Investigate the joint effect of tobacco exposure and alteration of TNF-α CD8,MDA and GSH levels in blood of Iraqi smokers

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Abstract: Background: Cigarette smoke cause alteration in body biological factors, so we hypothesized that the TNF, CD8, MDA and GSH will change in smoker's blood compared to non smoker subjects. **Methods**: blood samples were obtained from Iraqi smokers and non smoker subjects. Levels of TNF-α and CD8 were assessed by ELISA whereas MDA and GSH were measured via optical density using microplate reader. **Results**: levels of TNF- α and CD8 were significantly increased (P<0.01) at age group (26-35) years, while MDA were significantly increased(P<0.01) and GSH were significantly decreased (P<0.01) at the age more than 56 year, also association with consumption more than one pack per day, and with duration of smoking more than 20 year, compared with others smoker groups. **Conclusions**: The results were demonstrated increase TNF-α, CD8, MDA levels and decrease GSH levels in blood of smokers

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1.Introduction

As it is known cigarette smoking is associated with alterations of body defenses whether weakening of the antioxidant defense systems or disable the immune system (Costenbader and Karlson, 2006). Many changes are result of exposure to the negatively influence of up to 4700 different baleful substances (Anonymous, 2014), which leading to high production of cytokines like tumor necrosis factor α (TNF-α) (Borgerdingand Klus, 2005). Also characterized by proliferation numbers of eosinophils, neutrophil, mast cells, and has been associated with increased expression of IL and high numbers of lymphocytes such as CD8 and CD4 (Glader *et al*., 2006). Free radicals may be produced by the human body, for instance by neutrophils, which produce reactive oxygen species and reactive nitrogen species, with a high oxidizing power (Cedergren *et al*., 2007). The accumulation of free radicals leads to an altered oxidative status that may end in the oxidation and damage of biomolecules such as lipids, proteins or DNA (Valko *et al.*, 2007). In addition of smoking may enhance oxidative stress not only through the production of reactive oxygen radicals but also through sapping of the antioxidant defense systems (Surapaneniand and Venkataramana, 2007), and modification of oxidative stress marker levels as (MDA) and (GSH), quite like to thecase of infected with hurtful viral and bacteria, indicating to the state of alert experienced by the body as a result of exposure to these toxins (Mahapatra *et al*. 2008). Therefore, the principal aim of our study was to investigate the joint effect of tobacco exposure and alteration the levels of TNF-α CD8, MDA and GSH in blood of Iraqi smokers and variation of this levels with the age of smokers, duration of smoking and pack number consumption per day by smokers.

2. Materials and methods

Subject

Eight ml of venous blood sample was obtained from 150 volunteers of heavy smokers for at less one pack per day and a period of not less than 5 years and 50 volunteers of non-smokers as control, each sample was divided in to two parts, first 4 ml were collected with EDTA anticoagulant tubes and centrifuged at 1000 x g for 10 min at 4°C, then transferred the top plasma layer to a new tube stored at -20°C for measurement malondialdehyde (MDA) and glutathione (GSH). Another 4 ml of blood were put into a vacationer sterile tube, let to stand for two hours at room temperature, then centrifuged at 3000 rpm for 5 minutes, serum separated, removed and kept at −20°C until required.

Measurement of CD8 and TNF-α levels

The levels of TNF-α and CD8 were measured by Enzyme-Linked Immunosorbent assay (ELISA) technique. using TNF-α ELISA kit (Invitrogen. USA) and CD8 ELISA Kit (My bio source. USA), according to Pegram *et al.* (2015) and Aukrust (1994) respectively. The levels were measured in nanogram and picogram / ml, normal values of TNF-α and CD8 are (12.5-21.5) and (35.4-65.4) respectively.

Measurement of MDA:

Malondialdehyde (MDA) was measured using thiobarbituric acid (TBA) and MDA assay kit (abcam. USA) according to Qiao (2016),for blank, water was used instead of the sample. The levels were measured in nmol/ml, normal values (6.5-15.5).

