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Analyzing Heavy Metal Levels and Biochemical Indicators in Workers at Local Electrical Generators in Karbala Province of Iraq

Rana Abbas, Hassan Jameel*, Ibtisam Abbas
Department of Biology, College of Science, Kerbala University, Kerbala, IRAQ

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Abstract
Biological materials, such as human blood serum, hair, and nails, are commonly utilized as indicators to assess environmental contamination by heavy metals. The World Health Organization has recommended these biological samples for global ecological monitoring. Concentrations of heavy elemental lead (Pb), cadmium (Cd), and zinc (Zn), as well as oxidative stress markers 8-OHDG, malondialdehyde (MDA), and total antioxidant capacity (T-AOC) were measured in the serum sample. Fingernails and scalp hair were also collected from electrical generator workers of varying ages residing in the Karbala province of Iraq. Atomic absorption spectrophotometers were used to measure the amounts of Cd, Pb, and Zn. Also, 8-OHdG, MDA, and T-AOC were determined using the ELISA method. Data analysis showed significant differences in the average Cd, Zn, and Pb levels in electrical generator workers' serum, hair, and nails compared to the control groups. The Cd concentration in the worker's serum measured 4.428 mg/l, while the standard group's level was 2.590 mg/l. The Cd levels in the worker's hair were 2.517mg/l. However, the amount in the control group was 0.880 mg/l. The concentration of Cd in the nails of the worker was 1.155 mg/l, and the level for the control group was 0.378 mg/l. The Zn levels for the worker were as follows: 0.849mg/l in the serum, 54.97mg/l in the hair, and 186.6mg/l in the nail. In comparison, the Zn concentrations for the control group were 0.511 mg/l in the serum, 29.12 mg/l in the hair, and 1.209 mg/L in the nail. The results showed a significant rise in the Pb, Cd, and Zn levels in serum, hair, and nails of the workers exposed to the generator's diesel exhaust fumes in contrast to the control group, highlighting the importance of our study in raising awareness about the consequences of heavy metal pollution for the environment and human health.

Keywords: Serum, hair, nails, heavy metals, 8-OHDG, malondialdehyde, and total antioxidant capacity.

تحليل تركيز المعادن الثقيلة بعض المؤشرات الكيموحيوية للعاملين في المولدات الكهربائية في محافظة كربلاء في العراق

رنا عباس ، حسن جميل* ، ابتسام عباس
قسم علوم الحياة, كلية العلوم, جامعة كربلاء ,كربلاء , العراق

