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Effects of Short-Term Formalin Exposure on Serum Magnesium and Zinc Levels among Students at Nnamdi Azikiwe University, Nigeria.

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ABSTRACT: Background: Formalin remains the conventional fixative for cadaver preservation in medical education and is extensively used during anatomical dissections. Despite its utility, formalin exposure poses potential health risks to medical students, particularly through mechanisms involving oxidative stress and disruption of essential mineral balance.

Objectives: This study assessed the effect of short-term (3-hour) formalin exposure on serum magnesium and zinc levels among medical students at Nnamdi Azikiwe University.

Methods: A cross-sectional study was conducted among 90 participants aged 18–35 years, comprising 45 formalin-exposed medical students from the College of Health Sciences, Nnewi, and 45 unexposed controls. Blood samples from exposed students were collected after a 3-hour cadaveric dissection, while control samples were obtained without formalin exposure. Serum magnesium and zinc were measured using Atomic Absorption Spectrophotometry, and blood pressure was assessed with a standard sphygmomanometer. Data were analysed using independent t-tests and Pearson's correlation, with statistical significance set at $p < 0.05$.

Results: Mean serum magnesium and zinc levels were significantly lower in the exposed group [Mg: 3.46 ± 1.21 ppm; Zn: 0.30 ± 0.11 ppm] compared with the control group [Mg: 6.31 ± 1.70 ppm; Zn: 0.64 ± 0.18 ppm] ($p < 0.05$). Although systolic blood pressure was higher among exposed students, the difference was not statistically significant ($p > 0.05$). No significant correlation was observed between serum magnesium levels and diastolic blood pressure.

Conclusion: The results showed decreased serum zinc and magnesium levels, which may imply that short-term formalin exposure disrupts mineral homeostasis and predisposes exposed students to micronutrient deficiencies.

Keywords: *Serum Magnesium, Serum Zinc, Atomic Absorption Spectrophotometry, Formaldehyde.*

INTRODUCTION

Formalin is an aqueous solution of formaldehyde, typically containing 37–40% formaldehyde stabilized with methanol to prevent polymerization (Aronson, 2016; Lee et al., 2023). Formaldehyde is a highly reactive, colourless gas with a pungent odour, widely manufactured as formalin for its potent bactericidal, fungicidal, virucidal, and preservative properties (McKeen, 2018; Zhang et al., 2020). Consequently, it is extensively used in medical and biological laboratories, particularly as a fixative for cadaver preservation during anatomical dissections. Human exposure occurs both exogenously, through occupational and environmental sources such as laboratories, building materials, cosmetics, and textiles, and endogenously via normal metabolic pathways, including amino acid metabolism and demethylation reactions (Kaden et al., 2010; Lee et al., 2023). Although endogenously produced formaldehyde is rapidly metabolized to formate and excreted, exogenous exposure at higher concentrations has been linked to mucosal irritation, cytotoxicity, oxidative stress, and carcinogenicity, leading to its classification as a human carcinogen (Dhar, 2020).

Magnesium and zinc are essential micronutrients critical for cellular metabolism, enzymatic activity, and physiological homeostasis. Magnesium, predominantly intracellular, functions as a cofactor for over 300 enzymes and is central to energy production, nucleic acid synthesis, neuromuscular function, cardiovascular regulation, and electrolyte balance (de Baaij et al., 2015; Fiorentini et al., 2021). Zinc is equally vital, serving as a structural or catalytic component of thousands of proteins involved in immune defense, oxidative stress regulation, DNA repair, and cell signaling (Maret, 2013; Chasapis et al., 2020). Deficiencies in either element are associated with adverse outcomes, including cardiovascular disorders, metabolic dysfunction, impaired immunity, neurological disturbances, and increased susceptibility to infections (Razzaque, 2018; Maywald and Rink, 2022). Maintaining adequate serum levels of these elements is therefore essential for systemic health.

