African Scientist Vol. 24, No. 1 March 31, 2023
Printed in Nigeria

1595-6881/2023 $80.00 + 0.00

https://africansciientistjournal.org

AFS2022045/24105

Association of Total Antioxidant Capacity Status and Malondialdehyde with C-Reactive Protein Levels in Bronze Foundry Workers in Benin City, South-South, Nigeria

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(Received December 28, 2022; Accepted in revised form February 27, 2023)

ABSTRACT: Occupational exposure to bronze borne toxicant is entirely unregulated in many countries. The foundry industry is known to contain a lot of toxic metals and the exposure of foundry workers to these metals may result in pro-oxidant/antioxidant imbalance and can act as an intermediate in the formation of an oxidative stress state. This aim of this study was to investigate the levels of total antioxidant capacity, malondialdehyde and C-reactive protein among bronze foundry workers in Benin City, South-South Nigeria. A total of eighty (80) consenting participants were recruited for this study; they included foundry workers (bronze casters), randomly selected individuals around the foundry site (environmental) and apparently healthy unexposed individuals (control) which were age and sex matched in a comparative cross-sectional study. Occupational activities and anthropometric data of participants were collected using a questionnaire. MDA and TAC levels were evaluated using colorimetric methods while CRP levels was evaluated using enzyme-linked immunosorbent assay (ELISA). Data were analyzed using ANOVA and a p-value < 0.05 was considered significant. Only MDA (malondialdehyde) levels showed no significant difference within the groups. TAC levels in bronze casters (18.18±1.00) were found to be lower when compared to the environmental (36.7± 5.73) and control (22.21±1.10). CRP levels were found to be higher in control (16.39±1.10) and environmental (11.21 ±0.91) compared to the bronze casters (8.38±0.61). Continuous exposure leading to accumulation of these bronze associated toxicants can induce the production of increased levels of free radicals which may reduce TAC levels and increase CRP levels.

Keywords: Total antioxidant capacity, Malondialdehyde, C-Reactive protein, Oxidative stress, Bronze Casters, Bronze

Introduction

The bronze casting tradition, which was started in the 14th century by Oba Oguola and put under the hereditary rule of the Ine N'igun Eronmwon, is one of the most distinctive features of the old Kingdom of Benin, which is now the capital of Edo State in Nigeria (Nwachukwu, 2012). As a result, indigenous technology has facilitated the creation of cultural items such as commemorative bronze heads, free-standing figures and groups, plaques in relief, bells and rattle-staff, little expressive masks and plates worn on the belt as badge of offices, animals, jewelries, etc. For the people of Benin, these items serve socioeconomic, cultural, and religious purposes (Nwachukwu, 2012). A foundry is a type of factory where metal castings are made. Metals are melted into a liquid, poured into a mould, and the mould material is removed once the metal has set and is cooling (Degarmo *et al*., 2003). As a result of their exposure to these metals, foundry employees may experience pro-oxidant/antioxidant imbalance and oxidative stress, which may alter their levels of total antioxidant capacity (TAC), malondialdehyde (MDA), and C-reactive protein (Soleimani *et al*., 2015). The hazardous substances found in the primary material utilised by employees in bronze foundries, bronze metal, can cause oxidative stress, which can result in higher levels of these parameters (Erel, 2004).

Any molecule that, when supplied at low concentrations in comparison to those of an oxidizable substrate (proteins, lipids, carbohydrates, and DNA), considerably slows down or stops that substrate from oxidizing is referred to be an antioxidant. Antioxidants' primary purpose is to shield the body from the harm that free radicals cause (Crnogaj, 2017). Free radicals may be produced in cells and tissues as a result of weakened antioxidant defenses or from internal or external causes, such as inflammation, illness, or metabolism. In any instance, an increase in the generation of free radicals might cause oxidative damage (Lobo *et al*., 2010).

