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## Estimation the Levels of Some Inflammatory Markers Among Young Iraqi Smokers and Non Smokers

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### Abstract

In this study it was found that a significant decrease in the level of leptin in young Iraqi smokers ( $16 \pm 0.7$  ng/mL) compared to non smokers ( $24.2 \pm 4.5$  ng/mL) while, B2 microglobulin and CRP was significantly increased in the smokers ( $1.2 \pm 0.3$   $\mu$ g/mL), ( $4.07 \pm 0.02$  mg/L) respectively, compared to non smokers ( $0.6 \pm 0.9$   $\mu$ g/mL), ( $2.88 \pm 0.002$  mg/L) respectively, the presented data indicates the effect of smoking on these immunological markers.

**Keywords:** leptin, CRP, B2 microglobulin, nicotine effects

تقييم مستوى بعض المؤشرات المناعية بين الشباب المدخنين وغير المدخنين العراقيين

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### الخلاصة

في هذه الدراسة وجد ان انخفاض معدل leptin انخفاضاً معنوياً بين المدخنين العراقيين الشباب ( $16 \pm 0.7$  ng/mL) مقارنة بغير المدخنين ( $24.2 \pm 4.5$  ng / mL) ، بينما ارتفع بفارق معنوي كل من مستوى البروتينين B2 وال CRP بين المدخنين ( $1.2 \pm 0.3$   $\mu$ g/mL) ، ( $4.07 \pm 0.02$  mg/L) ، على التوالي مقارنة بغير المدخنين ( $0.6 \pm 0.9$   $\mu$ g/mL) ، ( $2.88 \pm 0.002$  mg/L) ، على التوالي، مما يؤكد تأثير التدخين على مستوى بعض المؤشرات المناعية.

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## Introduction

Cigarettes contain various toxic compounds that affect health and immune response, some reports indicated that tobacco suppress the immune system and this may have a therapeutic potential as an anti-inflammatory agent [1]. Others indicated tobacco contain 28 toxic compound as nicotine, carbon monoxide, carcinogenic benzoic and the elevated levels of these compound may increase risk to several cancers and coronary heart diseases [2]. Innate and acquired immune response are altered too by smoking, however this depends on other factors as sex, age, duration of smoking and dose of nicotine and tar [1,2]. Some reports indicated the increase in susceptibility to infection by smoking is due to changes in the humeral and cellular immune response [1].

Koc. and his colleges in 2008, tested 54 blood sample from smokers and 26 sample from non smokers for the level of leptin, using radio immune assay tests, they found a significance decrease in leptin level of smokers when compared to non smokers [3]; others indicated no significant difference between the two groups [4].

Similar studies was done to measure the level of C- reactive protein (CRP) among smokers and non smokers, Dijik and his colleges in 2013 found CRP level was significantly elevated after smoking in chronic obstructive pulmonary disease patients [5], same result was obtained by other researchers among young Iraqi individuals smokers, who showed elevated level of CRP. [6].

On the other hand B2 macroglobulin which is usually increase in peripheral arterial disease, [7] was found to increase significantly in smoking patients with renal dysfunction. [8, 9]

It was noted that most of the available data was controversial, therefore this research was planned to measure the level of CRP, leptin, and B2 microglobulin among healthy young male Iraqi individuals, these are important markers for inflammatory reactions.

## Materials and Methods

Blood samples were collected on day time from 30 healthy male young Iraqi individuals heavy smokers (> 10 cigarettes / day) for more than a year, their ages range was 18- 23 years old and another 30 samples from healthy non smokers, which were age, sex matched, All the studied groups looked healthy and no recent or acute disease, and their body weight was within the normal known range.

Sera were separated from the blood samples as usual by centrifugation at 2000 rpm for 3 minutes, and kept at 4°C to be tested within two days by serological tests..

