

Research Article

Leucocytes and CD4 Counts of Petrol Attendants in Ekpoma, Edo State, Nigeria

Babatope IO^{1*}, Jesuyon DO¹, Jesuyon OMA², Omolumen LE³

¹Department of Haematology and Blood Transfusion Science, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria

²Department of Animal Production and Health, Federal University, Oye-Ekiti, Ekiti State, Nigeria

³Department of Chemical Pathology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria

*Correspondence author: Babatope IO, Department of Haematology and Blood Transfusion Science, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Edo State, Nigeria; Email: babatope_olaniyi@yahoo.com

Citation: Babatope IO, et al. Leucocytes and CD4 Counts of Petrol Attendants in Ekpoma, Edo State, Nigeria. J Clin Immunol Microbiol. 2024;5(2):1-8.

<http://dx.doi.org/10.46889/JCIM.2024.5205>

Received Date: 20-06-2024

Accepted Date: 15-07-2024

Published Date: 22-07-2024



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Abstract

White Blood Cells (WBCs), also called leucocytes or leukocytes, are the cells of the immune system that are involved in protecting the body against both infectious diseases and foreign invaders. The overall aim of this study is to determine the leucocytes and CD4 counts of Petrol attendants in Ekpoma, Edo State. A total of fifty (50) petrol attendants aged 18-60years were recruited for this study while fifty (50) apparently healthy subjects served as control. The leucocyte counts were performed using Sysmex haematology autoanalyzer while the CD4 count was carried out using flow cytometry. The mean values of WBC total count of the control and test subjects were 5.57 ± 1.82 and 5.60 ± 1.2 respectively. However, statistical comparison did not reveal any significant difference ($P > 0.05$) between the two groups. With respect to differential leucocytes count, the mean values of the neutrophils (%) of the control and test subjects were 38.70 ± 8.97 and 41.05 ± 8.71 respectively. Similarly, statistical comparison did not reveal any significant difference ($P > 0.05$). Furthermore, the mean values of the lymphocytes (%) of the control and test subjects were 50.81 ± 8.94 and 48.19 ± 8.83 respectively. Also, the statistical comparison between the two groups was not significant ($P > 0.05$). In addition, the MXD (%) values of the control and test subjects were 10.09 ± 3.50 and 10.74 ± 4.48 respectively. There was also no significant difference ($P > 0.05$) in the mean values of the MXD (%) of both subjects. In addition, the mean values of CD4 count of the test subjects was higher than the control, this did not reveal any statistically significant difference ($P > 0.05$). From our findings, there was no statistically significant difference in total leucocyte and CD4 counts of petrol attendants compared to control. However, the MXD (%) of the male subjects was significantly higher ($P < 0.05$) compared to their female counterparts. Further studies that will capture subjects that have been exposed to petroleum fumes for a longer period of time is hereby recommended.

Keywords: Leucocytes; CD4; Petrol; White Blood Cells (WBCs); Haematology

Introduction

White Blood Cells (WBCs), also called leucocytes, or leucocytes are the cells of the immune system that are involved in protecting the body against both infectious diseases and foreign invaders. All white blood cells are produced and derived from a multipotent cell in the bone marrow known as a haemopoietic stem cell. Leucocytes are found throughout the body, including the blood and lymphatic system [1]. All white blood cells have nuclei, which distinguishes them from other blood cells, the anucleated Red Blood Cells (RBCs) and platelets. Types of white blood cells can be classified in standard ways. Two pairs of broadest categories classify them either by structure (granulocytes or agranulocytes) or by cell division lineage (myeloid cells or lymphoid cells). The broadest categories can be further divided into five main types; neutrophils, eosinophils, basophils,

lymphocytes and monocytes [2]. These types are distinguished by their physical and functional characteristics. Monocytes and neutrophils are phagocytic. Further subtypes can be classified; for example, among lymphocytes, there are B-cells, T-cells and Natural Killer (NK) cells.

Cluster of Differentiation 4 (CD4) is a glycoprotein found on the surface of immune cells such as T-helper cells, monocytes, macrophages and dendritic cells. CD4+ T helper cells are white blood cells that are essential part of the human immune system. They are called helper cells because one of their main roles is to send signals to other types of immune cells, including CD8 killer cells, which then destroy the infectious particle [3].

