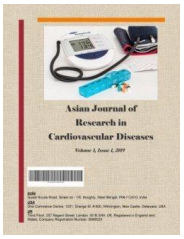

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Apolipoprotein B-48 and Tumour Necrosis Factor-alpha Levels in Male Welders in Nnewi, South-Eastern Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Welding processes produce toxic fumes consisting of gaseous and aerosol by-products which pose a risk to the male cardiovascular system. The rate of cardiovascular death is increasing globally, and there is existing evidence on cardiovascular impairment of welding fume constituents. This study sought to assess the effects of welding fume inhalation on the cardiovascular function of welders in Nnewi. A site-by-site cross-sectional study of 45 welders (aged between 18 and 50 years) who were exposed to welding fumes (Test group) and 45 age-matched non-welders (Control group) was carried out. The ages of the Test and Control subjects, as well as the years of exposure of the Test subjects were obtained via questionnaire. A single non-fasting venous blood (about 5 mls) was collected from the ante-cubital space from the subjects via venipuncture between 8:00 AM and 11:00 AM. Serum was separated following clotting and used for the investigation of the levels of Apolipoprotein B48 and Tumor Necrosis Factor-α among welders. Apolipoprotein B48 and Tumor

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Necrosis Factor-α levels were assayed by Enzyme Linked Immunosorbent Assay. Significantly elevated ($p<0.05$) levels of Tumor Necrosis Factor-α (22.43±3.92 pg/mL) was found in welders compared with controls (5.11±0.80 pg/mL). Longer duration of exposure was associated with increased levels of Tumor Necrosis Factor-α. From this study, the significant increase in TNF-α levels in these welders suggests that welding fume exposure may trigger systemic inflammation and atherogenic response. Welders therefore are at risk of developing cardiovascular disease.

Keywords: Apolipoprotein B-48; tumor necrosis factor-α; welding; cardiovascular disease.

1. INTRODUCTION

Welding is the most widely used technology for joining metals and alloys, and the processes of welding produce fumes made up of gaseous and aerosol by-products composed of metals, metal oxides and volatilized chemical species. These gaseous and aerosol by-products come from the base metals, welding electrode, or flux material [1]. Toxic substances such as chromium, nickel, arsenic, asbestos, manganese, silica, beryllium, cadmium, nitrogen oxides, phosgene, acrolein, fluorine compounds, carbon monoxide, cobalt, copper, lead, ozone, selenium and zinc, are the very many substances in welding smoke [1]. Welding generates individual ultrafine particles (particles smaller than 100 nm) that rapidly form larger chain-like aggregates in the fine particle size range. Mild steel welding generates fumes which are composed of 80% or more iron, some manganese, but no chromium or nickel. Fumes from stainless steel welding contain 20% of chromium and 10% of nickel [2]. Inhalation of particulate matter (PM) air pollution is associated with an increased risk of cardiovascular disease (CVD) [3-4].

Apolipoproteins are important components of lipoprotein particles. There is accumulating evidence that measurement of various forms of apolipoproteins may improve the prediction of the risk of cardiovascular disease [5-7]. Apolipoprotein B exists in two forms, apo B-48 and apo B-100. Apo B-48 is synthesized in the intestine, where it is complexed with dietary TG and free cholesterol absorbed from the gut lumen to form chylomicron particles, and metabolized in the liver. Apo B is essential for the binding of LDL particles to the LDL receptor, hence allowing cells to internalize LDL. The result is that cholesterol is absorbed. Excess circulating levels of apo B-containing particles is a main trigger in the atherogenic process [8].

The pro-inflammatory cytokine TNF-α is a polypeptide cytokine mainly produced by stimulated monocytes, macrophages, and T-lymphocyte subsets. Tumour Necrosis Factor-alpha, is considered to play a role in the development of cardiovascular disease [9]. High circulating levels of TNF-alpha has been implicated in the pathogenesis of several conditions including arthritis, coronary artery disease and myocardial dysfunction [10-12]. Elevated TNF-alpha levels have consistently been reported in patients with heart failure, and increasing concentrations of the cytokine are related with the severity of CVD and mortality rate in the patients [13-14].

