ALTERATIONS IN FULL BLOOD COUNT PARAMETERS AND SOME INFLAMMATORY BIOMARKERS OF OCCUPATIONALLY EXPOSED METAL RECYCLERS: A Case Control study

ABSTRACT

Background: Metal recycling is an integral industry that contributes significantly to sustainable resource management. However, metal recyclers are exposed to a range of occupational hazards, such as metal fumes and metal dust, which may impact their health. This study investigates the alterations in full blood count parameters and some inflammatory biomarkers in metal recyclers.

Method: Fifty (50) participants were enrolled from Benin City, south-south Nigeria; consisting of thirty (30) male metal recyclers matched with twenty (20) unexposed participants (control). Full blood count parameters such as hemoglobin concentration, white blood cell count, red blood cell count, platelet count, red cell indices, hematocrit, and red cell distribution width were determined using standard methods, the neutrophil-lymphocyte ratio was calculated from the full blood count parameters and high sensitivity C-reactive protein was analyzed using enzyme-linked immunosorbent assay (ELISA).

Results: Results obtained from this study showed a significant increase in the white blood cell count of exposed participants (7.17± 1.80×10 ^{3}µl) compared with control (5.08± 1.45×10^{3}µl) ($p< 0.001$). Platelet count was significantly elevated ($p< 0.048$) in the exposed group (248.73±129.13×10^{3}µl) compared to the control group (186.85± 53.29×10^{3}µl). Lymphocyte level was significantly reduced in the exposed group (43.10± 8.59%) compared to the control group (50.87± 8.69%) ($p< 0.05$). The red cell distribution width was significantly reduced in the exposed group (41.67± 5.10µm^{3}) compared to the control group (44.66 ± 5.16µm^{3}) ($p< 0.05$). The hs –C-reactive protein was significantly increased in the exposed group (9.73± 10.35µg/ml) compared to the control group (2.46±1.16µg/ml) ($p< 0.05$).

Conclusion: This study concludes that occupational exposure to metal recycling causes a significant increase in white blood cell count, platelet count, and high-sensitivity C-reactive protein and a significant decrease in lymphocytes and red cell distribution width of metal recyclers.

Keywords: Recycling, Metals, Full Blood Count, Inflammatory Biomarkers

INTRODUCTION

To minimize the demand for newly mined ores and protect natural resources, metal recycling is an approach that renders it easier to recycle, reuse, or remanufacture metal materials. The United Nations and the European Union (EU) have set sustainable development goals, and achieving these goals includes recycling metal from waste streams, as doing so ensures a future supply of rare earth elements and valuable metals. **(1)**

Processes involved in metal recycling include sorting, separating, and grinding/shredding, and depending on the concentration, chemical composition, and particle size, this process will produce several sorts of emissions that are sources of toxic metals. **(2,3,4,5,6,7,8).**

According to several studies **(3,9,10,11)** metal scrap and waste from electrical and electronic devices contain poisonous and allergenic metals that, when mechanically treated, could produce airborne emissions that could be harmful to both the environment and occupationally exposed workers.

Occupational exposure to specific hazards occurs when a condition or disease is more prevalent in a particular group of workers than in the general population **(12,13)** Researchers have developed and employed biomarkers which are quantifiable signs of a biological condition or state to improve the diagnosis of various disorders, assess their risks, and select the most effective treatment. Blood, serum, urine, or soft tissues are frequently used to measure and evaluate biomarkers **(14)**

In cases of metal toxicity, hematological markers are frequently utilized to assess health status **(15)**. Heavy metal toxicity to bone marrow precursors causes cell division, maturation-related

enzyme inhibition, red blood cell transport impairment, and immune-mediated cell division, all of which can cause harm to the haematopoetic system **(16)**.

Evidence from animal research has shown that high concentrations of lead, cadmium, and chromium have been linked to changes in several red blood cell parameters, (17,18,19,20,21).

