Original Article

Comparison Of Serum Levels Of IL-6 And TNF-A Cytokines InSmokers And Non-Smokers According To Buerger's Wounds

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Abstract

Introduction: Thromboangiitis Obliterans or Buerger Disease is a non-atherosclerotic obstructivesyndrome of small to medium arteries of the upper and lower extremities that causes blockage, recurrent and progressive inflammation, and clotting of the walls of the arteries. It becomes. Thisdisease is usually associated with the use of tobacco derivatives, especially smoking, but it is also associated with the consumption of smokeless tobacco.

Method: 108 healthy smokers and non-smokers who participated in the recall of Buerger's diseasewere studied and clinical information on smoking and drug use was entered in the relevant forms.Patients' informed consent was obtained to participate in research and experiments on their bloodsamples. 10 cc of blood was taken from each patient and serum was isolated and used for relevanttests. ELISA method was used to measure the concentration of cytokines IL-6 and TNF-α. After data collection, SPSS software version 24 was used for data analysis.

Results: The mean age of Buerger patients in the present study was 49.67± 8.91 years. The most common clinical variables during the study, the results showed that in smokers there are significantchanges in serum IL-6 levels compared to non-smokers ($p < 0.05$).

Conclusion: Considering the significant relationship between immune system components and some clinical symptoms and the role of their abnormal function in Buerger disease, they can a criterion for determining the course of Buerger disease or based on them, treatment strategy determined.

Keywords: Smokers, Tobacco, IL-6, TNF-α Buerger disease, Immune system.

Introduction

Buerger's disease, like Von Winiwarter, made distinct clinical and pathological points about the disease in relation to atherosclerosis(1). In 1928, Allen and Brown examined 200 cases of TAO patients at the Mayo Clinic between 1922 and 1926 and described their clinical and pathological features. Most of these patients were Jewish men and all of them were professional smokers(2). Ithas been suggested that TAO is an inflammatory disease of the end of the arteries, which is characterized by T-dependent cellular immunity and B-dependent humoral immunity and is associated with the activation of macrophages and denitric cells in the intima(3). The production of various autoantibodies and cellular sensitivities against vascular components has been observed.Abnormalities in immune responses are believed to trigger

inflammation(3,4). High titers of endothelial cell antibodies are also seen in Buerger patients. In general, endothelial cells play an essential role in the migration of inflammatory cells at the site of inflammation through the expression of adhesive molecules on their surface (5). Vascular endothelial damage is an importantaspect of TAO disease. Following endocrine function and paracrine synthesis, endothelial cells secrete significant amounts of vasodilators and constrictors to maintain vascular structure and functional integrity. When this tissue is damaged, it loses its function and as a result, abnormal secretion of cytokines leads to altered endothelial permeability (6). More than 100 years after thromboangitis obliterans, the cause of the disease remains unclear (7). TAO, also known as Buerger's disease, is a non-atherosclerotic inflammatory disease and is common among smokers(8). The disease spontaneously progresses to tissue loss and complete amputation, and inthe 50s and 60s of the disease, the disease itself is limited and diminished(9).

First of all, smoking is known as the main cause and progression of this disease, but at the same time, it is not known as the cause and onset(10). There are hypotheses that the immune system may be involved in the disease(3,4,11). In general, most autoimmune diseases are closely related to abnormalities in the regulation of cytokines, increased levels of lymphocyte apoptosis, and thecontinued presence of immune complexes in the bloodstream and body fluids(12). There is currently little evidence of immune regulation abnormalities in TAO patients that affect humoral and cellular immunity. In the meantime, there have been reports of changes in the secretion level and functional pattern of some immune system factors, which confirms the hypotheses(13). Immunological studies indicate that during inflammation, various mediators are generally mediated by immune system cells, including proinflammatory cytokines such as IL-6 secretion and TNFαα, which intensify the immune response(14–16). All of this has focused our attention onthe production of cytokines and other circulatory factors in TAO patients. Thus, since these factorsaffect each other, in this study, we investigated the serum levels of IL-6 and TNF-α factors in smokers and non-smokers. It is hoped that information on the effects of smoking on the disease and its relationship to the functioning of the immune system will be obtained and a step will be taken to advance the recognition of this complication.

Material and methods

The samples of this study consist of two groups of smokers and non-smokers. Blood samples of non-smokers were collected from non-smokers who donated blood in Shiraz blood transfusion center and blood samples of smokers were collected from Imam Reza (AS) Hospital in Mashhad. Through a comprehensive questionnaire, we extracted information about each individual completed by the individual. In this study, 108 people, 57 non-smokers and 51 smokers, were studied. The mean age in non-smoking and smoking groups was 49.67 ±8.91 and 48.08 ±7.7, respectively. The number and mean age of participants in this study in the two groups of non- smokers and smokers can be seen in Table .1.

Table .1. Characteristics of the study population

Characteristics	smoker	non-smoker
n	51	57
Mean age	48/08 ± 7/7	49/67 ± 8/91

10 cc of blood was taken from each person by a professional technician and poured into metal-freetubes containing anticoagulants (EDTA). Then, in order to separate the blood plasma, the blood sample was centrifuged at 1000 g for 10 minutes. Blood cells were precipitated and plasma solutionwas placed on the surface. The maximum time allowed for plasma separation is two hours. levelsof IL-6 and TNF-α measured by ELISA of IL-6 and TNF-α kits purchased from KPG Iran. The results of this study were analyzed using SPSS 24 statistical software and using Chi-square (2χ), t-test and Mann-Whitney test (for abnormal data) with a significance level of $P < 0.05$.

