



Leucocytes and Cd4 Counts of Individuals Exposed to Wood Dust in Ekpoma, Edo State, Nigeria

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Abstract

Exposure to wood dust may result in external and internal health problems which may be immediate, short-term or long-term. This study was carried out to evaluate the leucocytes and CD4 counts of individuals exposed to wood dust in Ekpoma, Edo State, Nigeria. A total of fifty individuals exposed to wood dust aged 16-60 years and of both sexes were recruited for this study. Fifty apparently healthy subjects who were not exposed to wood dust served as control. The leucocyte counts were carried out using Sysmex KX-21N Autoanalyzer and the CD4 count was determined using Flow Cytometry. In this study, the results obtained showed that the CD4 count (cells/ μL) of the test subjects and control subjects were 912.60 ± 298.05 and 891.14 ± 304.61 respectively. Similarly, total leucocyte counts ($\times 10^3/\mu\text{L}$) of both test and control subjects were 5.44 ± 1.34 and 5.34 ± 1.74 respectively. Furthermore, LYM % of the test subjects and control subjects was 41.76 ± 10.87 and 49.48 ± 8.67 , NEUT % was 45.58 ± 10.19 and 37.74 ± 8.39 , while MXD % was 12.38 ± 4.96 and 11.65 ± 3.69 respectively. Neutrophil % was significantly higher while lymphocyte % was significantly lower. There was a statistically significant increase in CD4 count of female subjects compared to males. Age did not affect any of the parameters studied except total leucocyte count. Duration of exposure to wood dust did not affect any of the parameters studied. In conclusion, neutrophil %, lymphocyte % and CD4 count were variably affected by wood dust. Similarly, the WBC total count of subjects in the age bracket of 51 years and above was significantly higher compared to other age groups. However, duration of exposure to wood dust did not affect any of the parameters studied. We hereby recommend that individuals exposed to wood dust should be encouraged to use Personal Protective Equipment (PPE) such as face mask while working to reduce exposure.

Keywords: Leucocytes; CD4; Wood; Dust; Haematology

Introduction

Wood dust is made of wood shavings from machining wood; it refers to the tiny sized and powdery wood waste produced by sawing of wood [1]. About 10-13% of the total volume of the wood log is reduced to wood dust in milling operations; this dust

generally depends largely on the average width of the saw kern and the thickness of the timber sawed [2]. Particle sizes in the wood dust package are distributed from very fine to coarse [2]. Exposure to wood dust may result in external and internal health problems which may be immediate, short-term or long-term [3].

White blood cells, also called leucocytes are the cells of the immune system that are involved in protecting the body against both infectious diseases and foreign invaders [4]. All white blood cells are produced and derived from multipotent cells in the bone marrow known as haematopoietic stem cells and their levels of production are regulated by organs such as the spleen, liver, and kidneys [4]. Leucocytes are found throughout the body, including the blood and lymphatic system [5]. All white blood cells have nuclei, which distinguishes them from the other blood cells, the anucleated red blood cells (RBCs) and platelets [5]. White blood cells are part of the body's immune system [6]. They help the body fight infections and other diseases [6]. Types of white blood cells are granulocytes (neutrophils, eosinophils, and basophils), and agranulocytes (monocytes, and lymphocytes (T cells and B cells) [6].

The "CD" or cluster of differentiation is a protein expressed on the surface of the cells of the haematopoietic system [7]. CD4 cells are white blood cells that play an important role in the immune system [8]. CD4 cell count gives you an indication of the health of the immune system - the body's natural defense system against pathogens, infections and illnesses [9].

