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Leucocytes and CD4 Counts of Individuals Exposed to Roadside Dust in Ekpoma, Edo State, Nigeria

Babatope IO[1,*], Omoregie OI[1], Iyevhobu KO[2]

[1]*Department of Haematology and Blood Transfusion Science, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Nigeria*

[2]*Department of Medical Microbiology, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Nigeria*

ABSTRACT

Roadside dust is a combination of particulate matter generated from vehicular traffic and other human activities along the road. This study investigated the leucocytes and Cluster of Differentiation-4 (CD4) counts of individuals exposed to roadside dust in Ekpoma, Edo State. A total to 50 test subjects aged 18-65 years and of both sexes were recruited for this study, while 50 individuals not exposed to road-side dust served as controls. The leucocytes and CD4 counts were analyzed using Sysmex KX-21N Autoanalyzer and Flow cytometry method respectively. The results obtained revealed the total white blood cell counts of the subjects studied were 5.27±1.60 and 5.22±1.66 for the test and control subjects respectively. With respect to the differential leucocyte count, the mean values of the test and control subjects for NEUT %, LYM % and MXD % were 40.31±9.75 and 38.62±10.0, 47.9 ±9.15 and 50.7±9.86, 11.66±4.40 and 10.7±3.72 respectively. Furthermore, the mean values of the CD4 counts of the test and control subjects were 890.26±266.21 and 850.94±302.13 respectively. Statistical comparison of the mean values of total WBC, differential leucocytes count and CD4 counts and road side dust did not reveal any significant difference ($p>0.05$). With respect to age, gender and duration of exposure to road-side dust, the leucocytes and CD4 counts were not significantly affected. In conclusion, exposure to road dust did not affect the leucocytes and CD4 counts of our study subjects.

Keywords: Leucocytes, CD4, Roadside, Dust, Haematology.

INTRODUCTION

Road dust consists of solid particles that are generated by any mechanical processing of materials including crushing, grinding, rapid impact, handling, deterioration and decrepitation of organic and inorganic materials such as rock, ore and metal [1]. When this dust becomes airborne, primarily by friction of tyres moving on unpaved dirt roads and dust covered paved roads, it is referred to as road dust [1]. Since early 1990s, the environmental impact of dust emission and transportation and deposition has become a major concern [2]. Unpaved road often contributes a significant amount of atmospheric dust formed due to re-suspension of road material by truck and observed as a dust cloud behind the driving vehicle.

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***Corresponding Author**

Babatope IO

Department of Haematology and Blood Transfusion Science, Faculty of Medical Laboratory Science, Ambrose Alli University, Ekpoma, Nigeria, Tel: +2348022303311, Email: babatope_olaniyi@yahoo.com

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Leucocytes, also known as white blood cells, are a diverse group of cells that play a crucial role in the body's immune system and they are effectors and biomarkers of inflammation. They are produced and primarily reside in the bone marrow and can be found circulating throughout the bloodstream as well as in various tissues throughout the body [3]. There are several types of leucocytes, including lymphocytes, monocytes, and granulocytes. Lymphocytes are responsible for recognizing and responding to specific foreign antigens, while monocytes are involved in phagocytosis and removal of foreign substances. Granulocytes, such as neutrophils, eosinophils and basophils are involved in inflammatory responses and are particularly important in fighting infections caused by bacteria and parasites [4]. Leucocytes play critical roles in protecting the body against infections and other foreign substances and abnormalities in leucocyte function can lead to immune deficiencies and increased susceptibility to disease, leading to immune system failure and increased susceptibility to opportunistic infections [5].

The "CD" or Cluster of Differentiation is a protein expressed on the surface of the cells of the haematopoetic system [6]. The expression of these proteins is used in lymphocyte nomenclature. Over 300 'CD' molecules have been reported to far [7]. These proteins are often associated with the specific function of the cells. Cells with different functions express different CD molecules (for example: CD3+ cells are total T-lymphocytes, CD4+ cells are T-helper cells, CD8+ cells are cytotoxic T-lymphocytes and CD19+ are B-lymphocytes) [8]. CD4 cells are white blood cells that play an important role in the immune system [7]. CD4 cells count gives you an indication of the health of the immune system – the body's natural defense system against pathogens, infections and illnesses [8].

