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Effect of cigarette smoking based on hematological parameters: comparison between male smokers and non-smokers

[Sigara kullanımının hematolojik parametrelere etkisi: Sigara kullanan erkekler ile kullanmayan bireylerin karşılaştırılması]*

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ABSTRACT

Objective: The effects of cigarette smoking on human health are serious and in many cases, deadly. The aim of the present study was to assess the extent of adverse effects of cigarette smoking on biochemical characteristics of blood in male population of Quetta city in Pakistan.

Subjects and Method: One hundred and forty two male subjects participated in this study: smokers (n=71) and nonsmokers (n=71). The smokers were regularly consuming 11-20 cigarettes per day for at least 3 years. Complete blood cell count were measured by Nihon Codon fully automatic Hematological analyzer.

Results: The smokers had significantly higher levels of white blood cell ($p<0.027$), red blood cell ($p<0.011$), hemoglobin ($p<0.001$) and hematocrit ($p<0.006$), whereas mean corpuscular hemoglobin concentration ($p<0.009$) and platelet crit ($p<0.017$) were significantly lower.

Conclusion: In conclusion, our findings showed that continuous cigarette smoking has severe adverse affects on hematological parameters (*e.g.,* hemoglobin, hematocrit, WBC count, RBC count, and platelet crit) and these alterations might be associated with a greater risk for developing atherosclerosis, polycythemia vera, chronic obstructive pulmonary disease and/or cardiovascular diseases.

Key Words: Cigarette smoking, hematological parameters, blood cells count

Conflict of Interest: The autors declare that they have no competing financial interests.

ÖZET

Amaç: Sigara kullanımının insan sağlığı üzerine olan etkisi ciddi boyutlardadır, hatta birçok durumda ölüme neden olmaktadır. Çalışmanın amacı Pakistan'ın Quetta şehrinde yaşayan erkeklerde sigara kullanımının kanın biyokimyasal özelliklerine olan yan etkilerinin araştırılmasıdır.

Yöntem: Çalışmaya yüz kırk iki erkek birey katılmıştır: Sigara kullanan bireyler (n=71) ve sigara kullanmayan bireyler (n=71). Sigara içen bireyler en az 3 senedir düzenli olarak günde 11-20 sigara içen bireylerdir. Tam kan sayımı Nihon Codon tam otomatik hematolojik analizör ile yapılmıştır.

Bulgular: Sigara içen bireylerde beyaz kan hücreleri ($p<0.027$), kırmızı kan hücreleri ($p<0.011$), hemoglobin ($p<0.001$) ve hematokrit ($p<0.006$) düzeyleri belirgin bir şekilde yüksek, ortalama korpusküler hemoglobin derşimi ($p<0.009$) ve platelet crit ($p<0.017$) ise düşük bulunmuştur.

Sonuç: Çalışma bulguları sürekli sigara kullanımının hematolojik parametreler (hemoglobin, hematokrit, beyaz ve kırmızı kan hücrelerinin sayısı ve platelet crit vb.) üzerine ciddi yan etkilerinin olduğunu göstermektedir. Bu değişimler ateroskleroz, polisitemia vera, kronik obstrüktif akciğer hastalığı ve/vaya kardiyovasküler hastalıkların gelişimi ile ilişkili olabilir.

Anahtar Kelimeler: Sigara kullanımı, hematolojik parametreler, kan sayımı

Çıkar Çatışması: Yazarlar hiçbir mali çıkar çatışması bulunmadığını beyan eder.

Introduction:

Tobacco cigarette smoking is one of the major leading causes of death and essential public health challenge in world over [1-2]. Smoking has both acute and chronic effect on hematological parameters. There are more than 4000 chemicals found in cigarette smoke [3], and a cigarette smoker is exposed to a number of harmful substances including nicotine, free radicals, carbon monoxide and other gaseous products [4]. It is widely known that smokers have higher risk for cardiovascular diseases, hypertension, inflammation, stroke, clotting disorder, and respiratory disease [5-11]. Moreover, cigarette smoking accelerates pathogenesis in different type of cancers such as lung, pancreas, breast, liver and kidney [2, 6-7]. Similarly, it also enhances pH in stomach that resulted in peptic ulcers and gastric diseases [1, 7-8]. During past decade, it was suggested that cigarette smoking affect the blood characteristics as well that leads to death. For example, relation between smoking and white blood cell count has been well established [5, 7-8]. In a number of studies, it has been found that smokers have higher white blood cell counts than nonsmokers [6, 9-11]. Although in some earlier studies relationship between smoking and red blood cell was found in smokers [12]. Some scientists suggested that increase in hemoglobin level in blood of smokers could be a compensatory mechanism. However, some were of view that smoking does not increase in hemoglobin level in all smokers and this relates to tolerance potential of individual to different kind of diseases. Moreover, episodic duration of smoking and age of individual might have changed the adverse effects of smoking on blood characteristics of human being. In view of these reports, a comparative analysis of blood hematology of male smokers and nonsmokers from Quetta, Pakistan was carried out to assess the adverse effects of smoking on White Blood cells (WBC) counts, Red Blood cells (RBC), Hemoglobin (Hb), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Hematocrit (HCT), Red Cell Distribution Width (RDW), Platelets (PLT), Platelet Crit (PCT), Mean Platelet Volume (MPV) and Platelet Distribution Width (PDW).

