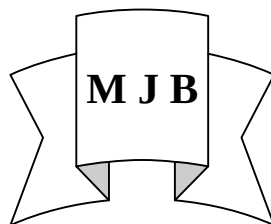


Study of systemic cellular immune responses in smokers

Azhar Ammran

Department of Biology College of Science Babylon University



Abstract

Phagocytic activity by using nitroblue tetrazolium reduction (NBT) and leukocyte migration inhibitory factor (LIF) were studied in thirty health persons (20 smokers and 10 in nonsmokers). Blood with anticoagulant were collected from them and used to study NBT and LIF. The results found that there is no significant differences between smokers and nonsmokers in NBT test. The main of LIF in smokers was 0.528 while in nonsmokers was 0.86 and high significant differences at level $P < 0.05$. this study found that smoking effect on specific cellular immune response but no effect on innate immune response.

الخلاصة

درست فعالية البلعمة وعامل تثبيط هجرة الخلايا في ٣٠ شخص طبيعى (٢٠ مدخنين و ١٠ غير مدخنين) وقد تم جمع الدم باستخدام مائع تخثر لدراسة كل من اختزال الصبغة لدراسة البلعمة وعامل تثبيط هجرة الخلايا للمفاوية لدراسة المناعة الخلوية المتخصصة وظهرت النتائج عدم وجود اختلاف معنوي في عملية البلعمة بين المدخنين وغير المدخنين ولكن هناك فرق معنوي كبير بمستوى معنوية $P < 0.05$ في اختبار تثبيط الهجرة نستنتج من هذه الدراسة ان التدخين يؤثر على استجابة المناعة الخلوية المتخصصة ولا يؤثر على المناعة غير المتخصصة.

Introduction

Tobacco smoking exerts many effects the majority of which are un available on almost all tissue or organ including the respiratory ,cardiovascular ,nervous endocrine and gastrointestinal system.(1).as far as the effect of smoking on the haematopiotic system is concerned ,lucocytosis has been will recognized (2).The lucocytosis includes neutrophil lymphocytosis and in a certain analysis monocytosis (3).Regarding cigarette smoking , it has been shown , in an animals study , that an accelerates release of neutrophil from the born marrow lead to lucocytosis. Therefore IL-8 seems to be a possible candidate cytokine responsible for lucocytosis in smokers ,while smoking tobacco , it

likely that neutrophil circulating in the blood vessels of the respiratory tract exposed to high concentration of nicotine, kind of alkaloid and major substance in tobacco smoke(4) .Some studies investigate that's cigarette tobacco smoke contain substances which initiate , promote and or accelerate tumor grant in experimental animals (5).The immune suppression exhibited by a water soluble condensate of tobacco smoke has been in vivo and in vitro.studies on the cellular basis of the immune suppression induced by water soluble cigarette showed a decrease in T-lymphocyte in the spleen of mice. A less marked suppression of b-cell function was noted in condensate treated . in contrast macrophage from water soluble cigarette treated animals enhanced the response of

normal t and b cell (6).The present work has been take to study the effect of smoking on some cellular immune response factors such as phagocytosis and leukocyte inhibition factors in human serum smokers and non smokers

Materials and Methods:

1- Samples:

The study involved 30 normal 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 with anticoagulant for phagocytosis, LIF test

NBT dye reduction test:

NBT test was performed by the method of (7), 0.1ml of blood was mixed with 0.1ml of NBT solution in a well of micro titer plate. The mixture was mixed gently and covered to ensure humidity, and incubated at 37C° for 15min. follow this with an equal period at room temperature.

Smear was then made , immersed with Wright stain , left for 5min. then rinsed with Wright stain buffer and allowed to dry. The slide was examined under oil immersion. 100 PMN was counted and then percentage of PMNs reduce NBT was recorded.

3.7.2: Leukocyte migration studies:

The leukocyte migration studies were done according to method of (8).

- 1- Two capillary tubes was filled with heparinized blood sample
- 2- The capillary tubes were centrifuged in haematocrite centrifuge for 5min. (1.5 min. in case of tonsils suspension).
- 3- The capillaries were broken at

The migration was read with and without sensitizer under light microscope using ocular micrometer to read the distance of migration.

The average of migration area with sensitizer (Mz) was related to the average of migration area in control (Mo) and expressed as follows:

$$\text{Migration index} = \text{Mz/Mo}$$

Statistical analysis :

Statistical analysis were performed using student s' test for parametric data .. by using SPSS 8.

Results

Assessment of phagocytosis

Nitro blue tetrazolium reduction dye (NBT). As illustrated in table (1) smoking has no significant effect on percent Polymorphoneuclear (PMN) positive for NBT in peripheral blood of smokers serum compare with non smoker serum control

Leukocyte inhibition factor (LIF).