According to reports, lead exposure has an impact on lymphocyte function, cellular immunity, humoral immune response, host resistance, and cytokine generation. Therefore, it was assumed that a considerable drop in lymphocytes might be caused by exposure to lead fumes at work **(22,23)**

The heavy metal cadmium is recognized to be poisonous and to be a powerful inflammatory trigger. According to **(24)**, cadmium is a known environmental pollutant that is also a health concern for the general public. Cadmium has been shown to activate several intercellular signaling pathways in immune cells and cause upregulation of inflammatory markers and mediators in micromolar concentrations **(25)**. Cadmium may also induce acute and/or chronic inflammatory responses in cardiac tissue, liver, lung, and the reproductive system, potentially resulting in tissue damage and even systemic inflammatory responses **(25,26)**

Hs-c reactive is a protein mostly produced in the liver by liver hepatocytes and released into the plasma **(27)**. By interacting with the humoral and cellular inflammatory effector systems, it can start the process of eliminating the targeted cells **(28)**. It serves as a tool for detecting inflammation, a sign of disease activity, and an aid in diagnosis **(29)**.

There have been some controversies regarding the relationship between hscrp levels and metals as some studies have been linked to a positive relationship between them, where heavy metals were found to be associated with an increased cardiovascular risk which ultimately led to an increase in the hscrp of the individuals **(30)**, whereas in a study carried out by **(31)**on Blood lead,

cadmium and mercury about homocysteine and C-reactive protein in women of reproductive age, hscrp was found to be negatively associated to the cadmium and lead levels.

The neutrophil-lymphocyte ratio (NLR) is another inflammatory biomarker that is calculated as a simple ratio between the lymphocyte and neutrophil counts measured in peripheral blood. It conjugates two phases of the immune system, the innate immune system and the adaptive immune system**(32)**.

MATERIALS AND METHODS

Study Area

The study was carried out in metal recycling workshops around Oredo, Ikpoba-okah, and Ovia North-East local government Areas in the metropolitan city of Benin, Edo state, Nigeria. Edo state has an estimated population of 1,147,166 according to the 2006 general census. She is bounded to the Northeast by Kogi state, to the east by Anambra, to the southeast by delta, and the west by Ondo state.

Research Design

This was a case-control study of occupationally exposed metal recyclers working in metal recycling industries in the Benin metropolis and was matched with apparently healthy nonexposed participants**.**

Inclusion Criteria

Male metal recyclers between the ages of 18 and 60 years carrying out informal (primitive) metal recycling without any history of chronic ailment who have been occupationally exposed to metal recycling.

Exclusion Criteria

Subjects with a history of any form of cancer, tobacco smoking, and alcoholism were excluded from the study, and individuals below 18 years and above 60 years of age.

Informed Consent

Subjects for this study were adults who were adequately briefed on the research protocol and informed consent was obtained before sample collection. The informed consent form used for this study was clearly explained to the participants in English and their native language.

Ethical Approval

Approval was obtained from the College of Medical Sciences Ethical Committee of the University of Benin with **REC Approval No: CMS/REC/2023/372**

Collection of Blood Samples

4.5ml of blood was collected from all the participants into potassium Ethylene Diamine Tetra acetic acid (K3 EDTA) and plain container. The sample in the plain container was separated into serum using the centrifuge and the sample in the EDTA container was used for full blood count and metal analysis.

Determination of Full Blood Count

Full blood count was analyzed immediately after collection using the SYSMEX KX-1N hematology analyzer. Calibration and standardization of the equipment, processing, and analysis were done strictly according to the manufacturer's instructions.

High sensitivity-C- reactive Protein (hs-CRP)

HS- CRP analysis was analyzed using the Accu-bind enzyme-linked immunoassay (ELISA) microwells with product code 3125-300.

Neutrophil-lymphocyte Ratio

The neutrophil-lymphocyte ratio was calculated by dividing the absolute neutrophil by the absolute lymphocyte.

$$\text{Neutrophil lymphocyte ratio (NLR)} = \frac{\text{Absolute neutrophil}}{\text{Absolute lymphocyte}}$$

Statistical Analysis

This was carried out with the aid of Statistical Package for Social Sciences (SPSS) version 21. The mean, standard deviation, and correlation coefficient were determined using this package.