Studies on the toxicological effects of welding fumes on human cardiovascular system are still controversial. Kim et al. found increased C-reactive protein (CRP) 16 hours after exposure to welding fumes in 37 workers [15]. Palmer et al. reported that no statistical differences in the TNF-α level in sputum and blood following the welding exposure have been reported in humans [16]. Scharrer et al. reported a significant decrease of endothelin-1 after 1 hour of exposure to welding fumes of 3.5 mg/m^3 in 20 non-smoking, healthy volunteers, but they observed no changes in leukocyte count, CRP, TNF-α, Interleukin-6 (IL-6), or Interleukin-8 (IL-8) [17]. Jarvela et al. found a slight, acute inflammatory effect indicated by an increase of leukocytes and neutrophils in blood and a decrease of Interleukin-1β, which were measured before and after work shifts in 20 workers. They did not observe changes of concentrations of CRP, IL-6, IL-8 or TNF-α [18].

This study therefore is designed to evaluate the possible effects of exposure to welding fumes and airborne particles in metal workers in welding halls and shops on the systemic inflammatory and cardiac parameters.

2. MATERIALS AND METHODS

2.1 Study Design

A total of 90 adult male volunteers aged between 18 – 50 years were recruited for this study by convenient sampling technique, comprising of 45 individuals as test group (welders) and 45

individuals as control group. The control population were age-matched volunteers who were not exposed to welding fumes (office workers, traders and students), who do not smoke more than 5 sticks daily and who do not consume more than 180 cl of beer daily.

2.2 Inclusion Criteria

Subjects with no history of pulmonary and CVD prior to employment as a welder, who are not drug addicts or excessive smokers/alcohol users during the previous one year and with no history of pulmonary or heart surgery were included in the study.

2.3 Exclusion Criteria

Subjects with job duration less than one year, above the age of 50 years, with hypertension and diabetes and with history of pulmonary or heart surgery prior to employment were excluded from the study.

2.4 Specimen Collection

About 5 ml of a single non-fasting venous blood was collected from the ante-cubital space from the subjects via venipuncture, and dispensed into plain containers. Blood was delivered to the laboratory where it was centrifuged, following clotting. Centrifugation was performed at 3000 rpm for 5 minutes using bench centrifuge, and serum separated and stored frozen, until the time of assay.

2.5 Analytical Methods

The serum Apo-B48 and TNF-Alpha assays were done using standard Enzyme Linked Immuno-Sorbent Assay - double antibody sandwich principle [19].

2.6 Statistical Analysis

The data from this study were subjected to statistical analysis using the Statistical Package for Social Sciences (SPSS) version 23 and presented as mean ± standard deviation. Student's t-test (independent t-test) was used to test difference in mean values. The tests of significance of variation within and among groups were compared using ANOVA at 95% level of confidence and post-hoc (Tukey) analysis was used to compare multiple variables. The results obtained were presented in tables for clarity. The p- value ≤ 0.05 was considered statistically significant. Correlation was performed using the Pearson's correlation.

3. RESULTS

Table 1 shows that there was no significant difference between the mean age of the test and control subjects (p= 0.703). Also, there was no significant difference when the levels of apolipoprotein B48 of the test subjects was compared with the control. Tumor necrosis factor- alpha (22.43±3.92 pg/mL) of the test subjects was significantly higher (p = 0.000) when compared with the control subjects (5.11±0.80 pg/mL).

Table 2 shows the correlation between duration of exposure and apolipoprotein B48, as well as tumor necrosis factor- alpha of the subjects in the study. There was a weak, negative relationship between duration of exposure and apolipoprotein B48 level of the subjects in the study, which was not statistically significant (p= 0.615) while there was a weak, negative relationship between duration of exposure and tumor necrosis factor- alpha level of the subjects in the study, which was not statistically significant (p= 0.859).

