51% to 90%	58% to 97%	23% to 51%	65% to 90%
No growth keratitis	46	32	79%	83%	44%	80%
			65% to 84%	66% to 92%	29% to 53%	54% to 83%

Table 2 shows that the sensitivity of PCR in detecting bacterial, fungal, mixed keratitis and no growth keratitis was 94%, 86%, 88%, 79% respectively while the specificity of the PCR was 90%, 82%, 95% and 83% respectively. The positive predictive value was the highest for bacterial keratitis (85%) and the lowest for mixed infection keratitis (38%), however the negative predictive value was the highest for mixed infection keratitis (87%) and the lowest for bacterial keratitis (40%).

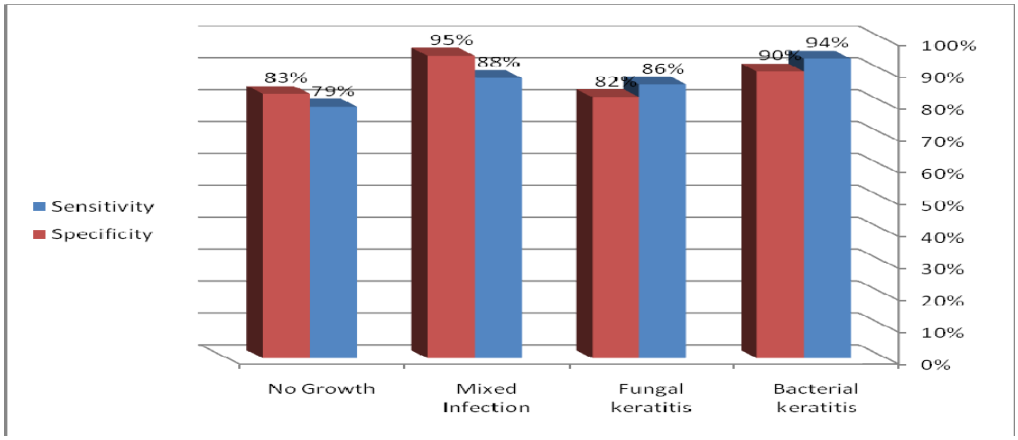

Figure (10): Comparison of sensitivity and specificity of the PCR assay for the detectionof microbial keratitis against the gold standard culture technique.

Table (3): Comparison of culture and polymerase chain reaction results for detection of pathogens in bacterial corneal ulcer

Bacterial isolates	Culture results	PCR results	Sensitivity (%) (95% CI)	Specificity (%) (95% CI)	Positive predictive value (%) (95% CI)	Negative predictive value (%) (95% CI)
Staphylococcus aureus	26	22	85% 68% to 90%	80% 58% to 85%	82% 62% to87%	36% 26% to 44%
Staphylococcus epidermidis	30	23	79% 58% to 84%	77% 57% to 85%	78% 67% to 81%	43% 40% to 54
Pseudomonas spp.	10	8	92% 77% to 95%	89% 73% to 92%	85% 69% to 93%	46% 33% to 55%

Table 3 shows that the sensitivity of PCR in detecting *S. aureus*, *S. epidermidis* and *Pseudomonas* was 85%, 79%, 92% respectively while the specificity was 80%, 77% and 89% respectively. The positive predictive value and the negative predictive value were nearly equivalent for the 3 organismas.

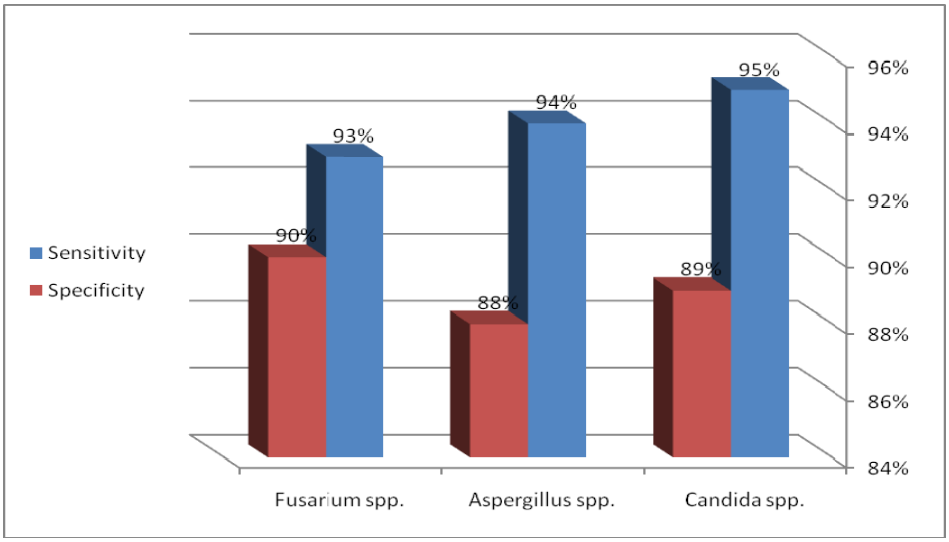

Figure (11): Comparison of sensitivity and specificity of the PCR assay for the detection of *Candida*, *Aspergillus* and *Fusarium*.

4. Discussion

Microbial culture remains the gold standard for identification of pathogens causing corneal ulcers [13]. The importance of correct identification of pathogen is increased in parts of the world where fungal keratitis is common, as choice of correct antimicrobial requires distinguishing fungal from bacterial etiology. The appearance of the ulceration is unreliable in distinguishing

fungal from bacterial ulcer [14]. Microbial culture is a relatively sensitive diagnostic test, with growth seen in about 70% of cases [15]. The PCR is a powerful technique for amplifying infinite quantities of nucleic acids for further analysis. PCR is an extremely sensitive technique able to detect single copies of pathogen DNA in complex mixtures. PCR has been successfully applied to the diagnosis of many ocular conditions [16]. The availability of DNA primer sets that effectively recognize all bacteria or all fungi suggests this technique may have utility for diagnosis of microbial keratitis. **Knox** and associates [17] studied 10 patients with culture positive microbial keratitis and 17 with culture-negative keratitis. Eight of 10 patients who were culture-positive in that study were PCR-positive. None of 17 other keratitis patients were positive for bacterial products. **Rudolph** and associates [18] studied four patients with severe infectious keratitis and either negative cultures or culture results incompatible with the clinical course, direct sequencing of PCR products revealed unusual species in several cases. **Kumar** and associates [19] have studied the use of PCR in detecting fungal pathogens in keratitis. In that study, samples from four patients with mycotic keratitis were studied, along with other samples obtained directly from fungal cultures. The authors found the PCR combined with single-stranded conformational analysis allowed rapid and precise identification of unusual mycotic pathogens [20].