Fuel is a complex mixture of volatile flammable liquid hydrocarbons which include benzene, toluene and xylene. Fuel attendants, also called fitting station attendants, gas station attendants or gas tenders perform duties at fuel filling stations by refilling fuel tanks [4]. Fuel attendants inhale and make contact with these flammable petrochemicals. Several volatile aromatic hydrocarbons are present in the atmosphere of petrol service station as a result of emission of vapours during dispensing, loading, unloading and transportation of petrol [5]. Occupational exposure to petroleum products and fumes have been reported to have toxic effects on various organs and body systems with high impact of the human respiratory system [5]. Organs such as the heart, lungs, skin and kidneys are affected by these toxic effects remitting in various diseases and different forms of genotoxic, mutagenic, immunotoxic, carcinogenic and neurotoxic manifestations [7,8]. Therefore, this present study is aimed at determining the total leucocyte and CD4 counts of petrol attendants in Ekpoma, Edo State, Nigeria.

Material and Methods

Study Area

This study was carried out in Ekpoma. Ekpoma is the headquarters of Esan West Local Government Area in Edo State which falls within the rain forest/savannah transitional zone of South Western Nigeria. The area lies between latitudes 6° 43' and 6° 45' North of the Equator and longitudes 6° 51' and 6° 51' East of the Greenwich Meridian. Ekpoma has a land area of 923 square kilometers with a population of 170,123 people as at the 2006 census [9]. The town has an official post office and it is home of Ambrose Alli University.

Study Population

A total of fifty (50) petrol attendants between 18-60 years of age and of both sexes were recruited for this study while fifty (50) apparently healthy subjects that do not dispense petrol served as control.

Ethical Approval

Ethical approval was obtained from the Health Research Ethics Committee (NHREC Registration Number: NHREC 12/06/2013) of Ambrose Alli University, Ekpoma. Informed Consent was sought from each participant before sample collection.

Sampling Criteria

Inclusion Criteria: Apparently healthy petrol attendants who gave their consent were included in this study.

Exclusion Criteria: Individuals who did not meet the inclusion criteria were excluded from the study.

Sample Collection

About 4ml of blood was collected from each subject (test and control) via venepuncture and dispensed into Ethylene Diamine Tetra Acetic Acid (EDTA) bottle and mixed immediately by reverse inversion method. All the field samples were placed in cold transport boxes with a temperature range of 20°C-80°C before they were transported to the laboratory for analysis. All samples were collected between 9.00am - 12.00 noon each day. Samples were analysed with minimal delay and not longer than 6 hours.

Sample Analysis

Leucocytes counts using Sysmex KX-21N autoanalyzer White blood cells (leucocytes) counts (that is total and differential leucocyte counts) were analysed using the Haematology autoanalyzer (Sysmex Corporation, Kobe, Japan). The Sysmex KX-21N is an automatic, 19 - parameters, 3 - part differential blood cell counter. The procedure was carried out according to the

manufacturer's instructions. The principle of this method is based on then DC (Direct Current) Detection method.

CD4 Count

CD4 cells counts were determined by flow cytometry using Partec cyflow counter adapted to single platform technology [10]. Forward and side scatter signals were measured using a linear scale. To ensure the optical alignment of the equipment and fluorescence compensation settings, count check bead green were run every day and the count was compared with the manufacture's range.

Statistical Analysis

The results obtained were presented as mean $\pm$ standard deviation. Statistical analysis was carried out using Student's t-test and one way Analysis of Variance (ANOVA). $P < 0.05$ was considered significant.

Results

Leucocytes and CD4 Counts of Petrol Attendants in Ekpoma

The total leucocytes and CD4 of Petrol Attendants in Ekpoma is shown in Table 1. The means values of the WBC total count of the control and test subjects were 5.57 ± 1.82 and 5.60 ± 1.2 respectively. However, statistical comparison did not reveal any significant difference ($P > 0.05$) between the two groups. With respect to differential leucocytes count, the mean values of the neutrophils (%) of the control and test subjects were 38.70 ± 8.91 and 41.05 ± 8.71 respectively. Similarly, statistical comparison did not reveal any significant difference ($P > 0.05$). Furthermore, the mean values of the lymphocytes (%) of the control and test subjects were 50.8 ± 8.94 and 48.19 ± 8.83 respectively. Also, the statistical comparison between the two groups was not significant ($P > 0.05$). In additional, the MXD (%) values of the control and test subjects were 10.09 ± 3.50 and 10.74 ± 4.48 respectively. There was also no significant difference ($P > 0.05$) in the mean values of the MXD (%) of both subjects. In addition, the mean values of the CD4 count of the control and test subjects were 865.50 ± 295.23 and 911.76 ± 335.70 . Although the CD4 count of the test subjects was higher than the control, this did not reveal any statistically significant difference ($P > 0.05$).

