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EVALUATION OF OXIDATIVE STRESS BIOMARKERS MDA AND SOD IN OCCUPATIONALLY EXPOSED ALUMINUM WORKERS IN ONITSHA, ANAMBRA STATE, NIGERIA

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Abstract

***Aim**: This research study was conducted to evaluate oxidative stress biomarkers using malondialdehyde (MDA) and superoxide dismutase (SOD) in aluminum workers exposed to occupational hazards.*
***Research Method:** The participants were recruited from an industrial area in Onitsha and divided into two groups: an exposure group composed of workers occupationally exposed to aluminum and a control group without exposure. The method of Gutteridge and Wilkins, (1982) was used for the estimation of malondialdehyde levels. SOD activity was measured according to the method as described by Misra and Fridovich, 1972. Data were analyzed using SPSS version 25 (IBM, USA, 2018). Independent T-test, descriptive, statistics, and Spearman correlation were used to analyze the data.*

***Results**: The results showed a significant increase in MDA in the aluminum workers group (3.53±1.04) as compared to the control group (1.65±0.58) ($p<0.005$). The SOD levels were reduced in the aluminum workers (21.55±3.85) as compared with the control samples (24.12±3.54) ($p<0.005$), indicating an elevation of oxidative stress. The study results revealed a significant positive correlation between MDA levels, age and duration of exposure. The SOD levels revealed a non-significant negative correlation with age and a significant correlation with duration of exposure.*

***Conclusion:** The findings indicate that long-term occupational exposure to aluminum can lead to increased oxidative stress, as indicated by higher MDA levels and lower SOD levels*

Keywords: *MDA, SOD, Oxidative Stress*

INTRODUCTION

Oxidative Stress is defined as an imbalance between oxidants and antioxidants in favor of the oxidants, leading to a disruption of redox signaling and control and/or molecular damage. (Sies 2020). Reactive oxygen and nitrogen species (ROS/RNS) produced under oxidative stress are known to damage all cellular biomolecules (lipids, sugars, proteins, and polynucleotides) (Zakorvick, 2020). Research has revealed a widespread involvement of oxidative stress in a number of disease processes, including cancer, cardiovascular disease (CVD), atherosclerosis, diabetes, arthritis, neurodegenerative disorders, and pulmonary, renal, and hepatic diseases (Granger and Kvietys, 2015). Thus, oxidative stress markers are important tools to assess the biological redox status, disease state and progression, and the health-enhancing effects of antioxidants in humans (Ghezzi, 2020).

Malondialdehyde (MDA) is a three carbon, low molecular weight aldehyde that can be produced from free radical attack on polysaturated fatty acids of biological membranes and used for monitoring lipid peroxidation in biological samples (Burg *et al*., 2010). Exposure to mixed chemicals increases lipid peroxidation due to free radical generation and causes antioxidant enzymes depletion, and alterations in antioxidant defense system.
Superoxide dismutase (SOD) is an enzyme that alternately catalyzes the dismutation (or partitioning) of the superoxide (O_2^-) radical into either ordinary molecular oxygen (O_2) or hydrogen peroxide (H_2O_2). Superoxide dismutases (SODs) are a group of metalloenzymes that are found in all kingdoms of life. SODs form the front line of defense against reactive oxygen species (ROS)-mediated injury. (Kangralkar, Paril and Bandivadar, 2010). Superoxide ion is produced as a by-product of oxygen metabolism and, if not regulated, causes many types of cell damage. Hydrogen peroxide is also damaging, but less so and is degraded by enzymes such as catalase. Thus, SOD is an important antioxidant defense in nearly all living cells exposed to oxygen.

Aluminum workers are exposed to high levels of aluminum and other toxic substances in their work environments, which can lead to oxidative stress and negatively impact their immune systems. According to the Agency for Toxic Substances and Disease Registry (2008), exposure to high levels of aluminum may result in respiratory and neurological problems. Also, Aluminum exposure has been associated with respiratory issues, such as lung fibrosis and reduced lung function (Zhou *et al*. 2021). Prolonged exposure to high levels of aluminum has also been associated with bone disorders, kidney damage, hormonal imbalances and cancers (McClure *et al*. 2020). The purpose of this study was to investigate the relationship between occupational exposure to aluminum and the levels of oxidative stress biomarkers in these workers. Presently there is no known report on the oxidative stress biomarkers in occupationally exposed aluminum workers in Onitsha metropolis. Hence, by measuring the levels of MDA, SOD, the study is aimed to determine the extent to which aluminum exposure affects oxidative stress in this population.