In our study, microbial culture yielded an organism in 70% of cases (104 cases out of a total 150 cases). This is similar to the overall 63% bacterial and fungal culture-positive rate described in a study involved 3,298 eyes with microbial keratitis in India [21]. However contrary to their study, the majority of culture ulcers in egyptian population were bacterial (76 cases), where in India the majority of population yielded fungal culture. Of the 76 samples culture-positive for bacteria, 73 (96%) were PCR-positive and out of the 19 samples culture-positive for fungi 17 (89.5%) were PCR-positive for fungi. Thus PCR appeared to have a higher yield in bacterial ulcer than in fungal ulcer. Our results were contrary to a study by **Elma *et al.*** [22], including 108 samples, 56 were culture-positive, 25 for bacteria and 31 for fungi. Nineteen of 25 bacterial culture-positive samples were positive by PCR (76%), and 29 of 31 samples culture-positive for fungi were positive by PCR (94%). Our results showed that matching of DNA genotyping results with cultured organisms was better for fungal species than bacteria (23/26 and 53/66 respectively). This lower concordance rate with bacterial ulcer may be due to detection of normal ocular surface biota by PCR. Our study revealed 12.7 % (19 cases) culture positivity, *Aspergillus* being the most common fungus isolated by culture (48.4%). This is similar to the study by **Vengayil *et al.*** [23] in which *Aspergillus* was also the most common isolate. In our study, of the 4 ulcers culture-positive for *Fusarium* spp., 3 (75%) matched PCR results; while for *Candida* spp., 6 (85.7%) out of the 7 culture-positive were PCR-positive, and for *Aspergillus* spp. 14 (93.3%) out of the 15 culture-positive were PCR positive. The sensitivity of PCR in detecting *Candida*, *Aspergillus* and *Fusarium* was 95%, 94%, 93% respectively while the specificity was 89%, 88% and 90% respectively. The sensitivity of PCR in detecting *S. aureus*, *S. epidermidis* and *Pseudomonas* was 85%, 79%, 92% respectively while the specificity was 80%, 77% and 89% respectively. Interpretation of discrepant culture and PCR results is somehow unclear, yet in an active corneal ulcer situation it may be sensible to consider culture positive or PCR positive of highly virulent organism such as *S. aureus* or *Pseudomonas* as evidence of infection with those microbes. In a study by **Vengayil *et al.*** [23], the sensitivity of PCR for detection of mycotic keratitis was found to be 70%, the specificity 56.7%, the predictive value of the positive test was 35% and that of the negative test was 85%. However in our study, the sensitivity of PCR in detecting fungal keratitis was 86%, while the specificity of the PCR was 82%. Our positive predictive value was the highest for bacterial keratitis (85%), however the negative predictive value was the highest for mixed infection keratitis (87%). In addition, the positive predictive value for fungal keratitis was 75% but the negative predictive value was 43% opposite to what was obtained by **Vengayil *et al.*** [23]**.** In contrast, **Alexandrakis *et al*.** [24] reported a sensitivity of 89% and specificity of 88% for their PCR technique used for bacterial keratitis detection and this is in agreement with our results Also our results for fungal keratitis are near to what is obtained by **Zunaina *et al.*** [25]**,** who reported 91% sensitivity and 95% specificity in the detection of fungal aetiology in microbial keratitis by PCR. PCR reliably distinguishes bacterial from fungal pathogen [26]. Of the 46 culture-negative samples, 32 (69.5%) were PCR-positive suggestive of potential pathogens, (one bacteria, one fungal and 30 mixed) and 14 PCR-negative.

PCR results in this study seemed quite promising. However, the disparity between culture and PCR results may be explained by the fact that the culture positivity requires viable organisms, whereas a PCR-based test can detect both viable and nonviable organisms. PCR test can theoretically be positive even if only a single copy of target DNA is present. The high positivity of PCR in already treated cases in comparison to culture, reiterates the difficulty in getting a positive culture from non viable organisms in the sample.

In another study, **Ferrer *et al.*** [4] highlighted the benefit of time factor in diagnosing fungal corneal ulcer. Although their PCR assay produced results in 8 hours, culture confirmation took almost 10 days. Our study was thus very much comparable to theirs because the PCR method used by us yielded results in 4 to 8 hours, depending on the number of cycles repeated. This is a major advantage of the technique, especially when compared to culture where it took at least 5 to 7 days for a positive growth in our setup. Although various advantages have been attributed to PCR due to its rapidity and widespread applicability to bacteria and fungi, the technique has various reported complexities and drawbacks, as evidenced from our study also. Some of the limitations are logistic and some technical. Among them is the difficulty in optimization, especially in case of fungi, apart from the difficulty in differentiating between active and latent infections, viable, and nonviable cells. Moreover, the DNA sequence has to be known in advance, and the high sensitivity could lead to false-positive results.

In a well-developed modern laboratory, the gold standard of a bacterial culture should ideally be replaceable today with a reliable and reproducible PCR technique as the new gold standard. This is not to say that PCR negates the undeniable role of a bacterial culture—after all, the conventional as well as the rapid sensitivity testing, so essential for diagnosis and initiation of correct therapy (even after some time lag), are entirely dependent on culture, not on PCR [27]. PCR remains a technically complex procedure involving skilled hands, expertise, and a fair degree of experience, in addition to the learning curve and standardization in relation to individual laboratories. The cost–benefit ratio of PCR should prove efficacious in the developed world. A cost effectivity

factor would be arrived at differently by different personnel even in the same institution, but even that one patient saved would surely calculate it differently. What the future holds in store as the next viable gold standard remains to be seen. Apart from these, the unavoidable cost of the investigation at least as of today limits its widespread use [28].

Corresponding author
Rania A. Khattab
Microbiology and Immunology Department, Faculty of Pharmacy, Cairo University, Cairo, Egypt
Khattab500@yahoo.com lady_ok@yahoo.com

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Meretrix Meretrix: Active Components and Their Bioactivities

Wenyan Xie[1], Chen Chen[2], Xiaoshuang Liu[1], Bo Wang[1], Ying Sun[1], Maocang Yan[3], Xiaoying Zhang[1*]

[1.] College of Veterinary Medicine, Northwest A & F University, Yangling 712100, China;
[2.] Shaanxi Key Laboratory of Resource Biology, Shaanxi University of Technology, Hanzhong 723000, China;
[3.] Zhejiang Mariculture Research Institute, Wenzhou 325005, China.
E-mail: zhang.xy@nwsuaf.edu.cn

Abstract: The clam *Meretrix meretrix* Linnaeus (*M. meretrix*, Veneridae), is a popular edible shellfish with abundant nutrition and valuable medical properties widely distributed in eastern Asia. As a kind of popular sea food diet, many bioactive components such as peptides, proteins, enzymes, polysaccharide, minerals, essential vitamins, essential amino acids and enzyme inhibitors, have been purified from *M. meretrix*, which are considered to be responsible for its nutritional and medicinal functions including anticancer, antioxidant, antihyperglycemia, antihperlipemia, reduce swelling and detoxification effects. This article reviewed the nutritional constituents, bioactive compounds and pharmacological effects of *M. meretrix* to provide further support and evidence for its medicinal and nutritional use.
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Keywords: *Meretrix meretrix (M meretrix)*; clam; nutrition; bioactive components

1. Introduction

Meretrix meretrix Linnaeus (*M. meretrix*, *Meretrix*, Veneridae), commonly known as Asiatic hard clam, is a historically marine food and a valuable source of traditional Chinese medicine (TCM). *M. meretrix* prefers the estuarine and coastal ecosystems and is widely distributed in coastal areas of South and Southeast Asia, including China, Korea, Japan and India (Jayabal, 1986; Ho, 1994). There are a total of 38 species and subspecies in the Genus *Meretrix* including *M. meretrix*, *M. casta*, *M. lamarckiI*, *M. lamarckii*, etc. *M. lurosia* has been considered as a synonym of *M. meretrix* according to the systematization (Pan BP *et al*., 2006).

M. meretrix was documented in the ancient Chinese pharmacopeia *Compendium of material* (the 16th century, by LI Shizhen) which stated *M. meretrix* could diminish inflammation, treat typhoid fever, hangover and relieve pains. Another ancient Chinese medicinal book *Treatise on Fevers* (the 2th century, by ZHANG Zhongjing) stated its special activities of eliminating cyst and detoxification. Many bioactive components such as peptides, proteins, enzyme and enzyme inhibitors have been purified and identified from *M. meretrix* in the recent years, and their functional effects including antihypertensive, hypolipidemic, antineoplastic and antioxidant effects have been proved (Xu *et al*., 1999; Zhao *et al*., 1997; Wei *et al*., 2007 and Huang *et al*., 2005). The clam shells are rich in calcium and have been applied to poultry industry. The calcium oxide derived from *M. meretrix* shells has been demonstrated to be an active biodiesel production catalyst (Viriya-empikul *et al*., 2010). Besides, *M. meretrix* shell can be used for decorative and ornamental purposes.