Table 3 shows the correlation between age and apolipoprotein B48, as well as tumor necrosis factor- alpha of the subjects in the study. There was a weak, negative relationship between age and apolipoprotein B48 level of the subjects in the study, which was not statistically significant (p= 0.307) while there was a weak, negative relationship between age and tumor necrosis factor- alpha level of the subjects in the study, which was not statistically significant (P = 0.591).

Table 4 shows the correlation between age and apolipoprotein B48, as well as tumor necrosis factor- alpha of the control subjects in the study. Also, there was a weak, negative relationship between age and apolipoprotein B48 level of the subjects in the study, which was not statistically significant (p = 0.248) while there was a weak, negative relationship between age and tumor necrosis factor- alpha level of the subjects in the study, which was not statistically significant (p = 0.722).

Table 5 shows the values of apolipoprotein B48 and tumor necrosis factor- alpha (TNF- α) based on different age groups of the test subjects in the study. The levels of apolipoprotein B48 and tumor necrosis factor- alpha shows no statistical significant difference (p = 0.484, p = 0.167, respectively) when age group 18-28 (years) was

compared with 29-39 (years) and 40-50 (years) in the study.

Table 6 shows the values of apolipoprotein B48 and tumor necrosis factor- alpha (TNF- α) based duration of exposure of the subjects in the study. The apolipoprotein B48 levels was not statistically significant (p= 0.547) when group 1-6 (years) was compared with 6-10 (years) and ≥11 (years). The tumor necrosis factor-alpha shows no statistical significant difference (p= 0.161) when group 1-6 (years) was compared with 6-10 (years) and ≥11 (years) in the study.

Table 1. The levels of apolipoprotein B48 and tumor necrosis factor-alpha of the subjects in the study (Mean±SD)

Parameters	Test subjects (N= 45)	Control subjects (N=45)	t- value	P- value
Age (Years)	31.93±9.56	31.13±10.00	-0.383	0.703
APO B48 (pg/ml)	1118.24±127.62	1174.96±161.29	-1.850	0.068
TNF-α (pg/ml)	22.43±3.92	5.11±0.80	29.016	0.000*

*Key: N: Number of subjects; p≤0.05: *Statistically significant; Apo B48: Apolipoprotein B48; TNF-α: Tumor necrosis factor- alpha; Degree of freedom (df): 88*

Table 2. The correlation between duration of exposure and biochemical parameters of the subjects in the study

Parameters	r (Pearson correlation coefficient)	P- value
Duration of exposure vs APOB48	-0.077	0.615
Duration of exposure vs TNF-α	-0.027	0.859

*Key: N: Number of subjects (45), P≤0.05: *Statistically significant; Apo B48: Apolipoprotein B48; TNF-α: Tumor necrosis factor- alpha*

Table 3. The correlation between age and the biochemical parameters of the test subjects in the study

Parameters	r (Pearson correlation coefficient)	P- value
Age vs APOB48	-0.156	0.307
Age vs TNF-α	-0.082	0.591

*Key: N: Number of subjects (45); P≤0.05: *Statistically significant; Apo B48: Apolipoprotein B48; TNF-α: Tumor necrosis factor- alpha*

Table 4. The correlation between age and the biochemical parameters of the control subjects in the study

Parameters	r (Pearson correlation coefficient)	P- value
Age vs APOB48	-0.176	0.248
Age vs TNF-α	-0.055	0.722

*Key: N: Number of subjects (45); P≤0.05: *Statistically significant; Apo B48: Apolipoprotein B48; TNF-α: Tumor necrosis factor- alpha*

Table 5. The mean (±SD) values of biochemical parameters based on different age groups of the subjects (welders) in the study