In Nigeria, the situation of unpaved roads is a significant issue. According to a report by the Nigerian Institute of Transportation Technology, over 80% of the federal and state roads in Nigeria are unpaved or in poor condition [9]. Potentially toxic pollutants consist of brake and tyre wear, combustion emissions and fly ash from asphalt. Heavy metals such as Zn, Cu, Pb, Ni, Cr and Cd primarily originate from such vehicular traffic while Fe, Al and Mn primarily originate from surrounding oils [10]. Lead is known to be responsible for deficits in neurobehaviour and cognitive development in children [11]. Reports have also found lead exposure to result in dysfunction of the reproductive system, as well as microcytyic anaemia resulting in conditions such as hypertension and chronic renal failure [12]. Hence, this study was aimed at evaluating the leucocytes and CD4 counts of individuals exposed to roadside dust in Ekpoma, Edo state, Nigeria.

MATERIALS AND METHODS

Study Area

This study was carried out in Ekpoma. Ekpoma is the home to the state- owned University known as Ambrose Alli University. Ekpoma has a land area of 923 square kilometres. The area lies between latitudes 60 43I and 60 45I North of the Equator and longitudes 60 5I and 60 8I East of the Greenwich Meridian. Ekpoma has a population of 170, 123 people as at the 2006 census [13]. Ekpoma is a town in Edo state which falls within the rain forest/savannah transitional zone of South Western Nigeria. Ekpoma is the administrative headquarters of Esan West Local Government Area of Edo State, Nigeria. The town has an official post office. Majority of the people residing in Ekpoma are Students, Lecturers/ Teachers, Civil servants, Farmers, Traders, Business men/ women and Self-employed.

Study population

A total of fifty (50) subjects exposed to roadside were recruited for this study while another fifty (50) apparently healthy subjects not exposed to roadside dust served as control subjects. All the participants were aged 18-65 years and of both sexes.

Ethical approval

Ethical approval was obtained from the Health Research Ethics Committee of Ambrose Alli University, Ekpoma. Informed consent was sought from each participant.

Inclusion Criteria

Individuals between the ages of 18 and 65 years living in Ekpoma that are exposed to road side dust were included in this study.

Exclusion Criteria

Individuals who are not exposed to road side dust were excluded from the 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 inversion technique. All the field samples were placed in cold transport boxes (temperature range of 2°C-8°C) before they were transported to the laboratory for analysis. All the 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

Haematology Assay using Sysmex KX-21N autoanalyzer

The leucocytes parameters 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 cells counter. The procedure was carried out according to the manufacturer's instructions. The principle of this method is based on the Direct Current (DC) detection method.

CD4 Count

CD4 cells counts were determined by flow cytometry using Partec cyflow counter 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

Leucocytes and CD4 counts of the subjects studied

The total leucocytes and CD4 counts of the subject studied is shown in table 1. The mean values of the WBC total count of the test and control subjects were 5.27 ± 1.60 and 5.22 ± 1.66 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 LYM (%) of the test and control subjects were 47.9 ± 9.15 and 50.7 ± 9.86 respectively. Similarly, statistical comparison did not reveal any significant difference (P>0.05). Furthermore, the mean values of the MXD (%) of the test and control subjects were 11.66 ± 4.40 and 10.7 ± 3.72 respectively. Also, the statistical comparison between the two groups was not significant (P>0.05). In addition, the NEUT (%) values of the test and control subjects were 40.31 ± 9.75 and 38.62 ± 10.0 respectively. There was also no significant difference (P >0.05) in the mean values of both subjects. In a similar fashion, the mean values of the CD4 count of the test and control subjects were 890.26 ± 266.21 and 850.94 ± 302.13, this did not reveal any statistically significant difference (P>0.05).

