SCREENING OF HEMOGLOBIN DISORDERS AMONG SCHOOLS' CHILDREN IN NAJRAN, KSA

Jobran Miree J AlQahtani*

*Assistant Professor of Pediatrics and The Dean of College of Medicine, Najran University, Saudi Arabia.
jobranm@hotmail.com

Abstract: Hemoglobin disorders are group of inherited worldwide disorders with high prevalence in Saudi Arabia. The aim of the study was to investigate the Hb disorders among school children in Najran province. 826 male primary school students were screened for hemoglobin disorders using hemoglobin electrophoresis. The children were sub-grouped according to the tribe's home and to the consanguinity of their parents. 23 samples (2.8 %) showed abnormal patterns; 18 samples (2.2%) showed the Hb S pattern and five samples (0.6%) showed increased Hb A2 level and decreased Hb A level of thalassemia patterns. In conclusion, the southern areas of Saudi Arabia represent the tribes' home of most cases and further studies are needed to confirm that Najran original tribes are free from the hemoglobinopathies.

[Jobran Miree J AlQahtani. **Screening of Hemoglobin Disorders in Najran Schools' Children, KSA**. Nature and Science 2012;10(4):56-62]. (ISSN: 1545-0740). http://www.sciencepub.net/nature. 9

Keywords: Hemoglobin disorders; thalassemia; Hb S; school children

1. Introduction

Hemoglobinopathies are group of inherited worldwide disorders with high prevalence in Saudi Arabia (Al-Hawsawi et al., 2003). The inherited hemoglobin disorders constitute a significant public health problem that may lead to various acute and chronic disorders, several of which may be lethal. Sickle cell anemia and thalassemia are the most common hemoglobin variants where there are structural abnormalities in their globin chains. Approximately 5% of the world's population carries trait genes for haemoglobin disorders, mainly, sickle-cell disease and thalassaemia. Over 300 000 babies with severe haemoglobin disorders are born each year (WHO, 2011). These inherited disorders may cause life-threatening disorders, chronic disability and exhaustion to family members (Al-Hawsawi et al., 2003).

Normal hemoglobin molecule is an assembly of heam and four globin chains, two α and two non - α globin chains (β, γ and δ) (Hoffbrand and Pettit, 2006). The structure of hemoglobin changes during development. Embryonic hemoglobin is replaced by fetal hemoglobin (Hb F) shortly before birth which in turn is replaced by adult hemoglobin (Hb A) over the first year of life (Weatherall, 2001).

Each of the different globin chain is controlled by distinct genes; two genes exist for the α and γ chains and one for each of the other chains (Steinberg et al., 2001).

The genes for α-like globin chains are found in a cluster at the tip of the short arm of chromosome 16, while those for β-like chains are found on chromosome 11. The complete nucleotide sequence of these regions has been determined and the molecular pathology of most hemoglobin disorders is well defined (Balgir, 2008).

Sickle-cell anemia (disease)

Sickle-cell disease (SCD) is an autosomal co-dominant genetic blood disorder (Platt et al., 1994), that is caused by mutations in the β globin gene resulting in the production of a structurally abnormal β globin chain due to valine for glutamic acid substitution at position 6 of the β chain (Langlois et al., 2008).

This abnormal synthesized hemoglobin "HbS" forms a gel-like polymer at conditions of low oxygen tension leading to a modification in the shape of the red blood cell from a smooth, biconcave shape into a crescent shape. The misshapen cells with decreased flexibility can block small blood vessels, impairing blood flow (WHO, 2011).

The life expectancy of patients is shortened, with studies reporting an average life expectancy of 42 in males and 48 in females (Platt et al., 1994).

Two cardinal pathophysiologic features of sickle cell disease represent the main reasons of clinical characteristics: chronic hemolytic anemia and vasoocclusion. The polymerization of the hemoglobin S molecule (Hb S) within the red blood cells upon deoxygenation causes the red blood cells to change from the usual biconcave disc to an irregular sickled or crescent shape. Upon reoxygenation, the red cell initially resumes a normal configuration but after repeated cycles, the erythrocyte is damaged permanently, resulting in red cell dehydration

and erythrocyte destruction. Sickled red blood cells also have a propensity to adhere to the walls of blood vessels and are susceptible to hemolysis, causing chronic anemia (Ashley-Koch et al., 2001).