Emerging evidence indicates that formaldehyde exposure may disrupt trace element homeostasis through oxidative stress, inflammation, altered protein binding, and interference with micronutrient metabolism (Tesfaye et al., 2020; Igharo et al., 2014). Sub-acute and occupational exposure has been associated with altered zinc and copper levels, impaired antioxidant defenses, and systemic metabolic effects, while experimental and epidemiological studies also suggest potential cardiovascular consequences, including elevated blood pressure (Ebojele and Iyawe, 2022; Wang et al., 2023). However, data on the impact of short-term formalin exposure on serum magnesium and zinc levels, particularly among medical students during cadaveric dissection, remain limited. This study therefore aimed to evaluate the effect of short-term formalin exposure on serum magnesium, serum zinc, and blood pressure among students at Nnamdi Azikiwe University.

MATERIALS AND METHOD

Study Site

The study was conducted among undergraduate students of the College of Health Sciences and Technology, Nnamdi Azikiwe University, where students in the early phases of medical training are routinely exposed to formalin during cadaveric dissection practicals.

Study Design and Population

This cross-sectional study involved 90 undergraduate students from Nnamdi Azikiwe University. The exposed group comprised 45 students (male and female) from the College of Health Sciences, Nnewi campus, who routinely encountered formalin during cadaveric practicals, while 45 students from the Faculty of Arts, Awka campus, served as the unexposed control group. The study assessed the effect of short-term (3-hour) formalin exposure on serum magnesium and zinc levels. Demographic data were collected using a structured questionnaire following informed consent. Five millilitres of venous blood were collected from each participant into plain containers, allowed to clot, and centrifuged to obtain serum, which was subsequently analysed for magnesium and zinc concentrations.

Inclusion Criteria

The study included undergraduate students from Nnamdi Azikiwe University, Nnewi campus, who consented to participate and were routinely exposed to formalin. The control group comprised students from the Faculty of Arts, Awka campus, with no prior formalin exposure who also provided consent. All participants were generally healthy and not on any prescribed or non-prescribed medications known to affect serum magnesium or zinc levels.

Sample Size Determination

The sample size was determined using G*Power software version 3.1.9.4 (Universität Düsseldorf, Germany). An analysis for the difference between two dependent means (matched pairs) was performed with an alpha of 0.05, a power of 0.95, and an effect size of 0.05. This yielded a minimum sample size of 45 per group, providing 96% power to detect a difference of 0.45 at a significance level of 0.05. Accordingly, a total of 90 participants were recruited for the study.

Ethical Approval

Ethical approval was obtained from the Ethics Committee of the Faculty of Medical Laboratory Science, Nnamdi Azikiwe University, in accordance with the Helsinki

Declaration of the World Medical Association for research involving human participants. Participant confidentiality was strictly maintained throughout the study.

Sample Collection

Venous blood (2 mL) was collected from each participant using a sterilized syringe and dispensed into plain, labelled containers indicating the participant's name, sample number, and date of collection. Samples were centrifuged to separate serum, which was then pipetted into cryovials and stored at –20 °C until analysis.

Analysis of Serum Magnesium and Zinc

Serum magnesium and zinc concentrations were determined using an Agilent FS240AA Atomic Absorption Spectrophotometer (AAS) following the APHA (1995) method. The AAS works by aspirating the sample into a flame, atomizing the elements, and measuring the light absorbed at the element-specific wavelength, which is proportional to its concentration.

Preparation of Standards

Daily reference solutions were prepared by diluting stock metal solutions in water containing 1.5 mL concentrated nitric acid per litre. Calibration blanks contained all reagents except the metal standards. Calibration curves were constructed by plotting absorbance against standard concentrations.

Sample Digestion

One millilitre of serum was mixed with 4 mL of distilled water, boiled at 37 °C for 20 minutes, and subsequently analysed using the FS240AA AAS.

Metals	Wavelength (nm)	Slit (nm)	Flame
Zinc	213.9	0.2	Air acetylene
Magnesium	285.2	0.2	Air acetylene

Stock Standard Solution

1000 mg/l, of stock metal solution was dissolved in a minimum volume of (1+1) $HNO3$. Diluted to 1 litre with 1% (v/v) HCL, appropriate dilution was carried out to produce 2, 4 and 6pm working solution

RESULTS

Table 4.1. Serum Magnesium and Zinc Levels (ppm) in Students Exposed and Unexposed to Short-Term (3 Hours) Formalin (Mean ± SD)