*Email: rana.a@s.uokerbala.edu.iq

الخلاصة

تم استخدم مصل الدم و الشعر و الاظافر كعينات لتحديد التلوث البيئي بالمعادن الثقيلة. اوصت منظمة الصحة العالمية باستخدام هذه العينات البيولوجية لرصد التلوث البيئي العالمي. تم قياس تركيز المعادن الثقيلة مثل الرصاص و الزنك و الكادميوم باستخدام جهاز المطياف الذري, كذلك تم قياس مؤشرات الاجهاد التأكسدي 8-OHdG و المالونديالديهايد MDA و السعة الكلية لمضادات الاكسدة T-AOC باستخدام طريقة(ELISA) المقايسة الامتصاصية المناعية للإنزيم المرتبط في مصل دم العاملين في المولدات الكهربائية و الغير العاملين. تم جمع عينات الشعر و الاظافر من العاملين و الغير العاملين في المولدات الكهربائية من مختلف الاعمار في محافظة كربلاء في العراق. أظهرت نتائج التحليل اختلافات كبيرة في مستويات الكادميوم و الرصاص و الزنك في مصل الدم و الشعر و الاظافر العاملين وغير العاملين في المولدات الكهربائية. بلغ تركيز الكادميوم في مصل العاملين في المولدات الكهربائية 4.428 ملغ/لتر، بينما كان مستوى مجموعة التحكم 2.590 ملغ/لتر. كانت مستويات الكادميوم في شعر العاملين 2.517 ملغ/لتر، بينما كانت مستويات مجموعة التحكم 0.880 ملغ/لتر. تركيز الكادميوم في أظافر العاملين كان 1.155 ملغ/لتر، بينما كان مستوى المجموعة التحكم 0.378 ملغ/لتر. كان مستوي الزنك في العاملين كما يلي: 0.849 ملغ/لتر في المصل، 54.97 ملغ/لتر في الشعر، و186.6 ملغ/لتر في الاظافر .بالمقابل، كان تركيز الزنك لمجموعة التحكم 0.511 ملغ/لتر في المصل، 29.12 ملغ/لتر في الشعر، و1.209 ملغ/لتر في الاظافر .كان تركيز الرصاص في مصل دم العمال أعلى بكثير، حيث بلغ 63.83 ملغ/لتر، مقارنة بـ 27.72 ملغ/لتر في مجموعة التحكم. كذلك أظهرت النتائج بان مستويات الرصاص عالية في شعر العاملين، حيث كانت 3.880 ملغ/لتر، بينما كانت 1.209 ملغ/لتر في مجموعة التحكم .وبالمثل، كانت تركيز الرصاص في أظافر العاملين 1.666 ملغ/لتر، بينما لوحظت 0.480 ملغ/لتر مجموعة التحكم. أظهرت النتائج ارتفاعاً كبيراً في مستويات الرصاص و الزنك و الكادميوم في مصل الدم و الشعر و الاظافر لدى العاملين في المولدات الكهربائية المعرضين لدخان عوادم الديزل مقارنة بالمجموعة الغير عاملة, مما يظهر أهمية هذه الدراسة في زيادة الوعي حول تأثير التلوث بالمعادن الثقيلة على البيئة و صحة الانسان.

1. Introduction

The growing issue of environmental pollution from heavy metals has become a significant fear due to its harmful impacts. While some heavy metals are essential, such as zinc, non-essential ones, like cadmium and lead, can have adverse health effects, including increased susceptibility to infection, autoimmune diseases, and cancer, even in small amounts[1, 2].

One of the most hazardous and poisonous metals found in the crust of the earth is lead. Lead causes oxidative stress, which damages DNA, antioxidant defense mechanisms, and cellular membranes. It can impact different body areas, as well as the liver, brain, blood arteries, lungs, and tests. It's important to note that lead-based neurotoxicity can result from not only acute exposure to elevated levels of lead [3]. Cadmium exposure can occur through consuming contaminated food or water, inhaling polluted dust (particularly for workers in primary metal industries or cadmium-polluted areas), or smoking cigarettes[4, 5]. Since this metal cannot penetrate the skin, dermal exposure is not considered a health issue[6]. Cadmium disrupts cellular processes by impairing mitochondrial function, inhibiting antioxidant enzymes, and potentially interacting with DNA. This reduces energy production, oxidative damage, and impaired nucleic acid synthesis[7].

Previous research has shown that electrical generators negatively affect the surrounding environment and people, as evidenced by elevated blood parameters and liver enzyme levels in generator workers. Furthermore, the observed increase in specific heavy metal concentrations beyond normal thresholds underscores the need for immediate and expert intervention to address these changes and prevent undesirable consequences for human health [8]. Most living tissues have elevated levels of heavy metals due to increased quantities in their surroundings. Continuous exposure to many toxic and harmful vapours from diesel power generators puts workers at risk for various health issues [9]. Consequently, the overall goal of this study was to assess the possible health along with environmental hazards associated with occupational exposure to heavy metals among electrical generator workers due to their link with heavy metal contaminants in the workplace.

2. Materials and Methods

2.1 Study Population

Biological samples (Blood, nails, and hair) were collected from 50 electrical generator workers and 20 control participants. Additionally, a sample of the ash from the electrical generator was collected. The worker group was between 14 and 67 years old, while the control group was between 14 and 68. The control group was selected randomly regardless of their work.

2.2. Sample Collections

Ten ml of blood was drawn from each individual and placed into a gel collection tube. The tube was then transported to the laboratory and centrifuged for ten minutes at 3,000 rpm. The serum was then collected using a micropipette and was stored at -20°C in a deep freezer until the tests were performed.

