THE IMPACT OF VEHICULAR EMISSION ON ERYTHROCYTE LEVEL AND RED CELL INDICES OF OCCUPATIONALLY EXPOSED SUBJECTS IN A GIVEN CITY.

Abstracts

Background

The association of combustive emission has been inter-related with hematologic parameters and a likelihood to pro-inflammatory state. This research is aimed at assessing the impact of vehicular emission on erythrocyte level and red cell indices of occupationally exposed subjects. **Method**: The level of haemoglobin concentration, haematocrit, red blood cells, mean cell volume, mean cell haemoglobin, mean cell haemoglobin concentration, and red cell distribution width were determined in four hundred subjects with the aid of Mythic 22 haematology autoanalyser. **Results**: The comparison of the haemoglobin and haematocrit shows that the value of mean ± standard error value of generator exposed and mechanics respectively were significantly higher than the other groups ($p< 0.00$). On the otherhand, the red blood cell count for generator exposed and mechanics ranked higher than the other groups ($p< 0.00$). The mean cell volume (MCV) of generator exposed and mechanics were 86.57±0.08 fl and 84.49±1.04 fl respectively while control and drivers had values of 93.24±1.13 fl and 93.22±1.13 fl respectively ($p< 0.00$). The mechanics recorded a mean cell haemoglobin (MCH) of 26.92±0.40 pg which was significantly lower ($p< 0.04$) than the control (30.37±0.47 pg), generator exposed (39.68±6.38 pg) and drivers (30.42±0.47 pg). **Conclusion**: The differences amongst the groups and none of the groups were within the medically acceptable ranges which is a pointer to the fact that there might be an underlying inflammatory condition which might be due to occupational exposure.

Keywords: Combustion emission, red blood cell, inflammatory, generator-exposed, drivers, control, mechanics, vehicular, haematocrit, haemoglobin, occupationally-exposed, red cell indices.

1.0 Introduction

Engine emissions are gases and particulates that are expelled by automobiles or other combustion-powered mechanical devices. The global economy relies on fossil fuel to function effectively [1]. Some of the emitted substances are particulate matter (PM), carbon monoxide (CO), carbon dioxide (CO_2), nitrogen dioxide (NO_2), sulfur dioxide (SO_2), ozone, polycyclic aromatic hydrocarbons (PAH) etc.

Emission from exhausts leads to poisoning of body cells thereby altering the pathways in the human body. The alteration might be connected to constriction of vessels and inflammatory pathophysiological response [2]. A selection of the health effects of these include mortality, disorders in the airways, cardiovascular diseases, formation of malignant cells, elevated stress of oxidative cells, mental challenge, dysfunction of endothelium and allergic reactions [3] [4] .

The association of combustive emission has been inter-related with hematologic parameters and a likelihood to pro-inflammatory state. It has been documented [5] that erythrocytes, haemoglobin, mean cell volume and mean cell haemoglobin concentration are decreased in fumes exposed subjects.

This decrease may be connected to aftermath of benzene oxide and xylene compounds [6]. Benzene is documented to initiate pancytopenia which could lead to bone marrow aplasia [7]. Previous studies documented increase in erythrocytes, haemoglobin, mean cell volume and mean cell haemoglobin concentration in gasoline exposed groups [8] [9].

The deleterious constituents especially those in petrol, is linked with alteration in blood and decrease in haematocrit level through the induction of hypoplasia in animal models [7]. CEEs are activates in the marrows of blood and the cytotoxic harms are seen through alteration in the functioning of deoxyribonucleic acid. Petroleum related fumes leads to depression of cells of the marrow leading to deficient generation of erythrocytes and some cellular components [10].

Materials and methods

Study population

A Comparative cross-sectional study will be carried out on a random sample of exposed subjects in Benin City, South-South Nigeria. The groups will include commercial bus drivers, mechanics, generator/plant houses workers as well as controls (individuals not exposed occupationally).

Inclusion criteria of exposed group

Subjects who are working regularly for at least one years in CEE-related occupation (at risk of emission from generator and/or vehicular exhaust).

Exclusion criteria of exposed group

Subjects such as chronic diseases (e.g. cancer, hepatitis, HIV/AIDS, *Mycobacterium tuberculosis*, *Mycobacterium leprae* etc.), renal disease, hepatic disease, diabetes mellitus, blood donation in the past 6 months, clinical evidence of haemorrhage, cardiovascular disease, deficiencies (iron or folate or vitamin B12 deficiency etc.), chemotherapy, concomitant infections, individuals living or working near benzene industries/filling stations (fuel/diesel/kerosene), refinery workers, obesed and underweight individuals were excluded from the study. Individuals below 20 and above 60 years were not considered as well. Interestingly, sample collection and analysis was done before outbreak of coronavirus disease 19 (COVID 19) in Nigeria and most countries of the world and as such consideration was not given for this.

Inclusion criteria

Individuals that have continuous activities in any of these occupational exposures will be enlisted after meeting the research selection criteria. Staff and student at University of Benin who were not occupationally exposed to vehicle exhaust/generator at their workplace/classes and; belonged to the same age group as the included subjects will be enlisted.