MATERIALS AND METHODS

Study Design and Participant Recruitment

This is a comparative study of oxidative stress biomarker malondialdehyde (MDA), superoxide dismutase (SOD), between occupationally exposed aluminium workers and unexposed subjects. A total of 72 male participants (test and control groups) aged between 20 and 40 years working and living in Onitsha were randomly selected at Oguta road, Anambra state.Test subjects comprise of occupationally exposed male aluminum workers with employment duration of 3 years and above. Control subjects were healthy male individuals with no occupational exposure and no hobby involving aluminum work or welding. The unexposed participants have no previous demographic and medical history of incidence of cancer. Informed consent was obtained from all individuals after being educated on the benefit of the study and completing of a structured questionnaire. Venous blood sample of 5ml was collected from test subjects and control subjects using standard phlebotomy procedures into plain tubes. The samples were allowed to clot and retract. Centrifugation done at 3000 r.p.m for 10min. Serum was extracted and stored at minus 20°C. SOD activity was measured according to the method as described by Misra and Fridovich, 1972. The method of Gutteridge and Wilkins, (1982) was used for the estimation of malondealdehyde levels.

Inclusion Criteria

Test subjects comprise of occupationally exposed male aluminum workers with employment duration of 3 years and above. Control subjects were healthy male individuals with no occupational exposure and no hobby involving aluminum work or welding. The unexposed participants have no previous demographic and medical history of incidence of cancer.

Exclusion Criteria

Aluminum workers who are not exposed were not considered suitable for the study. Subject with history of any form of cancer, tobacco smoking, chemotherapy, HIV infection, drug usage and alcoholism were excluded from the study.

Ethical Approval

Ethical approval was obtained from Ethics Committee of Nnamdi Azikiwe University Teaching Hospital NAUTH, Nnewi, Anambra State.

Statistical Analysis

Data were analyzed using SPSS version 25 (IBM, USA, 2018). Independent T-test, descriptive, statistics, and Spearman correlation were used to analyze the data obtained and values were presented using mean and standard deviation. Values to be considered significant were set at $p<0.05$.

RESULTS

Table 1 presents the levels of malondialdehyde, superoxide dismutase, in occupationally exposed aluminum workers and non-aluminium workers. This result shows that oxidative stress biomarker MDA level was significantly elevated ($p<0.05$) in the test group (3.53±1.04) when compared with the control (1.65±0.58), while the SOD levels was significantly decreased ($p< 0.05$) in the test group (21.55±3.85) when compared with the control group (24.12±3.54).

Table 2 presents the correlation between age and serum levels of MDA, SOD, in non- aluminum workers. The results shows that MDA had a non-significant correlation with age($p>0.05$) ,SOD had a non-significant correlation with age ($p>0.05$)
Table 3 presents the correlation between age and duration of exposure respectively with serum levels of MDA, SOD, respectively in aluminum workers. This result reveals a significant positive correlation between MDA levels and age and duration of exposure ($p<0.05$). The SOD levels revealed a weak non-significant correlation with age ($p>0.05$) and a significant correlation with duration of exposure ($p<0.05$).

Table 1: Levels of malondialdehyde, superoxide dismutase, in occupationally exposed aluminium **workers and non-**aluminium **workers**

Variables	Control group	Test group	P-value	T-value
Malondialdehyde level (nmol/L)	1.65±0.58	3.53±1.04	0.000**	9.444
Superoxide Dismutase (U/ml)	24.12±3.54	21.55±3.85	0.005*	-2.952

Table 2: correlation between age and serum levels of MDA, SOD, in non- aluminum **workers**

		Age (years)
Malondialdehyde level (nmol/L)	Correlation Coefficient	0.357
	Sig. (2-tailed)	0.122
	N	36
Superoxide Dismutase (U/ml)	Correlation Coefficient	-0.243
	Sig. (2-tailed)	0.301
	N	36

Table 3: correlation between age and serum levels of MDA, SOD, in aluminum **workers**

		Age (Years)	Duration of exposure
Malondialdehyde level (nmol/L)	Correlation Coefficient	0.666**	0.476**
	Sig. (2-tailed)	0.000	0.003
	N	36	36
Superoxide Dismutase (U/ml)	Correlation Coefficient	-0.298	-0.356*
	Sig. (2-tailed)	0.077	0.033
	N	36	36