Table 1. Leucocytes and CD4 counts of the subjects studied

Parameters	Test Subjects (Mean±SD) n=50	Control subjects (Mean±SD) n=50	t- value	p-value
WBC total (x10^3/µL)	5.27±1.60	5.22±1.66	0.17	0.8595
LYM %	47.96±9.15	50.75±9.86	-1.46	0.1453
MXD %	11.66±4.40	10.73±3.72	1.14	0.2568
NEUT %	40.31±9.75	38.62±10.05	0.85	0.3950
CD4 Count (cells/µL)	890.26±266.21	850.94±302.13	0.69	0.4915

KEYS: WBC- White Blood Cells; MXD - WBC-Middle Cells Count; NEUT - Neutrophils; LYM - Lymphocytes; MXD - Middle Cells; 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)

Total leucocytes and CD4 counts of individuals exposed to roadside dust in relation to age

The total leucocytes and CD4 counts of individuals exposed to roadside dust in relation to age is shown in Table 2. The mean values of the total WBC count of the subjects belonging to the age groups of 18-20 years, 21-25 years, 26-30 years and 31 years old and above were 5.22 ± 1.46, 5.34 ± 1.89, 4.70 ± 0.1.66 and 5.64 ± 1.12 respectively. The statistical comparison did not reveal any significant difference (P>0.05). Similarly, the differential leucocyte counts of the subjects according to the respective age groups of 18-20 years, 21-25 years, 26-30 years and 31 years and above were 43.25 ± 7.46, 47.47 ± 10.0, 51.65 ± 8.84 and 48.35 ± 8.51 for lymphocytes (%) and 47.74 ± 8.08, 40.93 ± 11.0, 35.55 ± 8.31 and 39.15 ± 7.84 for NEUT %. Statistical comparison among the age groups did not reveal any statistical difference (P>0.05) for both the LYM % and NEUT %. Furthermore, the MXD % mean values for the age groups were 9.0 ± 3.85, 11.48 ± 3.20, 12.8 ± 4.98 and 12.44 ± 5.40. The statistical analysis showed no significant difference (P>0.05). In the same vein, the mean values of CD4 count (cells/µL) were 899.57 ± 292.5, 859.05 ± 199.89, 826.1 ± 253.16 and 973.7 ± 360.28 for the age groups of 18-20 years, 21-25 years, 25-30 years, and 31 years and above respectively. Statistical analysis also showed no significant difference (P<0.05).

Table 2. Total leucocytes and CD4 counts of individuals exposed to roadside dust with respect to age

Parameters	18-21years (Mean±SD) (n=32)	22-25years (Mean±SD) (n=4)	26-30years (Mean±SD) (n=12)	31years and above (Mean±SD) (n=14)	F-value	P-value
WBC total (x10^3/µL)	5.22±1.46^a	5.34±1.89^b	4.70±1.66^c	5.64±1.125^a	0.66	0.5797
LYM %	43.25±7.46^a	47.47±10.0^a	51.65±8.84^a	48.35±8.51^a	1.19	0.3211
NEUT %	47.74±8.08^a	40.93±11.0^a	35.55±8.31^a	39.15±7.84^a	2.43	0.0763
MXD %	9.0±3.85^a	11.48±3.32^b	12.80±4.98^c	12.44±5.40^a	1.25	0.3015
CD4 Count (cells/µL)	899.57±292.5^a	859.05±199.89^b	826.10±253.16^c	973.7±340.28^c	0.73	0.5390

KEYS: WBC-White Blood Cells; MXD - WBC-Middle Cells Count; NEUT- Neutrophils; LYM- Lymphocytes; MXD- Middle Cells; 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)

Total leucocytes and CD4 counts of individuals exposed to roadside dust based on gender