Groups	APO B48 (pg/ml)	TNF-α (pg/ml)
18-28 (A)	1124.84±98.47	21.64±3.93
29-39 (B)	1138.07±158.71	23.93±4.24
40-50 (C)	1118.24±127.62	21.55±2.99
F- value	0.739	1.867
P- value	0.484	0.167
A vs B	0.952	0.208
A vs C	0.618	0.998
B vs C	0.470	0.269

*P≤0.05: *Statistically significant; N: Number of sample (A= 18, B= 16, C= 11); Apo B48: Apolipoprotein B48; TNF-α: Tumor necrosis factor- alpha*

Table 6. The mean (±SD) values of biochemical parameters based on duration of exposure of the subjects in the study

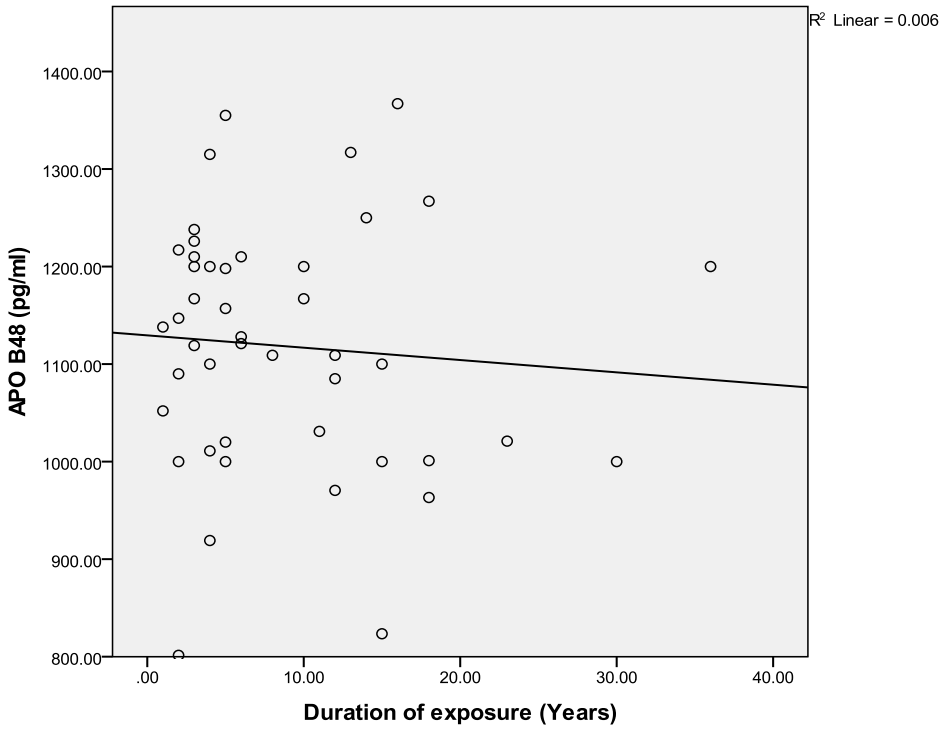
Groups (Years)	APO B48 (pg/ml)	TNF-α (pg/ml)
1-5 (A)	1127.64±129.59	23.18±3.89
6-10 (B)	1155.83±42.87	19.70±3.20
≥11 (C)	1094.73±143.75	22.48±3.97
F- value	0.612	1.909
P- value	0.547	0.161
A vs B	0.884	0.137
A vs C	0.708	0.839
B vs C	0.577	0.286

*P≤0.05: *Statistically significant; N: Number of sample (A= 21, B= 6, C= 18); APO B48: Apolipoprotein B48; TNF-α: Tumor necrosis factor- alpha*

4. DISCUSSION

Welding represents a unique occupational PM exposure because of the generation of inhalable metal fumes. When inhaled in the lungs, solubilized metals and/or bioactive and inflammatory cofactors induced by the pulmonary deposition of metal-rich welding particles may reach the circulatory system and enter vital organs, possibly producing functional alterations or damage. The goal of this study is to determine the potential influence of the inhalation of welding fume toxicants on the cardiovascular system.