Discussion

Malondialdehyde (MDA) is a compound that is derived from the peroxidation of polyunsaturated fatty acids (Cordiano *et al*., 2023). Malondealdehyde has been used as biomarker to measure lipid approximation in blood samples and occurs when reactive oxygen species (ROS) attack lipids in cell membranes, leading to their degradation(Cordiano *et al*., 2023). The results in this showed an increase in MDA levels in the test group (3.53±1.04) as compared to the control group (1.65±0.58) ($p<0.05$), this agrees with a report that an increase in free radicals causes overproduction of MDA (Gaweł *et al*., 2004). This result of elevated MDA levels indicates a presence of oxidative stress in subjects as a result of exposure to the aluminium toxic waste. Aluminium exposure enhance free radicals and change antioxidant capacity of the enzymes and cause lipid approximation (Rahimzadeh *et al*.,2022). Elevated lipid peroxidation can overwhelm the antioxidant defence system and trigger cell apoptosis and pathological processes leading to elevated serum MDA levels that reflect increased free radical production (Bergin et al., 2021). In summary, aluminum exposure can induce oxidative stress in the body, resulting in the generation of ROS, which in turn can cause lipid peroxidation and an increase in MDA levels. This increase

in MDA levels is indicative of oxidative damage to cell membranes and tissues caused by aluminum exposure (Marnett, 2002).

Also in this study, SOD levels in the test group was reduced as compared to the control group (21.55±3.85 vs 24.12±3.54; $p<0.05$). This agrees with a study by Yokel and McNamara (2001), aluminium-exposed rats exhibited reduced SOD activity compared to the control group. Aluminium toxicity has also been associated with a decrease in SOD activity, which is an important antioxidant enzyme that defends against oxidative stress. The findings is also in line with a study by Bimla *et al.*, (2005), where aluminium exposure resulted in a significant decrease in superoxide dismutase and catalase activity in both regions of the brain of developing and developed rat brain.SOD regulates oxidative stress, lipid metabolism, inflammation, and oxidation in cells. It can prevent lipid peroxidation, the oxidation of low-density lipoprotein in macrophages, lipid droplets' formation, and the adhesion of inflammatory cells into endothelial monolayers (Islam *et al*.,2022). This decrease in SOD levels coupled with the increase in MDA levels already observed in the test group, strongly indicates oxidative stress in the test group. In summary, aluminum exposure has been linked to oxidative stress in the body, and SOD is one of the key antioxidant enzymes that helps to counteract oxidative damage. When the body is exposed to aluminum, it can lead to an imbalance between reactive oxygen species (ROS) production and antioxidant defense mechanisms, resulting in decreased SOD activity or levels and impaired SOD expression in humans has been associated with several chronic diseases (Koltuksuz *et al*., 2000).

Also, the study showed correlation between age, duration of exposure on serum levels of MDA and SOD. This seeks to understand if older individuals or those with longer exposure durations tend to have higher levels of MDA and lower levels of SOD. The study results revealed a significant positive correlation between MDA levels, age and duration of exposure. This means that as age and duration of exposure increase, MDA levels also increase. MDA is formed as a byproduct of lipid peroxidation, which occurs when cell membranes are exposed to oxidative stress. Prolonged exposure to various factors such as environmental pollutants such as aluminium can lead to increased oxidative stress and subsequent lipid peroxidation (Rahal *et al*., 2014).As a result, MDA levels tend to rise with longer durations of exposure to these oxidative stressors (Rahal *et al*., 2014). Aging is associated with a decline in antioxidant defenses and an increase in oxidative stress. As individuals age, their bodies may become less efficient at neutralizing free radicals, leading to higher levels of oxidative damage and MDA production which accounts for the results in this study.

The SOD levels revealed a non-significant negative correlation with age and a significant correlation with duration of exposure. The negative correlation implies that as age increases, the levels of SOD tend to decrease slightly, and vice versa. However, because the correlation is not significant, it means that this observed relationship is not strong enough to confidently conclude that changes in age directly influence SOD levels, or vice versa. Also, SOD had a significant correlation with duration of exposure which implies that as the duration of exposure increases, the levels of SOD tend to decrease. In other words, longer exposure durations are associated with lower SOD levels. This finding suggests that prolonged exposure to the factor or substance may lead to a reduction in the body's antioxidant defense mechanisms, as reflected by lower SOD levels. It implies that extended exposure may impair the body's ability to neutralize harmful free radicals and mitigate oxidative stress, potentially increasing the risk of oxidative damage-related health issues. This agrees with the study by Yuan *et al.,* (2012), whose findings revealed that the activities of SOD decreased with increasing duration of lipid peroxidation.

Conclusion

The findings indicated that the test group exhibited an elevated level of MDA, along with a decrease in SOD levels when compared to the control groups. To summarize, prolonged exposure to aluminium can result in oxidative stress.

References

[1]. Burg A., Silberstein T., Yardeni G., Tavor D., Blumenfeld J., Zilbermann I. and Saphier O. (2010). Role of radicals in the lipid peroxidation products of commercial infant milk formula. *Jornal of agriculture and food chemistry.* **58**:2347–2350.

[2].Cordiano, R., Di Gioacchino, M., Mangifesta, R., Panzera, C., Gangemi, S. and Minciullo, P.L. (2023). Malondialdehyde as a Potential Oxidative Stress Marker for Allergy-Oriented Diseases: An Update. *Molecules*, 28, 5979.