2.3. Collecting and preparing sample materials

Hair samples were collected using sterilized scissors washed with ethanol. Nails were trimmed with clean, sterile scissors for sample collection. All the samples were placed in plastic bags before analysis. Hair and nail samples were collected from all individuals, giving 72 samples for the study. After the samples were digested with a mixture of acids, the concentrations of the selected metals were established using atomic absorption spectrophotometry [10, 11].

Each hair sample weighed three grams, which were then put into a pristine crucible. The samples were oven-dried partially to avoid an explosion. A concentrated solution of 6:1 perchloric acid and nitric acid, measuring 10 milliliters, was utilized to digest the dried hair samples. The mixture was heated until it evaporated, resulting in a water-clear solution. After digestion, each sample was transferred to a 100 mL volumetric flask and diluted with the necessary amount of distilled water [12].

A one-gram sample of dry nail material was incinerated for four hours at a temperature of 550 degrees Celsius in a furnace. The ashes were then dissolved in 10 ml of a solution to avoid excessive foaming, and a strong solution of nitric and perchloric acid was left overnight at room temperature. The samples were then heated to a temperature range of 160–180 °C until the mixture was reduced to 1 ml and clear. Finally, a solution of 0.1 N nitric acid was added to each sample solution and filled to a 50 ml volume using distilled water, following the procedure described by Abdul-Rahman [12].

2.4. Ethical Issues

The study was approved by the Department of Biology and the Scientific Committee of the Science College at Karbala University. The ethical approval reference number is 98 on 8/1/2024. All participants provided written consent after being fully informed about the objectives and potential benefits of participating in this research project.

2.5. Assessment of Serum 8-OHdG

Serum 8-OHdG levels were calculated by a human 8-hydroxydeoxyguanosine ELISA kit (Nanjing Pars Biochem; Cat No. PRS-01766hu) following the guidelines provided by the manufacturer.

2.6. Assessment of Serum Malondialdehyde

The level of serum malondialdehyde was measured by a human malondialdehyde kit from Nanjing Pars Biochem (Cat No. PRS-0099hu) following the guidelines provided by the manufacturer.

2.7. Assessment of the serum total antioxidant capacity

Total antioxidant capacity levels were measured by a human total antioxidant capacity kit from Nanjing Pars Biochem (Cat No. PRS- 01738hu) following the guidelines provided by the manufacturer.

2.8. Assessment of the cadmium, lead, and zinc

All samples were analyzed using an atomic absorption spectrophotometer to assess their amounts of Cd, Pb, and Zn.

2.9. Statistical Analysis

Graph Pad Prism software was utilized to analyze the data collected. Data were displayed as (mean ± SD). The t-test was applied for statistical analysis. Statistical significance was defined by p-values less than 0.05.

3. Results and Discussion

The study included 50 workers operating diesel-powered electrical generators and 22 healthy individuals as a control group in Karbala provinces. According to Table (1) below, the characteristics of the employees of major private electricity generators include height (172.50±1.21 cm), weight (81.64±2.63 Kg), body mass index (BMI) (26.86±0.94 Kg/m2), age (35.62±1.85 years), and mean length of employment (7.55±0.86 years).

Table 1: Demographic and background data of workers operating diesel-powered electrical generators and control group.

Characteristics	Control	Electrical generator workers
Age (years)	36 ± 3.52	35.62±1.85
Height (cm)	170.54±1.39	172.50±1.14
Weight (kg)	73.95±3.13	81.64±2.63
BMI (Kg/ m^2)	25.29±0.86	26.87±0.94
Duration of work (years)	-	7.55±0.86

Data are presented as Mean + Stander error.

Data analysis showed significant differences in the average Cd, Zn, and Pb levels in electrical generator workers' serum, hair, and nails compared to the control groups (Figures 1-3).