2. Pharmacological activities

2.1 Antitumor activity

Many antitumor ingredients including polypeptide, polysaccharide and nucleic acid (Table 1) have been purified and functionally confirmed from *M. meretrix*. They exhibit broad antitumor activities in various cancer cell lines of broad organs, and are also in limited *in vivo* trails.

2.2 Antioxidant activity

Proteins and peptides of antioxidant potentials have been confirmed from *M. meretrix*.

Three proteins (P1, 18kD; P2, 28kD and P3, 16kD) isolated from the meat of *M. meretrix* were evaluated for catalase (CAT) activity, superoxide dismutase (SOD) activity and inhibitory effect on lipid peroxidation (LPO) (Xiao *et al*., 2007). P2 exhibited the highest CAT activity (77.0 U/mg), while P3 showed the strongest SOD activity (68.8 U/mg) among the groups. P3 also demonstrated inhibitory effect against lipid peroxidation induced by Fe^{2+}.

After acid protease and trypsase treatments, the *M. meretrix* hydrolyzates exhibited hydroxy radical scavenging activities of 67.75% (Yan *et al.*, 2007) and 94.07% (Qiu *et al*., 2010), respectively. Furthermore, the acid-protease-induced hydrolyzates presented strong superoxide radical scavenging activity (62.79%) (Yan *et al*., 2007).

The antioxidant response of *M. meretrix* has been evaluated by exposing to tributyltin (TBT) of environmentally relevant concentration which could induce oxidative stress (Huang *et al*., 2005). After 2 days' exposure under TBT at the dose of 0.1ng/L, *M. meretrix* expressed approximately 0.2 μmol/min/mg additional glutathione S-transferase (GST) than control group, while the amount of glutathione

peroxidase (GPx) was elevated significantly after exposure for 20 days at the TBT dose of 10 ng/L, suggesting that *M. meretrix* antioxidant response could be enhanced after external challenges.

2.3 Immuno-modulatory activity

After hydrolytes of *M. meretrix* flesh of oral administration to mice at the dose of 20 g/kg for 7 days, the thymus weights of mice increased and the hemolysin antibody activity of mice were enhanced. In a sheep red blood cell (SRBC) induced delayed type hypersensitivity (DTH) model, the hydrolysates could depress DTH, while inhibited the clearance of carbon particles, suggesting that the immunologic function of hydrolysates of *M. meretrix* was alterable in different stages (Yu *et al*., 1991). It was also documented that *M. meretrix* polypeptide could improve mice immunity by promoting the growth of thymus and spleen (Zheng *et al*., 2008).

After polysaccharide of the oral administration isolated from *M. meretrix* to immune system damaged mice induced by cyclophosphamide, a series of immunological indicators, including the phagocytic power, the number of leukocyte, the level of hemolysin antibody, were ameliorated, and DTH reaction was enhanced in mice (Dou *et al*., 1999). *M. meretrix* polypeptide and crude extracts both played as immunosuppressor and immnopotentiator against excessive and inhibitory DTH, respectively (He *et al.*, 1995).

Ethanol extract of *M. meretrix* could enhance the expressions of T- and B-lymphocytes by 18% and 43%, respectively (Zhang *et al.*, 2005).

2.4 Antihyperglycemia and antihperlipemia activities

M. *meretrix* hydrolysate was administrated to diabetic mice and hyperlipidaemia rats for 4 and 8 days at the dose of 10 g/kg for 8 days, respectively. Compared to control groups, the contents of blood sugar decreased by 74.6 mg/dL and by 157.5 mg/dL, respectively. The concentration of triglyceride (TG) and the total cholesterol (TC) in serum of hyperlipaemia rats were reduced by 11.9 mg/dL and 56.1 mg/dL, respectively (Xu *et al*., 1999). Zhang *et al.* (1997) reported that the hydrolysate of *M. meretrix* soft tissue could also reduce the whole blood viscosity in both normal and experimental quail groups and could inhibit the platelet aggregation induced by adenosine diphosphate (ADP) in rabbits.

The polysaccharides extracted from *M. meretrix* also demonstrated antihyperglycemia activity in mesoxyalyurea- induced diabetic rats by remarkably decreasing the level of blood sugar and enhancing stress response on diabetics (Yuan *et al.*, 2007).

3. Chemical and nutritional constituents

M. meretrix is commonly consumed as sea food diet and contains multiple classes of physiological functional ingredients (Table 2) including proteins, polysaccharide, minerals, essential vitamins and essential amino acids (Table 3) (Gopalakrishnan *et al.*, 2009; Yang *et al*., 2007). The concentrations of calcium, magnesium, iron and copper are 175 μg/g, 29 μg/g, 1.75 μg/g and 0.69 μg/g, respectively. *M. meretrix* has high eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) contents of 6.9% and 7.2%, respectively (Yang *et al*., 2007). Taurine, as a kind of organic acid which is essential for cardiovascular function, development and function of skeletal muscle, the retina and the central nervous system, is widely distributed in *M. meretrix* tissues of the high contents of 2.3% and 0.4% from dry and wet *M. meretrix*, respectively (Gong *et al.*, 2003). Vitamin C and Vitamin E were also isolated from wet *M. meretrix* at the concentrations of 5.83μg/mg, 2.6μg/mg, respectively (Gopalakrishnan *et al.*, 2008).

4. Functional proteins, enzymes and enzyme inhibitors

4.1 Ferritin and metallothionein

Ferritin, which occurs in multiple forms in marine animals such as bivalve mollusk, plays an important role for iron storage and metabolism. A full-length ferritin subunit cDNA named MmeFer was cloned and characterized (Wang *et al.*, 2009). The increasing expression of MmeFer mRNA in different developmental stages of *M. meretrix* suggested that ferritin may be involved in shell formation.

Metallothioneins (MT) are cysteine-rich, low- molecular weight and inducible metal-binding proteins with key functions in metal homeostasis and deoxidation, and act as broad protective roles including detoxification of heavy metals, scavenging of free radicals and storing and carrying trace elements. Two MT genes, named *Mm- MT* and *MT*, with a 657bp and a 637bp full- length cDNA both containing an open reading frame of 231bp and encoding a protein of 76 amino acid residues were identified and cloned from *M. meretrix*, respectively (Gao *et al*., 2009; Wang *et al.*, 2010).

4.2 Lectin

Lectins are sugar-binding proteins which possess particular biological properties such as regulating of cell adhesion, glycoprotein synthetization and controlling of protein levels in blood. Kim *et al.* (1990) isolated and purified a carbohydrate-binding protein named MLA-1 from the hemolymph of the shellfish *Meretrix lusoria* (Veneridae), which exhibited hemagglutination and carbohydrate specificity. Zhao *et al*. (1992) isolated a specific sialic acid binding lectin (MML) from *M. meretrix*, which can widely agglutinate various kinds

