Assessment of Leucocytes and CD4 Parameters of Healthy Shisha Smokers in a University Town in South-South Nigeria

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Abstract. Shisha smoking is very popular among students of university, colleges and schools for the pleasant and relaxing experience. This study was aimed at assessing the leucocytes and CD4 parameters of healthy shisha smokers in a university town in South Nigeria. A total of fifty healthy shisha smokers aged 17-33 years and of both sexes were recruited for this study. Fifty (50) apparently healthy non-smokers served as control. Leucocytes parameters were assessed for WBC total count and differential leucocyte count (DLC) using Sysmex KX-2IN haematology autoanalyzer while CD4 count was performed using flow cytometry method. The result obtained from this study revealed that the mean WBC total count of shisha smokers (5.32 ± 1.89) and that of the control subjects (4.83 ± 1.29) did not show any statistically significant difference (P>0.5). With respect to DLC, the mean neutrophil count (%) of shisha smokers (51.80 ± 12.83) against control (43.01 ± 9.36) revealed a statistically significant increase (P<0.05), whereas the mean values of lymphocyte count (%) of shisha smokers (37.80 ± 11.63) against control (46.64 ± 8.51) revealed a statistically significant decrease (P<0.05). The results of the mean values of the CD4 count of shisha smokers (987.86 ± 227.52) compared to control (999.09 ± 307.40) did not reveal any significant difference (P>0.05). Based on gender and age, the mean values of WBC total count and DLC were not affected (P>0.05). In contrast, there was a statistical significant increase (P<0.05) in the CD4 count of female shisha smokers compared to their male counterparts. However, there was an insignificant difference (P>0.05) between CD4 and age. From the present study, we can conclude that shisha smoking caused alteration in the leucocyte counts of the subjects we studied, particularly DLC but not that of CD4 count.

Keywords: Shisha smokers, leucocyte counts, CD4 count, Ekpoma

Introduction

Shisha is a tobacco product that is smoked in a waterpipe, narghile or hookah (Akl *et al.*, 2010). Shisha smoking originated from the Middle East, South East Asia and Northern African (Akl *et al*., 2010). However, it is now becoming increasingly popular all over the world including Europe and North America (Maziak, 2011). Worldwide, it is estimated that 100 million people use hookah on a daily basis (Ward *et al*., 2005).

Shisha smoking has developed as a social event among the people who gather at parties, cafes and restaurants to smoke (Mugyenyi *et al*., 2018) and it is a well-known technique for inhaling tobacco among the youth for the past 20 years (Zyoud *et al*., 2014). Shisha smoking is very popular among students of universities, colleges and schools for the pleasant and relaxing experience (Naseem *et al*., 2022). Shisha smoking involves burning flavoured tobacco, known as molasses, using coal. When an individual breathes in from the mouthpiece, air is pulled through the apparatus into the tobacco and heated by the coal to produce smoke. As a result, the smoke contains components from both the tobacco and coal. These include

polycyclic aromatic hydrocarbons (PAH), volatile aldehydes, carbon monoxide (CO), nitric acid (NO), nicotine, furans and nanoparticles (Cobb *et al*., 2012).

A generally held notion is that shisha smoking is much less harmful than cigarette smoking due to the filtering effect of water (El-Zaatari *et al*., 2015). But there are numerous reports which have established that shisha smoking is several-fold more dangerous than smoking cigarettes (Naseem *et al*., 2022). The mucosa surfaces are in direct contact with the external environment and a major site of antigenic and toxic exposures in smokers. Toxins including endotoxins associated with tobacco smoke impact inflammation contributing to coronary heart disease, as well altering humoral and cell mediated immunity (Hasday *et al.*, 1999; Hansson, 2005). This present study was therefore aimed at assessing the leucocytes and CD4 parameters of healthy shisha smokers in a university town situated in South South Nigeria.

Materials and Methods

Study Area

Ekpoma is the administrative headquarters of Esan West Local Government Area of Edo State, Nigeria. It is located at latitude 6^0 45^1 N to 6^0 45^1 N of the Equator and longitude 6^0 08^1 E to 6^0 13^1 E of the Greenwich Meridian. It is moderately populated with about 190,000 people. Ekpoma is the home of Ambrose Alli University (World Gazetteer Nigeria, 2007).