According to a prior study, the sample population's serum zinc levels were estimated to be between 60 and 120 µg/dl, with males having levels between 59 and 125 µg/dl and females having levels between 50 and 103 µg/dl. [13]. According to the Centers for Disease Control and Prevention (CDC), healthy individuals without exposure to lead had typical blood lead levels of 0.9µg/dl. According to the guidelines, acceptable Pb blood levels are <5µg/dl for adults and <3.5 µg/dl for children, while the critical/hazardous levels are ≥70 µg/dl for adults and ≥20 µg/dl for children. In healthy, unexposed individuals, the normal range of Cd blood levels is 0.1 to 4 µg/dl [14].

Data analysis showed significant differences in the average cadmium, zinc, and lead levels in electrical generator workers' serum, hair, and nails compared to the control groups. The cadmium concentration in the worker's serum measured 4.428 mg/L, while 2.590 mg/L was the amount in the control group. The cadmium levels in the hair of the worker were 2.517

mg/L, but 0.880 mg/L was the amount in the control group. The concentration of cadmium in the nails of the worker was 1.155 mg/L, and the level for the control group was 0.378 mg/L. The zinc levels for the worker were as follows: 0.849mg/L in the serum, 54.97mg/L in the hair, and 186.6mg/L in the nail. In comparison, the zinc concentrations for the control group were 0.511 mg/L in the serum, 29.12 mg/L in the hair, and 1.209 mg/L in the nail.

The blood serum of the workers had a substantially higher lead concentration, measuring 63.83mg/L, compared to 27.72mg/L in the control group. The worker group also exhibited greater lead levels in their hair, at 3.880m g/L, versus 1.209 mg/L in the control group. Similarly, the lead concentration in the nails of the workers was 1.666 mg/L, surpassing the 0.480 mg/L observed in the control group.