Table 1. Antitumor components isolated from *M. meretrix*

Ingredient	Name	MW (Da)	Antitumor activities	Referen -ces
Polypeptide	Mercenene	≤ 10000	Strongly inhibit Sarcoma 180 (S180) and Krebs 2-breast carcinoma, Hale cell lines.	Schmeer *et al.* (1964, 1979)
	Mer2	N/A	Inhibit the proliferation of HepG2, Hela, QBC939, SPC-A-1 cell lines at dose of 80 μg /ml with inhibitory rate of 78.3%, 72.9%, 67.6% and 53.2%, respectively.	Fan *et al.* (2009)
	MGP_{0501}	15878	Inhibit the proliferation of K562, A549, HO8910 cell lines with IC_{50} of 32.03 μg/ml, 20.34 μg/ml and 29.13μg/ml respectively.	Wu *et al.* (2006)
	MGP_{0405}	9655	Inhibit the proliferation of B16, KB, A549, Hela, K562, BGC, HO8910 and SMMC-7721 cell lines with IC_{50} of 178μg/ml, 132μg/ml, 178μg/ml, 181μg/ml, 264μg/ml, 202μg/ml, 468μg/ml and 204μg/ml, respectively.	Zhang *et al.* (2009)
	MML	40000	Inhibit the growth of BEL-7402 which was transplanted into nude mice *in vivo* with IC_{50} of 52.2μg/mL.	Zhang *et al.* (2009)
	M2	18000	Inhibit the proliferation of BGC-823 cells and destroy their skeletal structures.	Liu *et al.* (2004)
Polysaccharide	N/A	N/A	Inhibit the growth of S180 *in vivo* (inhibitive rate of 43.64% at the dose of 100mg/kg), prolong the survival period of mice with EAC ascites carcinoma and hepatic carcinoma.	Wu *et al.* (2006)
Nucleic acid	N/A	N/A	Inhibit the proliferation of S180 and HepA with inhibitory rate of 43%-61% and 37%, respectively.	Zhang *et al.* (1990)

Note: N/A: data not available

of RBC agglutination activity. The Ca^{2+} dependent lectin is sensitive to high temperature (over 40 ℃) and to the extreme pH (over 8.5 or below 5.0). The MML was 59kDa and naturally consisted of two kinds of subunits of MML (WM 29 kDa, 30kDa) connected by-S-S- . The lectin was also reported to contain 5 percentage of sugar and have the ability of inhibiting or even killing cancer cells - human malignant lymphoblast (Raji cells).

4.3 Heat shock protein

MmeHsc71, a heat shock protein (HSP) (71.43 kDa), was found in *M. meretrix* and determined to be a member of the hsp70 family (Yue *et al.*, 2011). Based on the comparison of the spatial and temporal expression of MmeHsc71 in mRNA level between normal clams and *vibrio parahaemolyticus*- infected clams, MmeHsc71 mRNA could be found in all tested tissues including foot, hepatopancreas, mantle and gill. Moreover, the expression of MmeHsc71 mRNA in hepatopancreas of *vibrio parahaemolyticus*- infected clams was 2- fold of that of normal clams. This result can be further confirmed in a quantitative immunofluorescence analysis; the protein level of MmeHsc71 in *vibrio parahaemolyticus*- infected clams was higher than that of control group at 24 h post-infection, indicating that MmeHse71 may play a crucial role in mediating the immune responses of *M. meretrix* to bacterial challenges.

4.4 Adenosine diphosphate ribosylating protein

Adenosine diphosphate (ADP) - ribosylation plays a significant role in the posttranslational modification by transferring to its acceptor molecules mediated by the ADP-ribose moiety of β-NAD. Nakano *et al.* (2006) found a DNA ADP-ribosylation protein named CARP-1 in the hard clam *M. lamarckii*. After purification methods of ammonium sulfate fractionation, carboxymethyl-cellulose chromatography and CM52 column, a mass of molecular weight of 20 kDa was highly enriched. In addition, the DNA of ADP-ribosylating protein was purified and its cDNA was cloned which encodes 182 amino acids.

4.5 Lysozyme

Lysozymes have strong antimicrobial activity by damaging bacterial cell wall.

Lysozyme, named Mmelys, was cloned and sequenced from *M. meretrix*, and it consisted of a 15 amino acid signal peptide and an 131 amino acid mature protein. Mmelys presented high mRNA level and protein level in gill and hepatopancreas. Mmelys showed regressive inhibitory effect against *P. aeruginosa* and *M. luteus* at the purified enzyme doses of 375μg /mL and 250 μg /mL, respectively (Yue *et al.*, 2010).

4.6 Cathepsin B

Cathepsin B, belongs to the papain super family, is a key proteolytic in the nutrient metabolism of *M. meretrix*. It has been considered that cathepsin B can degrade β-amyloid precursor protein into harmless fragments, and can also possess endopeptidease, dipetidylcarboxy peptidase activities. The full length of cathepsin B (MmeCB) cDNA was cloned and it was constituted of 1647 bp, with an open reading frame of 1014 bp encoding a preproenzyme of 337 residues with Cys-114, His-282 and Asn-302 composing cathepsin B activity center (Wang *et al.*, 2008). No MmeCB mRNA was found in trochophore stage. In the later stages, detectable signals were found, suggesting that MmeCB may play a role in nutrient digestion. Further analysis showed that MmeCB may be also associated with other pathways of nutrient metabolism in larval epidermis. A recombinant fusion protein GST-MmeCB of high level was obtained from *Escherichia coli* and the recombinant MmeCB can degrade the selective substrate. The kinetic parameters of rMmeCB were calculated as follows: K_m, V_{max}, and k_{cat} were 6.11uM, 0.0174uM min^{-1} and 277.57s^{-1}, respectively. Further analysis showed that cathepsin B was probably involved in the nutrient digestion of *M. meretrix* (Yao *et al.*, 2010).

4.7Angiotensin converting enzyme inhibitor

The presence of Angiotensin converting enzyme (ACE) shows great importance in the regulation of blood pressure, which catalyzes the conversion of angiotensin I into activated angiotensin II. Angiotensin II has the properties of inducing aldosterone release; resulting in Na^+ entering cell, blood pressure raise. Inhibition of ACE may reduce the formation of angiotensin II and then release blood pressure. ACE-inhibitory peptides were derived from the meat of *Meretrix lusoria* hydrolyzed by Protamex (PX) of IC_{50} of 0.036mg/mL. Two peptides (E1, E2) purified by Sephadex G-25 column and RP-HPLC, E2, containing two amino acids residues (Tyr-Asn), showed higher inhibitory effect of IC_{50} of 51 μM (0.015 mg/ml) assayed by using a modified spectrophotometric method (Tsai *et al*., 2008).

4.8 Glutathione peroxidase

Glutathione peroxidase (GSH-Px), a kind of peroxidase, can protect the organism from oxidative damage by reducing lipid hydroperoxides to their corresponding alcohols and reducing free hydrogen peroxide to water. Wang *et al.* (2011) cloned glutathione peroxidase MmeGPx genes, which included two introns (723bp and 238bp) with an open reading frame of 711bp coding a protein of 76 amino acids.

4.9 Strombine dehydrogenase (SDH)

Lee *et al.* (2011) isolated and purified SDH from foot of *M. meretrix* by adopting ammonium sulfate precipitation, passing through sephacryl S-100 column and hydroxyapatite chromatography, and some other chromatographic programs. SDH is heat labile, and is of high enzyme activity under the optimal pH and temperature of 7.4–7.6 and 45℃–46℃, respectively. L-Alanine, glycine, and pyruvate are its preferred substrates.

Furthermore, a series of enzymes had been found in *M. meretrix*, including lactic dehydrogenase (Lee *et al.*, 2011), antioxidase (Wang *et al*., 2010), adenosine deaminase (Aikawa *et al*., 1966), alkaline phosphatase (Aikawa *et al.*, 1966), phosphohydrolase etc. (Umemori *et al*., 1967).

5. Prospective

Estuarine and coastal ecosystems provide productive aquatic resources since the unique environment where seawater meets and mixes with fresh water. As well, it is also good environment for the growth of bacteria and sediments, suggesting the bivalves need to develop their defense systems against potential pathogens and microorganisms which may induce them to synthetize unique chemical components of potential medicinal values.

Edible bivalves such as *M. meretrix* are in growing demand due to their abundant nutrition and valuable medical properties. *M. meretrix* was also elucidated as an excellent source for proteins, polysaccharide, minerals, essential vitamins and essential amino acids (Gopalakrishnan *et al.*, 2009; Yang *et al.*, 2007).

Many bioactive substances have been found in *M. meretrix*. However, related studies are still inadequate. Efforts should be made to further explore their bioactivities and medicinal functions as well as their mechanisms. TCM could provide a useful reference for this kind of investigations.

