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Lipid Profile and Malondialdehyde Levels of Mortuary Attendants Exposed to Embalming Chemicals in Some Mortuaries in South-South, Nigeria

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ABSTRACT

Background: Human exposure to embalming fluid containing formaldehyde is associated with multiple negative effects, and its exposure is known to cause oxidative stress in some vital organs like the liver, kidney, and lungs. The drought of basic data on the effect of embalming fluids on biochemical parameters, as well as safety precautions in the use of these chemicals, underscores the need for more extensive studies on embalming chemicals and their related health effects. **Aim**: This study evaluated the effect of embalming chemicals on lipid profile parameters and malondialdehyde (MDA) levels of mortuary attendants in some selected mortuaries in Bayelsa State, Nigeria. **Method**: In this work, a total of twenty-five (25) mortuary attendants, within the age range of 30-50 years, are exposed to embalming chemicals for a period of two (2) to twenty (20) years. Also, twenty-five (25) apparently healthy unexposed individuals within the same age range were recruited as controls. Blood samples were collected from the subjects and estimation of plasma lipids and malondialdehyde (MDA) levels were done using the spectrophotometric method. **Result**: The results showed that total cholesterol (4.67**±**0. 56) and triglyceride (1.91**±**0.86) levels of the mortuary attendants were slightly higher than the control (4.51**±**0.37 and 1.78**±**0.48 respectively), but was not significant (P>0.05). High-Density Lipoprotein (1.32**±**0.306) showed a statistically significant ($p<0.05$) increase in the exposed subjects when compared with the control (0.96**±**0.88). However, there was a significant ($p<0.05$) decrease in the level of Low-Density Lipoprotein (2.25**±**0.89) of mortuary attendants compared with the unexposed subjects (3.45**±**0.99). The serum malondialdehyde (3.27±0.530) level was significantly ($p<0.05$) higher in the exposed workers than unexposed subjects (1.78±0.303). Furthermore, there was a statistically significant ($p<0.05$) elevation in malondialdehyde (3.78±0.604)among exposed subjects with >5years duration compared with <2years (1.84±0.42). **Conclusion**: This study confirms that exposure to embalming fluid alters serum lipids and increases lipid peroxidation product malondialdehyde (MDA) in humans. This elevation is based on the prolonged duration of exposure. Thus, the use of personnel protective equipment is recommended for morticians.

INTRODUCTION

Occupational exposure to toxic chemicals in workplaces portends a serious risk to human health [1]. Mortuary workers are exposed to toxic chemicals such as preservatives, buffers, anticoagulants, germicides, fungicides, perfuming agents, hygroscopic agents, and dyes present in embalming fluids which are known to cause several health complications [2]. Some of these complications include; respiratory disorders, genotoxicity, dermatitis, eye blindness, autoimmunity, ocular irritations, corneal clouding, leukemia, nasopharyngeal cancers, congenital malformations, and menstrual irregularities [3].

Embalming is the process of temporarily preserving human remains to prevent decomposition and prepare them for public display at funerals [4]. It involves the application of chemicals to a dead human body in other to reduce the presence and growth of the microorganisms, retard organic decomposition, and restore acceptable physical appearance [5]. Embalming chemicals are majorly composed of preservatives, sanitizing, and disinfection agents and additives used in modern embalming [6]. Some essential constituents in embalming chemicals are buffers, anticoagulants, germicides, fungicides, perfuming agents, hygroscopic agents, and additives [2]. The goals of modern-day embalming are disinfection, preservation, and restoration of the body. Embalming fluids or chemicals are administered via arterial and intramuscular injection or sprayed on the body. The general principle is that embalming fluids act to fix cellular proteins [2]. Formaldehyde fixes tissues or cells by irreversibly connecting a primary amine group in protein molecule with nearby nitrogen or DNA molecule through CH2 linkage called Schiff base [6]. During the 19th and 20th centuries, arsenic-based solutions were the first widely recognized and regularly used embalming fluid, although they have since been replaced by more effective and less toxic formaldehyde [7].