Epidemiological studies indicated that exposure to welding fume particles may pose a risk for development of cardiovascular disease. Using two large cohorts from the Swedish National Censuses of 1970 and 1990, Sjorgen et al. observed a significant increase in mortality rate among welders due to ischemic heart disease [20]. Similarly, Ibfelt et al. sampled more than 10,000 metal workers in 75 welding companies in Denmark in a prospective cohort and noted a significant rise in hazard rate ratio for chronic ischemic heart disease in welders with increasing exposure to metal particles [21]. Studies of welders suggested potential mechanisms related to cardiovascular disease, including effects on heart-rate variability, aortic augmentation index (a marker of arterial stiffness), and markers of systemic inflammation and oxidative stress [17,22].



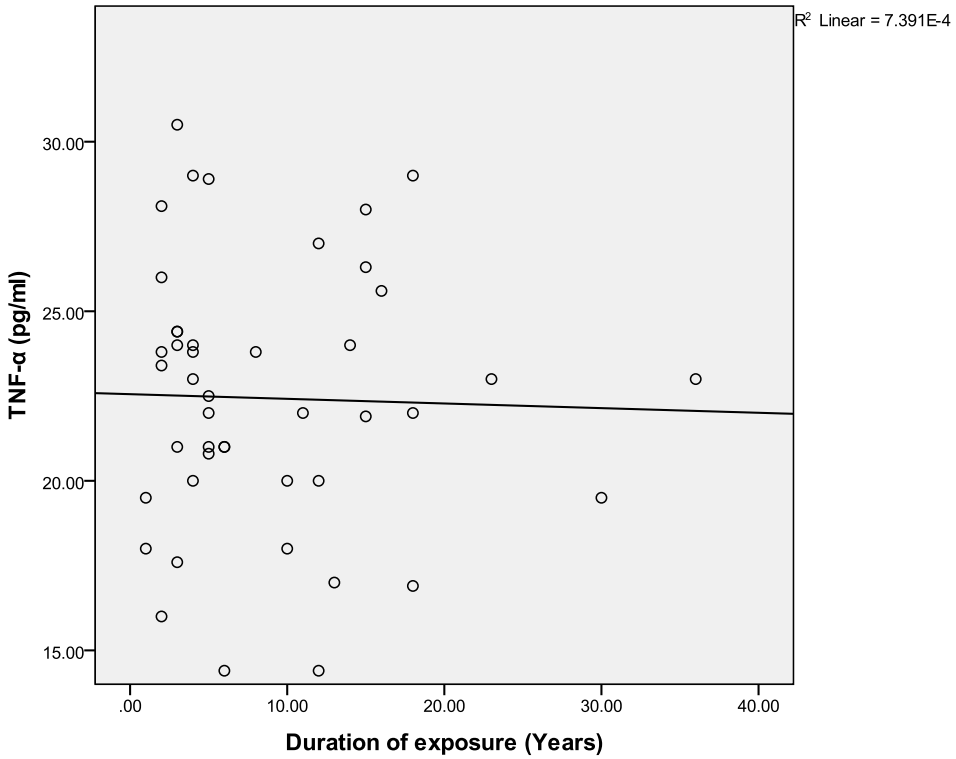


Fig. 1. Scatter plot showing the correlation between duration of exposure and TNF-α level of the subjects (Welders) in the study