Table 2. Mainly functional ingredients of *M. meretrix* (wet weight)

Constitution	Content (%)	References
Crude protein	10.5-15.54	Yang *et al.* (2007); Li *et al.* (2010)
Crude fat	1.07-6.78	Zhang *et al.* (2006); Li *et al.* (2010)
Carbohy-drate	4.14-8.3	Li *et al.*(2010); Kang *et al.* (2008)
Moisture	76.39-80.2	Yang *et al.* (2007); Li *et al.* (2010)
Ash	12.8-22.4	Kang *et al.* (2008); Zhang *et al.* (2006)

Table 3. Essential amino acids in *M. meretrix* (wet weight)

Amino acid	Content (mg/g)
Isoleucine	36.4
Leucine	52.1
Threonine	26.9
Valine	26.5
Tyrosine	32.1
Tryptophan	7.6
Lysine	42.2
Methionine	26.7

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Corresponding Author:

Prof. Dr. Xiao-Ying Zhang
College of Veterinary Medicine
Northwest A & F University (North Campus)
Yangling, Shaanxi Province, 712100, China
Tel. & Fax: +86 29 87091239
E-mail: zhang.xy@nwsuaf.edu.cn

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Presenting the model of inter-region freight transportation in Iran Road transportation network

Gholam Ali Shafabakhsh[1*], Mohsen Sadeghi[2], Ehsan Kashi[3*]

1 Semnan University, Faculty of Civil Engineering Assistant Professor, Semnan, I. R. of Iran
2 Semnan University, PHD student, Faculty of Civil Engineering, Semnan, I. R. of Iran
[3] Semnan University, PHD student, Faculty of Civil Engineering, Semnan, I. R. of Iran
shafabakhsh@semnan.ac.ir

Abstract : Interregional trips include passenger and freight trips. To predict passenger trips, four- stage method is used including trip production, trip distribution, modal split and assignment. Four-stage model is not more efficient for freight transportation. In this paper, mathematical modeling is presented based on entropy and reduction of intervals and freight transportation time. For this model input-output relationships between the regions and road transportation network flows were considered. To solve this model, the data from the Iran's transportation master plan has been used. Finally, the results of solving model were compared with the observations and the model was evaluated. The results showed that the presented model had good accuracy in estimating the percent of different kinds of transported freight and the accuracy of the model in estimation of freight transportation matrix between the regions and with the separation of different freight were suitable.
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Keywords: Freight transportation, distribution model, Entropy, math modeling

1. Introduction

Transportation is the basis of a linking bridge that various parts of societies by passing from it move toward permanent development. Today, transportation is infrastructure section of economy affecting economical development process and is the basis of trade exchanges and economical and social development key. It is obvious that there is a direct relationship between the efficiency in transportation and public efficiency of economy and economical growth and transportation is a mediator between agricultural, industrial, trade and service activities at national and international level. Another group considered transportation the heart of development and believed that transportation has effective role in distribution of incomes and reduction of social and economical inequalities and reduction of poverty and difference of income between the rural and urban people. The studies carried out at macro economical level of some of the countries showed that investment in transportation increased economical growth of the countries and by increasing the social output in private investment provided investment in transportation infrastructures. Transportation industry in Iran during various periods had some problems and its main aim was the attempt to achieve a better position and considering the special position of the country in the region, creating an efficient transportation system had special position in socio-economic development. Now, about 500 million tons freight are transported between the provinces in Iran annually and this subject shows the economical exchanges between the provinces of Iran.

Among all the freight exchanges done in Iran between the regions, about 95% are done by road transportation and 5% are done by rail transportation. Although this is due to the lack of incomplete railway network in Iran, irregular planning and the lack of considering combinational transportation. Finally, in Iran considering the existing condition, more inclination is toward road transportation of freight and the holders of freight due to some reasons as trip time, costs and comfort prefer to use this method to transport their freight.

Thus, modeling the distribution of different kinds of freight between some regions in Iran can be an interesting subject being investigated in this study.

2. Literature review

Modeling freight transportation as a subset of transportation comprehensive plans in terms of progress, importance and planning to the modeling in urban trips is very slow. However, Izard (1960) was the starter of freight transportation modeling and had a good progress[8] but Leontief and Strout (1963) and Wilson (1970) didn't have good progress in the execution and estimation of the issues with great scales [11,14].

Maybe, the main reason is the shortage of the models being used, the lack of access of good data about freight in transportation network and freight loading between the regions. Since 2002, there were some statistics in the form of bill of lading about

freight transportation in Iran but the data is not published with some details and they are not used compared to the existing data about urban trips for estimation of the model. The road network data were not completely available to be used for modeling freight transportation network.

In the plans presented during 1980s regarding the construction of freight transportation models Boyce and Hewings (1981), Batten and Boyce (1986), Kim et al (1983) it is attempted to consider the data of freight[2,1,9]. The structure of the presented plan Kim et al (2002) was regarding the effect of earthquake on inter regional freight transportation[10] while, Fernandez et al (2003) presented a comprehensive model in which the balance of supply and demand was shown in intercity freight transportation[4]. Lin and Kockelman (2002) followed the discussion of freight transportation and land use [12]. DeJong et al (2004) reviewed different kinds of freight transportation [3]. Ham et al (2005) presented a comprehensive model for freight transportation as multidimensional and by Lagrange algorithm solved it [6]. Vigan, Southworth (2005) dealt with the problems and errors of different kinds of freight transportation [15].

Considering the previous studies, such models are evident. The regions with low budget assignment had structural progress (provinces, metropolis, countries, etc) should predict which of the parts of inter regional road network are faced with more freight transportation issues. Better predications enter the market on the behalf of freight transportation companies with considerable money. Answering the questions of the priority of the development of infrastructures and other kinds of development require a better attitude to the increase of network dimensions.

3. Methodology

A country can be divided into some economical divisions each consisting of one or more small regions and agricultural, industrial and mineral activities. There is a region for each region of economical models covering economical relations and entrance-exit relations of freight from the region. In this study, the aim was to predict the transportation of inter regional freight based on the type of freight. To achieve the mentioned aim, a model should be made and used requiring some criteria, to make exchange model of the freight inter regional, the following criteria are considered:

- Forming a coherent model of transportation network and transporting various freight inter regional as the effect of network disturbance is observed.
- Estimation of model parameters of the existing data in special time

In this study, the conditions and data of the model were explained. Iran zoning for freight transportation, classification of different kinds of freight and definition of road arterial network are initial actions that should be done. In the next stage, the related model is presented and its characteristics are analyzed. Then, the estimation of the variables and results was explained and finally the results of the model were displayed briefly and the general predications of the model were compared with the data being used in estimation process to show the conformity of this model.

3.1. Zoning

Normally, the following criteria are used for the definition of the regions.

The regions system should be in conformity with the country divisions namely regarding the statistics of population and production. Thus, the regions are defined as: A set of smaller units that can be collected by various ways as the results of these studies are compared with each other.

- The regions should be homogenous as possible. This case is easily about urban regions. Because the regions are bigger and each one were including different kinds of installations or residents. Thus, homogeneity should be balanced in terms of the aims of the study and based on two variables of the region size and country divisions.

Fig 1: Zoning freight transportation based on gravity centers in Iran

- The regions should show the natural zone of influence and influence zone of its centrality and interior networks of the region. This case should be

observed in appearance of the regions as it shows the interior characteristics of the region.

- The regions shouldn't be the same and their sizes should be in conformity with the trip time units. Thus, more compressed regions should have smaller dimensions.

Each region is determined by a equilibrium or gravity center. In urban studies, gravity centers are virtual determining the average trip cost to another place in each region. Normally, these places are related to a specific location but it is not necessarily the same. These places are linked to the network by a channel showing the average cost of link to a node is in the real network (road). In intercity studies, bigger regions are used and this process is vice versa, it means that the central point shows the production and absorption trip centers and it is determined first. Then, the region boundary is determined based on influence zone of central point considering the influence zone of central points of the neighboring regions. Figure 1 shows the zoning including 56 zones.

