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ORIGINAL ARTICLE

Utility of World Health Organization Haematological Toxicity Scale in the Assessment of Smokers in Port Harcourt, Nigeria

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Running Title: WHO haematological toxicity scale and evaluation of smokers

Abstract

Introduction: This study was aimed at evaluating some haematological parameters among smokers in Port Harcourt, Rivers State and to ascertain if the effect of smoking can be assessed using the World Health Organization Haematological toxicity scale.

Materials and Methods: A cross-sectional study which involved 100 individuals (50 smokers and 50 non-smokers) within the ages of 20-45 years was used. Blood samples were collected by venipuncture into an EDTA anticoagulant bottle for haematological analysis of selected haematology parameters. The samples were processed using haematology auto analyser- SYSMEX KX-21. Statistical analysis was done using Graph Pad prism version 8.02 for windows. Comparisons of mean and standard deviation were made for the various parameters using student's t-test.

Results: The mean values of haemaglobin for smokers (14.50 ± 1.773 g/dL) was significantly elevated when compared with 11.74 ±1.15 g/dL in non-smokers. ($p \leq 0.0001$). There was no statistically significant difference in the mean values of leucocyte count in smokers compared to non-smokers ($p \geq 0.05$). The mean value of granulocyte count in smokers ($4.52 \pm 1.28 \times 10^9$/L) was significantly higher than that of non-smokers ($3.81 \pm .0.72 \times 10^9$/L) ($p \leq 0.0008$). The mean platelet count of smokers ($236.0 \pm 64.65 \times 10^9$/L) was significantly raised when compared with that of non-smokers ($217.7 \pm 42.71 \times 10^9$/L). In the control group, 8% were found to be anaemic corresponding to Grade 2 of the WHO toxicity scale (8-9.4g/dL) whereas all of the smokers were one hundred percent (100%) in grade 0 (≥ 11.0 g/dL). For the white blood cell count, majority of the control subjects were 90% grade 2 (3.0-3.9×109/L) whereas among the smokers, they were 100% in grade 0. For the platelets, all the control subjects were 100% grade 0 ($\geq \times 10^9$/L) while 2% of the

smokers (test) fell into grade 1 (75-99×10^9/L) of the WHO toxicity scale (ie mild thrombocytopenia).
Conclusion: We concluded that smoking causes a significant elevation of haemogobin and granulocytes and at the same time elevated platelet counts whereas 2% of smokers fell into grade 1 of the toxicity scale. This effect may be more pronounced if a longer duration of smoking and a larger sample is considered in further studies.

Keywords: WHO toxicity scale; Haematological Parameters,smokers.

Introduction

Cigarette smoking has been considered as the single most significant cause of preventable morbidity and premature death. People smoke for several reasons, some smoke for enjoyment or social reinforcement and some to alleviate stress (1). For many young people, smoking usually begins for psychological reasons such as parental smoking, curiosity, rebelliousness and assertion of independence. Once it becomes regular, the pharmacological properties of nicotine are a major influence on the persistence of the habit (2).

The World Health Organization (WHO) estimates that there are about 1.1 billion smokers in the world, one third of which are aged between 15-20 years. Most of these smokers are in the developing countries (800 million) and 700 million are men. Tobacco use is usually accepted in many segments of Nigerian society (3).

It is the most important modifiable risk factor for coronary artery disease, chronic obstructive pulmonary disease, hypertension and carcinoma originating in the nasopharynx, bronchus etc.

It has been estimated that an average of 7 minutes of life is lost for each cigarette smoked, within the smoking period. A person who begins smoking at the age of 15 years has an average of 8 years of reduced longevity and one starting after 25 years of age faces an average 4 years reduction (4). Coronary heart disease, cancer and various respiratory diseases account for the majority of excess mortality related to cigarette smoking. Smokers average a 16 fold increased risk of acquiring lung cancer, a 12 fold increased risk of acquiring chronic obstructive pulmonary disease (COPD) and a 2 fold increased risk of having a myocardial infarction as compared to non-smokers (2).

Since early 1950, several studies have shown that a direct relation between smoking and hematological parameters. Muhammed et al., (5) studied 142 male subjects, 71 smokers and 71 non-smokers. Complete blood cell count was measured and it was found that smokers had significantly higher levels of white blood cell count, red blood cell count, hemoglobin and hematocrit, whereas mean corpuscular haemoglobin concentration and platelet count were significantly lower (5).