Study Population

A total of fifty (50) healthy shisha smokers aged 18-33 years and of both sexes were enrolled into this study while fifty (50) apparently healthy, young adults non- shisha smokers served as the control group. Subjects' data were obtained using a structured questionnaire.

Ethical Approval

Ethical clearance was obtained from the Health Research Ethics Committee (NHREC registration number: NHREC/12/06/2013) of Ambrose Alli University, Ekpoma, Edo State, Nigeria. Informed consent was obtained from the participants.

Blood Collection

Four milliliters (4ml) of whole blood was aseptically withdrawn by venepuncture from the antecubital vein using vacutainer needles into EDTA vacutainers of 4ml. Blood samples for leucocytes and CD4 counts were collected at the same time (that is between 9.00am and 12noon). All the laboratory investigations were carried out with minimal delay and not exceeding six (6) hours of sample collection.

Specimen Analyses

White blood cells (leucocytes) counts (that is total and differential leucocytes counts) were analysed using automated haematology analyzer, Sysmex KX-21N autoanalyzer (Sysmex Corporation, Kobe, Japan). The Sysmex KX-21N is an automatic, 19-parameters, 3-part differential blood cells counter.

CD4 count was determined using Partec cyflow counter (Sysmex Partec GmbH, Görlitz, Germany) according to the manufacturer's instructions. The cyflow counter is based on the simultaneous measurement of multiple physical characteristics of CD4 T-cells in a single file as it flows through a light source, usually a laser beam. The counter separated the CD4 T-lymphocytes from monocytes bearing cells and noise using a gating system. In order to ensure accuracy and precision, count check beads green was used as control.

Statistical Analysis

Differences between the means of the two (2) groups were compared by Student's unpaired t-test while one-way ANOVA was used to analyze three or more groups. $P<0.05$ was considered significant.

Results

Table 1 shows the leucocytes and CD4 parameters of the study subjects. The mean WBC total count of shisha smokers was 5.32 ± 1.89 against 4.83 ± 1.29 for control. There was no significant difference between the two groups ($P>0.05$). With respect to differential leucocyte counts (DLC), the mean neutrophil count (%) of shisha smokers and control were 51.80 ± 12.83 and 43.01 ± 9.36 respectively. Statistical comparison revealed a significant increase ($P<0.05$) in the mean values of Neutrophil count (%) of shisha smokers compared to control. Similarly, the mean values of the lymphocyte count (%) of shisha smokers versus control were 37.80 ± 11.63 and 46.64 ± 8.51 respectively. In contrast, there was a statistically significant decrease in the lymphocyte count (%) of shisha smokers compared to control. The mean values of middle cells count (%) did not reveal any statistical difference ($P>0.05$). Furthermore, the mean values of Neutrophil count (#), Lymphocyte count (#) and middle cells (#) of shisha smokers were 2.90 ± 1.72, 1.90 ± 0.65 and 0.51 ± 0.23 against 2.09±0.81, 2.25±0.65 and 0.51±0.23 for the control group respectively. Statistical inference was similar to what was obtained for Neutrophil count (%), lymphocyte count (%) and middle cell count (%). The results of the mean values of the CD4 count of shisha smokers was 897.86 ± 227.52 in comparison to 999.09 ± 307.40 for control. Statistical comparison between the two means did not reveal any significant difference ($P>0.05$).