3.2.Freight classification

Freight classification can be done by various methods and the classification shouldn't be not very big or small. The freight classification in this paper is based on classification in bill of lading of road maintenance and road transportation. This classification is in accordance with Table 1. This classification is including 27 types of freight.

3.3. Road transportation network

Transportation network in this study is including arterial roads. The data of transportation networks including arterial paths are relate to arterial paths based on the data of road maintenance and road transportation. To analyze the freight transportation with heavy vehicles, a road network with 61 nodes and 227 links are made. The nodes and links were defined based on the intersection of the roads and the interval between these intersections.

The capacity of road links for highways and freeways in which the going and coming path are separate, 10000 trucks/passing line and for the rest of roads including major and minor ways 7000 trucks, passing lines, day are assumed. Fig. 2 shows the arterial network of Iran roads in 2007.

Table 1: The classification of different kinds of transported freight with road transportation

No.	Freight type	No.	Freight type	No.	Freight type
1	Grains	10	Cotton	19	Construction materials
2	Rice	11	Sugar	20	Mineral
3	Cereals	12	Edible oil	21	Fuel
4	Vegetables	13	Flour	22	Chemical
5	Fruit	14	Fast food	23	Textile
6	Live stock	15	Steel	24	Detergent
7	Poultry	16	Metals	25	Car and machineries
8	Fertilizer	17	Coal	26	Paper and wood
9	Tea	18	Cement	27	Durable freight

Fig 2: Arterial road network in Iran in 2007

3.4. The recommended model for freight distribution and solution

In this stage, we deal with the needs of the model, its construction and its limitations. The recommended math model is based on non-linear objective function and five limitations and in the following their design is mentioned.

3.4.1. Model requirements and preparing the data

Freight transportation is based on economical relations and the volume of entrance and exit freight of the regions; m shows the type of freight moving between r regions. This model of freight transportation based on enter-exit of freight of regions can predict the costs of freight exchange between the regions and freight transportation in the network by various transportation methods. The

predicted freight are attributed for exchange between the regions in accordance with a simple criterion of the minimum distance of the methods, paths and links. This model is formulated as an optimization issue with the limitation and is solved by Matlab software.

3.4.2. Objective function

The math relations of freight transportation model between the regions were obtained of the combination of Leontief, Strout, Wilson and Ham. The exit and entrance of the freight for each region is considered by entrance-exist regional models in analysis duration as one year. The existing issue is the predication of the exchanges between each pair of region in accordance with the section-freight and transportation networks.

The first assumption is that all the authorities in transporting in case of not having the information about transportation costs are inclined to make the distances and transportation time to the minimum and the second assumption is that there are some factors that cause the dispersion of freight transportation on origins and destinations. Thus, transportation methods can be depicted by Entropy functions. Thus, objective function is defined as:

$$\min z = \sum_a \int_0^{f_a} d_a(\varphi) d\varphi - \sum_m \frac{1}{\lambda^m} \sum_{ij} x_{ij}^m \ln\left(\frac{x_i^m}{x_{ij}^m}\right) - \sum_{im} \frac{x_i^m}{\theta_i^m} \ln\left(\frac{x_i}{x_i^m}\right) \quad (1)$$

In equation 1, the parameters are defined as:

f_a^w : Flow in Ton on link a

$d_a(f_a)$: The distance of freight transportation (km) on link a

x_{ij}^m : Freight exchange (Ton), type m from region i to region j

λ^m : Sensitivity parameter in regions for type m freight

x_i^m : Total freight exit of type m from region i(Ton)in a year

x_i : Total exit freight of region i (Ton) in a year

θ_i^m : Sensitivity parameter of production or storing the type m freight in region i

3.4.3. Limitations

After defining the objective function, the limitations should be defined as following. For this initial model, the distances of links are considered fixed or in case of the presence of linkhes with more traffic, the length is considered bigger than the real length effectively to show the extra operational costs of traffic density. To show this effective distance, the old function of trip time-volume (BPR) was used that by increasing the ratio of the flow to the capacity and as it reaches 1, positive power of trip time is increased. This function is investigated of power zero, 3, 6 and sensitivity analysis is done for it. During the use of this model, it is possible that to show critical events as earthquake, etc some of the links are deleted from the network completely or some of the paths are reduced. The limitations of the objective function defined in the previous item can be defined as:

The first limitation shows that the flow of each link is equal to the sum of the path flows on all the paths between all the regions by link a

$$\sum_m \sum_{ijr} s_{ijr}^m \delta_{ijr}^m = f_a$$

For all a links (2)

s_{ijr}^m : It denotes exit flow (Ton) of type m freight from region i to region j on path r

δ_{ijr}^m : If path r uses a link from i to j path, this parameter is 1, otherwise it is 0.

f_a : Flow in Ton on link a

The second limitation shows that the amount of exit and entered freight of type m and to region i should be equal to the sum of the freight exchange between region i and all the regions j.

$\sum_j x_{ij}^m = x_i^m$ For each freight type m and all regions i (3) The third limitation showed that total exit freight from a region and entered to a region is equal to the sum of different kinds of exit and entrance freight to region i.

$\sum_m x_i^m = x_i$ For all the regions i (4)

The final limitation showed that the path flows should be non negative and this means that the flows of links should be non-negative.

$s_{ijr}^m \geq 0$ For each freight m and all regions i,j and all paths r (5)

3.4.4. Solution algorithm

Innovative searching methods are methods presenting an answer close to optimized answer at optimal time for a problem by searching among the possible answers. Normally, there is no reason that

the obtained answer is the best answer and we can not even compare the closeness of the optimized answer with the real optimized answer.

One of the super innovative methods being used in recent years for solving math planning and the problems with integer numbers is genetic algorithm method. The researchers by observing the conformity, resistance, self-repair, guide, production and other characteristics of natural systems and reflecting in the point that how the nature solves the problems and they were thinking about imitating natural methods for solving complex problems and the design of systems. The main idea of genetic algorithm is formed based on Darwin evolution theory. Rechenberg (1973) by presenting evolutionary calculation methods being inspired by nature to solve the hard problems took the first step [13]. But the initial principles of genetics algorithm were presented by Holland et al. They were inclined to the conformity of natural systems for modeling artificial systems. These studies formed genetics algorithm. Holland (1975) indicated the math basics of genetic algorithm in his famous book [7]. Goldberg (1989) showed that genetic algorithms are research methods based on genetic mechanic and natural selection [5].

These methods rapidly after a small part of searching space is converged to optimized answers and are used successfully for complex optimization problems in engineering. Algorithm is an artificial resistance of a good test method with genetic process such as production, mutation and crossover for forming a better answer from one repetition to another repetition and reaching an optimized answer.

Considering the presence of some tools such as Matlab software with the capability of solving these issues by various algorithms, to solve this problem, this software and genetic algorithm module were used.

4. Results

The results of solving model can be summarized in three sections. The first section is about the determining the share of each of different kinds of freight based on the model and its comparison with the observations.

In this section, R^2 between the observations and estimations is 0.89. Table 2 shows the percent of each of different kinds of freight in two cases of observation and estimation.

