

Effects of Cigarette and Shisha Smoking on Hematological Parameters: An analytic case-control study

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Abstract— Cigarette smoking is one of the major causes of cancer and cardiovascular diseases leading to millions of premature deaths each year all over the world. Scientists have identified about 4,000 different substances in tobacco all of which have certain degree of toxic effects. At least 43 of them known carcinogens.

Objective: The aim of this study is to assess the extent of adverse effect of cigarette and shisha on haematological parameters in male population of Khartoum State – Sudan.

Subject and Methods: One hundred and fifty male subjects participated in this study, cigarette smoker (n= 50), shisha smoker (n= 50) and non-smoker (n= 50). The smokers were regularly smoking at least for 10 years. Fresh peripheral blood samples from healthy adult non-smokers and smokers (males) were collected and analysed for Red Blood Cells (RBC) count, haemoglobin (Hb) content, packed cell volume (PCV), MCV, MCH and MCHC, total and differential leucocytes (WBC) counts and total platelets count by using fully automatic haematological analyser.

Results: The smokers of cigarette and shisha had significantly higher level of Hb, HCT, RBCs, TWBC count and MCHC. However, platelets counts were significantly lower in cigarette smokers than that of non-smokers. Study shows that the duration of smoking had no significant effect on haematological parameters except Hb.

Conclusion: It concluded that smoking alters haematological parameter that is injurious to health.

Key words: Cigarette, Shisha, Smoking, Haematology

I. INTRODUCTION

Smoking is the most important public health problem. Many studies conducted have proved its deleterious effects on many organ systems mainly respiratory, reticulo-endothelial system and cardiovascular systems.¹ According to data reported from the World Health Organization ², there is about 2.4 billion people worldwide that have consumed tobacco in the forms of smoking, chewing, snuffing or dipping. WHO also estimates that tobacco-related deaths will amount to 8.3 million in 2030 and one billion deaths during the 21st century. ² Water-pipe (WP) is a classical device used for tobacco smoking attached with water bowl.³ WP usage has a history about 400 years with the different names like as a shisha, narghile, hookah chillum and arghile.^{4,5} Cigarette smoke contains a variety of other compounds including oxidants and free radicals that are capable of initiating or promoting oxidative damage⁶ leading to various degenerative pulmonary and cardiovascular diseases as well as cancer⁷ Although the effect of smoking on haematological parameters has been studied previously but the literature is limited and controversial. The effect of carbon monoxide on haemoglobin, its binding and

dissociation with oxygen and occurrence of hypoxia have been reported by many authors.^{7,8} However, there are paucities in studies on the effect of cigarette and WP smoking on hematological parameters in both human and animals. So this study aimed to investigate the effect of cigarette and WP smoking on hematological parameters in population of Khartoum State.

II. METHODOLOGY

An analytical case control study was conducted by measuring red blood cells, white blood cells and platelets count in Sudanese cigarette and shisha smokers in Khartoum State. Study was conducted in period in 1st half of year 2015.

Sample size: Sample size was calculated 35 subjects for each of the three group at alpha error 0.05 and power 80% assuming minimum difference of means in haemoglobine concentration to be detected with Standard deviation 3 ± 4 . So for the study purpose 50 cigarette smokers, 50 shisha smokers as study group and 50 non-smokers as control group were taken

Study population: This study was carried out on Sudanese volunteers from both smoker (for study group) and non-smoker (for control) groups according to the following inclusion and exclusion criteria.

Inclusion Criteria: Control group are males, age range from 18-50 years and apparently healthy individuals. Inclusion criteria for study group was regular smokers (both cigarette and shisha) for atleast 10 years, males, age range from 18-50 years otherwise apparently healthy individuals.

Exclusion Criteria: Any individual have any disease on examination/investigation in any of control or study group was excluded from the study. And any individual who smoke both cigar and shisha were excluded form study group.

Method of data collection: Predesigned and pretested questioner was used to obtain biosocial information of participants like age, smoking dose, smoking duration and other diseases related to it.

Sample processing: Three ml K₃EDTA anticoagulated venous blood was withdrawn using 5ml disposable syringe, all samples were checked for clots and hemolysis and mixed well before analysis. These samples were then subjected to apparatus analysis, 50µl from each sample was sucked by apparatus needle. Immediately the result of each sample was obtained, results were kept until they were statistically analyzed.