Estimation of leptin level was done by enzyme linked immunosorbent assay (ELISA) test (DRG, USA): Sandwich ELISA kit was used to estimate the level of leptin, that the wells are coated with monoclonal antibodies against leptin antigenic site, then sera samples were added to the wells (15µL) and incubated for 30 minutes after washing, 15µL of rabbit anti- leptin antibodies were added and incubated for 30 minutes, then washed and 15µL of anti rabbit peroxidase conjugate was added for detection of bounded leptin. The absorbances were read at 450nm, and levels of leptin were estimated from the standard curve [9], the procedure was done according to the manufacture instructions.

ELISA test for B2 microglobulin level estimation (DRG,USA): B2 micoglobulin levels were estimated using sandwich ELISA kit, mouse monoclonal antibody coated the wells, 10 µL of each sample was diluted 100 fold in kit dilution buffer and 10 µL was added to the wells and incubated for 30 min. at 37°C, after washing the conjugate sheep anti-B2 microglobulin was added in volume of 10 µL to each well to be incubated for 30 min. at 37°C, finally wells were washed and 10 µL of the stopping reagent was added, color developed and the absorbance's were read at 450nm, standard curve was drawn and results calculated from the curve [9], the procedure was done according to the manufacture instructions.

ELISA test for CRP level estimation(DRG,USA): ELISA test was used for estimation the level of CRP, utilizing mouse monoclonal antibodies directed against CRP as solid phase immobilization on micro titer wells, sera samples were diluted 100 folds and added to the wells in volume of 10 µL to be

incubated at 37°C for 30min, after washing 10 µL of the conjugate goat anti- CRP ( horseradish peroxidase) was added, and incubated as previously, then washed and 10 µL of the stopping reagent was added for 20 min, then the absorbance's were read at 450 nm, standard curve was done, samples results were calculated from standard curve[9], the procedure was done according to the manufacture instructions.

Statistical analysis: The mean, standard deviation and ANOVA tests were used in the statistical analysis. The p value that was considered statistically significant was < 0.05.

## Results and discussion

Leptin is a hormone that plays important role in food intake, increase energy, decrease appetite and regulates the immune response. Both innate and adaptive immune responses are regulated by leptin .It has a proinflammatory effect activating Th1 response, regulate the proliferation of naive and memory T cells, furthermore, it promotes production of other proinflammatory cytokines as TNF, IL2 and IL6 and it has also a role in some autoimmune diseases[3,9].

The effect of smoking on leptin level was studied by some researchers, however different results were obtained depending on the studied groups, some indicated in healthy young male, the levels of leptin were significantly decreased independently on the amount of body fat, and high amount of smoking was associated with lower leptin level [3], others indicated a gender difference, and high amount of smoking in male is associated with decrease in leptin level [10],

Table 1 shows the mean levels of leptin and B2 microglobulin in sera of 30 young Iraqi male smokers compared to 30 non smokers. It was clear that the level of leptin was significantly lower in smokers ( $16 \pm 0.7$ ng/mL) compared to non smokers ( $24.2 \pm 4.5$ ng/mL), this agrees with other studies, who indicated that nicotine might indirectly reduce leptin secretion in male via enhanced plasma catecholamine concentration [10,11], however others did not find any significant differences between the two groups[4],this may be due to the sex differences as there studied group was female and male and in the presented study all the samples were from young Iraqi males only, this sexual differences was indicated by many studies [ 3,4,10, 11].

Table 1- Levels of leptin and B2 microglobulin among young male healthy smokers compared to non smokers.

Samples (n)	Leptin (ng/mL) Mean $\pm$ S.D	B2 Microglobulin ( $\mu$ g/mL) Mean $\pm$ S.D
Smokers 30	16 $\pm$ 0.7	1.2 $\pm$ 0.3
Non smokers 30	24.2 $\pm$ 4.5	0.6 $\pm$ 0.1

P <0.05 significant for smokers and non smokers for both leptin and B2 microglobulin.

B2 microglobulin is a competent of MHC class 1 molecules, present on all nucleated cells; it is considered as a marker of various neoplastic, inflammatory and infections conditions [9]. Its level rise in some autoimmune diseases and plays important role in renal failure [2, 8, 9]. In the presented study B2 microglobulin level was significantly increased in the smokers (1.2  $\pm$  0.3  $\mu$ g/mL) compared to non smokers (0.6  $\pm$  0.1  $\mu$ g/mL). Similar results were reported, in which the level of B2microglobulin was increased significantly in smoker patients with renal failure [2,8],that smoking results in inhalation of more cadmium which in turn results in changes in proximal tubular function characterized by an increased excretion of B2 microglobulin [2].