Results

Age distribution

The non-smokers participating in this study are 57 people with a mean age of 49.67 ±8.91 years. Also, 51 smokers with a mean age of 48.08 ±7.7 participated in this study. As the results show (t
= 0.98, df = 106, P = 0.327) there is no significant difference between the mean age of the non- smoking and smoking groups.

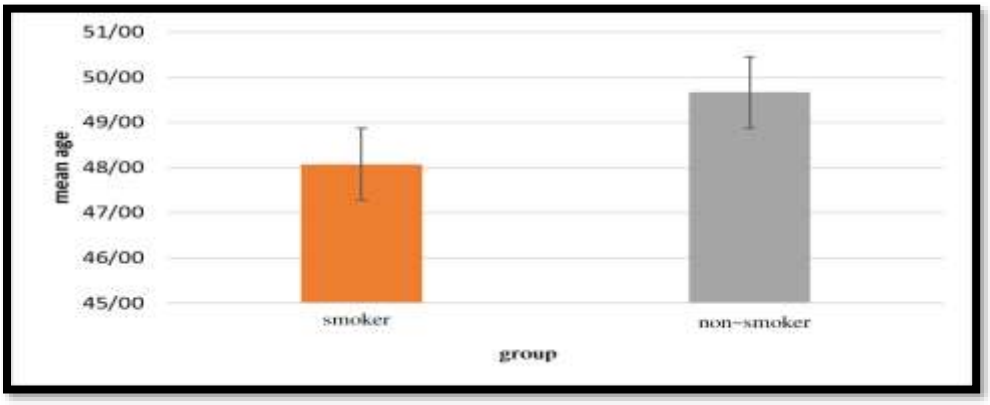


Diagram .1: Age distribution (year) in smokers and non-smokers

Weight distribution

The non-smokers participating in this study are 57 people with an average of 70.26 ±12.28. Also,51 smokers with an average of 75.2 ±13.56 participated in this study. As the results show (t = 1.98,df = 106, P = 0.048) the average weight of the smoking group is higher than the non-smoking group.

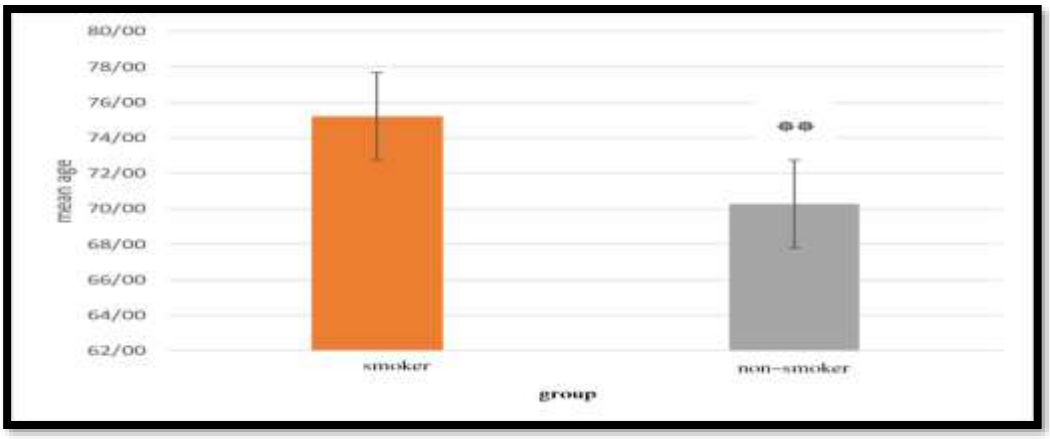


Diagram .2: Weight distribution in smokers and non-smokers

BMI distribution

The non-smokers participating in this study are 57 people with an average of 25.21± 3.74. Also, 51 non-smokers with an average of 26.45± 4.18 participated in this study. As the results show (t =1.62, df = 106, P = 0.108) there is no significant difference between the mean BMI of the non- smoking and smoking groups.

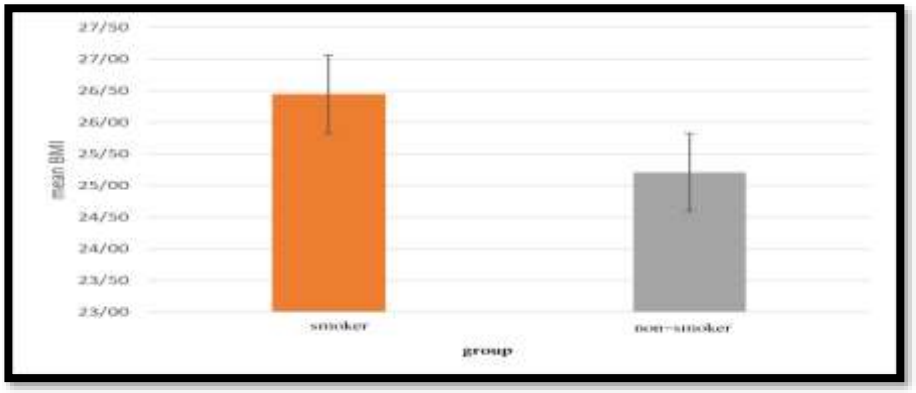


Diagram .3: BMI distribution in smokers and non-smokers

Distribution of white blood cells (WBC)

The non-smokers participating in this study are 57 people with a mean of 6.56 ±1.72. Also, 51smokers with an average of 6.12 ±1.52 participated in this study. As the results show (t = 1.38, df
= 106, P = 0.170, Z = 1.77, P = 0.077), where Z is the Mann-Whitney standardized statistic. Thereis no significant difference between the mean WBC of the non-smoking and smoking groups.