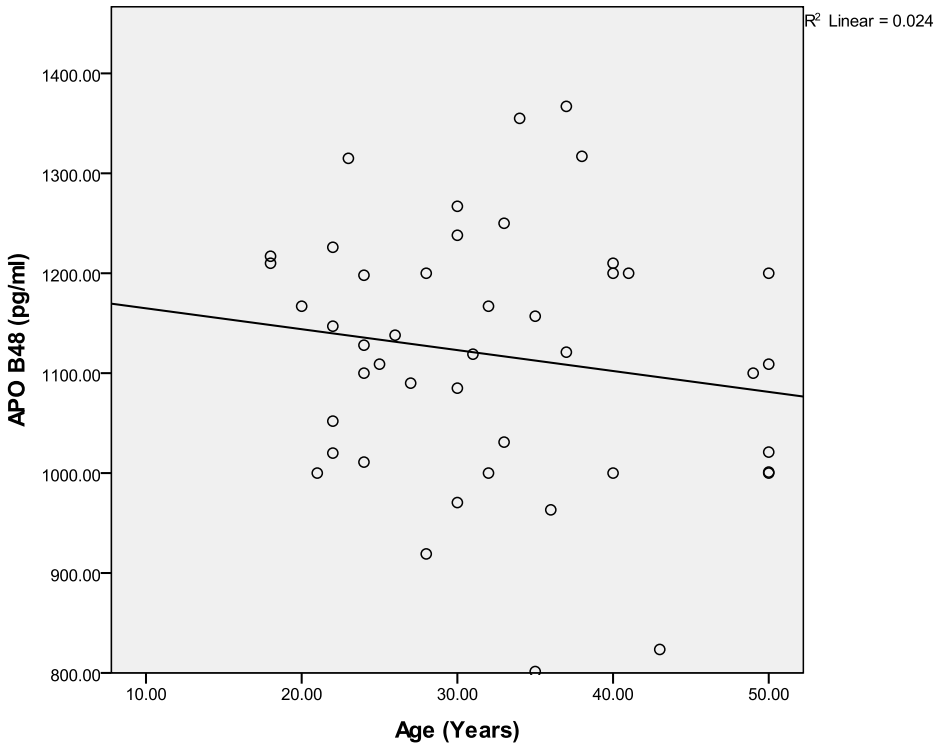


Fig. 2. Scatter plot showing the correlation between age and TNF-α level of the subjects (Welders) in the study

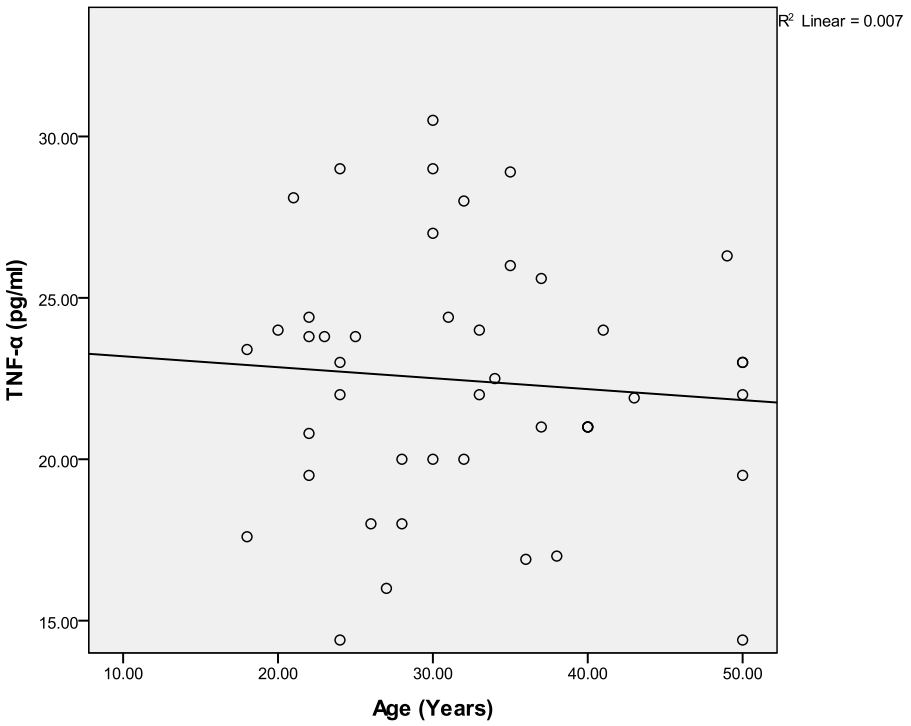


Fig. 3. Scatter plot showing the correlation between age and TNF-α level of the subjects (Welders) in the study