The embalming fluid of today typically contains a mixture of formaldehyde, methanol, ethanol, phenol glutaraldehyde, glycerine, oil of wintergreen, eosine solution, phenoxyethanol, and other solutions [8]. Of all the chemicals used in modern-day embalming, formaldehyde has been found to have the most important exposure concern. It is absorbed via the respiratory and gastrointestinal routes [3]. It has been reported that long-term exposure to formaldehyde at concentrations exceeding the national standard exerts a variety of toxic effects on the nasopharynx, lung, brain, and skin, and also on the hematopoietic organs bone marrow (BM) and

the spleen [8]. On the basis of epidemiological studies, the International Agency for Research on Cancer (IARC) classified formaldehyde as a human leukemogenic [8], while some studies on humans and animals showed that occupational exposure to formaldehyde can disrupt hematopoietic function and lead to hematopoietic toxicity [9, 10].

Lipids are organic substances relatively insoluble in water but soluble in organic solvents and are utilized by living cells. They are structural components in cells and are involved in metabolic and hormonal pathways [11]. Measurement of serum lipids levels has been the prime index of cardiovascular disease (CVD). However, several "atherogenic indices" such as TC/HDL-C and LDL-C/HDL-C ratios are considered risk indicators with greater predictive value than isolated parameters used independently, particularly LDL-C [12]. TG/HDL-C ratio also has been reported as a significant predictor of extensive coronary heart disease [13].

Malondialdehyde (MDA) is a final lipid peroxidation product and it is the most widely used indicator of the degree of the oxidation process in body fluids [14]. Lipid peroxidation is a well-established mechanism of cellular injury in humans and is used as an indicator of oxidative stress in cells and tissues. Lipid peroxides derived from polyunsaturated fatty acids are unstable and can be decomposed to form a complex series of compounds. These include reactive carbonyl compound, which is the most abundant malondialdehyde (MDA) [15]. The measurement of MDA is widely used as an indicator of lipid peroxidation and increased levels of the peroxidation products have been associated with a variety of acute, chronic pathophysiological processes in the human as well as animal models [16].

This study was conducted to evaluate the effects of embalming chemicals on plasma lipids and malondialdehyde levels of mortuary workers in some mortuaries in South-South, Nigeria.

METHODS

Study Population

A total of fifty (50) subjects were recruited for the study, which comprises twenty-five (25) mortuary attendants exposed to embalming chemicals and twenty-five (25) apparently healthy unexposed subjects who served as a control group. All the fifty (50) subjects used for the study were male with an age range of 20–50 years. Subjects who work in the morgue and consented to

the study without a known medical history of any metabolic disorder were included in the study. Subjects who do not consent to the study and with a medical history of known metabolic disorder were excluded from the study. Also excluded are chronic cigarette smokers and chronic alcohol drinkers. The ethical clearance was approved by the research ethical committee of Federal Medical Centre (FMC) Yenagoa, Niger Delta University Teaching Hospital Okolobiri (NDUTH), and other private mortuaries in South-South, Nigeria.

Blood Sample Collection

Seven (7) milliliters of blood sample was collected from each of the fifty (50) subjects after fulfilling the inclusion and exclusion criteria. Five (5) milliliters of the blood sample were dispensed into an Ethylene diamine tetraacetic acid (EDTA) sample tube. The remaining two (2) milliliters were dispensed into a plain dry glass tube. The blood samples were transported to the Research Laboratory of Medical Laboratory Science, Niger Delta University in a cool box containing ice bags. The samples were centrifuged at 3000rpm for 10minutes to obtain the clear serum and plasma. They were separated into separate plastic dry sample tubes and stored at -20^0C and analysis were done within 48hours of sample collection. Total cholesterol (TC), High-Density Lipoprotein (HDL), and triglyceride (TRIG) were measured using reagents from Randox Diagnostic kits as specified by Randox Diagnostics (Switzerland). Serum Lipid peroxide analysis was carried out by determining the concentration of MDA formed using the method of Varshney and Kale [17].

