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The effect of smoking on the inflammatory Interleukine-6 (IL-6) serum level

A Research
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بسم الله الرحمن الرحيم

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا ۖ إِنَّكَ أَنتَ الْعَلِيمُ الْحَكِيمُ (32)

صدق الله العلي العظيم

سورة البقرة :الآية 32

Dedication

To who was present with me at all times in

my heart and mind

To prophet of peace

"Mohammed"

Peace and prayer be on him and his

purified family

To ours great families who encouraged us

To all our friends

Hawraa Abody *Shahad Ali* *Baneen Ali*

Acknowledgment

First of all , praise to "Allah", who enabled me to overcome all the difficulties that were associated with this work till I brought it to the present state . Peace and prayer be on the most honorable

Mohammed and his purified family.

I would like to express my sincere gratitude to Dr. Ghosoun Mohammed- ali Wadai ,our supervisor for her support , encouragement , and useful advise that she provided during this study special thank go to the head of the department Dr. Nazar Hamza for his encouragement , and advise. I would like to thank Dean of Collage of Biotechnology and the staff of Medical department with my gratitude.

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Last, but not least, great gratitude and deep appreciation go to our families who were always listening to my complaints ,frustration, and for believing in me. I am the person who I am today because of

you – Thank you

Abstract

Cigarette smoking affects a wide range of immunological functions in human and experimental animals including both humoral and cell mediated immune responses. This study was done to estimate the concentration of IL-6 serum level as inflammatory indicator in cigarette smokers to find out the effects of smoking. This study was performed the concentration of IL-6 serum level by using (49) blood samples collected of Qadisiyah university healthy students during the period from November 2017-february 2018. The selected samples were classified into two groups: the first group included (20) healthy students that are non-smoking; the second group included (29) smoking students by using the ELISA technique (Sandwich ELISA formate).The result showed low concentration of IL-6 serum level in the patients (46.4pg/ml) significantly at ($P < 0.000$), when compared with those of healthy (96.1pg/ml).

Conclusion: the smoker group represented decrease in the concentration of IL-6 serum level and this condition clarify the smokers' susceptibility to infection by various disease specifically the respiratory tract infection.

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Introduction:

Smoking is one of the most common addictions of modern times. It has been implicated as an etiological agent for various chronic diseases including variety of infections, cancers, heart diseases, and chronic lung diseases, and so is a major 'preventable' cause of morbidity and mortality worldwide (1). Smoking is now increasing rapidly throughout the developing world and is one of the biggest threats to current and future world health (2). Most adult smokers start smoking regularly some time before 18 years of age tobacco use will cause about 18% of all deaths in developed countries and about 11% of all deaths in developing countries(3).

Smoking and Disease

It is well-known that smokers are more susceptible than non-smokers to multiple bacterial infections than are non smokers. Such infections can be life-threatening and both active smokers as well as those exposed to second hand smoke toxins are at increased risk. This important relationship between smoking and ill-health may not be universally appreciated (4, 5), chronic pulmonary obstructive disease (COPD) (6) and so, referred Alexander et.al.,to smoking as inducer for lung diseases(7)

The smoking and immunity:

Cigarette smoking affects a wide range of immunological functions in human and experimental animals including both humoral and cell mediated immune responses(8,9).The effects of smoking on innate immune function are less well studied however; accumulating data suggest that cigarette smoke compromises the immune system and increases susceptibility to infections (10).

Chronic cigarette smoke affects T cell responses in human (11, 12). 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 (13).

The effects of smoking on inflammation:

Acute effects of cigarette smoking (ACS) induces a wide range of (pro) inflammatory responses. All three models (human, animal, and in vitro) studied the effect of ACS on NE, leukotrienes, and IL-6.

An inflammatory response is a general defense mechanism of host immune system to combat invading pathogens [1]. Acute inflammation is a rapid and self-limiting process in which tightly controlled inflammation is critical for host defence, wound healing and maintenance of tissue homeostasis. When the inflammatory cells are not able to eliminate pathogens, however, acute inflammation changes into chronic inflammation that is associated with several disorders including chronic obstructive pulmonary disease (COPD) and lung cancer (LC) [2].

IL-6, which plays a role in innate and adaptive immunity, was also studied in all models. Alveolar macrophage IL-6 activity was decreased after in vitro smoke exposure and IL-6 degradation was increased in BALF of rats (14) No effect of ACS was found on human blood levels of IL-6 (15), suggesting that ACS may have a depressive effect only locally in the bronchial tree or that is compensated for by IL-6 production by other cells.Exposure to cigarette smoke increases oxidative which may lead to vascular inflammation.Current study aimed to investigate the relationship between IL-6 serum concentration as an inflammatory markers and smoking in an attempt to provide explanations for smoking-mediated morbidity and mortality.