Handling of blood sample: Three ml (3 ml) venous blood sample was collected in EDTA anticoagulant blood container in proper way and gently mixed in the hematology mixture and immediately (not more than one hour). The sample was then analyzed by Sysmex KX-21N automated hematology analyzer.

CBC: (complete blood count): Evaluations of the blood cell count were performed by Sysmex automated hematological analyzer, which could perform 18 hematological parameters with high accuracy and precision. Principally Sysmex analyzer is based on the electronic resistance (impedance) detection method for counting and sizing recognition of the leukocytes, erythrocytes and platelet. Through using three preliminary hydraulic systems for WBCs, RBCs, platelet and hemoglobin

and display the mode of the cells blood count results on the liquid crystal displayer (LCD) with histogram and printed out the results in thermal paper.⁹

Quality control of SYSMIX KX 21: All quality control of the machine done in instructed manner. The daily, weekly and monthly maintenance and calibration used to ensure quality assurance. Then before using the apparatus one of the last day samples was re-analysed for delta check.

Ethical consideration: Before collecting the sample, written informed consent was taken from the participants and ideal blood collection procedure was order to safe them.

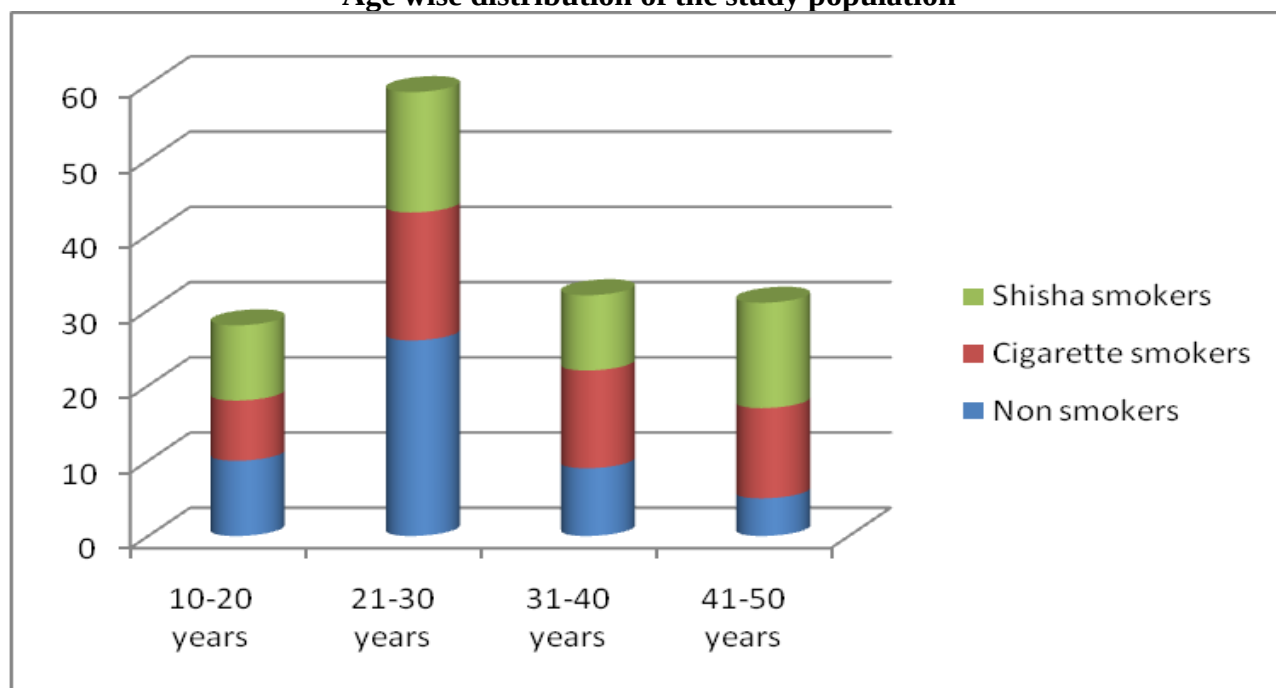
Data analysis: All results were analyzed and inferred by using Statistical Software Primer (Version 6)

III. RESULTS

Out of total 150 participants of the study, majority i.e. 59 (39.33%) were of 21-30 years of age group, followed by 31-40 years (21.33%), 41-50 years (20.67%) and 11-20 years (18.67%). (Table 1)

When the distribution of participants according to age was further analyzed it was observed that proportion of participants as per age in various group i.e. non smoker, smoker, and Shisha smoker was not found with significant variation ($p>0.05$). So these three groups were well comparable in age wise distribution (Table 1)

Figure 1
Age wise distribution of the study population



Chi-square = 8.506 with 6 degrees of freedom;

P = 0.203

LS=NS

RBC and its related parameters: RBC count, Hb content, MCHC and haematocrit was found significantly increased in smokers (cigarette as well as in shisha smokers) than non smokers. Although MCV and MCH were also found increased in smokers than non-smokers but both were not found significant in cigarette smokers and found significantly increased in Shisha smokers. (Table 1 & 2).