Male students exposed to formalin had significantly lower serum magnesium (3.07 ± 0.88 ppm) and zinc (0.30 ± 0.10 ppm) compared with their controls (Mg: 6.13 ± 1.46 ppm; Zn: 0.63 ± 0.19 ppm, $P < 0.05$). Similarly, exposed female students showed reduced magnesium (3.78 ± 1.36 ppm) and zinc (0.29 ± 0.12 ppm) levels relative to controls (Mg: 6.45 ± 1.88 ppm; Zn: 0.66 ± 0.17 ppm, $P < 0.05$). Overall, combined male and female participants exposed to formalin had significantly lower serum magnesium (3.46 ± 1.21 ppm) and zinc (0.30 ± 0.11 ppm) compared with the control group (Mg: 6.31 ± 1.70 ppm; Zn: 0.64 ± 0.18 ppm, $P < 0.05$).

Table 4.1. The values of Magnesium (ppm) and Zinc (ppm) of students exposed to short term (3 hours) formalin and those not exposed (Mean ± SD).

Variables	Test	Control	t-value	p-value
Mg (ppm) Males	3.07 ± 0.88	6.13 ± 1.46	-8.042	0.001
Zn (ppm) Males	0.30 ± 0.10	0.63 ± 0.19	-6.780	0.001
Mg (ppm) Females	3.78 ± 1.36	6.45 ± 1.88	-5.757	0.001
Zn (ppm) Females	0.29 ± 0.12	0.66 ± 0.17	-8.682	0.001
Mg (ppm) Both	3.46 ± 1.21	6.31 ± 1.70	-8.042	0.001
Zn (ppm) Both	0.30 ± 0.11	0.64 ± 0.18	-13.394	0.001

Sample sizes: Test – males: 20, females: 25, total: 45; Control – males: 20, females: 25, total: 45 Statistically significant at $p < 0.05$

Table 4.2. Mean values of body mass index (BMI), systolic and diastolic blood pressure of students exposed and unexposed to short-term (3 hours) formalin (Mean ± SD).

No significant differences were observed in BMI, systolic, or diastolic blood pressure between students exposed to short-term formalin (25.87 ± 4.84 kg/m²; 119.29 ± 7.60 mmHg; 82.97 ± 15.78 mmHg) and unexposed students (26.45 ± 5.49 kg/m²; 116.50 ± 9.10 mmHg; 76.44 ± 9.69 mmHg) ($p > 0.05$).

Table 4.2 The mean values of the systolic and diastolic blood pressure of students exposed to short term (3 hours) formalin and students not exposed (Mean ± SD)

Variables	Test N=45	Control N=45	T-value	P-value
BMI	25.87± 4.84	26.45 ± 5.49	-0.531	0.596
SBP	119.29 ± 7.60	116.5 ± 9.10	1.571	0.120
DBP	82.97 ± 15.78	76.44 ± 9.69	2.368	0.020

Sample sizes: Test – males: 20, females: 25, total: 45; Control – males: 20, females: 25, total: 45

Key:

BMI: Basal Metabolic Index

DBP: Diastolic Blood Pressure

SBP: Systolic Blood Pressure

Table 4.3. Correlation between serum zinc (ppm), serum magnesium (ppm), body mass index (BMI), systolic blood pressure (SBP), and diastolic blood pressure (DBP) in students exposed to short-term (3 hours) formalin.

No significant correlations were observed between serum magnesium and BMI (r = 0.08, p = 0.959), SBP (r = -0.129, p = 0.398), or DBP (r = -0.141, p = 0.354). Similarly, serum zinc showed no significant correlation with BMI (r = -0.059, p = 0.702), SBP (r = -0.058, p = 0.704), or DBP (r = -0.106, p = 0.487).

Table 4.3. Relationship between the levels of serum Zinc (ppm) and serum Magnesium (ppm), body mass index, systolic and diastolic blood pressure of the students exposed to short-term (3 hours) formalin.