Age range

All participants will be adult (20-60 years). Informed consent will be obtained from all the voluntary participants on random basis (complete randomized sampling).

Administration of questionnaire

Structured questionnaire will be administered to everyone to gather demographic data. The details of the questionnaire will include personal data, occupational history, smoking habits, medical history; exposure to irradiation/chemotherapy/electronic waste and others.

Ethical approval

The ethical approval "number: HA-737/30" was obtained from the Health Planning, Research and Statistics unit of Edo State Ministry of Health, Benin City, Nigeria and informed consent was obtained from each participant. A letter introducing me as a student researcher "*introduction to subjects*/*to whom it may concern*" was provided by the Department of Medical Laboratory Science, University of Benin.

Sample size determination

The sample size was calculated using the formula:

$$N = \frac{Z^2 P (1 - P)}{d^2}$$

Where n = sample size, Z = z statistic for a level of confidence, P = expected prevalence or proportion, d = precision (Daniel, 1999).

According to Ali *et al*. (2015), the prevalence due to automobile exhaust emission was 8.80% in subjects.

P = 8.80 % (= 0.088)

Z = 1.96 for the level of confidence of 95 %, d = 5 % = (0.05).

$$N = \frac{Z^2 P (1-P)}{d^2} = \frac{1.96 \text{ X } 1.96 \text{ X } 0.088(1-0.088)}{0.05 \text{ X } 0.05} = 123.40 = 124.0 \text{ subjects (approximately).}$$

For the purpose of this research, three hundred (300) occupationally exposed subjects (OESs) and one hundred (100) apparently healthy controls were used as sample size.

The sampling was carried in the order of generator/plant houses: 100 subjects; commercial bus drivers: 100 subjects; automobile mechanics: 100 subjects and apparently healthy controls: 100 subjects.

Collection of sample

Five (5) ml of blood was collected carefully from the cubital vein using a vacuutainer bottle, needle and holder while applying tourniquet lightly over the arm until blood flow was established under negative pressure. The K_3EDTA sample was used for full blood cell count

Processing of sample

It will be carried out at the Hematology and Blood Transfusion unit, Medical Laboratory Science Department, University of Benin, Physiology Department, University of Benin, Medical Laboratory Services unit, University of Benin Teaching Hospital; as well as other research centers.

Analysis of data

This will be done with the aid of Graphpad Prism v7.03, California, USA. Data obtained from this research work was presented using mean, standard error, ANOVA, student t- test, bar chart, histogram, line graphs as circumstances warrants and conclusion will be drawn at $p < 0.05$.

Result

As seen in table 1, the comparison of the haemoglobin (HGB) and haematocrit (HCT shows that the value of mean ± standard error value (M±SEV) of generator exposed and mechanics respectively were significantly higher than the other groups ($p< 0.00$). On the otherhand, the red blood cell count (RBC) for generator exposed and mechanics ranked higher than the other groups ($p< 0.00$). The mean cell volume (MCV) of generator exposed and mechanics were 86.57±0.08 fl and 84.49±1.04 fl respectively while control and drivers had values of 93.24±1.13 fl and 93.22±1.13 fl respectively ($p< 0.00$). The mechanics recorded a mean cell haemoglobin (MCH) of 26.92±0.40 pg which was significantly lower ($p< 0.04$) than the control (30.37±0.47 pg), generator exposed (39.68±6.38 pg) and drivers (30.42±0.47 pg). The mean cell haemoglobin concentration (g/dl) of generator exposed subjects were significantly higher ($p< 0.00$) than the other groups (32.53±0.17, 34.92±1.21, 32.53±0.17 and 31.59±0.17 for controls, generator exposed, drivers and mechanics respectively). When the red cell distribution width of generator exposed groups were statistically tested against the other subjects, it was statistically higher than others ($p< 0.01$).

Table 1: The haemoglobin, haematocrit, red blood cell and red cell indices of subjects.

SUBJECT	PARAMETER							
	HGB (g/dl)	**HCT (%)**	**RBC ($X10^6$ /ul)**	**MCV (fl)**	**MCH (pg)**	**MCHC (g/dl)**	**RDWC**	**RDWS**
Controls n= 100	11.07± 0.22[a]	34.24±0.52[a]	3.83±0.08[a]	93.24±1.13[b]	30.37±0.47[a]	32.53±0.17[a]	14.82±0.23[a]	50.42±0.63[b]
Generator exposed n= 100	12.74±0.25[b]	39.93±2.20[b]	4.29±.20[b]	86.57±0.80[a]	39.68±6.38[b]	34.92±1.21[b]	26.16±5.47[b]	44.17±1.81[a]
Drivers n= 100	11.06±0.21[a]	33.91±0.53[a]	3.67±0.65[a]	93.22±1.13[b]	30.42±0.47[a]	32.53±0.17[a]	14.82±0.23[a]	50.52± 0.63[b]
Mechanics n= 100	11.50±0.21[a]	36.08±6.28[a]	4.32±.08[b]	84.49±1.04[a]	26.92±0.40[a]	31.59±0.17[a]	15.45±0.22[a]	48.26±0.80[b]
p-value	0.00	0.00	0.00	0.00	0.04	0.00	0.01	0.00

HGB: haemoglobin, HCT: haematocrit, RBC: red blood cell, MCV: mean cell volume, MCH: mean cell haemoglobin, MCHC: mean cell haemoglobin concentration, RDWC: RDWS red cell distribution width:

Key: Values with same superscript are not significantly different from each other.