The total leucocytes and CD4 counts of individuals exposed to roadside dust based on gender is shown in Table 3. The results of the mean values of WBC total count of the subjects were 5.42 ± 1.84 for the males and 5.01 ± 1.03 for the females. Statistical comparison between both sexes revealed no significant difference (P>0.05) in the two groups. For the LYM %, the mean values for the male and female subjects were 49.70 ± 9.34 and 44.88 ± 8.15 respectively and statistical comparison did not reveal any significant difference (P>0.05). On the other hand, the mean values of the NEUT % of the male and female subjects were 38.47 ± 10.36 and 43.57 ± 7.79 respectively and the statistical comparison between these two groups was not significant (P>0.05). Furthermore, the MXD % values of the subjects were 11.76 ± 3.85 for the males and 11.5 ± 5.34 for the females and the statistical analysis also did not reveal any significant difference. Likewise, the results of the mean values of the CD4 counts of the male and female subjects were 872.09 ± 264.92 and 791.23 ± 122.11. Although the CD4 count of the male subjects was higher than the female subjects, this did not reveal any statistically significant difference (P>0.05).

Table 3. Total leucocytes and CD4 counts of individuals exposed to roadside dust based on gender

Parameters	Male Subjects (Mean±SD) n=28	Female subjects (Mean±SD) n=22	t-value	p-value
WBC total (x10^3/µL)	5.42±1.84	5.01±1.03	1.00	0.3221
LYM %	49.70±9.34	44.88±8.15	1.90	0.0644
NEUT %	38.47±10.36	43.57±7.79	-1.96	0.0559
MXD %	11.76±3.85	11.5±5.34	0.18	0.8560
CD4 Count (cells/µL)	872.09±264.92	791.23±122.11	1.39	0.1691

KEYS: WBC- White Blood Cells; MXD- WBC-Middle Cells Count; NEUT- Neutrophils; LYM- Lymphocytes; MXD - Middle Cells; 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)

Total leucocytes and CD4 counts of individuals exposed to roadside dust according to duration of exposure

The total leucocytes and CD4 counts of individuals exposed to roadside dust based on duration of exposure is summarized in Table 4. The WBC total count (x10^3/L) based on duration of exposure were 4.70 ± 0.14, 5.0 ± 0.18, 5.19 ± 0.46 and 5.37 ± 1.74 for the periods of less than 1 month, 1-2 months, 3-5 months and 6 months and above respectively. The statistical comparison showed no significance (P>0.05) among the various periods. Similarly, the mean values for the LYM % were 45.80 ± 9.19, 52.77 ± 2.95, 49.62 ± 9.97 and 47.04 ± 9.44. Statistical analysis did not reveal any significant difference. Likewise, the mean values for the NEUT % were 40.20 ± 14.9, 35.52 ± 3.91, 38.32 ± 9.16 and 41.46 ± 10.23 for the periods of less than 1 month, 1-2 months, 3-5 months and 6 months and above respectively. There was also no significant difference as revealed by the statistical analysis. Also, the mean values for the MXD % were 14.0 ± 5.79, 11.7 ± 1.86, 12.06 ± 3.09 and 11.41 ± 4.93 for the various time periods. The statistical analysis also did not show any significant difference. In addition, the mean values of the CD4 counts of the different duration of exposures were 843.50 ± 61.51, 1059.50 ± 249.01, 856.40 ± 209.21 and 883.05 ± 289.03 respectively. Statistical comparison also did not reveal any significant difference (P>0.05).

Table 4. Total leucocytes and CD4 counts of individuals exposed to roadside dust according to duration of exposure

Parameters	Less than 1 month (Mean±SD) (n=2)	1-2 months (Mean±SD) (n=4)	3-5 months (Mean±SD) (n=10)	6 months and above (Mean±SD) (n=34)	F-value	P-value
WBC total (x10^3/µL)	4.70±0.14a	5.0±1.18a	5.19±1.46a	5.37±1.74a	0.16	0.9190
LYM %	45.80±9.19a	52.7±2.95a	49.6±9.97a	47.0±9.44a	0.61	0.6093
MXD %	40.0±5.79a	11.7±1.86a	12.0±3.09a	11.4±4.96a	0.24	0.8676
NEUT %	40.2±14.9a	35.5±3.91a	38.3±9.16a	41.4±10.23a	0.60	0.6153
CD4 Count (cells/µL)	843.5±61.51a	1059.5±249.01a	856.4±209.21a	883.05±289.03a	0.60	0.6140