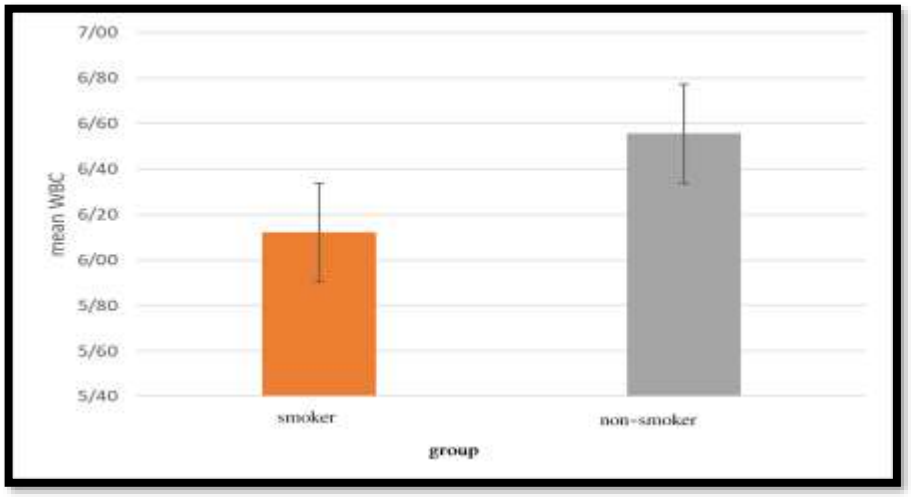


Diagram.4: Distribution of WBC in smokers and non-smokers

Distribution of red blood cells (RBC)

The non-smokers participating in this study are 57 with a mean of 5.11 ± 0.43. Also, 51 smokers with a mean of 5.18 ±0.38 participated in this study. As the results show (t = 0.81, df = 106, P = 0.419, Z = 1.09, P = 0.275). There was no significant difference between the mean RBCs of the non-smoking and smoking groups.

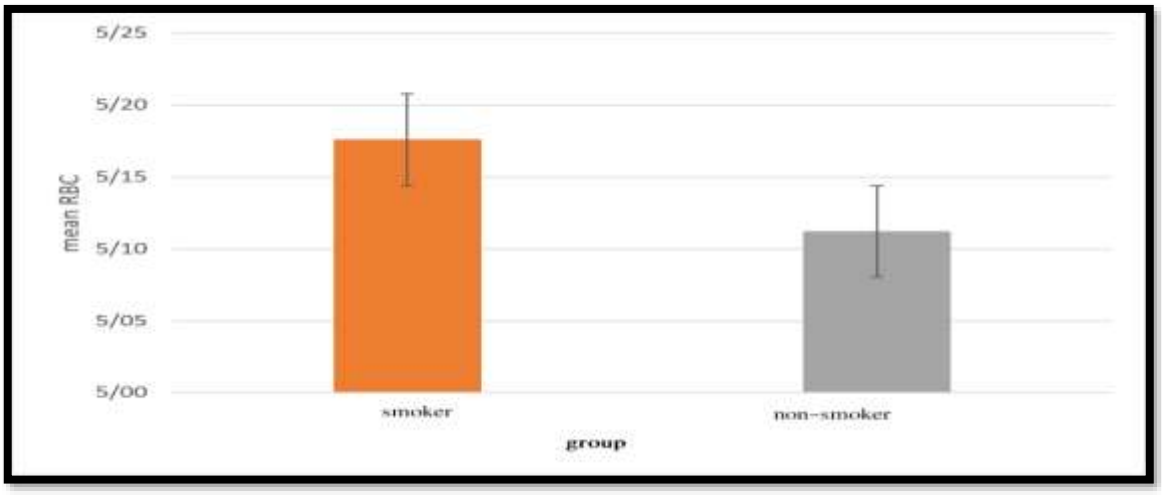
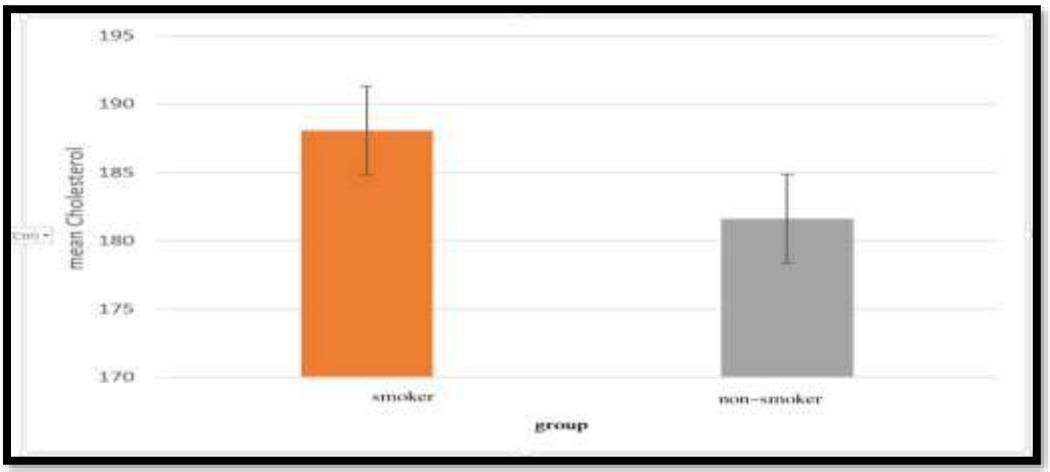