The leucocytes and CD4 counts of shisha smokers according to gender is summarized in table 2. The results of the WBC total count of shisha smokers and control for male and female subjects were 5.01 ± 1.30 and 5.32 ± 1.89 respectively. However, statistical comparison did not reveal any statistical significance ($P>0.05$) between the two groups. On the other hand, the mean values of Neutrophils count (%), lymphocytes count (%) and middle cells count (%) of the male shisha smokers were 51.80 ± 12.83, 37.80 ± 11.63 and 10.41 ± 3.96 against 42.74 ± 7.84, 46.41 ± 8.61 and 10.84 ± 5.12 for female shisha smokers respectively. Statistical comparison between both sexes for Neutrophils count (%), lymphocytes count (%) and middle cells count (%) also did not reveal any significant difference ($P>0.05$). Ditto for the mean values of Neutrophils (#), lymphocytes (#) and middle cells (#). Furthermore, the results of CD4 counts of shisha smokers between the male and female sexes were 837.45 ± 217.02 and 862.76 ± 212.20 respectively. In contrast, there was a statistical significant increase ($P<0.05$) in the CD4 count of female shisha smokers compared to their male counterparts.

Table 3 presents the leucocytes and CD4 counts of shisha smokers with respect to age. The mean values of the WBC total counts of shisha smokers according to the various age groups of 18-20 years, 21-23 years, 24-26 years and 27 years and above were 5.20 ± 0.58, 4.66 ± 1.08, 4.56 ± 1.37 and 5.63 ± 2.16 respectively. Statistically comparison across the four age groups did not reveal any statistical significant difference ($P>0.05$). Similarly, statistical comparison of shisha smokers across the four age groups for Neutrophils (%), lymphocytse (%) and middle cells (%) was not significant ($P>0.05$). Ditto for Neutrophils (#), Lymphocytes (#) and middle cells (#). With respect to CD4, there was also no significant difference ($P>0.05$) between the CD4 count of shisha smokers and age.

Table 1. Leucocytes and CD4 counts of the study population

Parameters	Control subjects Mean ± SD n = 50	Test subjects Mean ± SD n = 50	t-value	p-value
WBC total count (x10^3/µl)	4.83±1.29	5.32±1.89	1.540	0.127
NEUT %	43.01±9.36	51.80±12.83	3.913	0.000
LYM %	46.64±8.51	37.80±11.63	4.338	0.000
MXD %	10.33±3.01	10.41±3.96	0.111	0.912
NEUT #	2.09±0.81	2.90±1.72	3.019	0.003
LYM #	2.25±0.62	1.90±0.62	2.762	0.000
MXD #	0.51±0.23	0.51±0.19	0.095	0.924
CD4	999.09±307.40	897.86±227.52	1.902	0.061

Note: SD - Standard deviation; n - Sample size; WBC - White Blood Cell; NEUT % - Neutrophil %; LYM % - Lymphocyte %; MXD % - Middle cells %; NEUT # - Neutrophil (Absolute count); LYM # - Lymphocyte (Absolute count); MXD # - Middle cells (Absolute count); CD4 - Cluster of differentiation 4

Table 2. Leucocytes and CD4 counts of shisha smokers according to gender

Parameters	Male subjects Mean ± S.D (n = 41)	Female subjects Mean ± S.D (n = 9)	t-value	p-value
WBC total count (x 10^3/µl)	5.01±1.30	5.32±1.89	0.797	0.429
Lymphocytes (%)	37.80±11,63	46.41±8.61	0.767	0.447
MXD (%)	10.41±3.96	10.84±5.12	0.289	0.774
Neutrophils (%)	51.80±12.83	42.74±7.84	0.862	0.393
Lymphocyte (#)	1.90±0.19	2.47±1.61	0.335	0.739
MXD (#)	0.51±0.19	0.57±0.21	0.335	0.739
Neutrophil (#)	2.90±1.72	2.27±1.50	0.786	0.436
CD4 count	837.45±217.02	862.76±212.20	1.402	0.001

Note: SD - Standard deviation; n - Sample size; MXD % - Middle cells %; MXD # - Middle cells (Absolute number)