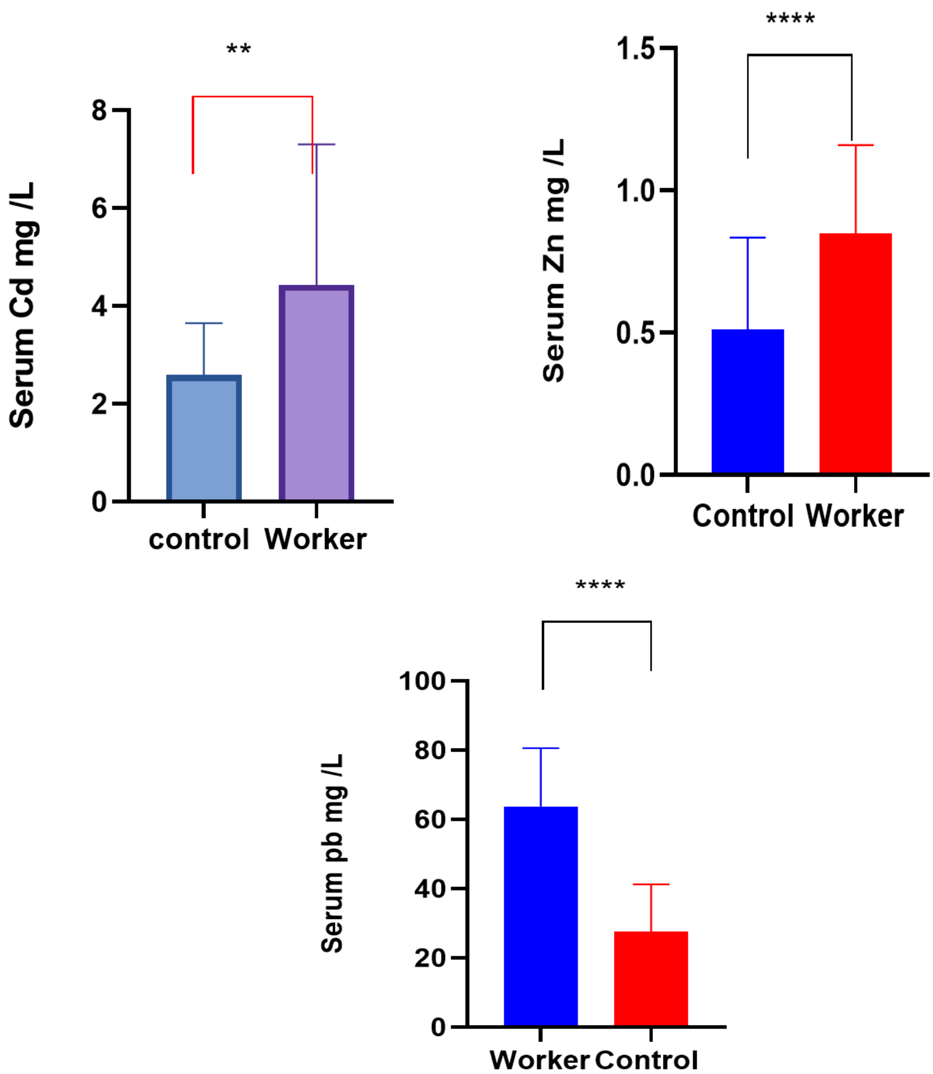

Figure 1: Comparison of serum Cd, Zn, and Pb levels between electrical generator workers and the control group. Data are presented as mean + standard division.

The statistical analysis demonstrated remarkable discrepancies in 8-OHDG concentrations in the workers' blood serum, which measured 29.92 mg/L, while the control group had a lower level.

Furthermore, the present investigation uncovered a substantial difference in serum malondialdehyde between the electric generator workers, which was 0.589 mg/L, and the control group, which was 0.639 mg/L. However, no notable difference existed in the serum levels of total antioxidant capacity among the studied group; the worker was 0.484 mg/L, and the control group was 0.508 mg/L.