Parameters	P/R	BMI (kg/m)	SBP (mmHg)	DBP (mmHg)
Magnesium (ppm)	R	0.08	-0.129	-0.141
	P	0.959	0.398	0.354
Zinc (ppm)	R	-0.059	-0.058	-0.106
	P	0.702	0.704	0.487

Sample sizes: Test – males: 20, females: 25, total: 45; Control – males: 20, females: 25, total: 45

Key:

BMI: Basal Metabolic Index

DBP: Diastolic Blood Pressure

SBP: Systolic Blood Pressure

DISCUSSION

Despite its widespread use, formalin is a known human carcinogen that causes respiratory and skin toxicity and may also disrupt magnesium and zinc homeostasis, impairing immune and antioxidant functions. In this study, mean serum magnesium and zinc levels showed significantly decrease in participants exposed to short-term formalin compared to unexposed students. Which is in tandem with Igharo et al. (2014), who reported decreases in Zn, Mg, Cu, Cr, and Fe, with a paradoxical rise in Se, in workers exposed to formaldehyde. Similarly, Boniamin et al. (2024) found significant reductions in serum magnesium and zinc among formaldehyde-induced hepatic and renal damaged subjects. Igharo et al. (2014) also reported a significant reduction in serum zinc (Zn), copper (Cu), chromium (Cr), and iron (Fe) levels among individuals occupationally exposed to formalin compared with non-exposed controls, indicating a markedly depressed micronutrient status in exposed subjects. The observed depletion of essential trace elements suggests that formaldehyde toxicity disrupts mineral homeostasis, potentially predisposing exposed individuals to adverse pathophysiological outcomes, including inflammation and carcinogenesis. Zinc, in particular, has received considerable attention in cancer research, with substantial evidence linking zinc deficiency to increased cancer risk in humans. Reduced zinc status has been documented in cancer patients relative to healthy individuals, and zinc is known to play critical roles in cellular metabolism and the regulation of malignant cell behavior. Previous studies have demonstrated decreased zinc content in leukemic leukocytes and shown that zinc deficiency enhances the carcinogenic effects of nitroso methylbenzylamine. Furthermore, zinc deficiency in humans has been associated with an increased risk of esophageal squamous cell carcinoma, underscoring the potential clinical relevance of formaldehyde-induced trace element depletion (Igharo et al., 2014).

Formaldehyde induces oxidative stress that consumes magnesium and zinc due to their antioxidant roles and may also impair intestinal absorption and metabolic regulation, resulting in reduced serum levels despite adequate dietary intake (Prasad, 2014; Gröber et al., 2015).

No statistically significant differences were observed in systolic blood pressure, diastolic blood pressure, or body mass index between participants exposed to short-term formalin and their unexposed counterparts. This suggests that brief exposure may not be sufficient to elicit measurable alterations in cardiovascular parameters or overall metabolic status, likely due to effective homeostatic and compensatory mechanisms that maintain blood pressure regulation and energy balance. Body mass index, in particular, is influenced by long-term dietary patterns, physical activity, and genetic factors (Hill, 2012), which are unlikely to be affected by acute chemical exposure. These findings are consistent with the report of Ebojele and Iyawe (2023), who similarly observed no significant changes in systolic blood pressure or BMI among medical students exposed to formaldehyde; however, their study documented a significant increase in diastolic blood pressure under conditions of chronic exposure, indicating that exposure duration may be a critical determinant of cardiovascular outcomes.

CONCLUSION

Short-term (3-hour) formalin exposure in undergraduate students significantly reduced serum magnesium and zinc levels, indicating that even brief exposure to formaldehyde can disrupt essential mineral homeostasis. Despite these biochemical changes, no significant effects were observed on blood pressure or body mass index, suggesting that short-term exposure does not acutely impact cardiovascular parameters or overall metabolic status. These findings highlight the importance of monitoring and minimizing formalin exposure in educational and laboratory settings to prevent potential long-term effects on mineral balance and related physiological functions. Additionally, ensuring adequate dietary intake and implementing protective measures, such as proper ventilation and personal protective equipment, may help mitigate the risk of trace element depletion associated with formaldehyde exposure.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest related to this study.

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Ethical Approval

Ethical approval for this study was obtained from the Ethics Committee of the Faculty of Medical Laboratory Science, Nnamdi Azikiwe University (Reference No.: FMLS/REC/025/074). The study was conducted in accordance with the ethical principles of the Declaration of Helsinki of the World Medical Association. All participants' information was handled with strict confidentiality throughout the study.