C reactive protein is acute phase protein synthesized by hepatocytes and considered important inflammatory marker, as it activates complement, as it is an opsinion that enhance phagocytosis. It was found that the elevated level of CRP may be considered as a marker for many inflammatory diseases as well as risk of progression to type 1 diabetes [9, 10].

Figure-1 shows the level of CRP in sera of smokers and non smokers, it was clear that the level of this protein was significantly higher in smokers (4.07 $\pm$ 0.05mg/L) compared to non smokers (2.8 $\pm$ 0.02mg/L), this agrees with many reports. Dijk and his colleges found that the CPR level increased directly after smoking by 0.13mg/dl and after 35minutes return to normal level in chronic obstructive pulmonary patients [5]. Another report indicated a positive association between smoking status and elevated CRP in young Iraqi individuals, particularly after months of smoking, they indicated that no safe level of smoking in youth for both sexes [6]

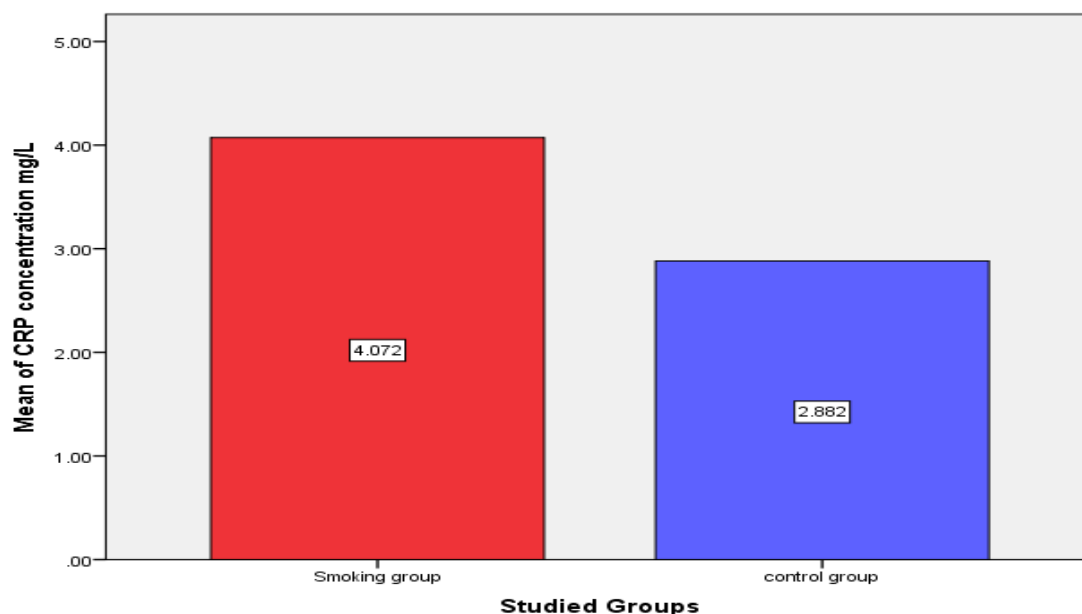


Figure 1- Mean levels of CRP in sera of smokers ( $4.07 \pm 0.05$  mg/L) and non smokers ( $2.08 \pm 0.02$  mg/L), P value was  $< 0.05$  indicating significant differences.

From the presented data, it is concluded that the toxic compounds in tobacco smoking results in immunological changes such as the increase level of leptin, B2microglobulin and CRP, and those proteins are considered important immunological markers for the immunological status of the body and inflammatory cases, when their level increase it may break the immunological balance in the body resulting in different diseases. To find more precise data we recommend that to continue the study to search if the duration of smoking and smoking cigarettes with lower nicotine and tar would give same results or not, thus we recommend a study for that purpose with bigger study groups and different sexes.

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