Cigarette smoking causes more harm than good to the human system. Excessive smoking can result to conditions such as coronary heart diseases, cancer and various respiratory diseases etc. Smoking an average a 16 fold increased risk of having a myocodial infarction as compared to non-smokers (2).

Hematological toxicity is a decrease in bone marrow and blood cells, which may lead to infection, bleeding, or anemia (6). The National Cancer Institute (NCI) classifies five grades for blood toxicity, which refer to the severity of the adverse event (7).

In Port Harcourt, no published data has been encountered on the effect of smoking

on haematological parameters using WHO haematological toxicity scale as a reference point. This study aimed to highlight the dangers of smoking on the health of smokers especially as it affects the haematological parameters such as haemoglobin, leucocyte, granulocyte and platelets among smokers in Rivers State, Nigeria.

Materials and Methods

Study Area

This research work was conducted in Abuja estate, in creek road, Port Harcourt, Rivers State. The choice of this area for the study was as a result of so many young adults who were found engaging in active smoking,

Study Population and Design

The study was cross sectional in nature. The study population comprised of young adult male subjects of the age group of 20-25 years. The duration of smoking in years and the numbers of cigarettes smoked per day was also considered. A total of one hundred (100) Individuals participated in this study, they were divided into two (2) groups, test groups (smokers) and control group (non smokers). The test group included fifty (50) healthy male, smokers aged between 20-45 years, residing in the Abuja Estate of Creek road, Port Harcourt,, Rivers State. The control group subjects were fifty (50) non smokers residing in Port Harcourt metropolis, Rivers State, aged between 20-45 years. Informed consent was taken from all the subjects. Sample taken from study subjects were analysed for haematological parameters such as haemoglobin concentration, total Leucocyte count (TLC), granulocytes and platelets count

Inclusion Criteria

The subjects were selected under the following criteria.

Inclusion criteria for Test group (50 smokers): Age (20-45 years),gender (Male), duration of smoking (2 years and above) and frequency of smoking (up to 5 cigarettes per day)

Exclusion Criteria were as follows: gender (Female), frequency of smoking < 3 Cigarettes per day, smokers with major respiratory problems, age (above 45 years), pregnant women and teenagers below the age of eighteen and who are involved in the occupation of scavenging were excluded also and Subjects with history of diabetes mellitus, kidney disease, hypertension, heart diseases or any other disorder were also excluded

Blood Sample Collection

Five (5) milliliters of venous blood were collected from each subject using sterile hypodermic syringes and needles: the collected blood were dispensed into EDTA-anticoagulant bottles and properly mixed by several gentle inversions and taken to the laboratory for the determination of haemoglobin concentration, red blood cell count (RBC), total leucocyte count (TLC), and platelets count using the haematology auto analyzer.

Haematological Analysis

The collected blood was dispensed into EDTA-anticoagulated blood and was analyzed for the haematological parameters within 3 hours of blood collection. Results obtained were compared with the reference range for a normal/ healthy adult.

Full Blood Count Estimation

The full blood count haematological tests were done by using Hemo analyser – SYSMEX KX-21. The SYSMEX KX-21 runs 60 samples and displays on LCD Screen the particle distribution curves of WBC, RBC and Platelets.

Principle: Blood sample is aspirated, measured to predetermined volume, diluted at the specified ratio and then fed into each transducer. The transducer chamber has a minute hole called the aperture. On both

sides of the aperture there are the electrodes, between which flows direct current. Blood cells suspended in the diluted sample pass through the aperture, causing direct current resistance to the change between the electrodes. As direct current resistance changes blood cell size, it is detected as electric pulse. Blood cell count is calculated by counting the pulses, also analyzing a histogram makes it possible to obtain various analysis data, thus hemoglobin, RBC count, TLC and Platelets count were analysed. Procedures were followed as contained in the standard operating procedures (SOP).

Statistical Analysis

GraphPad version 8.02 for windows statistical package was used for data analysis of the data. Statistical tools such as mean and Standard deviation were used. Students independent sampled t-test was used to compare means of groups for inferential evaluation. The probability (p) value less than 0.05 was used and considered statistically significant

Results

One hundred human subjects (50 smokers and 50 non-smokers) participated in this study. Haematological tests were conducted in 50 male smokers and compared with age matched controls (50 male non-smokers). They were analyzed for the haematological results using the haematological results using haematology auto analyser. The results obtained were expressed as Mean ± Standard deviation.