The aim of the study: This study was done to estimate the concentration of IL-6 serum level as inflammatory indicator in cigarette and water pipe smokers to find out the effects of smoking.

Sample, Materials and Methods

Sample:

This study was performed by using (49) blood samples collected of Qadisiyah university healthy students during the period from November 2017-february 2018. The selected samples were classified into two groups: the first group included (20) healthy students that are non-smoking; the second group included (29) smoking students, the smoking students was sub-grouped into (16) cigarette smoking only; and (13) cigarette and water pipe smoking.

Table (1-1) : The distribution of Qadisiyah university students

	Gender	Nonsmokers	Cigarette smoker	Cigarette and water pipe smoker
1	Male	20	16	13

Material

1.Instrument &Equipments:

	Equipments	***Manufacturing***
1.	Centrifuge –universal 16 A	Hittch Germany
2.	ELISA system	Bio-test, Germany
3.	Fine and adjustable micropipettes	Gilson, France
4.	Freezer	Ishtar (Iraq)

5.	Oven	Olympus, Japan
6.	PH-meter	Thermo Electron , USA
7.	Refrigerator	Arcelik, Turkey
8.	Sensitive balance	Sartorius (U.S.A)

2. Plastic and Glass Wares

	Plastic and Glass Ware	***Manufacturing***
1.	Automatic pipette	Birhit, Finland
2.	Disposable syringes	Meheco, China
3.	Eppendorf Tubes (0.25, 0.5,1 and 1.5 ml)	Star Lab ,UK
4.	Eppendorf tube	EAPIF, Germany
5.	Gloves	Hungary
6.	Pasteur's pipettes	Volac (England)
7.	Pipette Tips (10 µl , 20 µl , 100 µl , 200 µl and 1 ml)	Star Lab, UK

3. Immunological Kit :

Use Interleukin-6 ELISA kit from Peprotech Company from USA

It is a Human IL-6 ELISA development kit contains Human IL-6 in a sandwich ELISA format in the range of 24-1500pg/ml.

ELISA Solutions Required:

- Block Buffer - 1% BSA in PBS with 0.05% NaN3.
- **Wash Buffer:** 0.005% Tween-20, 0.1% BSA in PBS.

Solutions Preparation:_

- **Phosphate Buffer Saline (PBS):** One tablet of PBS was dissolved in 200 ml distilled water and the pH was adjusted to 7.2, then the solution was autoclaved (121ºC, 15 pounds per square inch for 20 minutes) and stored in the refrigerator (4ºC) until use

- Reagent Diluent1 - 0.1% Bovine Serum Albumine (BSA),5 0.05% Tween 20 in Tris-buffered Saline (20 mMTrizma base, 150 mMNaCl) pH 7.2 - 7.4, 0.2 µm filtered.
- Stop Solution - 2 N H2SO4
- Substrate Solution - 1:1 mixture of Color Reagent A (H2O2) and Color Reagent B (Tetra methyl benzidine)
- Tween 20

Methods:

Collection of Samples

The blood samples were collected from AL-qadisiyah university healthy students according to the blood aspirated technique, which is mentioned by (Baron *et al.*, 1995). The blood sample collected(1-5ml) was transferred to a plain tube, and then it was let to clot at room temperature (20-25 °C) for 15 minutes, centrifuged at 3,000 RPM for 10 minutes to separate the serum, then they were stored at -20ºC until assayed of the serum level.

RECOMMENDED MATERIALS

PLATE PREPARATION

1 - Dilute capture antibody with PBs to a concentration of 100µg/ml. immediately, add 100µ to each ELISA plate well. Seal the plate and incubate overnight at room temperature.

2 - Aspirate the wells to remove liquid and wash the plate 4 times using 300µl of wash buffer per well. After the last ash invert plate to remove residual buffer and blot on paper towel.

3 - Add 300µl block buffer to each well. Incubate for at least 1 hour at room temperature.

4 - Aspirate and wash plate 4 times.