Diagram .5: Distribution of RBC in smokers and non-smokers

Cholesterol distribution

The non-smokers participating in this study are 57 people with a mean of 181.61 ±31.92. Also, 51smokers with an average of 188.08 ±36.75 participated in this study. As the results show (t = 0.98,df = 106, P = 0.330), there is no significant difference between the mean cholesterol of the non- smoking and smoking groups.

Diagram 6: Distribution of cholesterol in smokers and non-smokers



Distribution of hemoglobin (HGB)

The non-smokers participating in this study are 57 people with a mean of 14.74± 1.18. Also 51 smokers with an average of 14.64 ±1.65 participated in this study. As the results show (t = 0.42, df = 106, P = 0.675) There was no significant difference between the mean HGB of non-smokersand smokers.

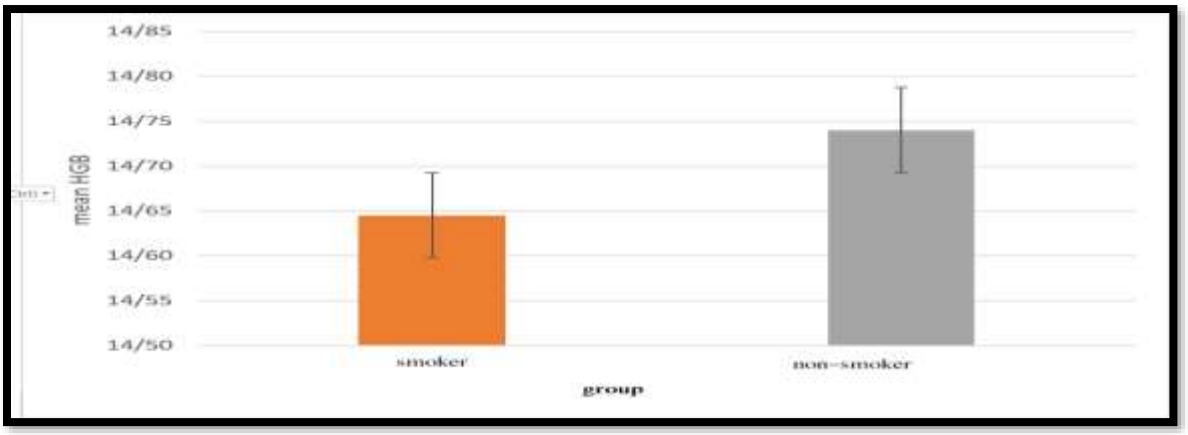


Diagram.7: Distribution of HGB in smokers and non-smokers

LYM_P distribution

The non-smokers participating in this study are 57 with an average of 34.75 ± 6.46. Also, 51 smokers with an average of 35.28 ±7.62 participated in this study. As the results show (t = 0.39, df = 106, P = 0.694) There is no significant difference between the mean LYM_P of non-smokersand smokers.

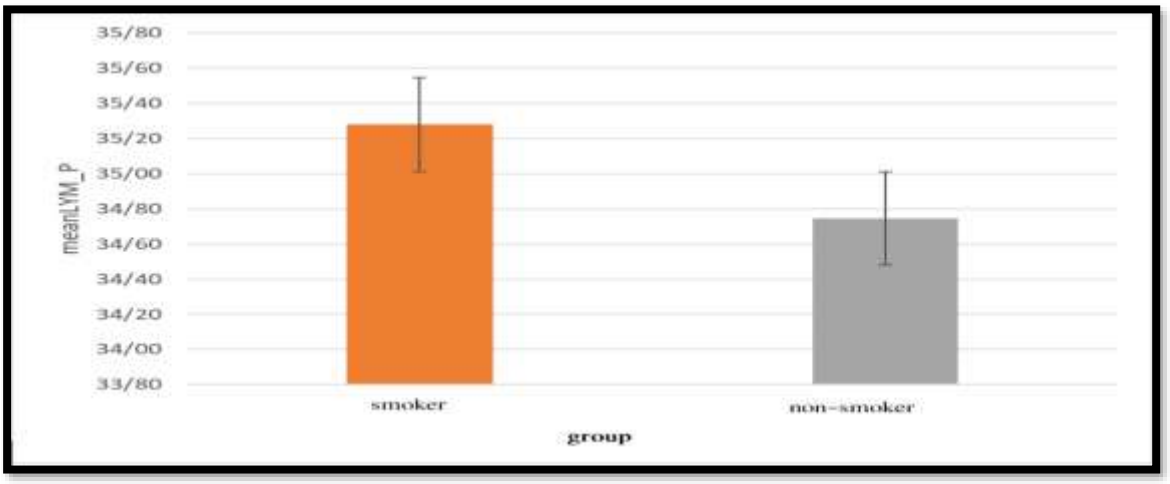


Diagram. 8: Distribution of LYM_P cholesterol in smokers and non-smokers

NEUT_P distribution

The non-smokers participating in this study are 57 with a mean of 53.87± 9.76. Also, 51 smokerswith a mean of 54.72 ±9.91 participated in this study. As the results show (t = 0.44, df = 100, P =0.664) There is no significant difference between the mean NEUT_P of non-smokers and smokers.