Table 1 shows the mean values of the parameters studied and comparison between test and control subject. The mean values of haemoglobin of the smokers (14.50 ± 1.773 gldl) was significantly elevated when compared with control subjects (11.74 ± 1.159 gldl) ($p \leq 0.0002$). Similarly the platelet count of the smokers (236 ± 64.5 × 10^9/l) was significantly elevated when compared with control value of (217.7 ± 42.71 × 10^9/l) ($p \leq 0.012$). There were no significant different in the values of leucocytes and granulocyte ($p \leq 0.05$). Separation of means according to age groups is shown in table 2, 3 and 4. The mean values follow the pattern of table 1.

Table 5 shows the haematological toxicity scale of smokers and comparison with standard WHO haematological toxicity scale. For haemoglobin value, the subjects scored zero percent (0%) in all the grades. Similar results was obtained for leucocytes and granulocytes. For platelet count, 98% of the smokers fall under Grade 0 toxicity while 2% fall into grade 1 (75-99×10^9/l) and 0% in other three categories of 2,3 and 4.

Table 1: Overall mean values of the parameters of test and control subjects.

Groups/parameters	Haemoglobin gldl	Leucocyte ×10^9/l	Granulocyte ×10^9/l	Platelets ×10^9/l
Control (n=50)	11.74±1.159	7.160 ±1.294	3.810 ±0.7232	217.7 ±42.71
Test (n=50)	14.50±1.773	7.706 ±1.636	4.524 ±1.276	236 ±64.65
t-test	2.341	1.598	3.113	2.291
p-value	0.003*	0.104^{ns}	0.0001***	0.004**

* = significant at P<0.01; ** = significant at P< 0.001; *** = significant at P< 0.001;
ns= not significant

Table 2: Mean comparison between test and control Groups for age range 20-29 years

Groups/parameters	Haemoglobin g/dL	Leucocyte $\times 10^9$/L	Granulocyte $\times 10^9$/L	Platelets $\times 10^9$/L
Control (N=50)	11.41 ±1.147	6.963 ±1.409	3.550 ±0.9739	210.6 ±52.50
Test (N=50)	14.33 ±1.484	7.900 ±0.5762	5.717 ±0.7441	256.3 ±34.40
T-test	41.66	15.25	4.531	1.847
P-value	0.51^{ns}	0.06^{ns}	0.57^{ns}	0.36^{ns}

ns = not significant.

Table 3: Mean comparison between test and control groups for age range 30-39 years

Groups/parameters	Haemoglobin g/dl	Leucocyte $\times 10^9$/L	Granulocyte $\times 10^9$/L	Platelets $\times 10^9$/L
Control (N=50)	11.84±0.9962	6.911±1.082	3.695±0.4743	228.6±45.38
Test (N=50)	14.59±2.246	7.395±1.491	4.291±1.346	234.8±72.28
T-test	4.928	1.175	1.833	0.3247
P-value	0.0001***	0.24^{ns}	0.07^{ns}	0.74^{ns}

ns = not significant. * = significant at P <0.0001

Table 4: Mean comparison between test and control groups for age range 40-49 years

Groups/parameters	Haemoglobin gldl	Leucocyte $\times 10^9$/L	Granulocyte $\times 10^9$/L	Platelets $\times 10^9$/L
Control (N=50)	11.77 ± 1.309	7.435 ±1.412	3.996 ±0.7790	211 .2±36.56
Test (N=50)	14.45 ±1.324	7.964 ±1.939	4.432 ±1.177	231.6 ±64.08
T-test	6.851	1.049	1.473	1.323
P-value	0.95^{ns}	0.14^{ns}	0.06^{ns}	0.01*

ns = not significant. * = significant at P <0.01

Table 5: WHO Haematological Toxicity Scale evaluation of the study participants

Parameter	Group	Grade 0	Grade1	Grade 2	Grade 3	Grade 4
Hb(gldl)	WHO	≥11.0	9.5-10.9	8.0-9.4	6.5-7.9	≤ 6.5
	Test	100%	0%	0%	0%	0%
	Control	86%	6%	8%	0%	0%
Leucocyte	WHO	≥4.0	3.0-3.9	2.0-2.9	1.0-1.9	≤ 1.0
x 10^9/L	Test	100%	0%	0%	0%	0%
	Control	10%	90%	0%	0%	0%
Granulocyte	WHO	≥2.0	1.5-1.9	1.0-1.4	0.5-0.9	≤ 0.5
x 10^9/L	Test	100%	0%	0%	0%	0%
	Control	100%	0%	0%	0%	0%
Platelets	WHO	≥100	75-99	50-74	25-49	≤ 2.5
x 10^9/L	Test	98%	2%	0%	0%	0%
	Control	100%	0%	0%	0%	0%