Table 3. Leucocytes and CD4 counts of shisha smokers with respect to age

Parameters	18-20 years (n=8)	21-23 years (n=11)	24-26 years (n=19)	27 years & above (n=12)	F-value	P-value
WBC total count (x 10^3/µl)	5.20±0.58^a	4.66±1.08^a	4.56±1.37^a	5.63±2.16	0.882	0.457
Lymphocytes (%)	36.82±8.42^a	40.21±9.33^a	38.24±10.46^a	37.37±12.19^a	0.123	0.946
MXD (%)	12.92±3.66^a	11.66±4.24^a	11.86±3.15^a	9.55±3.91^a	1.716	1.177
Neutrophils (%)	50.26±6.60^a	48.13±8.65^a	49.90±7.60^a	53.10±14.82^a	0.351	0.789
Lymphocytes (#)	1.74±0.32^a	1.80±0.34^a	1.74±0.72^a	1.96±0.73^a	0.349	0.790
MXD (#)	0.66±0.22^a	0.53±0.27^a	0.52±0.22^a	0.48±0.16^a	1.291	0.289
Neutrophils (#)	2.56±0.56^a	2.33±0.83^a	2.30±0.80^a	3.16±2.01^a	0.776	0.513
CD4 count	812.06±210.00^a	811.01±211.00^a	800.06±202.0^a	810.01±211.0^a	0.112	0.34

Note: n - Sample size; SD - Standard deviation; WBC - White Blood Cell; MXD % - Middle cells %; MXD # - Middle cells (Absolute count); CD4 - Cluster of Differentiation 4

Discussion

Recently, researchers have been showing more interest on the effects of smoking on haematological parameters (Acik *et al*., 2020). Our study demonstrated an increase (5.32±1.89) in the WBC total count of shisha smokers but this increase was not statistically significant (P>0.05) when compared with control (4.83±1.29). This finding is very much similar to the observations of Saad *et al*. (2018) who also found an increased WBC total count of 7.2±1.4 in comparison to control (6.9±1.2) that was not statistically significant (P>0.05). The statistically insignificant WBC total count of shisha smokers obtained our study may be due to the category of shisha smokers recruited for this study coupled with the fact that the duration of smoking and quantity of the shisha they smoked were not taken into account. Previous authors have found an association between the number of cigarettes smoked per day and WBC total count. For example, Lakshmi *et al.* (2014) demonstrated the effect of smoking and its intensity on haematological and lipid parameters among the subjects they studied. Other authors such as Kurtoglu *et al.* (2013) and Miri-Moghadam *et al*. (2014) also found similar results about WBC in smokers when the duration of smoking was taken into account. Kawada *et al*. (2015) also found an association between the number of cigarettes smoked per day and the WBC count. In addition, when the smokers' WBC count was compared on the basis of smoking intensity, an increase in WBC count was found (Shipa *et al*., 2017). Shipa *et al*. (2017) observed that the total WBC count in moderate and heavy smokers was significantly higher that of the mild smokers. Furthermore, Acik *et al*. (2020) established an association between smoking time and the number of cigarettes smoked per day and haematological parameters. Acik *et al*. (2020) opined that this is believed to be associated with greater and longer exposure to the toxic compounds in tobacco. Similarly, Naseem *et al*. (2020) confirmed that an increase in the period of shisha smoking increased the WBC counts of chain shisha smokers compared to light smokers.

However, our finding contradicted the previous reports of Silverman *et al*. (1975) who observed a significantly elevated total leucocyte levels in smokers. In another study carried out in Japanese male subjects, Watanabe *et al*. (2011) found that not only WBC counts but also tumour necrosis factor (TNF) system activities increased in current smokers in comparison to non-smokers. Similarly, Aula and Qadir (2012) demonstrated significant increase in leucocyte counts of smokers in relation to the control group or non-smokers. Furthermore, other previous authors such as Inal *et al*. (2014), Nadia *et al*. (2015), Higushi *et al*. (2016) and Naseem *et al*. (2022) have all established a positive link between shisha smoking and total white blood cells.A group of researchers have suggested that the increased leucocyte counts might be due to nicotine-induced released of catecholamines, resulting in an increase in blood leucocyte counts. In addition, the irritant effect of cigarette smoking on the 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 (Calapai *et al*., 2009). It is possible that increased number of leucocytes in peripheral blood of healthy smokers is linked with the phenomenon of cell movement from other lymphoid organs in the peripheral blood or that smoking decreases the ability of adhesion of these cells on the endothelium cells of blood vessels, which leads to general increase in the number of blood cells (Parry *et al*., 1997).