Because of both reduced deformability of sickle cells and increased adhesion to endothelial cells, the deformed red blood cells cause microcirculatory obstruction and prevent normal blood flow and decreased delivery of oxygen to organs and tissues resulting in the vasoocclusive crisis (Stuart and Nagel, 2004).

Some patients with sickle cell disease experience both chronic and episodic pain with severe disability, whereas others are able to lead relatively normal lives (McClish et al., 2005) according to the homo/heterozygosis of the disease and the co-inheritance with other genetic disorders such as with β thalassemia (i.e., S/ β thalassemia) or HbC (also known as SC disease) (ACOG , 2007).

Pain and swelling of hands and feet (hand-foot disease) is a frequent early presentation of sickle cell disease in infants and young children, and occurs as a result of aseptic necrosis of the small carpal and tarsal bones. The hemolysis leads to chronic anemia and predisposes the patient to aplastic crises (ACOG , 2007).

Painful crisis is the most common reason for hospitalization and acute chest syndrome is a cause of death in patients with this disease (Vichinsky et al., 2000). Cerebrovascular accidents , splenic dysfunction and iron overload with chronic transfusion, which can damage the liver are known complication that reduce the quality of life and decrease life expectancy by 25 to 30 years (Platt et al., 1994).

Thalassemia

There are two major types of thalassaemia, alpha and beta, which are named for the two protein chains that make up normal haemoglobin. Alpha and beta thalassaemia have both mild and severe forms (WHO, 2011).

Alpha thalassemia is one the most common inherited hemoglobin disorders results from defects in alpha-globin genes causing decreased or absent alpha globin chain production.

Alpha globin chain represents an essential structural and functional component of all hemoglobin types as in fetal hemoglobin (Hb F: $\alpha 2\gamma 2$), hemoglobin A2 (Hb A2: $\alpha 2\delta 2$) and adult hemoglobin (Hb A: $\alpha 2\beta 2$). The genetics of alpha-thalassemia are complex, as each normal person carries four functioning alpha globin genes (αα/αα). Two genes, alpha-1 (*HBA1*) and alpha-2 (*HBA2*), are present on each copy of chromosome 16.

The various degrees of alpha globin genes (αα/αα) injuries give different forms of alpha thalassemia disease.

A deletion or mutation of a single α globin gene (α^+ thalassemia) leaving the other α globin gene intact located on the same chromosome (designated α−/) gives a milder form than deletion of both α globin genes (α^0 thalassemia) located on the same chromosome (i.e.,−−/) (Langlois et al., 2008).

The defect in α globin gene resulting in decreased alpha-globin production, leading to an excess of γ chains in newborns and β chains in adults. The excess β chains form unstable tetramers (called Hemoglobin H or HbH of 4 beta chains) which have abnormal oxygen dissociation curves (Weatherall, 2001).

Alpha-thalassemia (α-thalassemia) is commonly found in Africa, the Middle East, India, Southeast Asia, with two symptomatic significant forms: hemoglobin Bart hydrops fetalis (Hb Bart) syndrome and hemoglobin H (HbH) disease. Hb Bart syndrome, results from serious mutations or deletions of all four alpha globin genes (−−/−−) resulting in complete loss of their function. The most severe form, is characterized by fetal generalized edema, ascites, pleural and pericardial effusions, and severe hypochromic anemia, which often lead to fetal or perinatal death (Powars et al., 2005).

Hemoglobin H (HbH) disease usually occurs due to loss of function of three alpha globin genes (−−/−α) which is characterized by moderate microcytic hypochromic hemolytic anemia with Heinz bodies, hepatosplenomegaly, mild jaundice, and sometimes thalassemia-like bone changes due to extramedullary hematopoiesis and due to the presence of Hb H (β4) the patient may suffer from an acute hemolytic attack after oxidative stress, infection or drug therapy (Powars et al., 2005).