Material and methods

Subjects

In present study we enrolled a total of 142 healthy adult male subjects including smokers (n=71) and non smokers as control (n=71). The smokers were regularly consuming minimum of 11 cigarettes per day for at least 3 years otherwise we consider them as non smokers. In our cohort, only male subjects passed the inclusion criteria where as none of the female passed the inclusion criteria of consuming minimum of 11 cigarettes for at least 3 years and were excluded from study. Informed consent was obtained from all the participants, and the study was approved by ethical committee of the university, prior to the start of study. The enrolled subjects did not had any serious health problem, no history of drug usage and none had donated or received blood in last 6 months. The clinical data, medical history and other relevant information were collected from subjects by personal interview.

Laboratory test

The blood samples were collected in the morning time 9.00 to 10.00 am after an overnight fasting for at least 10 hours. Venous blood samples were drawn in K3 EDTA (15%) Becton Dickinson Vacuum tubes and mixed gently. Complete Blood Count (CBC) was measured within 1-2 hours of blood sampling by using Nihon Codon fully automatic Hematological analyzer (Nihon Kohden, Japan). The Hematological analyzer was calibrated by standardized commercially available calibrators' kit. CBC count (WBC, RBC, Hb, MCV, MCH, MCHC, HCT, RDW, PLT, PCT, MPV, and PDW) were measured in this study.

Statistical analysis

Data analysis was performed using the Sigma state statistical software version 3.5. The Hematological values between smokers and nonsmokers were compared using unpaired "t" test. P value < 0.05 was considered for statistically significance.

Results

Table 1 shows general characteristics of smokers and non-smokers. The mean age of smokers and non-smokers were 33.85±7.28 and 32.19±7.17 years, respectively. We found that education level also play a role in smoking habit; for non-matriculate subjects, 29.57% were smoker and 23.94% were non-smokers whereas for graduate subjects, 30.98% were smoker and 38.02% were non-smokers. The smoking subjects were having weight of 69.94±15.25 and non-smokers were having 72.85±11.19 kg. The result of all hematological parameters between smokers and non-smokers are summarized in Table 2, and all parameters are expressed by Mean, Standard deviation (SEM) and P values.

Table 2 shows the changes in hematological parameters of smokers and non-smokers: WBC, RBC, Hb, and HCT were significantly high; MCHC and PCT were significantly low in smokers as compared to non-smokers; and MCV, MCH, RDW, PLT, MPV and PDW did not show any significant difference. Smokers had significantly higher level of WBC count than non-smokers control group (7.6×10^3/ µl vs 6.9×10^3/µl). The Red blood cell count also showed significant increase in smokers than non-smokers (5.3×10^3/µl vs 5.06×10^3/µl). Similarly, smokers had significantly higher mean HCT 49.67% than non-smokers 45.34% and hemoglobin values were also significantly higher in smokers 16.01 g/dl than non-smokers 14.71 g/dl. On the other hand mean MCHC 32.56 g/100 ml and PCT 0.13% was significantly

Table 1: General characteristics of smokers and non-smokers subjects

Characteristics	Smokers (n=71)	Non-smokers (n=71)
Age (years)	33.85 ±7.28	32.19±7.17
15-25	6 (8.45%)	16 (22.53%)
26-35	37 (52.11%)	36 (50.70%)
36-45	28 (39.43%)	19 (26.76%)
Education		
Below 10th grade	21 (29.57%)	17 (23.94%)
10th grade	10 (14.08%)	15 (21.12%)
12th grade	18 (25.35%)	12 (16.90%)
Graduation	22 (30.98%)	27 (38.02%)
Weight (kg)	69.94 ±15.25	72.85±11.19
Marital Status		
Married	39 (54.92%)	33 (46.47%)
Unmarried	32 (45.07%)	38 (53.52%)

Values are expressed in Mean and SD, Number and (%) of subjects

Table 2: Hematological results showing that WBC, RBC, Hb, HCT, MCHC and PCT are significantly different in smokers as compared to control