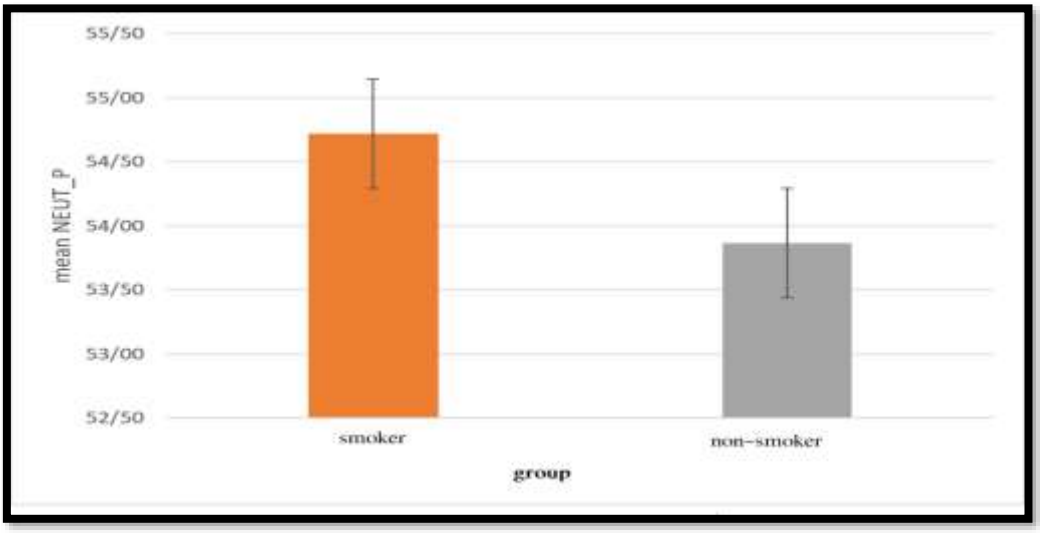


Diagram.9: Distribution of NEUT_P cholesterol in smokers and non-smokers

LYM_Sh distribution

The non-smokers participating in this study are 57 with a mean of 2.29± 0.73. Also, 51 smokerswith an average of 2.12 ±0.62 participated in this study. As the results show (t = 1.24, df = 106, P
= 0.219; Z = 1.48, P = 0.137) There is no significant difference between the mean LYM_Sh of thenon-smoking and smoking groups.

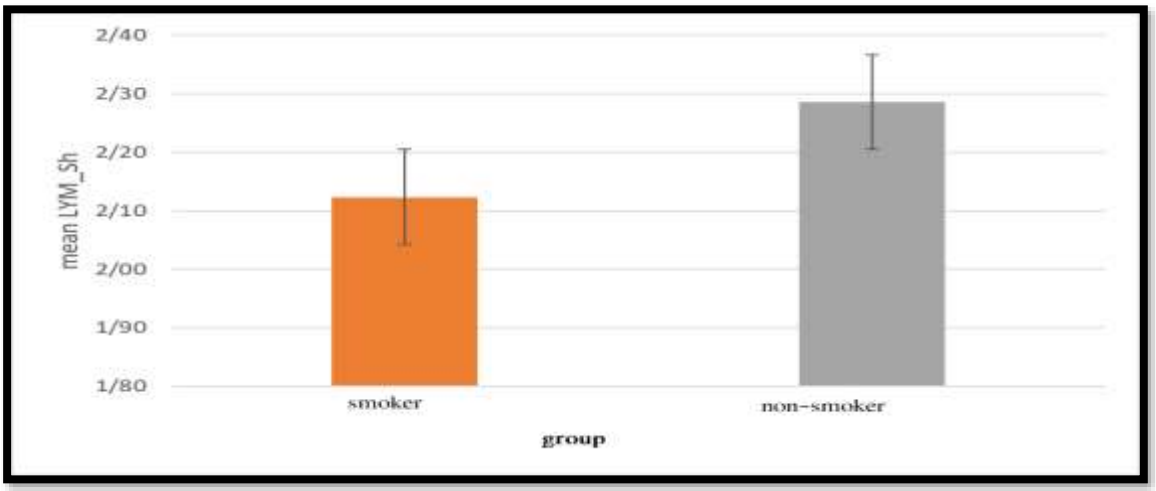


Diagram.10: Distribution of LYM_Sh in smokers and non-smokers

Distribution of NEUT_Sh

The non-smokers participating in this study are 57 people with an average of 3.54± 1.19. Also, 51smokers with an average of 3.4 ±1.22 participated in this study. As the results show (t = 0.59, df =100, P = 0.559; Z = 0.67, P = 0.503) There was no significant difference between the mean NEUT_Sh of the non-smoking and smoking groups.