A child who inherits two of the same trait genes - one from each parent - will be born with the disease. However, a child of two carriers has only a 25% chance of receiving two trait genes and developing the disease, and a 50% chance of being a carrier. Most carriers lead completely normal, healthy lives (WHO, 2011).

Beta thalassemias

Each normal individual has two copies of the β globin chain genes, the mutations of these genes inducing β thalassemia, result in

either decrease (β^+) or absence (β^0) in the formation the beta globin chain (Langlois et al., 2008).

According to its severity, β -thalassemia can be classified based on clinical symptoms into three main types: thalassemia major (TM), thalassemia trait (TT) and thalassemia intermedia (TI).

Thalassemia major (TM) is a severe form that requires transfusions from infancy for survival, whereas TT is usually asymptomatic. TI is used to indicate a clinical condition of intermediate gravity between TT and TM, which encompasses a wide phenotypic spectrum spanning from mild anemia to more severe anemia and these patients require only occasional blood transfusions, if any (Camaschella and Cappellini, 2004); (Weatherall, 2001). β -thalassemia major (TM) is the most severe form, which has either zero (β^0/β^0)or almost zero (β^0/β^+) β globin chain synthesis. It is characterized with severe hypochromic microcytic hemolytic anemia, hepatosplenomegaly and Hb electrophoresis or HPLC will show elevated HbA2. The affected individuals are lifelong transfusion-dependent,[(24)] although transfusion therapy prevents many of the complications of severe anemia, the body is unable to eliminate the excess iron contained in the transfused blood. Over time, the excess iron deposits in tissues and organs, resulting in damage and organ failure, rather than complications of the disease itself, and infectious complications (Langlois et al., 2008). Thalassaemias can be cured by a successful bone-marrow transplant, however this procedure is expensive and not readily available in most settings. Recently, gene therapy has been successfully applied to a patient with thalassaemia (WHO, 2011).

Beta-thalassemia intermedia is less clinically severe than thalassemia major. which encompasses a wide phenotypic spectrum spanning from mild anemia to more severe anemia and is distinct from major as patients require only occasional intermittent transfusions for intermedia (Camaschella and Cappellini, 2004); (Weatherall, 2001).

The combination HbE/ β –thalassemia results in a varied clinical expression ranging from severe transfusion dependence to a complete lack of symptoms (Nuntakarn et al., 2009) ; ; (Rees et al., 2009). The genotypes of β- and α- globin and expression of γ-globin are genetic modifiers of b-thalassemia (Thein , 2005) ;(Thein , 2008).

Also, some factors such as, alpha hemoglobin stabilizing protein (AHSP), (Kihm et al., 2002) ; (Lai et al., 2006) and heme-regulated initiation factor 2 alpha kinase (HRI), (Han et al., 2005) are also thought to contribute to the phenotypic diversity of TI (Chen et al., 2010). Due to the phenotypic diversity, and genotypic heterogeneity, the clinical severity of thalassemia intermedia cases are very difficult to predict from their genotypic data (Ho et al., 1998) ; (Murru et al., 1991). Carriers with one normal gene and one affected gene (genotypically represented as β / β+ or β / β0 depending on the type of β gene mutation) are clinically asymptomatic. These patients are described as having β –thalassemia trait or β -thalassemia minor. They may show mild or no anemia. Like α-thalassemia trait, the RBC count is often high. Because reduced production of β globin means an inability to generate as much normal HbA (α2 β2), they compensate by increasing production of other β -like chains, namely δ and γ, leading to increases in the levels of the minor hemoglobins HbA2 (α 2 δ 2) and HbF (α 2 γ 2) (Langlois et al., 2008).

2. Material and Methods

This study was carried out for random screening of schools' children, for inherited hemoglobin disorders in Najran province between June 2009 and February 2011. Fifteen primary schools for boys were geographically selected among 150 primary schools for boys to represent all regions of Najran area. Ethical clearance from Ethical Committee of Najran University Medical College, and from the educational administration in Najran area was obtained prior to conducting the study and an informed consent of family was taken before withdrawing blood sample from the students. A total of 826 male primary school students belonging to all rows were screened for detection of hemoglobin disorders. Complete family and personal history for each child were taken. The children were sub-grouped according to the age, tribe's home & parental consanguinity.