Total Leucocytes and CD4 Counts of Petrol Attendants Based on Gender in Ekpoma

The total leucocytes and CD4 counts of Petrol Attendants based on gender is shown in Table 2. The results of the mean values of WBC total count of the male and female subjects were 5.50 ± 1.32 and 5.73 ± 1.06 respectively. Statistical comparison between both sexes revealed a significant difference ($P < 0.05$) between the two groups. With respect to differential leucocytes count, the mean values of the neutrophils (%) of the male and female subjects were 41.61 ± 8.56 and 40.35 ± 9.04 respectively. However, statistical comparison did not reveal any significant difference ($P > 0.05$). Furthermore, the mean values of the lymphocytes (%) of the male and female subjects were 46.23 ± 8.51 and 50.59 ± 8.81 respectively. Also, the statistical comparison between these two groups was not significant ($P > 0.05$). Furthermore, the MXD (%) value of the males and female subjects were 12.16 ± 5.04 and 8.93 ± 2.81 respectively. In contrast, the statistical comparison between both sexes was significantly increased ($P > 0.05$) in male subjects compared to their female counterparts. The results of the mean values of the CD4 counts of the male and female subjects were 866.04 ± 354.84 and 969.95 ± 301.78 . Although the CD4 count of the male subjects was lower than the female subjects, this did not reveal any statistically significant difference ($P > 0.05$).

Total Leucocytes and CD4 Counts of Petrol Attendants According to Age

The result of the total leucocytes and CD4 counts of Petrol Attendants according to age is shown in table 3. Mean values of the total WBC count of the subjects belonging to the age groups of 18-27 years, 28-37 years, 38-47 years and 47 years old and above were 5.85 ± 1.02 , 5.38 ± 1.45 , 3.90 ± 0.55 and 6.25 ± 0.35 respectively. Statistical comparison revealed a significant increase in the WBC total count of the subjects in the age bracket of 18-27 years and 47 years old and above, while subjects belonging to the age group of 28-37 and 38-47 years recorded a progressive statistical significantly ($P < 0.05$) decreased values. Furthermore, the differential leucocyte counts of the studied subjects according to the age groups of 18-27 years, 38-47 years and 47 years and above were 39.89 ± 8.63 , 44.33 ± 10.21 , 43.19 ± 2.30 and 38.85 ± 0.75 for neutrophils (%) and 48.85 ± 9.53 , 43.30 ± 8.57 , 48.28 ± 5.03 and 47.70 ± 7.64 for lymphocytes (%). Statistical comparison among the age groups did not reveal any statistical difference ($P > 0.05$) for neutrophils (%) and lymphocytes (%). The MXD (%) mean values of 11.26 ± 4.30 , 9.13 ± 4.18 , 8.60 ± 2.82 and 16.45 ± 8.40 and the mean values of CD4 count (cells/ μ L) of 989.25 ± 339.60 , 905.00 ± 195.74 , 537.75 ± 338.09 and 460.50 ± 152.03 according to the respective ages were progressively decreased ($P < 0.05$).

Leucocytes and CD4 Counts of Petrol Attendants Based on Duration of Exposure

The total leucocytes and CD4 counts of Petrol attendants based on duration of exposure is summarized in Table 4. The WBC total count ($\times 10^3/\mu\text{L}$) based on period of exposure to petrol fumes were 5.76 ± 1.29 , 5.19 ± 0.84 , 6.13 ± 0.84 and 5.14 ± 1.31 for the periods of 1-6 months, 7-12 months, 1 year and above and 2 years and above respectively. The statistical comparison was not significant ($P > 0.05$) based on the various periods of exposure. Similarly, the differential leucocyte counts (%) with respect to the neutrophils %, lymphocytes % and MXD % were 41.51 ± 9.19 , 40.21 ± 7.43 , 37.35 ± 10.57 and 41.80 ± 8.68 ; 48.59 ± 9.14 , 47.90 ± 3.77 , 49.45 ± 9.91 and 47.16 ± 7.72 ; and 9.90 ± 3.87 , 11.88 ± 6.54 , 13.20 ± 3.24 and 10.83 ± 4.42 respectively. There was also no significant difference in DLC and periods of exposure. The mean values of the CD4 counts of the different duration of exposures of 1-6 months, 7-12 months, 1 year and above and 2 years and above were 987.08 ± 228.48 , 802.87 ± 387.51 , 984.50 ± 488.25 and 824.07 ± 337.63 respectively. Statistical comparison between CD4 counts and duration of exposure did not reveal any significant difference ($P > 0.05$).