To observe the antioxidant capacity of biological samples, total antioxidant capacity (TAC), a measurement of the quantity of free radicals scavenged by a test solution, is used (Rubio *et al*., 2016). Malondialdehyde (MDA), a low molecular weight aldehyde with the formal formula $CH_2(CHO)_2$, can be created by a variety of processes. It happens naturally and serves as a symptom of oxidative stress (Nair *et al*., 2007). Since people with various disorders have higher MDA levels, MDA has been regarded as a significant lipid peroxidation indicator over the past 20 years (Joosten, 2001). Lipid peroxidation is a series of processes that is started by free radicals and leads to the oxidative degradation of polyunsaturated lipids. Biological membrane components are the most frequent targets. These processes can be very harmful when they occur in biological membranes, including endoperoxides and aldehydes (Quim, 2009). MDA assessment is crucial for understanding pathological conditions, but it also has a significant impact on our understanding of the toxicological consequences of pollutants including metals, solvents, and xenobiotics on both people and animals. Studies examining the toxicity mechanism of various drugs have frequently used MDA measurement. Additionally, research has been done on the impact of a mixture including elements from the petroleum sector on the biochemical parameters and enzyme activity (Abd-Elghaffar *et al*., 2005). Malondialdehyde is produced when lipid hydroperoxides from polyunsaturated fatty acids are produced as a result of oxidative stress in cells (MDA). A notable illustration is the role of lipid peroxidation in cancer, which is brought on by the accumulation of many mutations in important growth regulating genes. These genetic alterations are brought on by DNA instability as well as mistakes in DNA replication brought on by exposure to exogenous genotoxins and reactive oxygen species. MDA is an endogenous product of lipid peroxidation and may play a significant role in DNA damage and mutation. According to Cirak *et al*. (2003), malignant brain tumours with higher blood and tissue MDA levels than healthy controls exhibit enhanced oxidative stress. An indicator of immune system activity is the acute-phase plasma protein known as C-Reactive Protein (CRP). Due to tissue damage, infection, and acute inflammation, a variety of proteins that are present in acute-phase plasma proteins experience fast changes in concentration. However, circulating levels of CRP are also somewhat increased in cancer and chronic inflammatory disorders as a result of a number of factors, most notably tissue damage and inflammation (Conventry *et al*., 2009).

Materials and methods

Study design: The study was set as a cross-sectional study with the intention of participant recruitment into the study groups. A total of eighty (80) participants comprising of occupationally exposed (bronze casters) (50), randomly selected individuals from foundry site (environmental participants) (20) and unexposed healthy participants (control participants) (10) were recruited for this study. Informed consent was obtained from each participant after proper notification and information on the nature of the research, risk involved, benefits as well as confidentiality, alongside with the administration of a questionnaire. Five milliliters (5mL) of venous blood were collected from the participants in each study group using standard phlebotomy techniques. The blood samples were allowed to clot, retract and were centrifuged at 3000 revolution per minute for 5 minutes. Serum obtained from each sample was stored in Eppendorf tubes, kept frozen (0^oC to -4^oC) before analysis. Frozen samples were, thawed to bring to room temperature and analyzed.

Laboratory analysis

Malondialdehyde determination using TBARS: Concentrations of MDA in the samples are estimated by thiobarbituric acid reactive substance (TBARS) assay (Varshney and Kale 1990). MDA which is formed from the breakdown of polyunsaturated fatty acid serves as a convenient marker for the determination of the extent of lipid peroxidation. Assay is based on the reaction of MDA with thiobarbituric acid (TBA), forming an MDA-2TBA adduct (Pink-red coloured complex) that absorbs light at 535nm.

Total antioxidant capacity (T-AOC) detection using a colorimetric assay kit: The total level of antioxidant macromolecules, antioxidant molecules and enzymes in a system that can eliminate all kinds of reactive oxygen species and prevent oxidative stress induced by reactive oxygen species, reflects the total antioxidant capacity in the system. Many antioxidants in the body can reduce Fe^{3+} to Fe^{2+} and Fe^{2+} can form stable complexes with phenanthroline substance. The antioxidant capacity (T-AOC) can be calculated by measuring the absorbance at 520nm.