Measurement of glutathione (GSH):

Measurement of GSH concentration was done by DTNB using GSH assay kit (Abnova-Taiwan) according to Anderson (1996) for blank, water was used instead of the sample. The levels were measured in mM/ml, normal values (10.5-18.5).

3. Statistical analysis

The Statistical Analysis System- SAS (2012) program was used to measure the effect of difference factors in study parameters. Least significant difference –LSD test (ANOVA) was used to significant compare between means in order to evaluate the significance of variability between smoker and non smoker (control) groups ($P<0.01$) significant, the data were presented in simple measure of mean ± SD (standard deviation).

4.Results

The results showed significant changes ($P<0.01$) in levels of TNF-α, CD8, MDA and GSH between all smoker groups when compared with non smokers, and these levels varied association with the age of smokers, duration of smoking and pack number consumption by smokers as shown in tables below, when it was studied the relationship between the age and smoking, the results showed significant alterations ($P<0.01$) in levels of TNF-α and CD8 among age groups. As shown in tables (1,2), the age group (26-35) years showed a high levels than other smokers groups. Otherwise, all smoker groups showed significant changes when it was compared with control group.

Table (1): Effect of age on smoker TNF- α levels, values are mean ± SD

Age groups	No. of smoker	TNF- α (pg/ml)	No. of non smoker	TNF- α (pg/ml)
14-25	19	20.75 ± 3.67	6	13.57 ± 1.26
26-35	32	39.39 ± 4.25	5	18.90 ± 2.74
36-45	38	32.70 ± 4.59	11	16.66 ± 2.58
46-55	32	30.73 ± 4.39	16	15.53 ± 1.17
More than 56	29	23.18 ± 3.01	12	13.17 ± 1.00

Table (2): Effect of age on smoker CD8 levels, values are mean ± SD

Age groups	No. of smoker	CD8 (ng/ml)	No. of non-smoker	CD8 (ng/ml)
14-25	19	59.72 ± 2.53	6	39.22 ± 7.33
26-35	32	94.09 ± 1.34	5	61.52 ± 2.34
36-45	38	84.65 ± 1.77	11	55.46 ± 2.72
46-55	32	72.38 ± 2.06	16	52.52 ± 1.73
More than 56	29	69.10 ± 2.55	12	52.53 ± 1.74

In addition, the results showed significant changes ($P<0.01$) in levels of MDA between age groups., the age group more than 56 years showed a high level of MDA than other age groups. As shown in tables (3).

Table (3): Effect of age on smoker MDA levels, values are mean ± SD

Age groups	No. of smoker	MDA (nmol/ml)	No. of non smoker	MDA (nmol/ml)
14-25	19	13.44 ± 0.78	6	7.23 ± 1.32
26-35	32	15.25 ± 1.19	5	9.92 ± 0.83
36-45	38	15.98 ± 0.94	11	11.52 ± 1.26
46-55	32	16.62 ± 1.11	16	12.76 ± 1.13
More than 56	29	20.70 ± 0.76	12	14.29 ± 0.84

While the results showed decrease in levels of GSH between age groups, the smokers with age of more than 56 years showed a lowest level than other age groups, as shown in tables (4).

Table (4): Effect of age on smoker GSH levels ,values are mean ± SD

Age groups	No. of smoker	GSH (mM/ml)	No. of Non smoker	GSH (mM/ml)
14-25	19	10.92 ± 191	6	15.8±2.4
26-35	32	10.57 ± 1.73	5	15.1 ±2.2
36-45	38	9.80 ± 1.47	11	13.1±2.7
46-55	32	8.69 ± 1.63	16	12.6±2.8
More than 56	29	7.64 ± 1.67	12	12.2±2.1

Also, it was observed significant changes (P<0.01) in level of TNF-α and CD8 association with smoking duration as shown in table (5), the groups of more than 20 year of smoking showed a higher levels of TNF-α and CD8 than other smoker groups. On the other hand, all smoker groups showed significant changes when compared with control group.