ELIZA PROTOCOL:

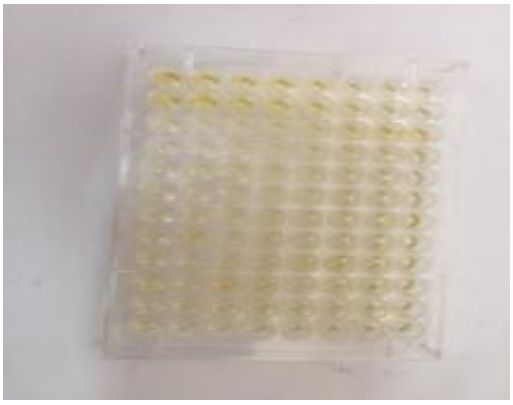
Standard/Sample: Dilute standard from 1.5ng/ml to zero in diluents immediately add 100µl of standard or sample to each well in duplicate .Incubate at room temperature for at least 2 hours.

Detection: Aspirate and wash plate 4 times. Dilute detection antibody in diluents to a concentration of 0.50µg/ml. Add 100µl per well. Incubate at room temperature for hours.

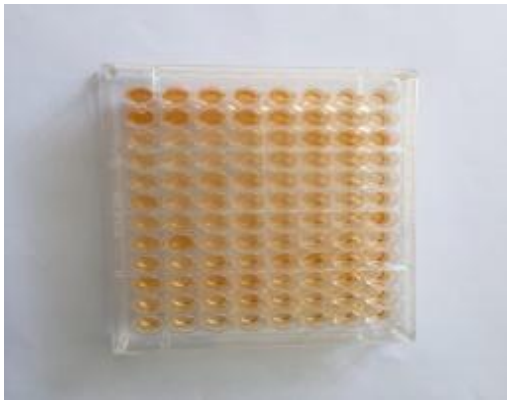
Avidin-HRP Conjugate: Aspirate and wash plate 4 times. Dilute 5.5µl of Avidin HRP-Conjugate. 1:2000 in diluents for total volume of 11ml. Add 100µl per well. Incubate 30 minutes at room temperature.

ABTS Substrate solution:

(ABTS Substrate should be at ambient temperature prior to use)



Aspirate and wash plate 4 times



. Ad 100µl of substrate solution to each well. Incubate at room temperature for color development. Monitor color development with an ELISA plate reader at 495 nm with wavelength correction set at 650nm.