Determination of CRP levels using Elabscience Quickey pro ELISA kit: This ELISA kit uses the Sandwich-ELISA principle. The micro-ELISA plate provided in this kit has been pre-coated with an antibody specific to Human CRP. Samples (or Standards) and Horseradish Peroxidase (HRP) linked antibody specific for Human CRP are added to the micro-ELISA plate wells. Human CRP in samples (or standards) combines with the coated antibody and HRP linked detection antibody specific to CRP. Excess conjugate and unbound sample or standard are washed from the plate. The substrate solution is added to each well. The enzyme-substrate reaction is terminated by the addition of stop solution and the color turns yellow. The optical density (OD) is measured spectrophotometrically at a wavelength of 450±2 nm. The concentration of Human CRP in the samples is then determined by comparing the OD of the samples to the standard curve.

*Statistical analysis***:** Statistical analysis including descriptive statistics were carried out using the Statistical Package for Social Scientists (SPSS) version 25.0 (IBM, USA). Data obtained were expressed as mean ± standard deviation for both test and control groups. The data generated were compared using one way analysis of variance (ANOVA) at 95% confidence intervals and a p value of less than 0.5 (> 0.5) was considered significant.

Results

Anthropometric indices of the bronze casters, environmental and control subjects are shown in Table 1. From the observation the mean age of the bronze casters was (47.12±15.748) environmental (42.58±1.73) and control (46.29±15.74).

Table 1: Age, sex, weight and body mass index of bronze casters and controls

Variable	**Bronze casters (n=50)**	**E. Cohorts (n=20)**	**Unexposed control (n=20)**
Age (Years)	47.12±15.75	42.58±1.73	46.29±15.74
Sex	Males	Males	Males
Nutritional Indicators	Yes (97.8%)	Yes (94.2%)	Yes (9.42%)
Mean Weight	44.31±22.27	79.92±1.67	67.20±125.06
Body mass index (Kg/m^2)	25.20±3.93	24.87±0.69	24.33± 4.27

Sex, mean weight and Body mass index are shown in Mean±SEM

The risk awareness levels and indicators among foundry workers (bronze casters) environmental and control groups are presented in Table 2. It was observed that (46%) of bronze casters, (34.6) environmental and (75%) of control lacked basic awareness of health hazards associated with bronze borne toxic substances. Practically, all participants, bronze casters (100 %) environmental (98.2 %) and control (90 %) indicated willingness and granted consent to participate in the toxicological study. Assessing the nature of exposure, it was observed that occupational and environmental exposures were high for bronze casters; but for the control group, occupational exposure was not indicated. Duration of exposure of ≥ 5 years was observed among bronze casters and the environmental group.

Table 2: Awareness of risk and occupational exposure pattern of study participants

Observation	Bronze Casters	Environmental cohorts	Control
Level of awareness of bronze borne toxic substances	Basic (64%) No basic (46%)	Basic (65.4%) No basic (34.6%)	Basic (15%) No basic (75%)
Nature of exposure	Occupational and environmental	Environmental	No occupational exposure
Duration of exposure to bronze casting material	≥ 5.0 years	≥ 5.0 years	Nil
% Using PPE while working (Apron, hand gloves and face masks)	Users (38.5%) Non users (46.1%)	Not applicable	Not applicable
Willingness to participate in bronze casting toxicological study (By granting informed consent)	Willing (YES)=100% Not wiling (No)= 0%	Willing (YES)=98.2% Not willing (NO)=1.8%	Willing (YES)=90% Not willing (NO)=8%