Table No. 1
Comparison of RBC and its related parameters in Non smokers and Cigarette smokers

S. No.	Parameters	Non smokers (N=50) Mean $\pm$ SD	Cigarette smokers (N=50) Mean $\pm$ SD	P Value	LS
1	HGB (gm/dl)	13.38 $\pm$ 1.23	14.61 $\pm$ 1.11	<0.001	S
2	HCT (%)	42.90 $\pm$ 2.52	45.85 $\pm$ 2.56	<0.001	S
3	RBCs(X106/mm ³)	5.06 $\pm$ 0.45	5.64 $\pm$ 0.56	<0.001	S
4	MCV(μ m ³)	85.43 $\pm$ 4.81	85.55 $\pm$ 5.14	0.870	NS
5	MCH (pg)	27.86 $\pm$ 2.13	28.34 $\pm$ 1.62	0.516	NS
6	MCHC(%)	30.66 $\pm$ 1.00	32.26 $\pm$ 1.34	<0.001	S
7	PLT(millions/mm)	253.04 $\pm$ 66.35	236.12 $\pm$ 67.21	0.081	NS
8	WBCs (X10 ³ cm ³)	4.97 $\pm$ 1.00	5.66 $\pm$ 1.50	0.002	S
9	LYM%	38.22 $\pm$ 4.98	38.73 $\pm$ 9.07	0.045	S
10	MXD%	10.07 $\pm$ 3.41	10.80 $\pm$ 5.03	0.177	NS
11	NEUT%.	51.68 $\pm$ 8.15 0	47.69 $\pm$ 10.75.	0.21	NS

Table No. 2
Comparison of RBC and its related parameters in Non smokers and Shisha smokers

S. No.	Parameters	Non smokers (N=50) Mean $\pm$ SD	Shisha smokers (N=50) Mean $\pm$ SD	P Value	LS
1	HGB (gm/dl)	13.38 $\pm$ 1.23	15.05 $\pm$ 0.92	<0.001	S
2	HCT (%)	42.90 $\pm$ 2.52	46.51 $\pm$ 2.877	<0.001	S
3	RBCs(X106/mm ³)	5.06 $\pm$ 0.45	5.72 $\pm$ 0.63	<0.001	S
4	MCV(μ m ³)	85.43 $\pm$ 4.81	86.45 $\pm$ 5.21	0.171	NS
5	MCH (pg)	27.86 $\pm$ 2.13	28.56 $\pm$ 2.09	0.023	S
6	MCHC(%)	30.66 $\pm$ 1.00	32.37 $\pm$ 1.51	<0.001	S
7	PLT(millions/mm)	253.04 $\pm$ 66.35	267.14 $\pm$ 88.0	0.263	NS
8	WBCs (X10 ³ cm ³)	4.97 $\pm$ 1.00	5.64 $\pm$ 1.81	0.011	S
9	LYM%	38.22 $\pm$ 4.98	37.39 $\pm$ 6.85	0.391	NS
10	MXD%	10.07 $\pm$ 3.41	10.39 $\pm$ 4.40	0.649	NS
11	NEUT%.	51.68 $\pm$ 8.15 0	52.44 $\pm$ 8.15	0.477	NS

When RBC count and its related parameters were compared with duration of smokers, it revealed that there were insignificant ($p>0.05$) changes in these parameters with time in cigarette smokers as well as in Shisha smokers except in haemoglobin content which significantly ($p<0.001$) increases with duration of both the type of smoking. (Table 3 & 4)