Table (5): Effect of smoking duration on smoker TNF- α and CD8 levels, values are mean ± SD.

Duration of smoking groups	No.	TNF- α (pg/ml)	CD8 (ng/ml)
5-10	22	19.50 ± 1.89	60.60 ± 3.34
11-15	44	25.23 ± 2.78	70.67 ± 2.65
16-20	46	32.42 ± 2.24	80.88 ± 5.58
More than 20	38	40.20 ± 2.92	93.01 ± 2.85

Also, the results clarified that a significant changes (P<0.01) in levels of MDA and GSH association with smoking duration as shown in table (6), the group of more than 20 years of smoking showed a highest level of MDA while the group of more than 20 years showed lowest level of GSH among the other smoker groups, moreover, all smoker groups showed significant changes as compared with control group.

Table (6): Effect of smoking duration on smoker MDA and GSH levels, values are mean ± SD

Duration of smoking groups	No.	MDA (nmol/ml)	GSH (mM/ml)
5-10	22	13.95 ± 1.50	12.33 ± 1.01
11-15	44	15.06 ± 0.94	10.69 ± 0.78
16-20	46	16.36 ± 0.77	8.94 ± 0.56
More than 20	38	20.02 ± 1.43	6.96 ± 1.06

The results of the present study showed significant changes (P<0.01) in level of TNF-α and CD8 between groups according to pack number consumption per day by smokers, as shown in table (7,8), the groups of one pack a day showed increase in levels of TNF-α and CD8 as compared with non-smoking persons, otherwise, groups of more than one pack showed a high level of TNF-α and CD8 when compared with one pack groups.

Table (7): Effect of pack number on smoker TNF- α and CD8 levels, values are mean ± SD.

Pack number smoking groups	No.	TNF- α (pg/ml)	CD8 (ng/ml)
One pack	16	18.49 ± 1.57	59.67 ± 2.63
More than pack	134	31.77 ± 0.69	80.05 ± 10.33

As well, the results observed significant changes (P<0.01) in levels of MDA and GSH association with pack number consumption per day by smokers as shown in table (8), the group of more than one pack a day showed a highest levels of MDA and low levels of GSH among the other smokers groups, moreover, all smoker groups showed significant changes (P<0.01) as compared with control group.

Table (8): Effect of pack number on smoker MDA and GSH levels, values are mean ± SD

Pack number smoking groups	No.	MDA (nmol/ml)	GSH (mM/ml)
One pack	16	13.15 ± 0.58	12.88 ± 0.77
More than pack	134	16.96 ± 2.26	9.05 ± 1.71

5.Discussion

However, the precise role of CD8 in smoking and the influence of cigarette smoke on CD8 remains unclear (Botelho *et al.*, 2010). but the increasing of CD8 level may be due to that the exposure to toxic ingredients of cigarette smoking, especially tar, N-nitrosamines, Carbon Monoxide… etc, which can stimulates the immune system, in addition the toxins effect involves in the cells of the body, which can promotes cell-mediated immunity such as T-cell including CD8 proliferation, although some *in vitro* research had indicated that soluble components extracted from cigarette smoke can significantly effect on proliferation

and activation of T cells (Arnson *et al*., 2010). Studies have suggested that that CD8 are inversely correlated with pulmonary function and may play a critical role in the smoking related pulmonary inflammation (Farhang *et al*., 2013) , also, the previous findings had demonstrated that CD8 display immune regulation functions (Boldison *et al*., 2014 and Tamara *et al*.,2016), moreover, present study indicated that there was an inverse correlation between the circulating CD8 and smoking index. It was noticed that the CD8 influenced by smoking in which observed significantly effect in all different cases, influenced by age, number of packs consumption and duration of smoking. Likewise, current results showed significant changes ($P<0.01$) in levels of CD8 which appeared very high in younger smokers compared with aged smokers, furthermore, the results showed that the number of packs and the smoking duration also effected on CD8 levels. This results are in agreement with Finkelstein *et al*. (1995) and Moszczynski *et al*. (2001) who observed increase levels of CD8 in smoker volunteers compared to nonsmoker. As well it was noticed that the TNF-α influenced by smoking in which observed significantly effect in all various cases, affected by age, number of packs consumption and duration of smoking. Elevated TNF-α level might be due to that the exposure to cigarette smoking toxins, especially tar, Benzene, Arsenic… etc, can stimulate the immune system, including proliferation of macrophages and natural killer cells which produce TNF-α.