Table 2: The difference of the percent of observed and estimated for different kinds of freight

Freight type	Observed percent	Estimated percent
Grains	5.6	9.14
Rice	0.9	0.81
Cereals	0.2	0.19
Vegetables	3	3.68
Fruit	2.5	2.28
Live stock	3.4	2.78
Poultry	0.6	0.42
Fertilizer	2.9	3.23
Tea	0.1	0.12
Cotton	0.4	0.32
Sugar	1	0.85
Edible oil	1.2	0.81
Flour	0.7	0.82
Fast food	3.1	3.58
Steel	11.8	7.81
Metals	1.6	1.30
Coal	0.7	0.50
Cement	10.9	13.89
Construction materials	12.2	10.26
Mineral	4.7	5.21
Fuel	12.3	11.36
Chemical	2.8	2.08
Textile	1.1	1.35
Detergent	0.6	0.62
Car and machineries	4.5	5.56
Paper and wood	2.3	2.38

Durable freight	8.8	8.66

As is shown in Table 2, the estimated values for the percent of the transported freight are acceptable considerably, only in some of the freight consisting of a great part of transportation in Iran such as grains and steel, the difference is considerable.

Second section of the results is the comparison of the flow of heavy vehicles on the links. In this section considering the presented model and BPR function with the equation $d_a^t = d_a(1+0.15(f_a/C_a)^p)$, in which p is parameter of link function, f_a: The observed volume on the link, Ca: link capacity, d_a free trip time and d_a^t:The final trip time. The investigation was done for three powers of 0, 3, 6 and the observed and calculated results were compared. For three cases R^2, 0.73, 0.51 and 0.81 were observed. Fig. 3 showed the dispersion between the observations and estimations for function parameter of link equal 6.

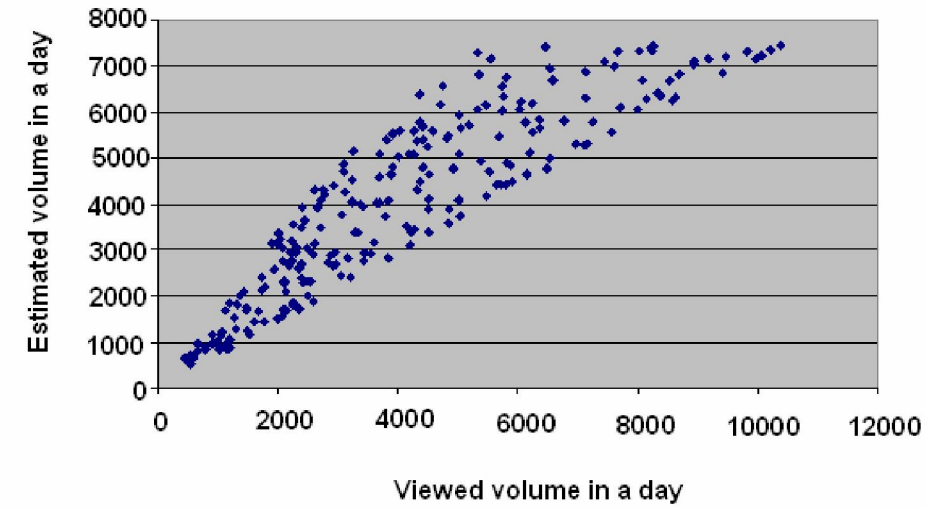

Fig 3: The comparison of the trip time of the links for two cases of observed and estimation with function parameter of link6

. The third section of the results of the presented model in this study is the comparison between the origin and destination matrix of each freight separately. As it was presented in the model, x_{ij}^m is the amount of transported freight of type m between two regions i, j. This matrix had 84672 members in this study. In the comparison of the observations and estimations for this variable, R^2 was 0.63. Although correlation coefficient is not very suitable in this case, it can be acceptable.

5. Conclusion

A mathematical model based on entropy objective function and reduction of flow in links was executed to predict the transportation of inter regional freight and transportation network flows based on the type of freight. Thus, this model is an alternative for the stages of trip distribution and traffic assignment of four-stage model of transportation planning. This model is done for transportation of freight with 27 type's freight and 56 regions and for simplified road network of Iran. This model was successfully solved by genetic algorithm in Matlab software. The required model was executed to estimate the transportation of the inter regional freight based on the type of freight, the links flow. It seems that this model kept the general nature of regions and different types of freight and the predicted transportation was in line with the observed transportations.

According to the results of model, the percent of different kinds of transported freight can be done and the elements of origin and destination matrix for each of the freight mentioned that regarding the first case, the results were rather acceptable but in the second case, the correlation coefficient of the estimated results was considerably different from the observed results.

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Investigation and Design Seawater Desalination with Solar Energy

Saeed Daneshmand [1], Ali Mortaji [2], Z. Mortaji [3]

[1,2.] Department of Mechanical Engineering, Majlesi Branch, Islamic Azad University, Isfahan, Iran
[3.] University of Ontario Institute of Technology, Canada
s.daneshmand@iaumajlesi.ac.ir

Abstract: While the global population rises, the supplement of fresh water is becoming a major concern. A number of seawater desalination approaches have been designed during the decades to contribute for overcoming fresh water shortage. Two kinds of these system have been introduced in this paper to be installed close the sea and in second one in dried regions such as deserts. This system is environmental friendly and cost effective. All the system equipment is designed to make from recycling materials.

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Keywords: Seawater desalination; Fresh water; Energy; Solar evaporation; Recycling material, Wind catcher

1. Introduction

The world's population is becoming increasingly concerned and different challenges occurred regarding to food and fresh water security in the world. Shortage of fresh water is a major issue since only less than 0.5% of available water resources in the earth are as fresh water [1]. Fresh water is playing a critical role in human life as well as in agriculture and industrial process. Moreover, about 97% of the earth is covered by sea and ocean's water, which are unsuitable for human desires. One of the main strategies to solve this problem is water management through investigation of new source of water supply such as water desalination. Nowadays, desalination from the limitless resources of earth's water is becoming one of the precious challenges in the world [2]. Currently, many researches are being performed about desalination systems especially in dried and mid-dried countries such as Middle Eastern countries [3]. All the different desalination approaches has been developed during the decades are based on thermal distillation, membrane separation, freezing, and electrophoresis. Among these methods Reverse Osmosis (RO), multi-stage flash (MSF), and multiple-effect distillation (MED) are more worldwide and leading systems [4, 5]. Different source of energies are applied in a desalinating system, some of the systems are very expensive due to the type of energy being used to desalinate [2]. The application of free solar energy maintains the overall system expenses very low down. Basically in a desalinating based on solar evaporation, the process starts by heating up the seawater through sun rays, and the produced water vapor later condense by a cool surface. The main purpose of this study is to design a solar desalination system using recycling material, in which the cost of system will reduced dramatically. The proposed plan is at a concept level and can be potentially implemented at an industrial scale. Two desalination systems are presented in this paper, the first one is useful to install close to seawater in general for humid region and the second system could be establish in dry climate such as deserts.

2. Material and Methods

System 1 and 2: The required components as shown in figure 1 are the following:

Component 1 - Larger Dish made out of transparent plastic. The UV plastic material comparing to all other transparent or clear plastics is preferable because it has green house effect and also has a high absorption for heat. However this is not necessarily recommended to be implemented at industrial scale due to its high cost. The edge of this dish is flat but gets deeper toward the centre. This component is identified by number 1 in figures 1 and 2 of this document [6].

Component 2- Smaller Cone connected to a pipe. This cone receives water drops and moves them into a main pipe. This component is identified by number 2 in Figures 1 and 2 of this document.

The numbers in figure describes the following parts or energy, 1: larger dish, 2: smaller cone, 3: earth, 4: water vapor, 5: water droplets, 6: solar rays [7], 7: air valve, 8: wind catcher [7], 9: wind, 10: pump, 11: water collected storage.