Blood collection

About 2-3 mL intravenous blood samples were collected using ethylene diamine tetra acetic acid (EDTA) as anticoagulant with disposable syringes and needles from each students after obtaining the informed/written consent in the presence of a doctor and the school supervisor. The collected blood samples were transported to the hematology department

at College of Medicine Najran Universityr under ice-cold conditions within 6-12 hours of collection.

Laboratory analysis

Laboratory investigations were carried out following the standard procedures after cross checking for quality control from time to time. Hematological parameters were studied using an automated Blood Cell Counter (COULTER® *LH 500* Hematology Analyzer - *Beckman Coulte*r, USA).

Hemoglobin electrophoresis was carried out at alkaline pH 8.6 using SAS-1plus, Helena Biosciences' SAS-2 gel processing system.

3. Results

A total of 826 subjects were studied: 23 samples (2.8 %) showed abnormal patterns; 18 samples (2.2%) showed the Hb S pattern and five samples (0.6%) showed increased Hb A2 level and decreased Hb A level of thalassemia patterns. No subject showed decreased Hb level, but 68% of subjects with HbS have microcytosis and hypochromia.

The studied population are further subdivided according to their original home into 8 subgroups including students from; Najran, Aseer, Jazan, northern, eastern, western and central areas of Saudi arabia in addition to Yemen (a country very closed to the southern areas; Najran, Aseer and Jazan) and the other countries. Najran as a tribes' home represented the largest proportion of studied population (53.1 %) who showed the higher frequency of HbS pattern (2.0 %) of total students and about (0.4 %) to both Aseer and Jazan for subjects having thalassemia disorder. The details are found in table 2.

The consanguinity degree between the parents of the hemoglobin disorders carriers were not significant as only 47.7% of the parents of the HbS-children were relatives, and non of the parents of thalassemia disorders children were relatives.

Table (1): Distribution of participant students among different tribes

	Tribe								
	Najran Area	Aseer Area	Eastern Area	Western Area	Jazan Area	Northern Area	Central Area	Yemen	Other Countries
% of participant students among different tribes	54.4 %	22.5 %	2 %	1.5 %	4.6 %	1.5 %	1.2 %	8.5 %	5.7 %

Table (2): The home of the tribes which carries hemoglobin disorders

		Tribe								
		Najran Area	Aseer Area	Eastern Area	Western Area	Jazan Area	Northern Area	Central Area	Yemen	Other Countries
Normal HB	% within Tribe	97.8 %	98.9 %	100.0 %	91.7 %	81.6 %	83.3 %	100.0 %	98.6 %	100.0 %
	% within Total	53.1 %	22.3 %	0.2 %	1.3 %	3.8 %	1.2 %	1.2 %	8.4 %	5.7 %
Abnormal HB	Sickle Cell Disease % within Tribe	2.0 %	0 %	0 %	8.3 %	13.2 %	16.7 %	0 %	1.4 %	0 %
	% within Total	1.1 %	0 %	0 %	0.1 %	0.6 %	0.2 %	0 %	0.1 %	0 %
	Thalassemia % within Tribe	0.2 %	1.1 %	0 %	0 %	5.3 %	0 %	0 %	0 %	0 %
	% within Total	0.1 %	0.2 %	0 %	0 %	0.2 %	0 %	0 %	0 %	0 %

4. Discussions

According to a global epidemiological database, haemoglobin disorders represent a significant health problem in 71% of 229 countries, which include 89% of all births worldwide. Over 330,000 affected infants are born annually (83% with sickle cell disorders, 17% with thalassaemias) (Modell and Darlison, 2008). In this study, hemoglobin disorders were found in 2.8% of screened school children in Najran area by gel electrophoresis. Most (73%) of these disorders showed sickle cell disorders while 27% have thalassemia disorders.

As There is no previous studies that reported high frequency, the higher frequency of sickle cell disorders among students from Najran area are probably due to their descent from Yemeni families that recently received citizenship.