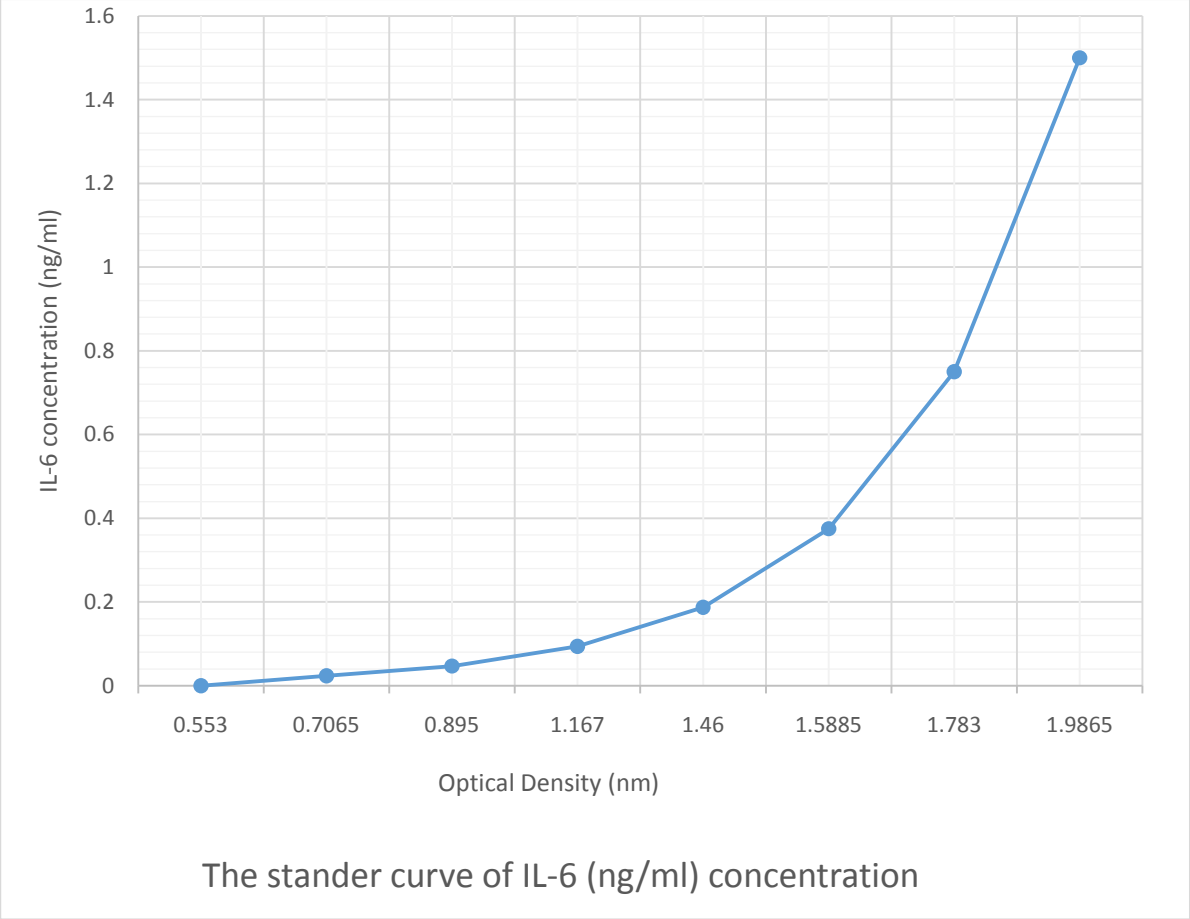
A **B**

A: The plate before added stop solution

B: The plate after added stop solution

Data Calculation:

Average the duplicate readings for each standard, control, and sample was subtracted from the average of zero standard optical density. Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the IL-6 concentrations versus the O.D. and the best fit line can be determined by regression analysis.



The stander curve of IL-6 (ng/ml) concentration

Statistical Analysis:

The statistical analysis system- SAS (2010) was used to study the effect of difference factors on study parameters. Least significant difference –LSD test was used to significant compare between means of cytokine (IL-6) serum level

Results and Discussion:

Smoking is a risk factor of many diseases and contributes to the economic burden worldwide (16).There is a need to assess its toxicity; however, toxic mechanisms underlying smoke related diseases are not yet completely understood.IL-6 has long been considered a general marker of inflammation.Our result explained decrease the concentration of IL-6 serum level as mention below table(1-2) the figure (3-1)

Table (2): Explain the Serum IL-6pg\ml Level for smoker in comparative with healthy

The mean of IL-6 concentration (pg/ml)

Group	No.	Mean ± SE(pg\ml)
		IL-6
Healthy	20	96.1± 13.9
Smoker	29	46.4 ± 11.9
P-Value	--	0.000
LSD Value	--	22 *
* (P<0.000)		

IL-6 levels in the smokers (46.4pg/ml) were significantly lower at (P < 0.000),when compared with those of the non smokers (96.1pg/ml).The present study differed from other study (17) that showed high concentrations of IL-6 but agree with other(18) this may be due to the immunomodulatory agent of tea

component in this study all smokers were drinking tea in many times per day (19)

Twigg and co-workers (20) showed that cigarette smoking decreases the secretion of the pro inflammatory cytokines such as IL-1and IL-6.The cytokines IL-1 and IL-6 are important in the host defence against infection disease.(21,22)

The nicotine modulates the production of inflammatory cytokines by alveolar macrophages [23]. In other study in which cultured macrophage cells we re exposed to cigarette smoke there was a delay in production of key cytokines such as IL-1beta and IL-6 .This was associated with a reduction in NF-κB activation [24], and so the chronic exposure of mice and rats to cigarette smoke or nicotine inhibits T cell responsiveness with decreased antibody response. This inhibition resulted from aberrant antigen-mediated signalling and depletion of calcium stores in animals that were exposed to nicotine [25].

Conclusion and Recommendations

Conclusion:

From this study we conclude that cigarette smoking has bad effect on the inflammatory response where the smoker group represented decrease in the concentration of IL-6 serum level in comparative with healthy group, and this condition clarify the smokers' susceptibility to infection by various disease specifically the respiratory tract infection.

Recommendations:

The Cigarette smoking has a major impact on health issues, and could be related to its ability to compromise the immune system, We recommend by stopping the smoking.

References:

1. Arcavi L. ; Benowitz N.L.(2004). Cigarette smoking and infection. Arch. Intern. Med. 164: 2206–2216.
2. Edwards R.(2004). The problem of tobacco smoking. B.M.J. 328:217–219.
3. World Health Organization.(2005).Global School-based Student Health Survey (GSHS) 2004 Core Questionnaire Module Rationale. WHO, Geneva .
4. Murphy T.F. (2006). Otitis media, bacterial colonization, and the smoking parent. Clin. Infect Dis. 42(7):904-906.
5. Nuorti J.P. ; Butler J.C.; Farley M.M.; Harrison L.H.; McGeer A.; Kolczak M.S.; Breiman R.F.(2000). Cigarette smoking and invasive pneumococcal disease. Active Bacterial Core Surveillance Team. N. Engl J.Med. 342(10):681-689.
6. Pauwels R. A.; and K. F. Rabe. (2004). Burden and clinical features of chronic obstructive pulmonary disease (COPD). Lancet 364: 613–620.

