

Studying of immunoglobulins ,C3 , C4 and IL- 8 concentrations in serum smokers and nonsmokers

دراسة تركيز الكلوبولينات المناعية ومكونات المتمم C3 و C4 و IL-8 في امصال المدخنين و غير المدخنين

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Abstract :

The present study was included fifty healthy person (smokers and nonsmokers).Sera were collected from them .IgA ,IgM ,IgG , complement 3 (C3),and complement4 (C4) concentrations were studied by using single radial immune diffusion .The results showed that IgA and IgG were decreased in smokers in comparison with non smoker while IgM ,C3 and C4 were increased in smokers . Interleukin IL-8 concentration was measured by using ELISA and found that Its concentration was increased in smokers .We found that cigarette smoking has affect on immunity in different forms .Some parameters were increased and other decreased in the same social individuals .

الخلاصة :

تضمنت الدراسة الحالية 50 شخص طبيعى مدخنين وغير مدخنين وقد تم جمع الامصال من هؤلاء الاشخاص واستخدمت لقياس تراكيز IgA و IgG و IgM و C3 و C4 باستخدام طريقة الانتشار المناعي الشعاعي المفرد . وقد اظهرت النتائج ان تركيز IgA و IgG قد قل بالمدخنين مقارنة بغير المدخنين . اما IgM و C3 و C4 فقد اظهرت النتائج زيادة معنوية . اما IL- 8 فقد تم قياس تركيزه باستخدام طريقة الاليزا و اظهرت النتائج قلت تركيزه في المدخنين بشكل معنوي . الدراسة الحالية وضحت بان للتدخين تاثير مختلف على مكونات المناعة فقد يؤدي الى تنشيط البعض ويثبط الاخر لافراد المجتمع الواحد .

Introduction :

Cigarette smoking affects a wide range of immunological functions in human and experimental animals including both humoral and cell mediated immune responses(16,17).Chronic cigarette smoke affects T cell responses in rodents (18, 4.) and human (15 ,14) . The molecular mechanism through which cigarette smoke affects the lymphocyte function is largely unknown .

Chronic exposure of rats to nicotine one of the major components of cigarettes inhibits antibody forming cell response and this immunosuppression is causally related to impairment of antigen mediated signaling in T cells (5) . Some investigator have provided evidence that nicotine activates dendritic cell and augments their capacity to stimulate T cell proliferation and cytokine secretion .These effects of nicotine may contribute to its influence on the progression of atherosclerotic lesions (2). Moreover nicotine was demonstrated to enhance the growth of *Legionella pneumophila* and cause a corresponding inhibition of IL-6 ,TNF- α and IL-12 in murine alveolar macrophage cell line (8) .

The aim of this study was to determine the concentrations of some immunoglobulins(IgG,IgA,IgM) , complement components(C3,C4) and IL-8 concentration in sera of smokers and nonsmokers human .

Materials and Methods:

1- Samples:

The study involved 50 healthy persons(age 18-40 years ,male) in Babylon province in December 2007 . Five ml of blood was collected by using sterile disposable syringe . The blood was put into AFMA disposable tubes without anticoagulant ,then the serum was collected after centrifuge at 2500rpm for five minutes and it was stored at -20 C° .

2- Identification of Igs by using single radial immune diffusion (SRID)

The purpose of all immunodiffusion techniques is to detect the reaction of antigen and antibody by the precipitation reaction .SRID was used to detect concentrations of Igs and C3,C4 (7). This test was prepared and used in according to with manufacturing company (Biomaghrab,Ref 80800 for12 test). The method is following :

- 1- The plate was opened ,if moisture was present it was allowed to evaporate
- 2- Well was filled with 5 µl of serum
- 3- Wet cotton was put in the plate center to avoid agarose dehydration and the plate was closed .
- 4- The plate was flat at room temperature for 48 hours.
- 5- The diameter was measured within 0.1mm with suitable device (eye piece) in microscope ,evaluate result using the table reference.