Discussion

In this study, we discovered that the groups belonging to control, drivers and mechanics did not differ significantly ($p > 0.05$) with reference to haemoglobin (HGB) concentration, haematocrit, red blood cells (RBC), mean cell volume (MCV), mean cell haemoglobin (MCH), mean cell haemoglobin concentration (MCHC) and red cell distribution width (RDWC). Available literature indicate that some components of vehicular emission are linked with relative drop in erythrocyte count. It is on record that anaemia affects about 1.62 billion individuals globally and this condition is linked with several impact on well-being as exemplified in higher mortality and cognitive disorders. Most developing nations are responsible for about 89% of all anemia-related issues [12]. According to World Health Organization, 50% of cases are due to iron deficiency anemia while the other 42% are linked to inflammatory origin (inflammatory anaemia). Inflammatory anaemia or anemia of inflammation (IA/AI) in some persons are linked to infection as observed in third world countries [13]. Though, infections are not the only known cause of inflammatory circumstances, literatures indicates that some conditions such as chronic kidney disease, congestive heart failure, chronic pulmonary disease, and obesity may result in AI [14]. On the otherhand, the values from generator exposed groups were significantly higher than those of the other groups. There might not be direct adducible cause of this difference, one thing that comes to mind is the fact that the subjects under investigation are apparently healthy groups that are occupationally exposed. Furthermore, exposure to vehicular air pollutants has also been incriminated in this circumstance. Fine particulate matter are equivalent to or less than 2.5 microns in diameter (PM2.5) and NO_2 causes systemic inflammation [15] with direct effect on bone marrow [16]. PM2.5 is a highly toxic pollutants, and acute exposure causes 2.8% increase in mortality rates in humans. Alongside, chronic exposure to PM2.5 is associated with cardiovascular disease and chronic obstructive pulmonary disease. This might also lead to oxidative stress-dependent inflammation in the bronchioles [17] [18]. Shortened half-life of red blood cells suppresses erythropoietin response to anaemia and the inhibition of erythroid cell differentiation by inflammatory mediators, further contributing to AI [13]. Oxidative stress has been connected with eryptosis or shortening of erythrocyte half-life [19]. Air pollution might affects hemoglobin (Hb) which might lead to anaemia as tenable in many age groups. There seems to be an established relationship between usage of biofuel and the likely risk of anaemia. This effect has been demonstrated in children and other age groups.

The findings from this research shows that exposure to increasing concentrations of vehicular emission such as PM2.5, nitric oxide and others were not directly linked with decrease in hemoglobin levels in some groups. Despite the fact that the generator-exposed groups had higher values than the other groups, there is indication that this is still low when compared to acceptable values medically. The direction of our findings indicate that vehicular pollution might be pointing to inflammatory process in the human body but this is not showcased in anaemia [20].

The constituents of vehicular emission is linked with rise in systemic inflammation [21] as well as chronic inflammation [22]. This cascade of events might have contributed to reduced haemoglobin levels and an increase in prevalence of anaemia as observed in the present study. It has been demonstrated that the levels of PM10 were associated with a reduction in haemoglobin concentration and erythrocyte counts but increased leucocyte level indicating proportionate association with inflammatory process and an inverse relationship with haemoglobin count [23]. Furthermore, [18] showed that an increased exposure to PM2.5 was associated with a 0.81 g/dL drop of haemoglobin in elders [18]. Some other volatile pollutants have also been linked to a decrease in haemoglobin and other haematological parameters leading to increase in pro-inflammatory cytokines [24]. Particulate matter and other pollutant produces inflammatory response that affects bodily physiology [26]. These impact are almost momentary because it initiates a rise in 1- to 3-day post-exposure to PM2.5, C-reactive protein levels and inflammatory markers [26]. Outdoor air pollution has been linked to red cell haemolysis which further give rise to anaemia. This episode might be caused by 3 mineral particles adsorbed after airborne pollution exposure [27].

Conclusion

From our findings, the were differences amongst the groups and none of the groups were within the medically acceptable ranges which is a pointer to the fact that there might be an underlying inflammatory condition which might be due to occupational exposure.

Erythropoietic stimulation leads to the reinforcement of the ability of the precursor red cell to generate new cell lines due to pressing demand. Red cell indices could predict a form of pathological condition or the other if they are on a marked high or low side. In as much as this work portrayed differences in some of the subjects, it did not pinpoint pathological abnormality. Though this research had some reasonable findings, there is need for further studies.

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