[3]. Gaweł, S., Wardas, M., Niedworok, E., and Wardas, P. (2004). Dialdehyd malonowy (MDA) jako wskaźnik procesów peroksydacji lipidów w organizmie [Malondialdehyde (MDA) as a lipid peroxidation marker]. *Wiadomosci lekarskie* . 57(9-10), 453–455.

[4]. Ghezzi, P. (2020). Environmental risk factors and their footprints in vivo—A proposal for the classification of oxidative stress biomarkers. *Redox Biology*, 34, 101442

[5]. Granger, D. N. and Kvietys, P. R. (2015). Reperfusion injury and reactive oxygen species: The evolution of a concept. *Redox biology*. **6**: 524-551.

[6]. Islam, M. N., Rauf, A., Fahad, F. I., Emran, T. B., Mitra, S., Olatunde, A., Shariati, M. A., Rebezov, M., Rengasamy, K. R. R., and Mubarak, M. S. (2022). Superoxide dismutase: An updated review on its health benefits and industrial applications. *Critical Reviews in Food Science and Nutrition*, 62(26), 7282-7300.

[7]. Kangralkar, V.A., Patil, S.D. and Bandivadekar, R.M. (2010). Oxidative stress and diabetes: A review. international journal of applied pharmaceutical sciences and research. **1**:38–45

[8]. Koltuksuz, U., Uz, E., Ozen, S., Aydinç, M., Karaman, A. and Akyol, Ö. (2000). Plasma superoxide dismutase activity and malondialdehyde level correlate with the extent of acute appendicitis. Journal of pediatric surgery international. **16**(8): 559–561.

[9]. Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S. and Dhama, K. (2014). Oxidative stress, prooxidants, and antioxidants: the interplay. *BioMed research international*. **2014**: 761-764.

[10]. Sies, H. (2020). Oxidative stress: a concept in redox biology and medicine. *Redox biology*. **4**: 180-183.

[11]. Zarkovic, N. (2020). Roles and Functions of ROS and RNS in Cellular Physiology and Pathology. *Cells*. 9(3):767.

[12]. Bergin, P., Leggett, A., Cardwell, C.R., Woodside, J.V., Thakkinstian, A., Maxwell, A.P. and McKay, G.J. (2021).The effects of vitamin E supplementation on malondialdehyde as a biomarker of oxidative stress in haemodialysis patients: A systematic review and meta-analysis. *BMC Nephrology.* **22**:126-130.

[13].Misra, H.P. and Fridovich I.(1972). The Role of Superoxide Anion in the Autoxidation of Epinephrine and a Simple Assay for Superoxide Dismutase. *Journal of Biological Chemistry.* **247**:31-70.

[14]. Gutteridge, J.M.C. and Wilkins, S.(1982).Copper-dependent hydroxyl radical damage to ascorbic acid: formation of a thiobarhuric acid reactive product. *Federation of European Biomedical Societies Letters.* **137** (2): 327-330

[15]. Marnett L. J. (2002). Oxy radicals, lipid peroxidation and DNA damage. Toxicology, 181-182, 219–222. https://doi.org/10.1016/s0300-483x(02)00448-1

[16]. Yokel, R. A., & McNamara, P. J. (2001). Aluminum toxicokinetics: an updated minireview. Pharmacology & Toxicology, 88(4), 159–167.

[17]. Rahimzadeh, M. R., Rahimzadeh, M. R., Kazemi, S., Amiri, R. J., Pirzadeh, M., & Moghadamnia, A. A. (2022). Aluminum poisoning with emphasis on its mechanism and treatment of intoxication. Emergency Medicine International, 2022, 13.

[18]. Bimla, N., & Priya, A. (2005). Oxidative damage following chronic aluminium exposure in adult and pup rat brains. Journal of Trace Elements in Medicine and Biology, 19, 203–208.

[19]. Yuan, C. Y., Lee, Y. J., & Hsu, G. S. W. (2012). Aluminum overload increases oxidative stress in four functional brain areas of neonatal rats. Journal of Biomedical Sciences, 19, 51.

[20]. Zhou, M., Yang, S., Wang, X., Zhang, X., Cen, X., Mu, G., Wang, D., Ma, J., Wang, B., & Chen, W. (2021). The association between urinary aluminum and lung function among an urban adult population: a repeated-measure longitudinal study. Chemosphere, 270, 129443.

[21]. McClure, E. S., Vasudevan, P., DeBono, N., Robinson, W. R., Marshall, S. W., & Richardson, D. (2020). Cancer and noncancer mortality among aluminum smelting workers in Badin, North Carolina. American Journal of Industrial Medicine, 63(9), 755–765.