3- Determination of IL-8 concentration in serum

IL-8 enzyme linked immunosorbent assay was used for determination in serum of smokers and nonsmokers . This test was done according to the manufacturing company (Beckman coulter Tm)as following

- 1- The components of the kit were equilibrated to room temperature before use
- 2- 50 µl of sample was added to each well of plate
- 3- The plate was incubated for 2hr at 18-25 C° while shaking .
- 4- The wells were washed (three cycles) washing buffer
- 5- 50 µl of biotinylated antibody and 100 µl of streptavidin HRP conjugate were added to the wells .
- 6- The plate was incubated 30 min at 18-25 C° while shaking .
- 7- The wells of plate were washed (three cycle) washing buffer
- 8- 100 µl of substrate was added to the wells and then incubated at 10 min at 16-25 C° while shaking .
- 9- 50 µl of stop solution was added to wells and absorbance was read at 450 nm .
- 10- The optical density was recorded and then the results were calculated by using linear equation . $\hat{y} = -49.64 + 251.4 X_i$ (1)

Statistical analysis :

Statistical analysis was performed using student s'T test for parametric data . Data were expressed as mean values \pm SD,N number of subjects .Analysis was done using SPSS software ,version 8 for windows . .

Results

Serum IgG and IgA levels were higher in nonsmokers than in smokers Table (1) and showed high significant at level $P < 0.05$,while IgM levels were increased in smokers compared to non smokers .

Serum C3 and C4 levels were found to be higher in smokers than in non smokers at level $P < 0.05$ Table (2) . Using ELISA method serum IL-8 concentration was determined in smoker and non smokers and we found that its concentration was significant decreased in smoker compared with non smokers at $P < 0.05$ Table (3) .

Table (1) Immunoglobulin concentrations(mg/dl) in smokers and non smokers

	Non smoker (N=20) Mean \pm Standard error	Smoker(N=30) Mean \pm Standard error	P-value
IgG	1313.22 \pm 50.9633	347.226 \pm 23.0333	0.000***
IgM	83.970 \pm 5.5268	256.683 \pm 11.0739	0.000***
IgA	266.16 \pm 8.0301	66.996 \pm 5.7085	0.000***

$P < 0.05$ is significant .

Table (1) C3 and C4 concentrations (mg/dl) in smokers and nonsmokers

	Non smoker(N=20) Mean \pm Standard error	Smoker(N=30) Mean \pm Standard error	P-value
C3	129.290 \pm 22.238	231.980 \pm 13.526	0.000***
C4	25.5700 \pm 1.7847	49.216 \pm 3.4341	0.000**

P < 0.05 is significant

Table (1) IL-8 concentrations (pg/ml) in smokers and nonsmokers

	Non smoker (N=20) Mean \pm Standard error	Smoker (N=30) Mean \pm Standard error	P-value
IL-8	134.38 \pm 8.3094	94.9950 \pm 4.8105	0.000***

P < 0.05 is significant

Discussion :

The result indicated that IgG ,IgA concentrations were highly decreased in smokers in compare with nonsmokers .In table (1) the mean of IgG concentrations in nonsmokers and smokers (1313.22 , 347.226), IgA concentrations (266.16 , 66.996) at the level (P < 0.05) these degree with (11 , 3) .IgM antibody predominates in early primary immune responses to most antigen (13) and this may be to the high components of cigarette some reference indicated that cigarette contains more than 4000 components .C3and C4 concentrations were highly increased in smoker than non smokers , table(2) this may be due to activation of alternative complement pathway . Although smoke does not alter C1 concentration indicating that it does not activate the classical complement pathway. Alternatively since C3 and C4 share a considerable degree of homology in the primary sequence of their alpha chains . This could render both molecules susceptible to smoke induced modifications by thiolester independent mechanism and this may be that smoke treatment produces a heterofore unrecognized form of C3 which has ability to activate alternative pathway without cleavage of its thiolester(6) . IL-8 levels were significantly lower at (P < 0.05)in the smokers (94.9950 pg/ml) when as compared with those of the non smokers (134.38 pg/ml) .IL-8 is produced by leukocyte and non leukocyte cells ,among these cells neutrophils produce a very small quantity of IL-8 .The present study differ form other study (10) that showed high concentrations of IL-8 but agree with (12) this may be due to the immunomodulatory agent of tea component in this study all smokers were drinking tea in many times per day (9) . From this study we conclude that cigarette smoking has affect on immunity in different forms .Some parameters were increased and other decreased in the same social individuals .

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