Table No. 3
Comparison of CBC in Non smokers and Cigarette smokers

S. No.	Parameters	Non smokers (N=50) Mean $\pm$ SD	Cigarette smokers (10 to 30 Years) Mean $\pm$ SD	Cigarette smokers (> 30 Years) Mean $\pm$ SD	P Value	LS
1	HGB (gm/dl)	13.68 $\pm$ 1.01	14.65 $\pm$ 1.12	15.5 $\pm$ 0.99	0.001	S
2	HCT (%)	45.85 $\pm$ 2.54	45.81 $\pm$ 2.34	45.89 $\pm$ 2.21	0.176	NS
3	RBCs(X106/mm ³)	5.58 $\pm$ 0.50	5.63 $\pm$ 0.57	5.71 $\pm$ 0.51	0.077	NS
4	MCV(μ m ³)	85.55 $\pm$ 5.21	85.24 $\pm$ 5.39	85.86 $\pm$ 5.15	0.772	NS
5	MCH (pg)	28.04 $\pm$ 1.54	28.1 $\pm$ 1.62	28.9 $\pm$ 1.60	0.069	NS
6	MCHC(%)	31.3 $\pm$ 1.34	32.23 $\pm$ 1.24	33.3 $\pm$ 1.15	0.216	NS
7	PLT(millions/mm)	230.43 $\pm$ 76.2	234.5 $\pm$ 75.98	243.4 $\pm$ 74.65	0.078	NS
8	WBCs (X10 ³ cm ³)	5.1 $\pm$ 1.50	5.6 $\pm$ 1.49	6.1 $\pm$ 1.44	0.098	NS
9	LYM%	37.9 $\pm$ 9.12	39.4 $\pm$ 9.07	38.9 $\pm$ 9.05	0.599	NS
10	MXD%	10.8 $\pm$ 5.10	10.6 $\pm$ 5.04	11.0 $\pm$ 5.08	0.097	NS
11	NEUT%.	47.0 $\pm$ 10.56	52.0 $\pm$ 10.49	44.0 $\pm$ 10.75	0.234	NS

Table No. 4
Comparison of CBC in Non smokers and Shisha smokers

S. No.	Parameters	Non smokers (N=50) Mean $\pm$ SD	Shisha smokers (10 to 30 Years) Mean $\pm$ SD	Shisha smokers (> 30 Years) Mean $\pm$ SD	P Value	LS
1	HGB (gm/dl)	14.6 $\pm$ 0.96	15.00 $\pm$ 0.98	15.57 $\pm$ 0.94	<0.001	S
2	HCT (%)	45.95 $\pm$ 2.74	46.57 $\pm$ 2.86	47.01 $\pm$ 2.78	0.146	NS
3	RBCs(X106/mm ³)	5.67 $\pm$ 0.67	5.69 $\pm$ 0.70	5.81 $\pm$ 0.65	0.069	NS
4	MCV(μ m ³)	85.41 $\pm$ 5.23	87.69 $\pm$ 5.14	86.27 $\pm$ 5.11	0.654	NS
5	MCH (pg)	28.31 $\pm$ 2.13	28.54 $\pm$ 2.09	28.85 $\pm$ 2.10	0.061	NS
6	MCHC(%)	31.45 $\pm$ 1.57	32.11 $\pm$ 1.48	33.56 $\pm$ 1.52	0.145	NS
7	PLT(millions/mm)	266.32 $\pm$ 85.4	267.2 $\pm$ 87.56	267.9 $\pm$ 88.09	0.254	NS
8	WBCs (X10 ³ cm ³)	5.47 $\pm$ 1.01	5.59 $\pm$ 1.09	5.86 $\pm$ 1.07	0.081	NS
9	LYM%	37.41 $\pm$ 6.85	36.54 $\pm$ 6.71	38.22 $\pm$ 6.92	0.354	NS
10	MXD%	10.51 $\pm$ 4.35	10.45 $\pm$ 4.40	10.21 $\pm$ 4.28	0.082	NS
11	NEUT%.	52.75 $\pm$ 8.14	53.13 $\pm$ 8.06	51.44 $\pm$ 7.98	0.071	NS

Total and differential WBC count: Analysis revealed a significant decrease in total WBC counts in smokers. However, lymphocytes count, neutrophils count and mixed white blood cells were insignificantly decreased in smokers than that of non-smokers. (Table 1 & 2)

When WBC count and its related parameters changes were compared with duration of smokers, it was revealed that total WBC counts, lymphocytes count and mixed white blood cells were higher in long-term smokers but the neutrophils count was reduced. But these changes were found not significant ($p>0.05$) in both the type of smoking. (Table 3 & 4)