Occupational exposure to wood dust particles has long been reported of its associated varying degrees of negative health effects due to different extractive chemicals present in the various timber species [10]. However, tropical hardwood is also reported to have higher levels of extractive chemicals of antihistamine, antioxidant and anti-inflammatory properties [10]. Leucocytes are effectors and biomarkers of inflammation [11]. The total white blood cells count in the circulation is associated with infection, coronary, cancer and all-cause mortality in human subjects [11]. In Nigeria, wood workers have for years been exposed to wood dust from mixed tropical hardwood species, with little or no protective equipment such as nose masks [12]. Furthermore, there are evidences which show that exposure to wood dust causes alteration in blood cell counts [13]. Despite the fact that various researches on the impact of wood dust on exposed people have been conducted, none of these studies specifically addressed the impact of wood dust on the leucocytes and CD4 count of people exposed to wood dust in our study area. Hence, this study was carried out to determine the leucocytes and CD4 counts of individuals exposed to wood dust in Ekpoma, Edo State, Nigeria.

Materials 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° 5' and 6° 8' 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 [24]. The town has an official post office and it is home of Ambrose Alli University.

Study population

A total of fifty (50) individuals exposed to wood dust between the ages of 16 and 60 years and of both sexes were recruited for this study. Similarly, Fifty (50) apparently healthy individuals not exposed to wood dust and between 16-60 years of age and of both sexes 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.

Inclusion and exclusion criteria

Apparently healthy and fit male and female Subjects exposed to wood dust who gave their consent were included in this study While individuals who were not exposed to wood dust and those who did not give their consent among others were excluded from this study.

Sample collection

About 4ml of blood was collected from each subject via venepuncture and dispensed in Ethylene Diamine Tetra Acetic Acid (E.D.T.A.) bottle and mixed immediately by reverse uniform inversion. All the field samples were placed in cold transport boxes with a temperature range of 2°C-8°C before they were transported to the laboratory for analysis. All samples were collected between 9.00 am - 12.00 noon each day. Samples were analysed with minimal delay and not longer than 6 hours.

Sample analyses

Leucocyte counts using Sysmex KX-21N autoanalyzer

The leucocyte counts were analyzed using Sysmex KX - 21N 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 (Partec GmbH, 2006) adapted to single platform technology. 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 manufacturer’s range.

Statistical analysis

The results obtained were presented as mean ± 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

Total leucocytes and CD4 counts of individuals exposed to wood dust in the study area

The result of the total leucocytes and CD4 counts of individuals exposed to wood dust in the study area is shown in table 1. The results obtained showed the mean values of WBC (x10³/μl) of the test subjects and control to be 5.44 ± 1.34 and 5.34 ± 1.74, NEUT (%) was 45.58 ± 10.19 and 37.74 ± 8.39, LYM (%) was 41.76 ± 10.87 and 49.48 ± 8.67, MXD (%) was 12.38 ± 4.96 and 11.65 ± 3.69 respectively. Similarly, the mean values of CD4 count (cell/μl) of the test subjects and control was 912.60 ± 298.05 and 891.14 ± 304.61 respectively. NEUT (%) was significantly higher (p < 0.05) in test subjects compared with control, while LYM (%) was significantly (p < 0.05) lower in test subjects compared with control. However, there was no significant difference (p > 0.05) in the CD count and other leucocytes counts of test subjects when compared with control.

Parameters	Test Subjects (Mean ± SD) n = 50	Control subjects (Mean ± SD) n = 50	t-value	p-value
WBC (x10 ³ /μL)	5.44 ± 1.34	5.34 ± 1.74	0.293	0.771
NEUT (%)	45.58 ± 10.19	37.74 ± 8.39	4.096	0.000
LYM (%)	41.78 ± 10.87	49.48 ± 8.67	3.984	0.000
MXD (%)	12.38 ± 4.96	11.65 ± 3.69	0.802	0.426
CD4 Count (cell/μl)	912.60 ± 298.05	891.14 ± 304.61	0.394	0.695

Table 1: Total leucocytes and CD4 counts of individuals exposed to wood dust in the study area.

KEYS: WBC- White blood cells; NEUT- Neutrophils; LYM- Lymphocytes; MXD- Middle cells; %- Percentage; μl- Micro litre; CD4- Cluster of differentiation 4.