Parameters#	Smokers (n=71)	Non Smoker (n=71)	P value
	Mean±SD	Mean±SD	
WBC ($10^3/\mu l$)	7.622 ±2.25	6.90 ±1.53	0.027*
RBC ($10^3/\mu l$)	5.38 ±0.87	5.06 ±0.59	0.011*
Hb (g/dl)	16. 01±2.45	14.71 ±1.43	0.001*
HCT	49.67 ±11.59	45.34 ±5.78	0.006*
MCV(fl)	90.26 ±14.23	88.61 ±8.62	0.404
MCH (pg)	28.19 ±4.10	28.84 ±2.97	0.283
MCHC (g/dl)	31.50 ±2.55	32.56±2.18	0.009*
RDW (%)	13.28 ±1.73	12.83±0.11	0.102
PLT ($10^3/\mu l$)	252.25 ±71.53	270.28 ±60.25	0.107
PCT (%)	0.12±0.036	0.13 ±0.036	0.017*
MPV (fl)	5.26 ±1.16	5.28 ±0.95	0.905
PDW (%)	17.26 ±1.00	17.33±0.94	0.662

* Statistically significant

Abbreviation used: White Blood cells (WBC), Red Blood cells (RBC), Hemoglobin (Hb), Hematocrit (HCT), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Hemoglobin Concentration (MCHC), Red Cell Distribution Width (RDW), Platelets (PLT), Platelet Crit (PCT), Mean Platelet Volume (MPV), and Platelet Distribution Width (PDW).

low in non-smokers than smokers 31.5 g/dl and 0.12% respectively.

Discussion

Tobacco smoking has been correlated to cause several major morphological and biochemical problems in individuals. In this study hematological parameters had been used for comparative analysis between smokers and non-smokers among local population of Quetta, Pakistan. The experimental results showed significant differences in hematological parameters of subjects; WBC, RBC, Hb and HCT were significantly high whereas MCHC and PCT were significantly low in smokers as compared to non-smokers. We did not find any significant difference in MCV, MCH, RDW, PLT, MPV and PDW level.

Although strong epidemiological evidence links cigarette smoking to cardiovascular disease, cancer and chronic obstructive pulmonary disease (COPD), the exact mechanisms of these links remain poorly understood. Some of the adverse effects of smoking include: initiation of endothelial injury [13], acceleration of coronary progression and new lesion formation [14] and overall alterations in lipid and hemostatic systems [15]. Detecting endothelial damage may be the most useful step in the early diagnosis of atherosclerosis. Although the endothelium releases many molecules into the circulation and arterial wall, not all of them are specific to the endothelium and are therefore of limited research or diagnostic potential [16].

WBC count is perhaps the most useful, inexpensive and simple biomarker for endothelial damage. We found that regular smokers had significantly higher WBC count compared to non-smokers (P=0.027). The high WBC count in male smokers in this study is consistent with other published reports [10, 17]. Freedman et al. observed that median total leukocyte count was 36% higher in current smokers as compared to non-smokers [9]. The mechanism for smoking-induced increase in WBC count is not clear. It has been suggested that inflammatory stimulation of the bronchial tract induces an increase in inflammatory markers in the blood but it has also been suggested that nicotine may induce an increase in blood lymphocyte counts [18-19]. While leukocytosis may simply be a marker of smoking-induced tissue damage, the high count can promote cardiovascular diseases through multiple pathologic mechanisms that mediate inflammation, plug the microvasculature, induce hypercoagulability and promote infarct expansion [2, 5, 10-19, 20]. In fact several studies have shown that WBC count is an independent predictor of atherosclerosis and cardiovascular disease [21-22]. The high WBC count in our male smoking subjects may also suggest that they might be at greater risk for developing atherosclerosis and cardiovascular diseases than non-smokers.

We observed that hemoglobin values were significantly high in smokers (P=0.001). Elevated levels of hemoglobin are correlated with increased numbers or sizes of RBCs. RBC values were significantly high in smokers than those of non-smokers (P=0.011) and are consistent with other investigations [1, 12, 20]. It is reported that high level RBC, WBC and Hematocrit are associated with blood viscosity and clotting in smokers [23-25]. High level of RBC is termed as polycythemia and very high RBC mass slows blood velocity and increase the risk of intravascular clotting, coronary vascular resistance, decreased coronary blood flow, and a predisposition to thrombosis [26]. It has been established that fibrinogen levels are higher in smokers than in non-smokers, and it has been estimated that the increasing risk of cardiac disease in smokers may be associated with high fibrinogen levels through arterial wall infiltration and effects on blood viscosity, platelet aggregation, and fibrin formation [9, 27-28].