Reports on differential blood count related with smoking are not consistent (Shipa *et al*., 2017). From the standpoint of differential leucocytes count (DLC), the mean neutrophils count (% and #) of shisha smokers in the study area revealed a statistically significant (P<0.05) increase compared to control. Our finding is in concordance with earlier reports of Aula and Qadir (2012) who demonstrated a significant increase in the neutrophils count of smokers compared to the control group or non-smokers. Similarly, Tanasan *et al*. (2012) reported that smoking caused elevation in neutrophils. Our results were also well in resonance with the

observations made by Acik *et al*. (2020) who reported the laboratory findings of their study revealed that smokers had higher levels of neutrophils count (%) of 5.4 x 10^3/µl compared to 4.1 x 10^3/µl (P<0.001) for non-smokers/control. As prior mentioned, a lot of reasons have been documented for the increase in leucocytes as well as neutrophils count. According to Inal *et al.* (2014), increased white blood cells (leucocytes) may be explained by a systemic inflammatory response. In contrast, some authors did not report elevated levels of neutrophils count in shisha smokers. For example, Kastelein *et al*. (2015) established no significant difference in the values of neutrophils between middle-aged smokers and non-smokers. In the same vein, Malenica *et al*. (2017) demonstrated that the values of granulocytes, which include neutrophils were not significantly increased. In addition, Nadia *et al*. (2015) reported that the neutrophils count (%) was not significantly increased in the subjects they studied.

In this study, the lymphocytes count (% and #) of shisha smokers was found to be statistically decreased (P<0.05) in relation to control (non-smokers). However, Nadia *et al*. (2015) reported a statistically non-significant difference with respect to lymphocytes count (% and #).In contrast, our finding disagreed with the previous reports by Silverman *et al*. (1975) who found significantly elevated levels of lymphocytes in smokers. Similarly, Tanasan *et al*. (2012) also reported that smoking caused elevation in lymphocytes. In addition, Aula and Qadir (2012) also documented that there was a statistically significantly increased levels in the lymphocytes count of smokers in relation to the control group or non-smokers. Furthermore, Kastelein *et al*. (2015) and Acik *et al*. (2020) established statistically significant larger values of lymphocytes in smokers in comparison to non-smokers. As aforementioned, some researchers suggested that increased leucocytes count might be due to nicotine-induced release of catecholamines, resulting in an increase in blood lymphocyte counts (Deutsch *et al*., 2007). Moreover, in 2017, a small group of researchers led by Nishizaki *et al.* (2017) suggested that lack of sleep could cause lymphocytopenia. However, they based their suggestion on a single case study. Other authors have attributed lymphocytopenia to be caused by malnutrition that is associated with corticosteroid use (Ng *et al*., 2006) as well as intense or prolonged physical exercise due to cortisol release (Robson *et al*., 1999). Additionally, alcohol exposure and particularly chronic heavy drinking affects all components of the adaptive immune system. According to Pasala *et al*. (2015), studies carried out in both humans and animal models determined that chronic alcohol abuse reduces the number of peripheral T-cells thereby disrupting the balance between different T-cell types as well as influencing T-cell activation, impairing T-cell functioning and promoting T-cell apoptosis. This report was supported by the findings of Dai *et al*. (2020) who observed that laboratory indicators such as lymphocyte counts of alcohol users was lower than that of non-alcohol group (P<0.05). Adams (2016) reported that the vogue called shisha smoking is rapidly pervading the society in Nigeria and as prior mentioned, it is commonly practiced by university undergraduates, adolescents and older population in restaurants and at hotels and at social gatherings. Therefore, we are of the opinion that the various locations where shisha is being smoked in Nigeria might promote the consumption of alcohol and its misuse among other things.