The present result are in agreement with Boström *et al*. (1999) who reported a raise level of TNF- α in the gingival crevicular fluid of smoker patients compared to nonsmoker with periodontal disease. Also, the result are consistent with Merghani *et al*. (2012) who indicated that the serum levels of TNF-α were significantly higher in the smoker group than in the nonsmoker group. The levels of TNF- α were also significantly higher in smoker of more one pack per day than one pack, that is in agreement with the study of Petrescu *et al*., (2010) who referred that the marked increase of TNF- α system activation with an increase in the number of cigarettes smoked per day.

MDA has been recognized as an important lipid peroxidation indicator, since subjects affected by several diseases have an increase of MDA levels (Ho *et al*.,2013). The involvement of lipid peroxidation in cancer which results from the accumulation of the multiple mutations in genes (Talarowska *et al*., 2014). These genetic changes are a consequence of instability of DNA replication which result from exposure to toxins and reactive oxygen species (Chu *et al*., 2013), thus, MDA can be used as one of the markers for diagnosis cancers (Giera *et al*., 2012). In the present study, MDA levels were increased among smoker subjects compared with non-smoker, moreover the study showed variation the levels of MDA among the groups of the pack per day, duration of smoking and the age. Many studies reported that the smoking lead to increase the MDA level in smokers compared with non-smokers (Nia and Bambang, 2011 ; Kahnamoei *et al*., 2014). So, study carried out by Jaggi and Abhay (2015) showed the effect of smoking on the MDA levels and the relation between the number of cigarette and the MDA levels, they found that the smoking leads to increase the MDA levels and there was a relation between the number of cigarette per day and MDA levels.

Moreover, GSH is fundamentally involved with many metabolic and biochemical mechanisms such as protein and prostaglandin synthesis, DNA synthesis and repair, maintenance of disulfide bonds in proteins, enzyme activation and amino acid transport across cell membranes (Kamerbeek *et al*., 2007), also, it plays an important role in a multitude of cellular processes, including cell differentiation, proliferation, and apoptosis (Bohanes *et al*.,2013). Low levels of GSH or deficiency to be contribute to oxidative stress which involved in diseases such as cancer, Parkinson's disease, and Alzheimer's disease liver disease, diabetes, anemia and heart attack (Zenger, 2004). In addition, imbalances in GSH levels affect immune system function, and are thought to play a role in the aging process (Mandal *et al*., 2015).

In the present study the GSH level was decrease among smoker subjects compared with non-smoker, the results also showed changes among the groups of the consumption of packs per day, duration of smoking and the age which lead to decrease the GSH levels. Study of Mahapatra *et al*. (2008) observed that the smoking leads to decrease GSH levels in smokers compared with non-smokers, and the study carried out by Diken *et al*. (2001) showed that the long smoking duration leads to decrease the GSH levels more then short smoking duration and in the two groups of smoker the GSH levels were decreased compared with non-smokers. In addition, Arvind and Suchetha (2013) referred that the chronic smoking leads to decrease the GSH level in smokers compared with non-smokers, and the deleterious effects of smoking in oxidative stress and subsequently in carcinogenesis. In concluded, the age with the pack number and smoking duration increased the risks of smoking by elevate the levels of TNF-α, CD8 and MDA, which were noticed significantly higher ($P<0.01$) and decreased in GSH activities which were significantly lower ($P<0.01$) in smokers than in nonsmokers.

6.Reference

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