Comparison between smokers and control group non smokers group was observed using independent-sample T test inhibition in the smokers compared with control

Discussion

Neutrophil are known to play an important role in inflammatory responses by virtue of their ability to perform a series of effectors function that collectively represent as a major mechanism of innate immunity against injury or infection .It has become obvious that the contribution made by neutrophil to has defense and natural immunity extends well beyond their traditional role as professional phagocytosis (9).NBT test is non specific cytochemical of neutrophil cytoplasm membrane function and membrane changes may be induced in vivo(10).As show in table (1) no significant effect was observed in comparing between smokers and non smokers serum. However the effect of nicotine on immune cell is incompletely

characterized and controversial .Some investigators have provided evidence that nicotine promotes inflammation. By contrast other studies indicates that nicotine may have immunosuppressive effective ,although these results were achieved with relatively high doses of nicotine(11). Leukocyte migration factors was originally described as T-cell product and later found to also be generated by pituitary cell (12).The table (2)show Leukocyte migration factors in smoker were significant compare with non smokers at level $P < 0.05$ this may

be that cigarette smoking inhibit the migration this, agreed with other study the role effect of cigarette smoke on endothelial function may be quite different from that of nicotine alone ,some results show a decrease in immune responses in the presence of nicotine (13).Degree effect of nicotine or tobacco cigarette is immunoresponssivnes depended on some factors such as periods of smoking ,kinds of cigarettes ,age and some others(6)

Table(1):Precent PMN positive for NBT in peripheral blood of smokers.

NO	Smokers Mean $\pm$ Standard error	P-value
Smokers 20	60.8000 $\pm$ 4.5431	0.000***
Non smokers 10	71.500 $\pm$ 5.0470	0.000***

*** $P < 0.05$ is highly significant .

Table (2):Systemic LIF in peripheral blood ofsmokers

No	Smokers Mean $\pm$ Standard error	P-value
Smokers 20	.5280 $\pm$ 4.498E-02	0.1
Non smokers 10	.8650 $\pm$ 6.336E-02	0.1

$P < 0.05$ is non significant

References

- 1-Benowitz,N.L.(1996). Parmacology of nicotin.Annu.PharmacolTaxicol.36:597-613.
- 2-Freedman,D.S.;Flanders W.D.;Barborisc J.J;Malaroger A.M.(1996)Cigarette smoking and leukocyte subpopulation

in men.Ann.Epidemiol.299-306

- 3-Schwarts J.and Weiss S.T.(1994).Cigarette smoking and periphirel blood lukocyte differentiate.Ann.Epidemiol.4:236-242

- 4-Iho S. ;Tanak Y.;Kobayashi C.;Muramatsu I.;Iwasaki H.; Nakamura K.;SasakiY.; Nakao K.;and Takahashi T. (2003) Nicotine induces human neutrophils to produce IL-8 through the generation of peroxynitrite and subsequent activation of Nf-K β .Journal of leukocyte
- 5-Hoffman D. and Wynder E.L.(1971). A study of tobaccocarcinogenesis .XI.Tumor initiators,accelerators and promoting activity of condensate ,fractions.Cancer,27:848
- 6-Jacobc.v.;Stelzer G.T. and Wallace J.H.(1980).The influence of cigarette tobacco smoke products on the immune response.Immunology ,48:612
- 7-Park,B.H. ; Fikrig,S.M. and Smithwick,E.M.(1968) Infection and nitro blue tetrazolium reduction by neutrophils. Lancet, 2, 532-534.
- 8-Soborg,M.(1969) Interaction of human peripheral lymphocytes and granulocytes in the migration-inhibitionreactions. Acta.Med.Scand 185:221.
- 9-Ryu,J.S. ; Kang,J.H. ; Jung,S.Y. ; Shin,M.H. ; Park,H. ; and Min,D.Y.(2004) Production of interlukin-8 by human neutrophils stimulated with Trichomonas vaginalis . Infection and immunity. 72(3): 1326-1332.
- 10-Gordon, P.A. ; Stuart, J. ; Lee, T.R. , Breeze,G.R. , and Pugh,R.N.H. (1975) The cytocenterifuge NBT test. J.Clin.Path. .28: 674-679.
- 11-Aicher A.; Heeschen C.; Mohaupt M. ;Cook J. ; Zeiher A. ; Dimmeler S. (2007).Nicotine strongly activates denderitic cell mediated adaptive immunity potential role for progression. Journal of the American heart ssociation
- 12-Riedemann,N.C. ; Guo,R.F ; Gao,H. ; Sun,L. ; Hoesel,M. ; Hollmann,T.J. ; Wetsel, R.A. ; Zetoune,F.S. ; Ward,P.A.(2004) Regulatory role of C5a on macrophage migration inhibitory factor release from neutrophils. The American Association of immunology. 173: 1355-1359.
- 13- 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.