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 (Almirall *et al*., 1999; Sopori, 2002; Arcavi & Benowitz, 2004).In the present study, the absolute CD4 count of shisha smokers was lower (897.86±227.52) in relation to control (999.09±307.40). However, statistical comparison between the two groups did not reveal any statistical significance (P>0.05). Our finding is in tandem with the earlier reports of Forsslund *et al*. (2014) who observed that the percentages of CD4 T-cells were lower in the blood of smoking that non-smokers. According to Sopori and Kozak (1998), chronic exposure to cigarette smoker caused T-cell unresponsiveness. Nicotine which is the major component impairs antigen mediated signal transduction in lymphocytes (Geng *et al*., 1995)

and induces a state of T-cell anergy (Geng *et al*., 1996). This T-cell anergy may account for or contribute to the immunosuppressive and anti-inflammatory properties of cigarette smoke. Furthermore, Sopori *et al*. (1998a and 1998b) demonstrated that nicotine inhibits the antibody forming cell responses through impairment of antigen mediated signaling in T-cells by suppressing the intracellular calcium responses. In contrast, Shipa *et al*. (2017) reported that the proportion of CD4 lymphocytes was significantly increased in smokers compared to non-smokers. They also found that the percentage of CD4 cells tends to increase with the number of cigarettes smoked per day. The reason for this increase is not clear. Nonetheless, Shipa *et al*. (2017) affirmatively reported that the mechanisms responsible for this effect was obscure but suggested that the increase in lymphocytes count they obtained in their study can be attributed to the stimulating effect of nicotine on lymphocytes among other factors.

With respect to gender, the WBC total count, neutrophils count (% and #), lymphocytes count (% and #) and middle cells count (% and #) of shisha smokers in comparison to control did not reveal any significant difference (P>0.05).However, our finding is contrary to the earlier reports of Lakshmi *et al.* (2014) and Iyar *et al*. (2014) who both reported a positive relationship between WBC count and male smokers. Furthermore, Malenica *et al*. (2017) found that cigarette smoking caused a significant increase (P<0.001) in white blood cells (P=0, 040) of male subjects in comparison to female smokers. Similarly, Shipa *et al*. (2017) observed a statistically significant difference between WBC count of smokers compared to non-smokers (control).From the standpoint of CD4 count and gender, the CD4 count of shisha smokers was significant increased in females (P<0.05) compared to males. The higher CD4 values observed in our study is comparable to the earlier reports of Prins *et al*. (1999) and Lugada *et al*. (2014). According to Grossman (1985), sex hormone effect could be the possible explanation for the observed gender difference in CD4 counts as the circulating lymphocytes have receptors for androgens and estrogen.

In terms of age, the WBC total count of shisha smokers was not statistically significant (P>0.05) in relation to control. Our result is supported by the findings of Silverman *et al*. (1975) who found no correlation between age and smoking. However, our finding is contradicted by the recent findings of Shipa *et al*. (2017) who found an elevated white blood cells in smokers compared to non-smokers in relation to different ages and smoking levels. Therefore, we are inclined to suggest that the WBC counts were increased in the various age categories because of the intensity or level of smoking by the subjects they recruited for their study. In addition, the neutrophils count (% and #), lymphocytes count (% and #), middle cells count (% and #) and CD absolute count of shisha smokers were also not statistically significant (P>0.05). With respect to the relationship between age and CD4 count, our finding is consistent with the previous reports of Lugada *et al.* (2004) and Aina *et al*. (2005).

In conclusion, the results obtained from this study have shown that the WBC total count of shisha smokers was increased in the population studied but the increase was not statistical significant. However, we observed that the neutrophils count (% and #) of shisha smokers was significantly increased in comparison to a statistically decreased lymphocytes count (% and #). The age and sex of shisha smokers did not affect the leucocyte counts of the subjects studied. On the other hand, the CD4 count of shisha smokers was affected by sex but not by age while the overall CD4 count was reduced in shisha smokers although this was not statistically significant.

Conflict of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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Authors' Contributions

Babatope, I.O. – Research idea and design, drafted the work.

Amaechi, R.A. – Reviewed the write-up.

Iyevhobu, K.O. – Data analysis.

Enakoya, D. and Ojiezele, G.O. – Field work (Questionnaires administration and sampling).

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