Keys: PPE: personal protective equipment

A total of 80 subjects comprising of 50 male bronze casters, 20 environmental subjects and 10 control subjects were used for this study. The levels of TAC, MDA and CRP in bronze casters, environmental and control group is indicated in the Table 3 below. There was a significant difference in the mean values of Total Antioxidant Capacity (TAC) (U/mL) in bronze casters (18.8±1.00) and environmental subjects (36.7±5.73) compared to the control subjects (22.21 ± 4.29) ($p < 0.05$). The mean values of Malondialdehyde (MDA) (mmol/mL) showed no significant difference in bronze casters (0.03± 0.00) and environmental subjects (0.02 ± 0.00) compared to the control subjects (0.01 ± 0.00) ($p < 0.05$). C-Reactive Protein (CRP) showed a significant difference in the mean values of bronze casters (8.38 ± 0.61) and environmental subjects (11.21 ± 0.91) compared to the control subjects (16.39 ± 1.10) ($p < 0.05$).

Table 3: Levels of MDA, TAC, CRP in bronze casters, environmental and control groups

Parameters	Bronze casters	Environmental	Control	f-value	p-value
TAC (U/mL)	18.18 ± 1.00	36.7 ± 5.73	22.21 ± 4.29	0.00	<0.0001
MDA (mmol/mL)	0.03 ± 0.00	0.02 ± 0.00	0.01 ± 0.00	0.18	0.56
CRP (ng/mL)	8.38 ± 0.61	11.21 ± 0.91	16.39 ± 1.10	0.23	<0.0001

Values are shown in Mean±SEM, $p<0.05$ is considered significant.

The correlation coefficient of Total antioxidant capacity, Malondialdehyde and C-Reactive protein is shown in Table 4. Among the bronze casters, there was a weak positive correlation between TAC and CRP and also between MDA and CRP. TAC and MDA showed a weak negative correlation. For the Environmental cohorts TAC/MDA and TAC/CRP both showed weak positive correlation while MDA showed a moderate correlation with CRP. For the control group, TAC showed a low negative correlation with MDA and also showed a moderate negative correlation with CRP. There was no significant correlation between MDA and CRP.

Table 4: Correlation coefficient of TAC, MDA and CRP

Group		TAC (U/mL)	MDA (mmol/mL)	CRP (ng/mL)
Bronze casters				
TAC (U/mL)	r value	1	-0.139	0.173
	p value		0.179	0.229
MDA (mmol/mL)	r value	-0.193	1	0.180
	p value	0.179		0.212
CRP (ng/mL)	r value	0.173	0.180	1
	p value	0.229	0.212	
Environmental				
TAC (U/mL)	r value	1	0.239	0.171
	p value		0.310	0.470
MDA (mmol/mL)	r value	0.239	1	0.375
	p value	0.310		0.103

Group		TAC (U/mL)	MDA (mmol/mL)	CRP (ng/mL)
CRP (ng/mL)	r value	0.171	0.375	1
	p value	0.470	0.103	
Control				
TAC (U/mL)	r value	1	-0.234	-0.500
	p value		0.489	0.117
MDA (mmol/mL)	r value	-0.234	1	0.051
	p value	0.489		0.882
CRP (ng/mL)	r value	-0.500	0.051	1
	p value	0.117	0.882	

Discussion

The toxicity of heavy metals on bronze workers was clearly seen in the increase of ROS that was observed from the slight although insignificant increase of MDA with a decrease in TAC. The increase in the production of ROS causes an increase in lipid peroxidation and production of MDA which is considered a good indicator to evaluate oxidative stress (Belch *et al*., 1991). As we mentioned earlier, heavy metals cause an increase in the formation of ROS, oxidative Stress and decrease antioxidant defenses (total antioxidant capacity) or cause to an increase in the processes that produce oxidants (Hussain *et al*., 1999). In this study, serum TAC of bronze worker level was significantly lower than controls activity. Foundry workers are occupationally exposed to heavy metals and oxides of metals such as copper, cadmium, mercury, lead, arsenic, etc. These metals have the ability to interact with biomolecules and alter physiological processes in the body which may lead to oxidative stress, through the generation of reactive oxygen species (ROS) and damage to lipids, proteins and DNA (Patra *et al.*, 2011). This study was designed to evaluate the levels of Total Antioxidant Capacity, Malondialdehyde and C-reactive protein levels among foundry workers (bronze casters), environmentally exposed and control group. Also, in terms of exposure to bronze casting materials (sand, metals, etc.) the environmental subjects were not in any way exposed in their chosen profession and the control group were not in any way exposed both in their chosen profession /job and home environment. This makes it possible for results obtained in the test group to be attributable to the job (foundry work). It was observed that the nutritional status of the groups was similar, which was as a result of the exclusion of confounding influence of nutrition on data obtained in this study. Dietary intake of antioxidants is imperative to protect cells from damage caused by free radicals (Ralman *et al.*, 2015).