Total leucocytes and CD4 counts of individuals exposed to wood dust based on sex

Table 2 showed the total leucocytes and CD4 counts of individuals exposed to wood dust based on sex. The results obtained showed the mean values of WBC (x10³/μl) in male and female test subjects to be 5.45 ± 1.41 and 5.07 ± 0.75, NEUT (%)

was 47.21 ± 9.56 and 45.17 ± 13.74, LYM (%) was 40.75 ± 9.96 and 45.35 ± 15.03, while MXD (%) was 12.41 ± 5.19 and 12.82 ± 3.96 respectively. Similarly, the mean values of CD4 count (cell/μl) in male and female test subjects was 765.50 ± 179.14 and 931.40 ± 312.95 respectively. CD4 count was significantly (p < 0.05) higher in female subjects compared with male subjects. However, there

was no significant difference ($p > 0.05$) in the total and differential leucocytes of male subjects compared with female subjects.

Parameters	Male (Mean ± SD) n = 45	Female (Mean ± SD) n = 5	t-value	p-value
WBC ($\times 10^3/\mu\text{L}$)	5.45 ± 1.41	5.07 ± 0.75	1.403	0.168
NEUT (%)	47.21 ± 9.56	45.17 ± 13.74	0.852	0.399
LYM (%)	40.75 ± 9.96	45.35 ± 15.03	1.717	0.093
MXD (%)	12.41 ± 5.19	12.82 ± 3.98	0.378	0.707
CD4 Count (cell/ μL)	765.50 ± 179.14	931.40 ± 312.95	3.458	0.001*

Table 2: Total leucocytes and CD4 counts of individuals exposed to wood dust based on sex.

KEYS: WBC- White blood cells; NEUT- Neutrophils; LYM- Lymphocytes; MXD- Middle cells; %- Percentage; μL - Micro litre; CD4- Cluster of differentiation 4.

Total leucocytes and CD4 counts of individuals exposed to wood dust according to age

Table 3 showed the total leucocytes and CD4 counts of individuals exposed to wood dust according to age. The results obtained showed that the mean values of WBC ($\times 10^3/\mu\text{L}$) of the test subjects in age group 16-30 years, 31-50 years and 51 years and above were 5.28 ± 0.96 , 5.32 ± 1.77 and 6.24 ± 0.35 , NEUT (%) was 47.64 ± 11.56 , 45.34 ± 9.13 and 45.30 ± 8.86 , LYM (%) was 41.07 ± 10.13 , 42.61 ± 12.39 and 43.44 ± 7.74 , while MXD (%) was $12.97 \pm$

4.25 , 12.07 ± 5.54 and 11.36 ± 5.61 respectively. Similarly, the mean values of CD4 count (cell/ μL) of the test subjects in age group 16-30 years, 31-50 years and 51 years and above were 890.23 ± 321.99 , 885.52 ± 212.59 and 1001.71 ± 410.27 respectively. Total leucocyte count was significantly higher ($p < 0.05$) in age group 51 years and above when compared with age group 15-30 years and 31-50 years respectively. However, there was no significant difference ($p > 0.05$) in CD4 count and other leucocytes counts in subjects with respect to age.

Parameters	15-30 years (Mean ± SD) n = 22	31-50 years (Mean ± SD) n = 21	51 years and above (Mean ± SD) n = 7	t-value	p-value
WBC ($\times 10^3/\mu\text{L}$)	5.28 ± 0.96^a	5.32 ± 1.77^a	6.24 ± 0.35^b	4.487	0.000
NEUT (%)	47.64 ± 11.56^a	45.34 ± 9.13^a	45.30 ± 8.86^a	0.752	0.461
LYM (%)	41.07 ± 10.13^a	42.61 ± 12.39^a	43.44 ± 7.74^a	0.850	0.406
MXD (%)	12.97 ± 4.25^a	12.07 ± 5.54^a	11.36 ± 5.61^a	0.634	0.533
CD4 Count (cell/ μL)	890.23 ± 321.99^a	885.52 ± 212.59^a	1001.71 ± 410.27^a	0.946	0.356

Table 3: Total leucocytes and CD4 counts of individuals exposed to wood dust according to age.