Discussion

World Health Organization haematological toxicity scale is often used in evaluating malignant disorders which has serious consequences on the haemopoietic and lymphoid tissues in the body. It is hypothesized that cigarette smoking has no toxic effect on the haematological parameters in those who engage in smoking. However, there is constant advert caveat that says "Smokers are liable to die young" or "Cigarette smoking is dangerous to your health". In this study, Smokers were placed on the WHO haematological toxicity scale in order to evaluate the impact of cigarette smoking on selected haematological parameters. The major findings in this study are as follows;1) Elevation of haemoglobin level in smokers. 2) mild granulocytosis and 3) mild thrombocytopenia.

The haemoglobin concentrations are increased in smokers as observed in this study. Cigarette smoking produces carbon-monoxide. The carboxy-haemoglobin (CoHb) found in smokers are known to interfere with oxygen transport and utilization. Smoking reduces tissue oxygen delivery leading to hypoxia. This hypoxia is a potent stimulus for the release of erythropoietin by interstitial cells in 85% of peritubular capillary bed in kidney and 15% of perivenous hepatocytes in liver. Erythropoietin increases the number of erythropoietin –sensitive committed stem cells in the bone marrow, that are converted to red blood cells precursors and subsequently to mature erythrocytes. It also promotes haemoglobin synthesis by increasing globin synthesis and potentiating amino levulinic acid synthetase thereby leading to an increase in red cell count and haemoglobin concentration. Studies conducted on red cell count and haemoglobin concentration by Sagone et al, (8) and Whitehead et al, (9) are corroborating with our study. Among control subjects, 6% were in group 1 of the WHO scale and 8% in grade 2 categories. This anaemic situation were not found among smokers as all of them fell into grade 0.

In this study, it has been observed that in smokers, total leucocyte count (TLC) is increased but not statistically significant.

The precise mechanism by which, cigarette smoking leads to an elevated TLC is not clear. The possible hypotheses put forward to explain this finding in smokers are as follows: Cigarette smoke contains many harmful components including acrotein, nicotine, acetaldehyde and formaldehyde produced from chemical reactions within the cigarette smoke. In addition, cigarette smoke produces structural changes in the respiratory tract. These changes include peribronchiolar inflammation and fibrosis, increased mucosal permeabililty, impairment of mucocilliary clearance, changes in pathogen adherence and disruption of the respiratory epithelium. These changes predispose to the development of upper and lower respiratory tract infections, which may amplify cigarette smoking induced lung inflammation. The systemic inflammatory response is characterized by the stimulation of the haematopoietic system, specifically the bone marrow resulting in the release of leucocytes into circulation aided by colony stimulating factors like granulocyte-monocyte colony stimulating factor (GM-CSF). This may lead to an increase in total leucocyte count in smokers. Quantitative analysis of different types of WBC clearly indicated that there is a relationship between leukocytes and lymphocytes. Lymphocytes increased with the increase of leukocytes in the smokers compared with non-smokers(7).

In this study, it has been observed that platelet count is elevated significantly. The increase in platelet count in smokers can be explained as follows: Cigarette smoke induced lung inflammation leading to stimulation of bone marrow, resulting in the release of platelets. Platelet activity and survival appear to be adversely affected by chronic smoking. Smoking causes acute and chronic inhibition of cyclo-oxygen which inhibits prostacylin and increases the biosynthesis of thromboxane A2. Thromboxane A2 is potent vasoconstrictor and platelet agonist. This may be contributing to higher platelet count in smokers. However,among the smokers, 2 percent fell into WHO group 1 which is platelet count between 75-79 x 10^9/l.The possible explanation to this is that in early smoking phase,there is inflammation which triggers the increase in the number of platelets but as the smoking becomes chronic and toxic to the cells, the platelet counts begin to decline and progressively leads to thrombocytopenia.

Conclusion

Smoking causes increase in heamoglobin value giving false feeling of well being.There was mild thrombocytopenia among smokers as 2% of the study participants were found in group 1(75-99×10^9/l) of the WHO haematological toxicity scale.

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