Sickle-cell disease (SCD) occurs more commonly in people (or their descendants) from parts of tropical and sub-tropical regions where malaria is or was common, as in the south west of Saudi Arabia (Wellems et al., 2009) as the protective effect of the sickle-cell disorder against infection with malaria is well known (Williams et al., 2005) ; (Aidoo et al., 2002) and it is known that the southern of Saudi Arabia is an endemic area for malaria (Saeed et al., 2002).

Reference to the original home of each child tribe. It was found that there is a highly significant correlation (P= .000; Pearson Chi-Square) between the subjects of hemoglobinopathies and the southern regions of Saudi Arabia; Najran, Asser and Jazan areas. It was considered that samples were withdrawn from schools covering the all regions of Najran area geographically.

A few cases were found to be originated from Northern and Western areas of Saudia Arabia and one from Yemen.

The sources of sickle cell disorders in Saudi Arabia arise from the central-west of Africa (Benin) and India. The Benin type has spread to north-western and south-western of Saudi Arabia, while further foci in eastern Saudi Arabia may have arisen initially from the Indus valley (Bain , 2001).

Contrary to expected, it was found that the incidence of hemoglobin disorders in children of non relative parents is more than of relative parents.

This is perhaps due to the increased awareness of the importance of pre-marital medical & laboratory work up and increased health education about the health risks that may result from marriage of relatives or within the tribe.

This assumption leads to decrease the total incidence of such disorders with a relative false increase in the portion for the children of non-relatives parents.

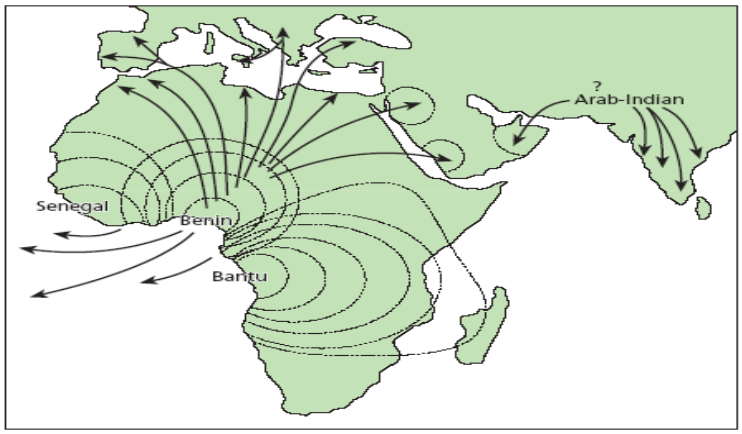

Fig.1 Multifocal origin and spread of the β^s gene (Bain , 2001).

We recommend investigating all children twice for hemoglobin disorders. One at birth for sickle cell disorders detection as it can be discovered early in the first few days and it is the most common hemoglobin disorder in Najran area. The second to be at the end of the first year for detection of other types of hemoglobinopathies “specially for thalassemia” as some may be misdiagnosed earlier, as early identification of some clinically significant hemoglobinopathies and the precise differentiation of hemoglobin variants are important to provide early comprehensive medical care to prevent some undesirable complications, assess prognosis, and offer genetic counseling (Kim , 1981).

Also, we have to increase health awareness among the population of Najran area to risks of relative’s marriage carrying hereditary disorders such as hemoglobinopathies and the marriage between the tribes reduces the incidence of such disorders more than marriage within the tribe. The most cost-effective strategy for reducing the burden of haemoglobin disorders is to complement disease management with prevention programs (WHO, 2011).

Acknowledgment:

This work was sponsored by Chair of the Prince Meshaal Bin Abdullah in the field of endemic diseases in Najran and supported by Najran University Program for Health and Medical Research Grants (NU 06/09).

I would like to express my deep thanks to both Dr. Mohamed M. Al-Khawanki; a lecturer of hematopathology & Mr. Maher I. Khalil for their valuable contribution in the practical part of this work, and Dr. Mohammad Hanafy; assistant professor of hematology for his great help.

Corresponding Author:
Dr. Jobran Miree J AlQahtani
Assistant professor and The Dean of College of Medicine, Najran University, Saudi Arabia.
E-mail: jobranm@hotmail.com

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3/13/2012