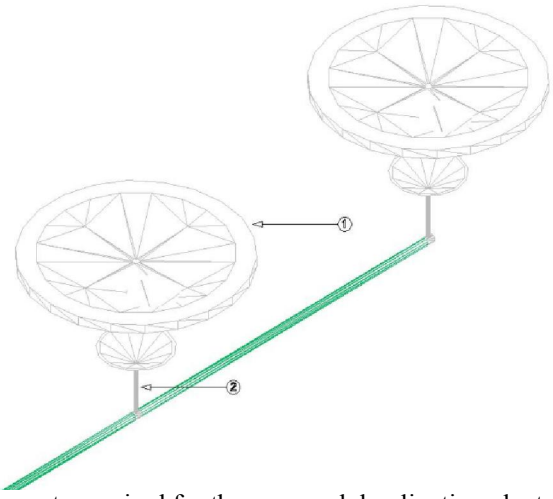

Figure 1. Components required for the proposed desalination plant

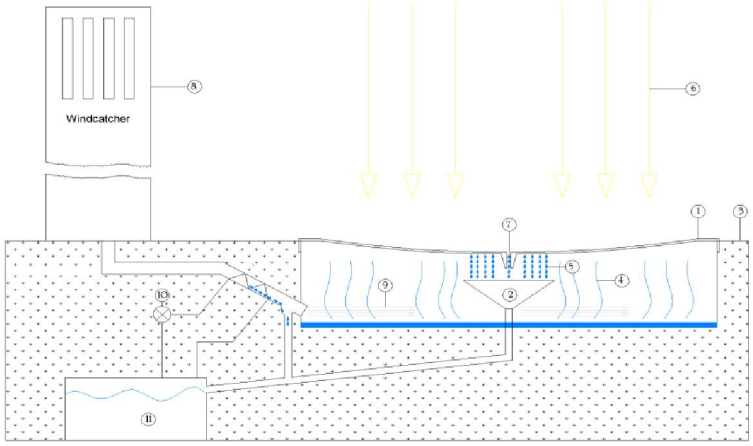

Figure 2. Desalination concept

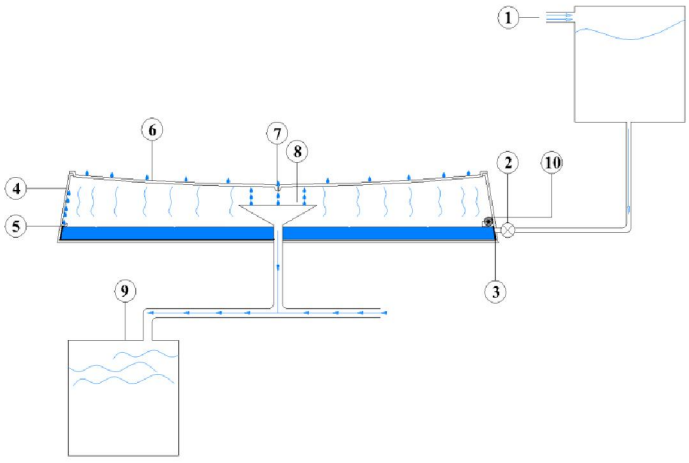

Figure 3. System 2 for water desalination useful for setting up in desert

3. Installation Guide

1. Dig a ditch in the sand by the sea as shown in figure 2. The dimension of this ditch depends upon the dimensions of components 1 and 2 described above.
2. Insert component 2 (smaller cone) into the ground such that part of the pipe is into the ground. This is shown as number 2 in figure 2 of this document.
3. Locate Component 1 (larger dish) to cover the ditch such that the flat edges are inserted into the ground [6]. This is shown as number 1 in figure 2 of this document.

4. Desalination Mechanism

Sand on the shoreline absorbs significant amount of water from the sea while also removing some of the salt and excess minerals from the water. Sun heat reflected toward the Larger Dish (shown as number 6 in Figure 2) as well as the water in the sand will cause the green house effect in the ditch. Moreover, the temperature difference between inside and outside of the ditch will cause the humidity inside the ditch to be evaporated and raised to the convex surface of the larger dish (Component 1) as desalinated water (shown as number 4 in figure 2) and will then slide toward the center, and finally will be poured into component 2 (shown as number 5 in figure 2) which is connected to a pipe linking to the final destination. The final destination (number 11) is storage for collecting the fresh water. In order to maximize production of our desalinated water system, the following extra parts added to the system. Number 7 is designed as an air valve in the center of the larger dish which will open due to the weight of collected rain and later it conducts the rain toward the smaller cone. Furthermore, number 8 is a wind catcher in order to increase the evaporation process and lastly, number 10 is a pump, which contributes the circulation of water around air entrance gate. This pump obtained its energy from wind turbine and this mechanism convert the humid air to dry air before let it go [8].

Moreover, the second system designed to be set up in dried regions such as lout and center deserts of Iran. The main mechanism of system 2 is similar to our previous system with some modification. Figure 3 represents the schematic of system 2 in which number 2 is an automatic valve to adjust the amount of water entrance. Next, the system applied a dark plate that attract the solar rays and transfer the involved energy [9] to water as heat source. Moreover, number 4 is a transparent plastic structure in order to catch the maximum duration of solar energy and number (10) 5 collect the droplets from slopped wall. The slopped water itself is helpful for easier collecting of the water droplet. Finally a fan is designed (number 10) that produce air circulation to increase the evaporation rate and obtained its energy from solar energy.

Number 1, 2, 3, 4, 5, 6, 7, 8, 9, and 10 describes the following respectively water entrance gate, automatic valve, dark plate, slopped wall, water droplet conductor, larger dish, air valve, smaller cone, water collector storage, and fan.

5. Conclusion

This paper has introduced an innovative and effective approach to desalinate water by proposing a cost effective concept, which is low not only in capital, but also maintenance, and operational cost. The system is also environmental friendly and does not require any fossil based energy and all system is made of recycling materials. The disadvantage maybe slow pace and the low capacity of produced desalinated water, and also the flavor that the resulted water will contain. Therefore, applicability of this system is mostly advantageous for irrigation, in particular any landscaping including plants and grass by the seaside. For future advances the growing of sea plants mostly algae in these systems could increase the quality of fresh water by reducing the nitrate from sea water [11]. Both systems include number of advantages such as application of wind catcher in system 1 has the main purpose as using fan in system 2. Both of these parts increase the overall yield of system by referring to formula [13]

$g = \Theta A (XS - X) / 3600$

g is amount of water evaporation.

$\Theta = (25 + 19 V)$ = evaporation coefficient (kg/m^2h)

V = velocity of air above the water surface (m/s)

A = water surface area (m^2)

XS = humidity ratio in saturated air at the same temperature as the water surface (kg/kg)
(Kg H_2O in kg dry air)

X = humidity ratio in the air (kg/kg)
(Kg H_2O in kg dry air).

For example, suppose there is no wind in area A, g would be equal to

$$g = (25 + 19 \times 0) \times 3.14 (0.019826 - 0.0115)/3600)$$

= 1.81 kg/s

and if the wind consider as 1 m/s, g is calculated 3.195 kg/s, almost the double amount of evaporation through using wind power. In addition, a number of important elements of seawater such as sodium, potassium, magnesium, and etc [12] will settle and could be collected and use for other purposes Figure 4.

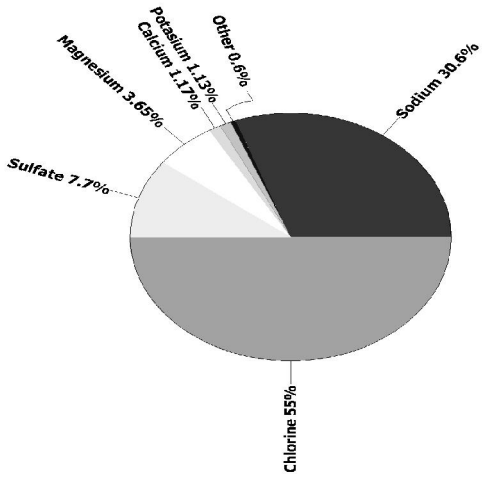

Figure 4. Amount of different elements in oceans [12]

Corresponding Author:
Ali Mortaji
Department of Mechanical Engineering, Majlesi Branch, Islamic Azad University, Isfahan, Iran
E-mail: ali.mortaji@gmail.com

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