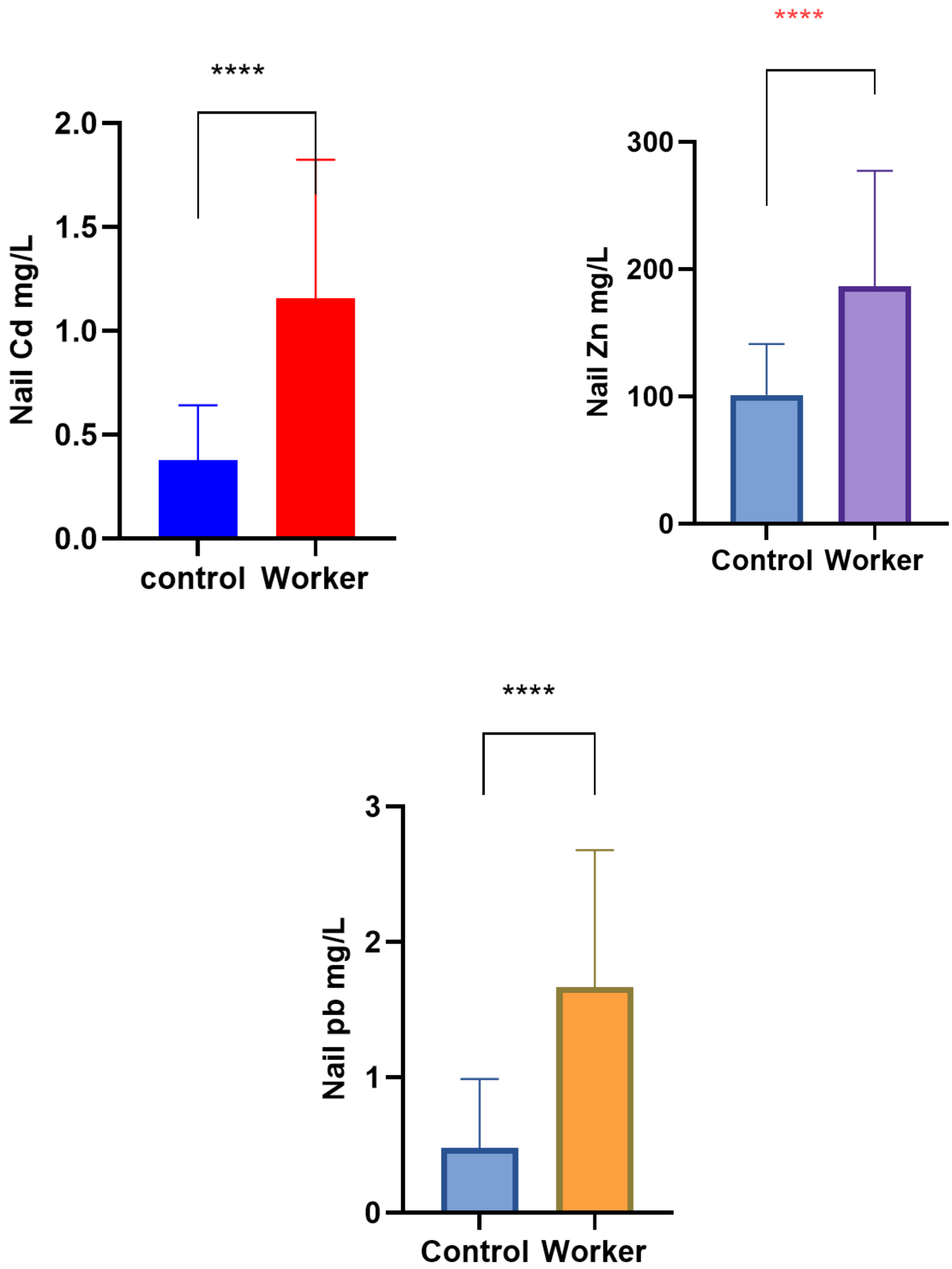

Figure 2: Comparison of Cd, Zn, and Pb levels in nails between electrical generator workers and the control group. Data are presented as mean + standard division.

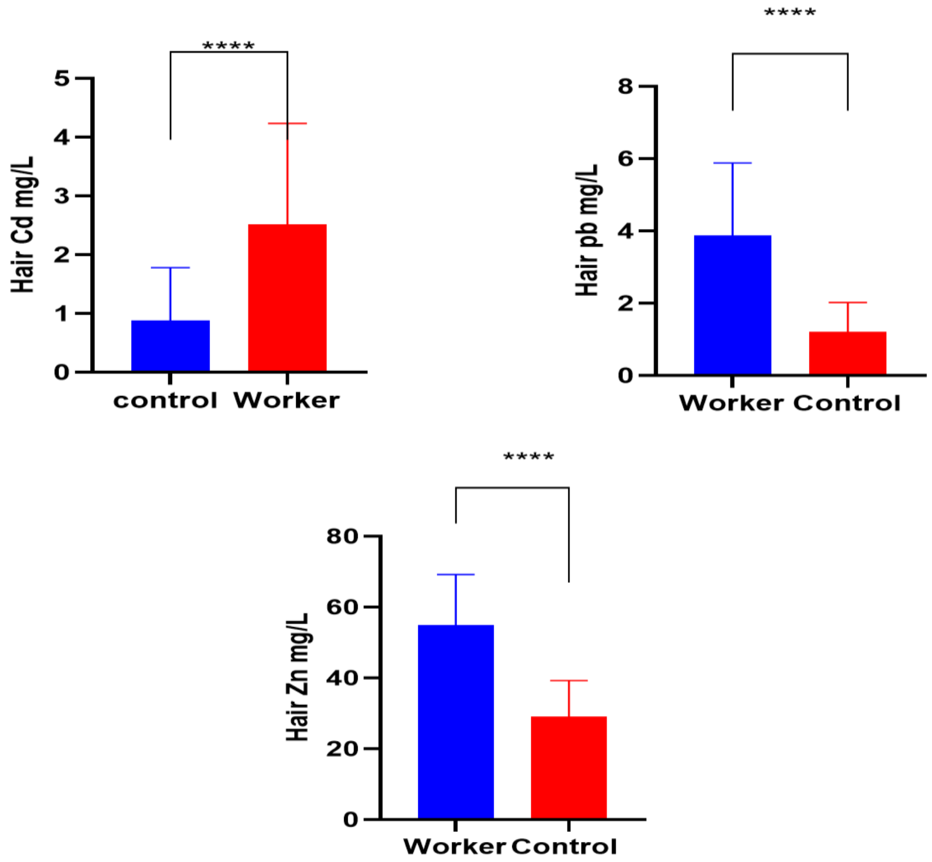

Figure 3: Comparison of Cd, Zn, and Pb levels in hair between electrical generator workers and the control group. Data are presented as mean ± standard division.