This elevation may lead to congenital heart disease, pulmonary fibrosis and elevated erythropoietin [1, 11-12, 20, 29]. The mechanism by which polycythemia causes thrombosis is still under investigation, but smoking cigarettes creates a unique condition of combined polycythemia to chronic hypoxia, leading to elevated red cell production due to an elevated carboxyhemoglobin level, with concomitant plasma volume reduction. Overall, thrombosis is a serious complication of polycythemia and can lead to death in up to 8.3% of patients [26, 30].

Hematocrit values were also significantly high in smokers than those of non-smokers (P=0.006) and consistent with previous findings [1, 11-12]. Higher levels of hematocrit may cause polycythemia vera (PV), a myeloproliferative disorder in which the RBCs are produced excessively by bone marrow, and also related to an increased risk of development of atherosclerosis and cardiovascular disease [30].

In cigarette smoking, carbon monoxide (CO) is produced by the incomplete combustion of carbon-containing material. CO has a very high affinity for hemoglobin relative to that for oxygen (approximately 200-fold) [31]. Thus, CO displaces oxygen from hemoglobin in red cells to produce carboxyhemoglobin (COHb), which reduces the release of oxygen to tissues [32]. Higher levels of hematocrit and hemoglobin have been demonstrated in smokers, and these increases are likely to be compensatory for exposure to CO [33]. Increased hematocrit and hemoglobin concentrations observed in smokers that may contribute to a hypercoagulable state [32, 34].

PCT were observed significantly low in smokers than non-smokers (P=0.017). Low PCT value indicates the platelet abnormality resulted by absence of a bone marrow response to a peripheral demand for platelets. We did not find any significant changes in platelets (PLT), mean platelet volume (MPV) or platelet distribution width (PDW) between smokers and non-smokers. The previous reports have shown that chronic smoking ca-

uses platelet activation and smoking cessation improves platelet function [35-36]. However, Butkiewicz et al. studied the effect of smoking on platelet activation and some morphological parameters including MPV and they did not find any effect of smoking on MPV [37]. Similarly, Arslan et al. investigated the effects of smoking on MPV in young healthy male population (smokers 56 and non-smokers 46) and they found no significant difference in MPV between the smoking and non-smoking healthy male participant [38].

MCV, MCH and MCHC are three main red blood cell indices that help in measuring the average size and hemoglobin composition of the red blood cells. Their values are derived from the Hb, HCT and RBC-count by mathematical calculations; MCV = [HCT (%) x 10/RBC (million/cmm)] fL; MCH = [Hb (g/dL)/RBC (million/cmm)] pg and MCHC = [Hb (g/dL)/HCT (%)] g/dL. We found increase in MCV and decrease in MCH and MCHC levels. MCV indicates the size of a red blood cell and presence of red cells smaller or larger than normal size means the person has anemia, elevated levels of MCV in our study indicates that subjects might suffer from megaloblastic, hemolytic, pernicious or macrocytic anemia usually caused by iron and folic acid deficiencies. MCH is the average weight of hemoglobin that is present inside single red blood cell whereas MCHC denotes the amount of hemoglobin in a specific volume of 'packed' red corpuscles or cells. We found significantly low value of MCHC ($p<0.009$) among smokers indicating hypochromic anemia and might be due to paucity of folic acid or vitamin B12 or thyroid problems. [39-40].

Conclusion

Our findings clearly show that continuous cigarette smoking has severe adverse affects on hematological parameters (*e.g.* hemoglobin, hematocrit, WBC count, RBC count and platelet crit). In our result RBC, WBC and HCT counts are significantly higher in smokers. Too many blood cells can make the smoker's blood more viscous so the blood does not flow efficiently and can contribute to the formation of clots. This can increase the risk of clotting complications, such as stroke, heart attack, deep vein thrombosis or pulmonary embolism. This study suggests that these biomarkers might be associated with a greater risk for developing atherosclerosis, polycythemia vera or cardiovascular diseases in smokers as compared to non-smokers. The implications of these relationship range from helping to guide the medical work-up in a smoker with leukocytosis or erythrocytosis to broadening our understanding of how smoking leads to atherosclerosis, polycythemia vera, chronic obstructive pulmonary disease and cardiovascular disease.

Reduction in smoking does improve measures of some of these biomarkers, demonstrating that these biomarkers are sensitive to change in smoking intake. Additional research is clearly necessary to determine if reduction is beneficial, if so to what extent smoking needs to be reduced for health benefits to occur and which biomarkers are most sensitive to measuring improved health. Reduction in smoking may be a good approach to engage subjects in treatment and may serve as a good stepping stone for individuals who are resistant to quitting.

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MA, MR, ZU design the study and writeup the manuscript. MA, TI, AM performed the laboratory work. SK, AC, MHA and MR analysed the data and critically revised the manuscript.

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