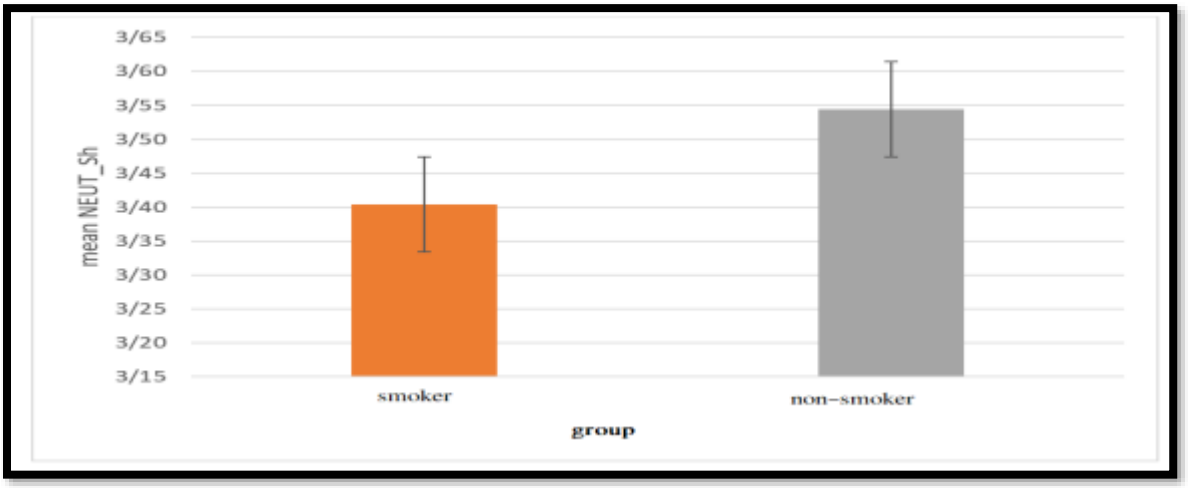


Diagram.11: Distribution of NEUT_Sh in smokers and non-smoker

Distribution of IL-6 and TNFα

To compare IL-6 and TNFα levels in smokers and non-smokers, first a standard curve was drawnand based on this curve, the concentration of the sample was predicted to be R2 of the fitted modelsequal to 0.9975.

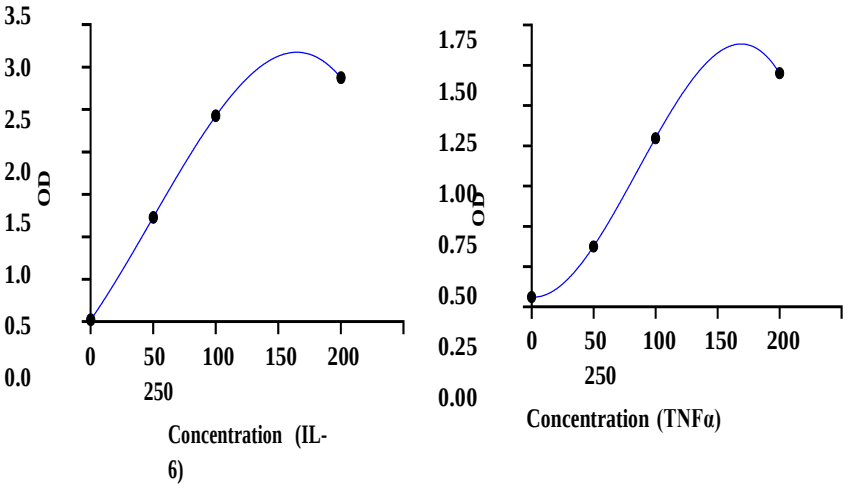


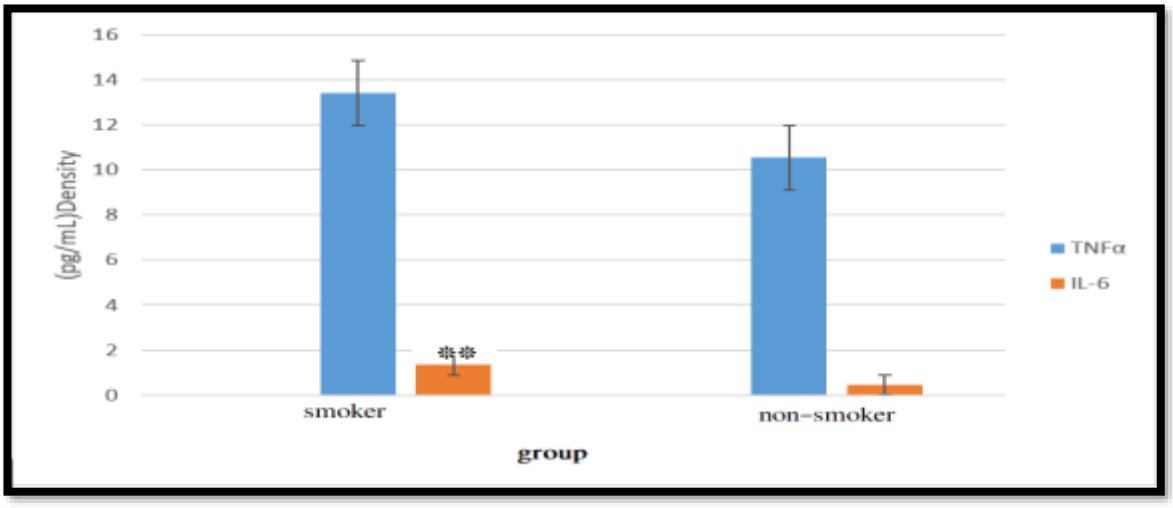
Diagram.12: Standard curves of IL-6 and TNFα