Parameters	Control Subjects (Mean $\pm$ SD) n = 50	Test Subjects (Mean $\pm$ SD) n = 50	t-value	p-value
WBC total ($\times 10^3/\mu\text{L}$)	5.57 $\pm$ 1.82	5.60 $\pm$ 1.21	0.01	0.9382
Neutrophils (%)	38.70 $\pm$ 8.97	41.05 $\pm$ 8.71	1.77	0.1869
Lymphocytes (%)	50.81 $\pm$ 8.94	48.19 $\pm$ 8.83	2.24	0.1380
MXD (%)	10.09 $\pm$ 3.50	10.74 $\pm$ 4.48	0.65	0.4218
CD4 Count (cells/ μL)	865.50 $\pm$ 295.23	911.76 $\pm$ 335.7	0.54	0.4661
WBC: White Blood Cells; MXD: WBC-middle Cells Count; CD4: CD4 T-lymphocytes; SD: Standard Deviation; P-value: Significance level, P-values greater than 0.05 shows non-significance between subject categories ($P > 0.05$)				

Table 1: Leucocytes and CD4 counts of petrol attendants in Ekpoma.

Parameters	Control Subjects (Mean $\pm$ SD) n = 50	Test Subjects (Mean $\pm$ SD) n = 50	t-value	p-value
WBC total ($\times 10^3/\mu\text{L}$)	5.501.32	5.73 $\pm$ 1.06	4.50	0.0500
Neutrophils (%)	41.61 $\pm$ 8.56	40.35 $\pm$ 9.04	0.25	0.6161
Lymphocytes (%)	46.23 $\pm$ 8.51	50.59 $\pm$ 8.81	3.13	0.0833
MXD (%)	12.16 $\pm$ 5.04	8.93 $\pm$ 2.81	7.22	0.0099
CD4 Count (cells/ μL)	866.04 $\pm$ 358.84	969.95 $\pm$ 301.78	1.19	0.2818
WBC: White Blood Cells; MXD: WBC-middle Cells Count; CD4: CD4 T-lymphocytes; SD: Standard Deviation; P-value: Significance level, P-values greater than 0.05 shows non-significance between subject categories ($P > 0.05$)				

Table 2: Total leucocytes and CD4 counts of petrol attendants based on gender in Ekpoma.

Age group (years)/ Parameter	18-27yrs (Mean $\pm$ SD) (n=32)	28-37yrs (Mean $\pm$ SD) (n=4)	38-47yrs (Mean $\pm$ SD) (n=12)	47yrs and above (Mean $\pm$ SD) (n=2)	F-value	p-value
WBC total ($\times 10^3/\mu\text{L}$)	5.85 $\pm$ 1.02a	5.38 $\pm$ 1.45b	3.90 $\pm$ 0.55c	6.25 $\pm$ 0.35a	4.11	0.0116
Neutrophils (%)	39.89 $\pm$ 8.63a	44.33 $\pm$ 10.21a	43.19 $\pm$ 2.30a	38.85 $\pm$ 0.75a	1.07	0.3697
Lymphocytes (%)	48.85 $\pm$ 9.53a	43.30 $\pm$ 8.57a	48.28 $\pm$ 5.03a	47.70 $\pm$ 7.64a	0.23	0.8723
MXD (%)	11.26 $\pm$ 4.30a	9.13 $\pm$ 4.18b	8.60 $\pm$ 2.82c	16.45 $\pm$ 8.40a	2.20	0.0460
CD4 Count (cells/ μL)	989.25 $\pm$ 339.60a	905.00 $\pm$ 195.74b	537.75 $\pm$ 338.09c	460.50 $\pm$ 152.03c	4.08	0.0120

Table 3: Total leucocytes and CD4 counts of petrol attendants with respect to age in Ekpoma.