RESULTS

Table 1: Sociodemographic Characteristics

SEX	**EXPOSED (N=30)**	**CONTROL (N=20)**	**X^2**	**P**
MALE	30	20	18.348	<0.05
AGE (18-45 YEARS)				
18 – 22 **23 – 28** **29 – 33** **34 – 38** **39 – ABOVE**	11 (36.7%) 4 (13.3%) 2 (6.7%) 6 (20%) 2 (10%)	10 (50%) 6 (30%) 4 (20%) 2 (10%) 2 (10%)	7.48	0.11
EDUCATIONAL VALUE				
FORMAL **INFORMAL**	0 30	20 0	- -	- -
YEARS OF EXPOSURE				
1 – 3 **4 – 7** **8 – 11** **12 – 15** **16 – 19** **20**	11 (36.7%) 13 (43.3%) 5 (16.7%) 1 (3.3%) - -	- - - - - -		
PROTECTIVE DEVICE				
YES **NO**	0 30	- -		

Table 2: *Mean Comparison of Full Blood Count Parameters of Exposed and Control Subjects*

Parameters	*Metal recyclers*	*Control*	*t*	*P Value*
WBC x $10^3 \mu l$	7.17 ±1.80	5.08 ± 1.45	4.344	0.000
Lymphocytes LYM%	43.10 ± 8.59	50.87 ±8.69	-3.119	0.003
Monocytes MON%	10.70± 7.62	7.96 ± 3.42	1.508	0.138
Eosinophil EOS%	3.68 ± 0.78	3.46 ± 0.89	0.913	0.366
Basophil BAS%	0.89 ± 0.49	0.87 ± 0.44	0.147	0.884
Neutrophil NEU%	41.15 ±11.27	45.79 ± 9.26	-1.526	0.134
Haemoglobin HGB g/dl	13.81±1.12	13.89±1.81	0.175	0.862
Red blood cell RBC × $10^6 \mu L$	4.74 ± 0.66	5.01 ± 0.81	0.147	0.884
MCH Pg.	31.25 ± 8.67	27.89 ± 2.20	1.69	0.097
MCHC g/dl	33.79 ± 2.02	34.47 ±1.01	-1.382	0.173
MCV μm^3	2.29±11.58	78.32±11.83	0.176	0.245
Haematocrit HCT %	40.68±3.33	44.66±5.16	0.350	0.728
RDWS%	41.67±5.10	44.66±5.16	2.024	0.048
Platelet PLT × $10^3 \mu L$	248.73±129.13	186.85±53.29	2.026	0.048

Values are shown in Mean ± SD, $p<0.05$ signifies statistical significance. MCV= Mean cell volume, MCHC= Mean cell hemoglobin concentration, MCH= Mean cell hemoglobin, RDWS= Red cell distribution width.

Table 3: *Mean Comparison of Inflammatory Markers of Exposed and Control Subject*

Parameters	*Metal recyclers*	*Control*	*t*	*P*	*Significance*
NLR	1.03±0.47	0.90±0.13	1.236	0.222	Not significant
hs CRP µg/ml	9.73±10.35	2.46±1.16	3.117	0.003	Highly Significant

Values are shown in Mean ± SD, $p<0.05$ signifies statistical significance

Table 4: shows the correlation between hs C reactive protein and neutrophil lymphocyte ratio (r=.796). There was a positive correlation between hs-C-reactive protein and neutrophil-lymphocyte ratio.

Table 4: *Correlation Between hs-C-reactive protein and Neutrophil Lymphocyte Ratio of the exposed group*

Dependent Variables	*n*	*r value*
NLR	30	.796*

r value = coefficient of significance, n sample size

DISCUSSION

Concerns regarding ambient air pollution levels have been raised by the discovery of metal recycling emissions as sources of toxic metals on a local and regional scale **(3,4,33,34)**. Metal recyclers are exposed to different metals that are carcinogenic and are listed as restricted hazardous substances in waste of electronic and electrical equipment (WEEE). This present study investigates alterations in full blood count parameters and some inflammatory biomarkers of occupationally exposed metal recyclers.