The mean IL-6 concentration of smokers participating in this study was 0.78± 1.18. Also, the meanconcentration of IL-6 in non-smokers participating in this study is 1.45± 0.72. As the results show

(t = 2.65, df = 59, P = 0.01; Z = 3.35, P = 0.001) the concentration of IL-6 in the non-smoking group is higher than the smoking group. The mean TNFα concentration of smokers participating in this study was 11.32± 7.9. Also, the mean concentration of TNFα in non-smokers participatingin this study is 13.4± 6.54. As the results show (t = 1.03, df = 53, P = 0.305; Z = 1.66, P = 0.097)there is no significant difference between the mean concentration of TNFα in the non-smoking andsmoking groups.

Diagram 4.13: Distribution of IL-6 and TNF-α concentrations in smokers and non-smokers

4.1.13. Obesity distribution



Among the non-smokers participating in this study, 52 have obesity less than 30 and 5 have obesitymore than 30. Also, out of 51 smokers participating in this study, 43 have obesity less than 30 and8 have obesity more than 30. There was no significant difference between obesity of non-smokersand smokers (P = 0.27, $\chi 2$ = 1.21).

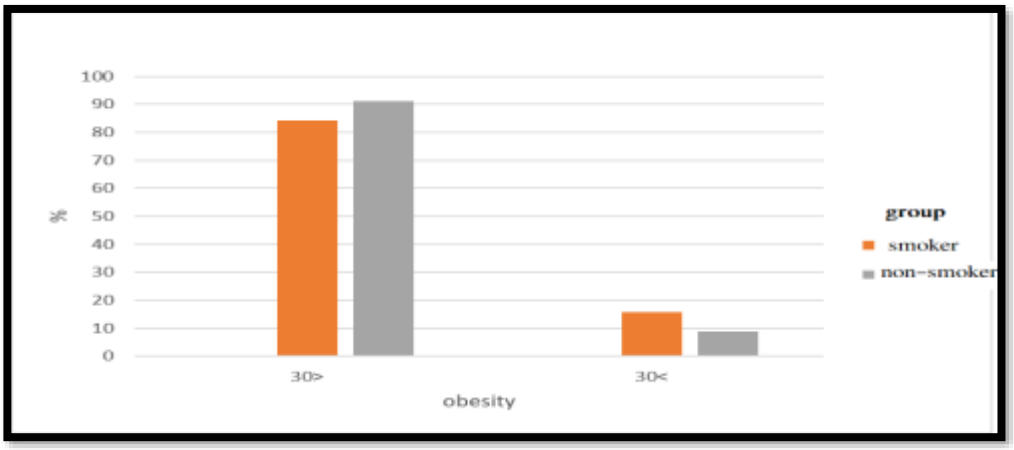


Diagram.14: Obesity distribution in smokers and non-smoke

Gender distribution

Among the non-smokers participating in this study, 56 are male and 1 is female. Also, out of 51 smokers participating in this study, 48 are male and 3 are female. There was no significant difference between the genders of the non-smokers and smokers (P = 0.257, $\chi 2$ = 1.29).