7. Alexander ;L.E; Shin ;S. and Hwang; J.H.(2015) Inflammatory Diseases of the Lung Induced by Conventional Cigarette Smoke. American College of Chest Physicians. 148(5): 1307 - 1322
8. Sopori M. (2002) Effects of cigarette smoke on the immune system .Nat.Rev. Immunol.2:372-377.
9. Sopori M. ; and Kozak W.(1998) Immunomodulary effects of cigarette smoke .J .Neuroimmunol . 83-148
10. Sopori M. .(2002). Effects of cigarette smoke on the immune system. Nat Rev Immunol. 2: 372–377.
11. Peterson B.H. ;Steimel L.F. ; and Callaghan J.T.(1983) Suppression of mitogeninduced lymphocyte transformation in cigarette smokers .Clin.Immunol.Immunopathol .27:135-140.
12. Silverman N.A.; Potvin C.; Alexander J.C. ;and Chretien P.B.(1975) In vitro lymphocyte reactivity and T cell levels in chronic cigarette smokers Clin.Exp. Immunol 22: 285-292.
13. Geng y.; Savage S.M.;Johanson L.J .; Seagrave J. ;and Sopori M. (1995).Effects of nicotine on the immune response .I. chronic exposure to nicotine impairs antigen receptor.
14. Pessina G.P. ;Paulesu L. ; Corradeschi F. ; et al. (1996). Pulmonary catabolism of interleukin- 6 evaluated by lung perfusion of normal and smoker rats. J.PharmPharmacol 48:1063–7.
15. Hockertz S. ;Emmendorffer A. ; Scherer G. ; et al. (1994). Acute effects of smoking and high experimental exposure to environmental tobacco smoke (ETS) on the immune system. Cell Bio lToxicol 10:177–90.
16. Chalouhi N. ; Ali M.S. ; Starke R.M. ; Jabbour P.M. ; Tjoumakaris S.I.; Gonzalez L.F. et al.(2012). Cigarette smoke and inflammation: role in cerebral aneurysm formation and rupture. Mediators Inflamm . 2012:271582.

17. Cesar-Neto J.B.; Duarte P.M. ;de Oliveira M.C.; Tambeli C.H. ; Sallum E.A. ; Nociti F.H. Jr. (2007). Smoking modulates interleukin-6:interleukin-10 and RANKL: osteoprotegerin ratios in the periodontal tissues. J. Periodontal Res 42:184- 91.
18. Matsunaga K.; Klein T.W.; Friedman H. and Yamamoto Y. (2001) Involvement of nicotine acetylcholine receptors in suppression of antimicrobial activity pneumophila infection by nicotine .J.Immunol .167.
19. Huang M.T.; Liu, Y.; Ramji, D.; Lo, C.Y.; Ghai G; Dushenkov S; Ho CT.(2006).Inhibitory effects of black tea the aflavin derivatives on 12-O-tetradecanoylphorbol-13-acetate-induced inflammation and arachidonic acid metabolism in mouse ears. MolNutr Food Res. 50(2):115-22.
20. Twigg H.L. III; Soliman D.M. ;Spain B.A. (1994). Impairedalveolar macrophage accessory cell function and reduced incidence of lymphocytic alveolitis in HIV-infected patients who smoke. AIDS. 8:611-618.
21. Smith K.A. (1988). Interleukin-2: inception, impact, and implications. Science. 240:1169-1176.
22. Luster M.I. ;Simeonova P.P. ; Gallucci R. ; Matheson J. (1999). Tumor necrosis factor-α and toxicology .Crit Rev Toxicol. 29:491-511.
23. Madretsma S. ;Wolters L.M. ;van Dijk J.P. ; Tak C.J. ; Feyerabend C. ;Wilson J.H. ; Zijlstra F.J.(1996). In-vivo effect of nicotine on cytokine production by human non-adherent mononuclear cells. Eur J .Gastro enterolHepatol. 8(10): 1017–20.
24. Birrell M.A. ; Wong S. ; Catley M.C. ; Belvisi M.G. (2007) May 31. Impact of tobacco–smoke on key signaling pathways in the innate immune response in lung macrophages. J. Cell Physiol. [Epub ahead ofprint].
25. Sopori M.L. ; Kozak W. ; Savage S.M. ; Geng Y.; Soszynski D. , KlugerM.J., Perryman E.K.;Snow G.E. . (1998). Psychoneuroendocrinology. 23(2): 189–204.