Duration of Exposure/ Parameter	1-6 months (Mean±SD) (n=24)	7-12 months (Mean±SD) (n=8)	1yr & above (Mean±SD) (n=4)	2 years (Mean±SD) (n=14)	F-value	p-value
WBC total (x10 ³ /μL)	5.76±1.29a	5.19±0.84a	6.13±0.84a	5.41±1.31a	0.81	0.4939
Neutrophils (%)	41.51±9.19a	40.21±7.43a	37.35±10.57a	41.80±8.68a	0.31	0.8188
Lymphocytes (%)	48.59±9.14a	47.90±3.77a	49.45±9.91a	47.16±7.72a	0.10	0.9573
MXD (%)	9.90±3.87a	11.88±6.54a	13.20±3.24a	10.83±4.42a	0.86	0.4707
CD4 Count (cells/μL)	987.08±228.48a	802.87±387.51a	984.50±488.25a	824.07±337.63a	1.07	0.3716

Table 4: Total leucocytes and CD4 counts of Petrol Attendants according to duration of exposure to petrol fumes.

Discussion

Literature abounds linking one's job to certain impalatable health outcomes [11]. There is evidence about health effects linked to low level dose exposures to volatile organics including benzene in Petrol Motor Spirit (PMS) [12,13]. In this study, the results of the WBC total count of the test subjects compared to control did not reveal any significant difference ($P>0.05$). Our findings is supported by the previous reports of Collins, et al., Tsai, et al., Uzma, et al., and Ezejiofor, who did not observed decreased blood cell counts on routine monitoring of workers exposed to low levels of benzene [11,14-16]. In contrast, other authors such as Okoro et al. (2006) reported a significant decrease ($P<0.01$) in white blood cell counts when compared with controls. The authors observed that the decrease was greater in those exposed for more than two years and they attributed this to be possibly caused as a result of pancytopenia and leucopenia. The observations of Okoro, et al., lends credence to the association between duration of exposure to these volatile organic substances and reduction in blood cell counts particularly white blood cells [4]. Marieb, observed that this may result in impaired migration of phagocytic cells, lower resistance to viruses, bacteria and foreign bodies [17]. The statistically insignificant WBC total count result obtained in our study may be due to the category of subjects we recruited for our study. The different study groups recruited by authors such as Okoro, et al., could be the possible explanation for the significant decreased WBC total count they reported [4]. In Ekpoma, Nigeria, we have observed that poor conditions of service, employers' consistent habit of recruiting young secondary school leavers on a temporary basis and the constant search for "greener pastures" by these young employers among other factors may be some of the reasons why we could not recruit subjects that had stayed longer years at their various duty posts. Hence, our inability to record any association between WBC total count and petrol attendants in the study area. Also, low customers traffic coupled with the semi-urban nature of Ekpoma may also be one of the factors responsible for this. This is because most of the filling stations' nature of business cannot be compared to urban areas where the volume of business transaction is more and this may be inversely related to amount to exposure to these fumes here.

With respect to differential leucocyte counts, the results obtained in the study did not reveal any significant difference ($P>0.05$) in the mean values of Neutrophils % and lymphocytes % observations of Ezejiorfor (2016) who found the granulocyte count (%) of oil workers to be consistently decreased significantly ($P<0.01$) with converse increases in the lymphocyte count % ($P<0.01$). On the other hand, Getu, et al., revealed that the absolute lymphocyte count % of petrol filling workers showed a significant increment compared with the control group [18]. Ezejiofor, reasoned that this may be caused by a possible functional aberration following haemopoietic toxicity of the oil workers [11].

The MXD test measures the combined levels of three types of white blood cells (monocyte, eosinophils and basophils) in the blood. In the present study, the mean values of the MXD % of the male subjects was significantly higher ($P<0.05$) than those of their female counterparts. Our finding is in consonance with the previous report of Uzma, et al., who observed an initial increase in the eosinophils counts of workers with less than ten years of exposure, which they claimed may be due to the effect of polluted air [16]. According to Moller, et al., there are chances for Eosinophilia to occur among peoples who are exposed to polluted air [19]. Similarly, Singh, et al., observed the value of eosinophil count was a little bit higher in their study group than control group when the duration of exposure was less than 10 years and on comparison, statistically significant ($P<0.05$) changes were seen. Furthermore, in another study, Pesatori, et al., revealed that the eosinophil count was inversely related to benzene exposure only among smokers [10]. According to Haen, et al., it was found that allergens - that is, substances causing immediate hypersensitivity reactions stimulate Eosinophils to produce secretions inhibiting the release of vasoactive amines from masT-

cells and basophils [20].