In this study, it was observed that metal recyclers are exposed to toxic metals particularly due to the occupational lifestyle of the primitive metal recyclers who work with near-zero safety. The result showed that the test participants who are occupationally exposed to metal recycling had an increased white blood cell count (WBC) compared to the control participants, this was in accordance with a study carried out by **(33)**on the association and interactions between heavy metals with white blood cell count and eosinophil count. The white blood cell count was seen

elevated in the test group than in the control group and there was a correlation between the elevated white blood cell count and a high concentration of lead in the blood. **(35)**also investigated immune reactions to different concentrations of Lead (Pb) exposure in guinea pigs and in this study the guinea pigs inhaled 0.1m, 0.2m, and 0.4m of aerosol Lead (Pb) for 1 hour, twice a week, after which blood samples were collected and analyzed. The results showed elevated levels of total white blood cell count supporting the proinflammatory effect of Lead (Pb) poisoning **(35)**.

The lymphocyte level in the test participants was significantly lower than the lymphocytes in the control participants. Research has shown that lead exposure has an impact on lymphocyte function, cellular immunity, humoral immune response, host resistance, and cytokine generation. Therefore, it was assumed that a considerable drop in lymphocytes might be caused by exposure to lead fumes at work. **(22,23)**

According to **(36)** the red cell distribution width (RDW) is a quantitative measure of the consistency in the size of circulating erythrocytes,such that higher red cell distribution width (RDW) reflects greater variability in the size of red blood cells. It is interesting to note that this study had a significantly reduced red cell distribution width (RDW) ($p<0.05$) compared to our controls. This study is not in agreement with previous studies carried out by **(37)**on the association of cadmium and lead exposure with red cell distribution width. The red cell distribution width may be reduced as a result of the physical activities metal recyclers go through due to their demanding job compared to individuals with sedentary lifestyles. This is in accordance with the work of **(38)** where an increase in physical activity was inversely related to the red cell distribution width.

Platelet value was significantly increased in the test group compared to the control. This is in agreement with the work of **(38)** where platelets were found to have a significant role in modulating clot formation and also considerable roles in inflammation and immune response. Platelets gather at the damaged site and adhere to white blood cells. Subsequently, they release cytokines and chemokines which are chemotactic for neutrophils and monocytes. These interactions result in the formation of platelet granulocyte or platelet leucocyte aggregates which triggers further inflammation. Researchers have shown that exposure to metals and metalloids causes pathological conditions, including immunotoxicity and inflammation-related diseases **(3,39)**

Serum levels of high sensitivity C reactive protein an inflammatory biomarker an inflammatory biomarker was significantly increased in metal recyclers compared with the unexposed population. This is in accordance with the work of **(40),** where blood lead level was associated with an elevated hs-CRP level, which could be supported by the study of **(41)** where elevated lead level induces oxidative stress and acted on various pathways through specific mediators such as interleukin (IL-6). Another investigation in Nigerian lead-exposed workers found a decreased immune status and significantly raised CRP ($p<0.001$) in response to elevated lead levels **(42)**. In another study by **(33)** blood lead levels, cadmium, and mercury were not associated with hs-crp concentrations although this study was carried out in pregnant women.

Neutrophil lymphocyte ratio which is also an inflammatory biomarker as high sensitivity C-reactive protein (hs-C-reactive protein). This may be due to C reactive protein being present in minute quantities in healthy individuals which increases quickly in inflammation within a few hours and decreases when the inflammation subsides **(43)**. However, in this present study, a

positive correlation was found between the neutrophil-lymphocyte ratio and hs C-reactive protein of the exposed metal recyclers (r=.796*).

5.1 Conclusion

Occupational exposure to metal recycling is a hazard seen to cause alterations in full blood count parameters such as white blood cell count, Lymphocyte count, red blood cell distribution width, platelet count and inflammatory biomarker, and high sensitivity C-reactive protein. The study concludes that exposure to toxic metals such as lead and cadmium during the metal recycling process might cause an increase in white blood cell count, platelet count, serum level of high sensitivity C-reactive protein, and a decrease in red blood cell distribution width.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that they have no known competing financial interests OR non-financial interests OR personal relationships that could have appeared to influence the work reported in this paper.

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