Several studies have shown that measurement of various forms of apolipoproteins may improve the prediction of the risk of cardiovascular disease [5-7]. Apolipoprotein B exists in two forms, apo B-48 and apo B-100. Apo B-48 is synthesized in the intestine, where it is complexed with dietary Triglyceride and free cholesterol absorbed from the gut lumen to form chylomicron particles, and metabolized in the liver. Apo B is essential for the binding of LDL particles to the LDL receptor, hence allowing cells to internalize LDL. The result is that cholesterol is absorbed. Excess circulating levels of apo B-containing particles is a main trigger in the atherogenic process [8].

This study reveals that there was no significant difference in Apolipoprotein B48 levels of welders when compared with the control population.

A few studies have investigated the inflammatory response induced by welding fumes with inconsistent results. Kim et al. reported that there were increased circulating C- reactive protein (CRP) levels 16 hours after exposure to welding fumes in 37 workers [15]. Palmer *et al*. reported that no statistical differences in the TNF-α levels in sputum and blood following the welding exposure have been reported in humans [16]. Scharrer *et al*. reported a significant decrease of endothelin-1 after 1 hour of exposure to welding fumes of 3.5 mg/m^3 in 20 non-smoking, healthy volunteers, but they observed no changes in leukocyte count, CRP, TNF-α, Interleukin-6 (IL-6), or Interleukin-8 (IL-8) [17]. Jarvela et al. found a slight, acute inflammatory effect indicated by an increase of leukocytes and neutrophils in blood and a decrease of Interleukin-1β, which were measured before and after work shifts in 20 workers. They did not observe changes of concentrations of CRP, IL-6, IL-8, or TNF-α [18]. A study by Stark et al. revealed that long-term exposure to mild steel welding is associated with local neutrophil inflammation of the lungs, as well as an increased expression of the gene encoding Vascular Endothelial Growth Factor (VEGF), and decreased expression of the gene encoding hemeoxygenase-1, which functions in a pathway involved in oxidative stress [23]. Tumour Necrosis Factor-alpha is a pro-inflammatory cytokine that can produce widespread deleterious effects when expressed in large amounts [10]. It is produced in the heart by both cardiac myocytes and resident macrophages under conditions of cardiac stress and is thought to be responsible for many of the untoward

manifestations of cardiac disease [9]. The over expression of TNF-alpha has been implicated in the pathogenesis of several conditions including Coronary Artery Disease and myocardial dysfunction [10].

Although the above earlier studies [16-18] reported no significant changes in the levels of the pro-inflammatory cytokine TNF-α among welders, this study sharply refutes these earlier studies, as the mean value of TNF-α of the study group was significantly higher as compared with the control subjects in the study. Since the study and control groups were comparable, the marked difference so obtained may have resulted from the exposure and could therefore signify inflammatory trigger of welding fume toxicants. The implication is CVD and myocardial dysfunction.

From this study, the significant increase in TNF-α level in these welders suggests that welding fume exposure may trigger systemic inflammation and atherogenic response. Welders therefore are at risk of developing cardiovascular disease.

5. CONCLUSION

Chronic welding fume inhalation caused a significant elevation in the pro-inflammatory cytokine TNF-α levels compared with controls. This may have resulted from the exposure to welding fume toxicants which cause systemic inflammation to the heart. Since increase in TNF-α levels have been correlated with CVD, this study therefore subscribes to the hypothesis that welding fume inhalation may have a potential for causing cardiovascular disease. Welders therefore are advised to apply precautionary measures such as limiting exposure time as well as strict adherence to respiratory protective devices.

CONSENT AND ETHICAL APPROVAL

The ethical approval for this research was obtained from the Human Research Ethics Committee of the Nnamdi Azikiwe University Teaching Hospital, Nnewi, Anambra State, Nigeria. The procedures were explained to the subjects, and written informed consent obtained from each subject before specimen collection.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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