Studies have been undertaken to evaluate the blood serum concentrations of various substances, including cadmium, zinc, and lead, among workers exposed to electrical generators. Aligned with the current results, the previous results suggest that generator workers may exhibit heightened levels of specific metallic elements like copper, zinc, cadmium, and lead in their blood serum, which can have health ramifications [8, 15]. Hair and nails are other body samples used in screening for metal exposure. Earlier studies have shown that individuals working in occupationally exposed workers have higher concentrations of zinc and lead in their scalp hair than the general population[16]. While heavy metals can be harmful to humans even in small quantities, the level of toxicity typically depends on their concentration within the body. It has been noted that heavy metals can cause site-specific damage through their interactions with DNA and nuclear proteins. Either "direct" or "indirect" damage can happen. When a metal causes "direct" damage, biomolecules undergo structural changes. Conversely, "indirect" damage results from producing reactive oxygen and nitrogen species, including endogenous oxidants, such as hydrogen peroxide, nitric oxide, and superoxide and hydroxyl radicals. Additionally, there is evidence that heavy metals can activate signaling pathways. [17].

Free radicals, which can damage DNA, upset sulfhydryl equilibrium, and cause lipid peroxidation, are released when metal poisoning occurs. Damage to membranes also affects metal-mediated calcium homeostasis, triggering different calcium-dependent mechanisms involving endonucleases. Since cadmium is known to cause cancer, free radical production has been thoroughly investigated for iron, copper, nickel, chromium, and cadmium[17].

Chronic cadmium exposure can cause kidney damage, including proximal tubule degeneration and atrophy, as well as disturbances in protein, amino acid, glucose, and phosphate metabolism. It also disrupts lipid composition, enhances lipid peroxidation, depletes antioxidant enzymes, and alters the metabolism of essential minerals, leading to cardiotoxicity and testicular toxicity through interference with zinc-protein complexes and apoptosis [18].

Lead is also a harmful environmental pollutant that highly affects various bodily organs. The respiratory and digestive systems are the primary organs that absorb lead, although the skin can absorb it. Due to oxidative, inflammatory, and immunological system modification, lead exposure can result in neurological, pulmonary, urinary, and cardiovascular diseases.[19, 20]. In addition, lead exposure can trigger oxidative stress, altering the levels of antioxidant molecules like glutathione and its oxidized form and disrupting the activity of antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase. Lead has a great affinity for the reactive sulfhydryl groups in glutathione, leading to decreased glutathione levels. Furthermore, lead can impair the antioxidant functions of certain metalloprotein enzymes, including glutathione peroxidase, catalase, and superoxide dismutase, ultimately resulting in oxidative damage in various organs through lipid peroxidation and reduced antioxidant capacity [21, 22].

Data from the current study also found a significant difference in serum malondialdehyde between the electric generator workers and the control group. However, no notable differences existed in the serum levels of total antioxidant capacity among the studied groups (Figure 4). These results matched previous findings reporting that exposure to gases emitted from electric generators significantly increased malondialdehyde levels in diesel generator workers [23, 24]. The increased levels of oxidative stress in the serum of workers operating diesel generators indicate an upsurge in local free radical manufacture. This shows free radical activity in cells is a sign of oxidative stress.