KEYS: WBC- White Blood Cells; MXD - WBC-Middle Cells Count; NEUT - Neutrophils; LYM - Lymphocytes; MXD - Middle Cells; 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)

DISCUSSION

Numerous research studies have explored the impact of road dust on public health [14-17]. In this study, the WBC total counts of the test subjects did not reveal any statistically significant difference (p>0.05). This finding is consistent with earlier research studies [18] that did not identify any significant correlations between dust exposure and alterations in white blood cell counts. In contrast, other authors, such as Zuurbie et al. [19], reported a notable decrease in white blood cell counts among subjects with prolonged exposure when compared to control groups. The observations made by these authors provide support for the connection between the duration of exposure to road dust and a reduction in blood cell counts, particularly white blood cells. The statistically insignificant WBC total count result obtained in our study may be due to the fact that the exposure time observed in our study is shorter than the exposure time observed in the other studies.

With respect to the differential leucocyte counts, the results of this study did not show any significant difference in the LYM % and NEUT % counts of the subjects studied. This finding contrasts the previous reports of Gao et al. [20] whose study suggested that short-term air pollution exposure, temperature, and relative humidity were associated with leucocyte distribution. Also, Steenhof et al. [18] reported an elevation in neutrophil count. Likewise, the investigations conducted by Salvi et al. [21] and Ma et al. [22] revealed a significant increase in lymphocyte count among the subjects they studied. Ma et al. [22] suggested that this could be attributed to the activation of specific components of the immune system in vivo, which subsequently participate in the inflammatory processes.

The WBC-middle cells count (MXD) test assesses the collective levels of three distinct white blood cell types (monocytes, eosinophils, and basophils) present in the bloodstream. In the context of this investigation, there was no noteworthy distinction observed in the MXD % between the subjects and the control group. Our findings are consistent with the prior findings of Steenhof et al. [18], who detected no reductions in eosinophils and basophils among the subjects in their study.

From this study, the CD4 count of individuals exposed to roadside dust did not exhibit any statistically significant disparity when contrasted with the control group. Conversely, in a separate study conducted by Rudd et al. [7], a decrease in CD4 cells was observed among the groups they studied.

With respect to gender, the mean value of the WBC total count of the female subjects was significantly higher than those of their male counterparts. Our finding is in agreement with the observations of Hu et al. [23] who reported slightly higher differences with the total leucocyte counts in female subjects. In this study, the results of NEUT % and LYM % 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 Gondalia et al. [24] who found that gender classification did not show any appreciable impact.

From the standpoint of age, the mean WBC total count values for individuals aged 31 years and above exceeded those of other age groups. This observation is in tandem with the findings of Ma et al. [22], who similarly noted age-related impacts on total leucocyte count in adults.

Based on the duration of exposure, the results of the WBC total count, differential leucocyte count, and CD4 count did not demonstrate any significant differences (p>0.05), indicating that the duration of exposure had no discernible impact on these parameters. Our finding is in consonance with the previous reports of Viehmann et al. [25] whose findings did not reveal a consistent relationship between duration of exposure and white blood cells changes. Nevertheless, our results contradicted the prior research conducted by Carlsen et al. [26] who conducted a cross-sectional study analyzing the association between residential proximity to major roadways and immune system parameters. Their study found that individuals living closer to roads with higher traffic volumes had altered leucocyte profiles and lower CD4 counts compared to those living farther away. These findings suggest a potential link between road dust exposure and immune system deregulation.

CONCLUSION

In conclusion, the total and differential white blood cells and CD4 counts of our study subjects did not show any significant differences. Similarly, duration of exposure did not affect any of the laboratory parameters studied.

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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