From this study, the results of the CD4 count of petrol attendants did not show any statistically significant difference ($P>0.05$) when compared with control. This is in line with the findings of Chen, et al., who reported that out of the four benzene - exposed groups studied, CD4(+) T-lymphocytes and CD4(+)/CD8(+) ratio did not show any statistically significant difference ($P>0.05$) [21]. However, in another study by Chen et al. (2012) they reported that the percentage of CD4 (+) T-cells in the experimental group decreased continuously in the second, third and fourth examinations of the subjects they studied. Similarly, Fayed, et al., stated that when compared with controls, benzene - exposed workers had significant decrease in CD4 cells [22]. Okoro, et al., (2006) indicated that the petroleum fumes caused a reduction in haematological indices which worsened with prolonged exposure [4]. Ogbomade, et al., also observed that the effect of exposure to petroleum products tend to increase with increasing exposure [23].

In terms of sex, the mean values of the WBC total count of the female subjects studied was significantly higher ($P<0.05$) than those of their male counterparts. Our finding is in agreement with the observations of Taylor, et al., and England and Bain who both reported slightly higher differences with the total leucocyte counts in healthy girl volunteers than in boys and in women than in men respectively [24,25]. On the other hand, other researchers such as Okoro, et al., reported that there was a significant decrease ($P<0.01$) in the white blood cells in both sexes of the test groups they studied when compared with control [4]. According to Marieb, this decrease may be caused by pancytopenia and leucopenia, which result in impaired migration of phagocytic cells, lower resistance to viruses, bacteria and foreign bodies [17]. In contrast, Okoro, et al., observed WBC total count that was greater in those exposed for more than two years [4]. While the duration of exposure to petroleum fumes may play a significant role in this, other reasons for this increase are not clear.

In this study, the results of the Neutrophils % and Lymphocyte % between the male and female subjects studied did not show any statistical significance ($P>0.05$). Our finding is in line with the previous report of Ezejiofor who found that gender classification did not show any appreciable impact [11]. However, Okoro, et al., reported that there was significant decrease ($P<0.01$) in white blood cell count in both sexes of test groups when compared with control [4]. The reason for this is not clear, but Ogbomade, et al., attributed this to duration of exposure to such fumes which tend to increase with increasing exposure [23]. Furthermore, Okoro, et al., indicated that a reduction in haematological indices was worsened by prolonged exposure [4].

From the standpoint of age, the mean values of the WBC total count of the subjects in the age brackets of 18-27 years and 47years and above revealed a statistically significant increase ($P<0.05$). Our finding concurred with previous reports of Ezejiofor, who inferred that age grouping suggests the health effects indicated by the observed variations are likely to rear up from age 40 years [11]. However, Fayed, et al., observed insignificant differences regarding age and suggested that the reduction in the numbers of lymphocytes and subpopulations did not occur because of immune system aging, but probably because of exposure to benzene [22].

With respect to the duration of exposure, the results of the WBC (total) count, differential leucocyte count and CD4 count were not significantly different ($P>0.05$) inferring that duration of exposure did not exert any effect on the aforementioned parameters. However, our findings disagreed with the previous work of Fayed, et al., who found that duration of occupational exposure to benzene showed a significant negative correlation with the WBC counts including all lymphocyte subtypes and neutrophils [22]. Similarly, Ezejiofor, reported statistically significant correlations for exposure grouping with most of the parameters suggesting that exposure period has a strong impact in defining the patterns of variations observed in the haematological indices among oil workers [11]. Furthermore, Ogbomade, et al., reported that the result of their study revealed an increase in the neutrophil count of individuals exposed to petrol fumes for a duration of 1-5 years and 6-10 years [23-28]. Their result also showed a significant decrease in the lymphocyte count.

Conclusion

In conclusion, there was no statistically significant difference in the leucocytes and CD4 counts of petrol attendants in Ekpoma, Edo State, Nigeria. However, the MXD % of the male subjects was significantly higher ($P<0.05$) compared to their female counterparts. Further studies that will capture subjects that have been exposed to petroleum fumes from two years and above is

hereby recommended.

Conflict of Interests

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

Funding

This research did not receive any grant from funding agencies in the public, commercial, or not-for-profit sectors.

Authors' Contributions

The entire study procedure was conducted with the involvement of all authors.

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