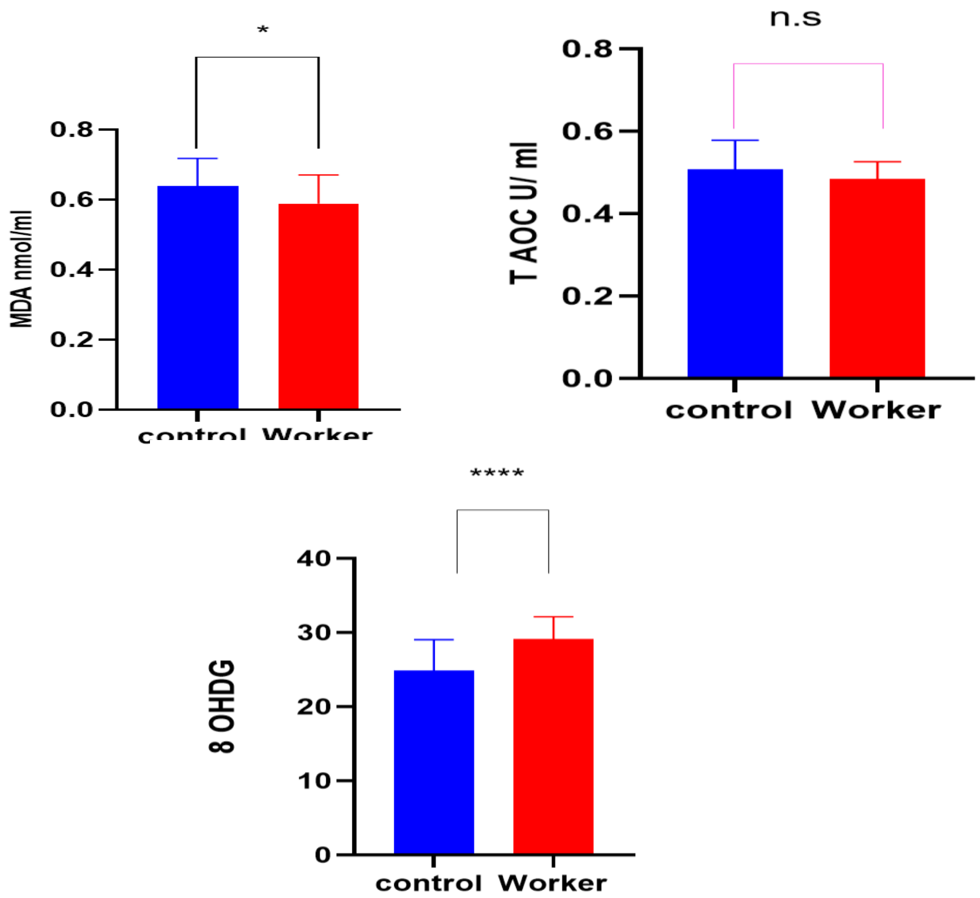

Figure 4: Comparison of serum MDA, T AOC, and 8-OHDG concentrations between electrical generator workers and the control group. Data are presented as mean + standard division.

Comparative statistical analysis between 8-hydroxydeoxyguanosine 8-OHDG levels in both the control and worker groups, 8-hydroxydeoxyguanosine 8OHDG, within the serum of the workers displayed considerable differences ($p < 0.0001$) (Figure 4). This phenomenon demonstrates that workers in the electric generator industry are at high risk due to exposure to hazardous metals. DNA damage from reactive oxygen species and DNA base modifications can alter genetic information. These types of damage, including Point mutations, chromosomal translocations, deletions, or insertions, may activate oncogenes or inactivate tumor suppressor genes, potentially initiating the carcinogenic process [25].
When carcinogenic agents enter the body and become activated, they are able to bind to DNA covalently, leading to DNA adduct formation. This exchange could potentially result in DNA strand breaks. Among the various photooxidation DNA outcomes, The 8-hydroxy-deoxyguanosine biomarker is persistent and sensitive, providing useful information for assessing the extent of DNA damage.[26]. According to Matsui[27], 8-OHdG played a vital part in the early stages of carcinogenesis. Oxidative DNA damage intervened by reactive oxygen species contributed to the development of certain diseases, including cancer[17].

4. Conclusion

The results showed that electrical generator workers demonstrated higher levels of certain heavy metals and increased serum concentrations of the oxidative stress markers 8-OHdG and malondialdehyde. These results suggest the need for expert involvement to address the health-related changes observed in this workforce to avoid adverse effects on human health.

Conflict of interest

The authors declare that they have no conflict of interest.

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