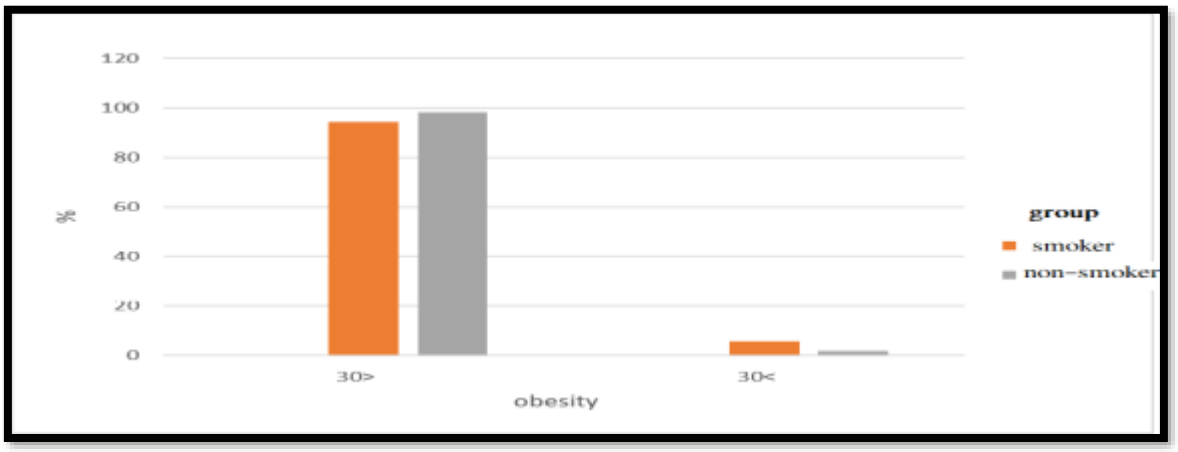


Diagram.15: Gender distribution in smokers and non-smokers

Discussion

Buerger Disease (Thromboangiitis Obliterans) is a non-atherosclerotic obstructive disorder of thearteries (arteries and veins) of small to medium upper and lower limbs(17,18). The cause of this blockage is recurrent and progressive inflammation and the formation of clots in the walls of the arteries (19). This disease is usually associated with the use of tobacco derivatives, especially smoking (20,21). But it is also associated with the consumption of smokeless tobacco. Symptomsinclude intermittent pain in the fingers and painful sores on the tips of the fingers and toes. Buerger disease has no definitive cure and amputation is sometimes necessary. The only way to prevent the rapid progression of the disease is to completely quit smoking and tobacco products(21,22). Despite the declining incidence of Buerger disease in North America and Western Europe, its prevalence is increasing in Central, Far Eastern, and Mediterranean societies. Our country, Iran, isone of the countries affected by this disease (4). The main causes of Buerger disease are not known,but smoking plays an important role in its occurrence (23). However, cases have been reported inthe review of articles that the person was not a smoker (24). Due to the fact that many smokers donot have this disease, other factors may be auxiliary or independent of Buerger etiologies. Numerous other factors such as drug use, bacterial-viral etiology, environmental conditions and genetics are considered as causative agents of this disease(9).

The current study investigated two cytokines TNF-α, IL-6, the main results of the present study, in comparison between smokers and non-smokers, showed that smokers show an increase in proinflammatory cytokines (significant increase in IL-6 but no significant increase in TNF- αlph)(14). In the study of R. Dellalibera-Joviliano and colleagues noted that the increase in TNF-α and IL-6 levels in patients with TAO was significant when compared to controls. The presentedresults indicate an increase in the production of cytokines in TAO, which probably contributes tothe inflammatory response observed on the vascular surface of patients, meaning that there may be a link between smoking and TAO (14). Ping Zheng and his team stated that TAO is associatedwith increased levels of the cytokine (TNF) -α, IL-6 (9,25). Previous studies have not systematically examined the extent of tobacco dependence in individuals with elevated cytokine levels(20,26). Using the study population with the longest recorded follow-up time, this study evaluated the levels of cytokines TNF-α, IL-6 in smokers and differed from those in non-smokers.The framework for this argument is that some researchers hypothesize that tobacco dependence and Buerger disease have a common

biological mechanism. Our hypothesis was that smokers would have higher cytokine levels. Masahiro Matsushita et al. Show that although Buerger diseaseis closely related to smoking, no objective analysis of smoke-related problems has been performed(27,31). Overall, this study examined further evidence, including data on the relationship between smoking status and body weight or BMI in Asian or Southeast Asian samples is low and is usuallynot representative of national studies (28,29). Few previous studies have examined the prevalence ofsmoking and the relationship between smoking and BMI and body weight in Asia or specifically in Thailand. However, there have been many studies in Western countries that have examined therelationship between smoking and body weight, and they typically find that current smokers havea BMI and lower body weight than former smokers or never(21-30). studies showed that the relationship between smoking status and macronutrients was statistically significant only in men.Compared to non-smokers, male smokers actually consumed more total energy (kcal), protein (g)and carbohydrates (g). This finding, along with other studies, shows that smokers consume more energy than non-smokers(21,29,30,32,33) .
In this study, non-smokers participating in this study are 57 people with an average of 70.26

±12.28. Also, 51 smokers with an average of 75.2 ±13.56 participated in this study. As the resultsshow ($t = 1.98$, $df = 106$, $P = 0.048$) the average weight of the smoking group is higher than the non-smoking group. Considering the available evidence on the relationship between smoking and immune responses and the role of these responses, especially cellular immune responses in smokers, the present study examined the levels of inflammatory cytokines in smokers and non- smokers. According to the results obtained from ELISA method, the average concentration of IL-6 in smokers participating in this study is 0.78±1.18. Also, the mean concentration of IL-6 in non-smokers participating in this study is 1.45± 0.72. As the results show ($t = 2.65$, $df = 59$, $P = 0.01$; $Z = 3.35$, $P = 0.001$) the concentration of IL-6 in the non-smoking group is higher than the smoking group. The mean TNFα concentration of smokers participating in this study was 11.32± 7.9.
Also, the mean concentration of TNFα in non-smokers participating in this study is 13.4± 6.54. Asthe results show ($t = 1.03$, $df = 53$, $P = 0.305$; $Z = 1.66$, $P = 0.097$) there is no significant differencebetween the mean concentration of TNFα in the non-smoking and smoking groups.

Conclusion

In the present study, which is one of the comprehensive studies on the samples of patients with Buerger's, 108 individuals (51 smokers and 57 non-smokers) in terms of clinical findings and findings related to hemorrhagic immunity and part of the findings Cellular cells were examined. The results showed that there was a significant change in IL-6 concentration in the group of smokers compared to the group of non-smokers. This result could be a link between immune cytokine levels and smoking, and may therefore contribute to the inflammatory processes of Buerger disease

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