KEYS: WBC- White blood cells; NEUT- Neutrophils; LYM- Lymphocytes; MXD- Middle cells; %- Percentage; μL - Micro litre; CD4- Cluster of differentiation 4.

Total leucocytes count and CD4 counts of individuals exposed to wood dust based on duration of exposure

Table 4 showed the total leucocytes and CD4 counts of individuals exposed to wood dust based on duration of exposure.

The results obtained showed that the mean values of WBC ($\times 10^3/\mu\text{L}$) of the subjects who have been exposed to wood dust for 1-5 years, 6-10 years, and 11 years and above were 5.37 ± 1.90 , 5.30 ± 0.99 and 5.57 ± 0.91 , NEUT (%) were 47.41 ± 10.12 , 45.33 ± 8.84

and 45.43 ± 9.31 , LYM (%) was 41.24 ± 8.13 , 43.44 ± 7.71 and 42.14 ± 12.92 , while MXD (%) was 12.73 ± 5.20 , 11.30 ± 5.60 and 12.01 ± 5.45 respectively. Similarly, the mean values of CD4 count (cell/ μ L) of the subjects who have been exposed to wood dust for 1-5 years,

6-10 years, and 11 years and above were 838.00 ± 220.98 , 869.00 ± 231.58 and 936.59 ± 304.08 respectively. There was no statistical significant difference ($p > 0.05$) in CD4 and leucocytes counts of subjects with respect to duration of exposure.

Parameters	1-5 years (Mean \pm SD) n = 19	6-10 years (Mean \pm SD) n = 17	11 years and above (Mean \pm SD) n = 14	F-value	p-value
WBC ($\times 10^3/\mu$ L)	5.37 ± 1.90^a	5.30 ± 1.90^a	5.57 ± 0.91^a	0.713	0.488
NEUT (%)	47.41 ± 10.12^a	45.33 ± 8.84^a	45.43 ± 9.31^a	0.413	0.685
LYM (%)	41.24 ± 8.13^a	43.44 ± 7.71^a	42.14 ± 12.92^a	0.105	0.918
MXD (%)	12.73 ± 5.20^a	11.30 ± 5.60^a	12.01 ± 5.45^a	0.534	0.602
CD4 Count (cell/ μ L)	838.00 ± 220.98^a	869.00 ± 231.58^a	936.59 ± 304.08^a	1.192	0.251

Table 4: Total leucocytes and CD4 counts of individuals exposed to wood dust based on duration of exposure.

KEYS: WBC- White blood cells; NEUT- Neutrophils; LYM- Lymphocytes; MXD- Middle cells; %- Percentage; μ - Micro litre; CD4- Cluster of differentiation 4.

Discussion

Occupational exposure to wood dust particles has long been reported of its associated varying degrees of negative health effects due to different extractive chemicals present in the various timber species [10]. However, tropical hardwood is also reported to have higher levels of extractive chemicals of antihistamine, antioxidant and anti-inflammatory properties [10]. Leucocytes are effectors and biomarkers of inflammation [11]. This study was carried out to evaluate the total leucocytes and CD4 counts of individuals exposed to wood dust in Ekpoma, Edo State, Nigeria. In this study, there was a significant decrease ($p > 0.05$) in lymphocyte count in subjects when compared with control. On the other hand, neutrophil count of exposed individuals was significantly increased ($p < 0.05$) compared with control. This indicates allergic response. This finding is in agreement with previous studies [14-16]. Tripathi, *et al.* [15] reported that there was significant variation in leucocytes of sawmill workers of Lucknow district, India. Weller, *et al.* [14] have found that the exposure to wood dust causes increase in Eosinophil count as well as Eosinophil-Lymphocyte interaction in immune response. Praveen, *et al.* [16] reported a significant increase in Neutrophil, Eosinophil and Lymphocyte count among individuals exposed to wood dust in comparison with control group. This abnormal variation of neutrophils, Eosinophils and Lymphocytes can lead to chronic inflammation. In this study, though there was

significant variation in differential leucocyte count but the total leucocyte count did not show any significant variation ($p > 0.05$) between the subjects when compared to control. This finding is comparable with previous studies [13,15,16] which reported no significant difference in the total leucocyte count of individuals exposed to wood dust compared with control. This might be due to adaptation of body immune response for long-term exposure to the irritant substances. This data shows that there is an immune response which is provoked due to wood dust exposure [16]. The rise in WBC count perhaps suggests a response to toxic effect of wood dust inhaled into the lungs [13].