Knowledge of the identified hazard was assessed among the casters in this study. A smaller amount of the studied population (46%) of the casters had basic awareness of the level of the hazards associated with the occupation. This finding is in contrast to the study conducted by Efegoma (2017) that reported 78.4% of good knowledge amongst the bronze casters on hazard associated with their jobs, only 40% were reported to use personal protective equipment while working. The lack of use of these PPE may be due to lack of provision by employers and that the PPE provided are mostly uncomfortable and do not provide comfort while working (Tanko and Anigbogu, 2012). In this present study, the observed statistical decline in the TAC levels of the bronze casters and environmentally exposed group compared with the unexposed group (control) may be linked to observed chronic exposure and this burden may have been further aggravated by the low risk of awareness and exposure patten detected as earlier described. Although this reduced levels in bronze casters still fall within the normal range.

The irregular or outright non-use of PPE observed in this study may have significantly raised the ingestion of bronze-borne particles among the bronze casters. These toxins may have induced the formation of increased levels of free radicals which are scavenged by TAC, thus reducing its levels by way of consumption. Antioxidants provide protection against deleterious metal-mediated free radical attacks (Valko *et al*., 2005). It has been reported that in the presence of high levels of free radicals, there would be a reduction in the activity of TAC (Lalhminghlui and Ganesh, 2018). Free radicals are closely associated with oxidative damage and antioxidants are reducing agents, which limit oxidative damage to biological structures by donating electrons to free radicals and passivating them (Herling *et al*., 2008).

Unexpectedly, the control group had elevated levels of CRP compared to the environmental and exposed group (bronze casters). However, this increase in CRP levels among the control group falls within the normal range of CRP values for humans and as such may not be significant in inducing inflammation. Concentration of CRP is a very useful non-specific biochemical marker of inflammation, and its measurement in bronze casters contributes to the assessment of their health and diagnosis of metal induced diseases (Ahmet *et al*., 2008). The exposure of the bronze casters to bronze-borne toxins may have induced oxidative stress in them because of the role of heavy metals in initiating oxidative stress. Comparing the association of the levels of these three (3) parameters it was observed in this study that the levels of TAC is inversely proportional to the levels of MDA and CRP. MDA and CRP are markers of oxidative stress through inflammation and lipid peroxidation respectively. TAC is involved in the stabilization of the oxidant-antioxidant levels in a biological system. This corroborates with the findings of a similar study (Ahmet *et al*., 2008) that showed significantly low levels of TAC in serum of patients with pneumonia with significantly elevated MDA levels, indicating increased oxidative stress in the blood of patients with pneumonia. Our results are supported by a study reported previously (Laskaj *et al*., 2017). It this study, it was observed that CRP concentration correlated with MDA but not TAC. In contrast to this study, it was observed that there was no association between the concentrations of TAC, MDA and CRP.

Conclusion

This study reveals that chronic occupational exposure to bronze-borne toxicants may be associated with reduced levels of TAC as a result of increased production of free radicals.

Recommendation

- There should be close medical supervision of the work place and constant enlightenment of the foundry workers on the use of PPE like coveralls, boots, nose mask etc.
- Newer and safer casting sites away from their homes which would have dust control measures and sound muffling of noisy processes should be built.
- A periodic health assessment scheme should be organized for the bronze casters

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