Platelets count: It was found in this study that there is increase in platelets counts in shisha smokers but the platelets counts was reduced in cigarette smokers. But this change was not found significant ($p>0.05$) in both the type of smoking. (Table 1 & 2)

When platelets counts changes were compared with duration of smoking it was revealed that there is increases in platelets counts in long-term smokers and decrease of platelets counts in short term smokers were evident but these changes were found not significant ($p>0.05$) in both the type of smoking. (Table 3 & 4)

IV. DISCUSSION

Since cigarette smoking causes several health problems in people, the observations of this study also clearly show that cigarette and WP smoking has severe adverse effects on hematological parameters (e.g. Hb, Hct, WBC, RBC, Plt count) among study population.

It was found in this study that total erythrocytes, hemoglobin concentration, haematocrit, WBC count and mean cell haemoglobin concentration was increased significantly in cigarette and WP smokers with ($p<0.001$) when compare with non-smokers. This indicated to that cigarette and shisha smoking can develop secondary polycythemia in the future as the result from increased levels of carboxyhemoglobin which causes clinically significant hypoxemia and account for the increased erythrocyte masses. These observations were well in resonance with Muhammad et al; (2013)¹⁰ and Pankaj et al; (2014).¹¹

Mean cell hemoglobin was increased significantly in shisha smokers when compare with non-smokers with ($p= 0.023$) in this study which is similar to observations made by Muhammad et al; (2013)¹⁰ who stated that shisha smoking causes elevation in mean cell hemoglobin. Moore and Pearson (1986)¹² and Moore (1988)¹³ also reported polycythemia in cigarette smokers

There was no significant difference in this study between cigarette smokers and non-smokers in mean cell volume, mean cell hemoglobin, platelets counts and MXDs%. These findings were very much similar to observations made by Pankaj; et al (2014).¹¹

Total leukocyte counts were significantly elevated in cigarette smokers and shisha smokers when compared with non-smokers. These observations were similar to observations made by other authors.^{14,15} Watanabe, (2011)¹⁶ found that not only WBC counts but also tumour necrosis factor (TNF) system activities increased in current smokers than non-smokers.

In this study there was not significant change in WBC count in smokers and nonsmokers. However other authors reported elevation of WBC counts like Tanasan et al; (2012)¹⁷, reported that smoking cause elevated in Neutrophils and lymphocytes. A group of researchers suggested that the increased

leukocyte count might be due to nicotine-induced release of catecholamines, resulting in an increase in blood lymphocyte counts. In addition, the irritant effect of cigarette smoke on respiratory tree with resultant inflammation might be a contributory factor for higher WBC count. Also, it has been suggested that inflammatory stimulation of the bronchial tract induces an increase in inflammatory markers in the blood circulation.¹⁸

Platelet counts in this study were reduced in cigarette smoking whereas it increased in shisha smokers compared to non-smokers. However there were mixed response from other authors^{19,20} some reported that platelet counts significantly more in smokers whereas some authors reported that platelet counts significantly lowered in smokers than non smokers. Whereas Tell et al¹⁹ reported that platelet counts significantly increased in adolescent who started smoking relatively early ages. Elevation of platelet counts in adolescent smokers indicates that these blood components may have an early role in the pathogenesis of arteriosclerosis. It has been proposed that fibrinogen links platelet receptors, which are preconditions for platelet aggregation and also promote hypercoagulable state as well as causing endothelial damage, disorganization and dysfunction. Aghaji et al²¹ reported that the platelet counts were higher for the regular WP smokers than non-smokers ($P= 0.004$). Whereas, Al-Dahr (2010)²² found that there was no significant difference between the platelet count and mean platelet volume in smokers compared to nonsmokers.

CONCLUSION

Erythrocyte count, hemoglobin concentration, hematocrit, leukocyte count and mean cell hemoglobin concentration increased in cigarette and shisha smokers compared to control group. But there was no change in mean cell volume for both cigarette and shisha smokers compared with non-smokers.

RECOMMENDATIONS

Increase awareness about the side effects of cigarette and shisha smoking. Introduce health education programs emphasizing on these bad habits should be implemented. And this programme should be directed towards target groups in the school children and university students. More research and advanced technique as Cell markers follow cytometer and gene study should be used to make the picture more illuminated in the future research.

CONFLICT OF INTEREST

None declared till now.

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