In the present study, a non-significantly increased CD4 count was observed in subjects exposed to wood dust compared to control. In contrast, the previous study of Gripenback, *et al.* [17] reported a significantly reduced numbers of CD4+ T-lymphocytes after exposure to wood dust, a phenomenon most probably caused by an influx into the lungs of large numbers of CD69-negative CD4+ peripheral blood T-lymphocytes. However, any distinct role of these cells remains unclear, especially as no cytokines were analyzed here. Previous studies on particulate exposure using the whole-body counter revealed signs of airway inflammation in particular, following wood dust exposure [17]. The importance of wood dust polysaccharide component in the dramatic lung accumulation

of eosinophils following wood dust exposure remains open to speculation. Interestingly, the cellulose component of pinewood dust, but not the fibre extract of the wood dust, has previously been implicated in the induction of pathological changes in the lung, including granulomatous inflammation and fibrosis [18].

In this study, CD4 count was significant ($p < 0.05$) higher in female test subjects compared to their counterparts male. There was no significant difference ($p > 0.05$) in the total and differential leucocytes of male subjects compared with female subjects. This finding is in harmony with the reports from previous studies by Oladepo., *et al.* [19] and Miri-Dashe., *et al.* [20] who in their separate studies reported that female subjects had higher CD4 counts than their male counterparts. This observation has also been reported in several other countries among Africans [21,22] and Caucasians like Uganda and Ethiopia [23]. A sex hormone effect is one possible explanation for the reported difference in CD4 counts between genders that has been suggested [23].

Furthermore, the results of this study showed that there was no significant difference ($p > 0.05$) in CD4 and total leucocytes counts of the test subjects in relation to age. This finding is in accord with the previous report by Afolabi., *et al.* [25] who reported that age did not have any significant effect ($p > 0.05$) on the CD4 count of adults. Bolaji., *et al.* [26] reported that age group 50-69 years has a significantly higher CD4 absolute count compared to other age groups which corroborated the finding of this study. Some factors which have been established to be associated with low CD4 cell counts include psychological stress among the elderly coupled with their constant exposure to wood dust could be the possible reason for this observation [19].

In this study, there was no significant difference ($p > 0.05$) in the CD4 and leucocytes counts of subjects with respect to duration of exposure. However, CD4 count, total and differential leucocyte counts were higher in subjects exposed to wood dust greater than 11 years compared with those exposed for 1-5 years. This finding is consonance with some previous studies of Mojiminiyi., *et al.* [13], Tripathi., *et al.* [15] and Praveen., *et al.* [16] which reported no significant difference in the CD4 and total leucocyte counts of individuals exposed to wood dust with respect to duration of exposure. This might be due to adaptation of body immune response to long-term exposure of the irritant substances.

According to Praveen., *et al.* [16], our data showed that there is an immune response which is provoked due to wood dust exposure.

Conclusion

In Conclusion, Lymphocyte (%) was significantly ($p < 0.05$) lower in subjects compared with control while Neutrophil (%) was significantly higher ($p < 0.05$). Total WBC was significantly higher ($p < 0.05$) in age group 51 years and above when compared with age group 15-30 years and 31-50 years respectively. CD4 count was significantly ($p < 0.05$) higher in female subjects compared with male subjects. There was no significant difference ($p > 0.05$) in CD4 count and leucocytes in subjects with respect to duration of exposure. It is hereby recommended that individuals exposed to wood dust should be encouraged to use personal protective equipment while working to reduced exposure.

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

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

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