Analysis of Biochemical Parameters

Determination of lipid profile Parameters: Lipid profile parameters: total cholesterol (TC), High-Density Lipoprotein Cholesterol (HDL-C), and Triglycerides (TG) were estimated by spectrophotometric methods using Randox reagent kits. Plasma Total Cholesterol concentration was measured by the enzymatic method as described by Tinder [18] and modified by Richmond, [19], and high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) were determined enzymatically after precipitation of other lipoproteins as described by Burstein *et al*., [20] and Assmann*et al*., [21] respectively, using Randox reagent kits.

Determination of Malondialdehyde: Serum Malondialdehyde (MDA) was measured by the method of Shah and Walker's, [22] using an auto-analyzer spectrophotometer. In this reaction, malondialdehyde conjugates with thiobarbituric acid (TBA) reagent under acidic conditions to generate a pink-colored product, and the absorbance was determined at 532 nm. Briefly, 1.0ml reagent 1 (17.5% TCA), reagent 2 (70% TCA) and regent 3 (Thiobarbituric acid 0.6%) was added to 1.0ml of serum and mixed. The reaction mixture was incubated in a boiling bath for 15 minutes, allowed to cool, and then let to stand at room temperature for another 20 minutes. Then the tubes were centrifuged at 2000 rpm for 15 minutes and the supernatant layer was read at 534 nm. Distilled water was used for the blank. The concentration of MDA (nmol/ml) was calculated by using the following formula: Concentration of the test= Abs (test) –Abs (blank) / 1.56 x 1000000

DATA ANALYSIS

Results obtained from the biochemical estimations were analyzed with SPSS version 23.0. Data presentations were in the form of tables. The mean and standard deviation of the mean values of lipid profile parameters of mortuary workers were compared with values of those not exposed to the embalming chemical using student t-test and ANOVA. The level of significance was set at $P<0.05$.

RESULTS

Table 4.1 shows the Comparison of the Mean level of Serum Lipid Profile Parameters among Mortuary Workers and the Control Group. The result showed that the mean values of total cholesterol (4.67±0. 56), High-density lipoprotein (1.32±0.306), and triglyceride (1.91±0.86) of the mortuary workers were slightly higher than the control group (4.51±0.37; 0.96±0.88and 1.78±0.48 respectively). However, there was no statistically significant ($P>0.05$) difference in the mean values of total cholesterol and triglyceride, while high-density lipoprotein (HDL) shows a statistically significant ($p<0.05$) difference. The mean value of low-density lipoprotein (2.25±0.89) shows a statistically significant ($P<0.05$) reduction in the exposed workers when compared with the control group (3.45±0.99).

Table 4.2 shows the comparison of the Level of Lipid peroxidation product Malondialdehyde among Mortuary Workers and Control Group. The result revealed that the mean value of malondialdehyde (3.27±0.530) of the mortuary workers exposed to the embalming chemical is significantly ($p<0.05$) higher than the control group (1.78±0.303).

Table 4.3 shows the Statistical results of the effect of duration of exposure to embalming chemicals on lipid profile parameters and malondialdehyde level of mortuary workers. The result revealed that the mean values of total cholesterol, high-density lipoprotein, and triglyceride levels showed a statistically non-significant ($p>0.05$) increase across the three groups of mortuary workers, while low-density lipoprotein shows a statistically non-significant ($p>0.05$) increase across the three groups of mortuary workers when compared with the control group. However, the mean value of MDA (3.78±0.604) showed a statistically significant ($p<0.05$) increase in the mortuary workers of > 5years exposure to embalming chemicals when compared with the control group (1.78±0.303).

Table 4.1: Comparison of Mean level of Serum Lipid Profile Parameters among Mortuary Workers and Control Group

PARAMETER	CONTROL n= 25 X ± SD	MORTUARY WORKERS (n=25) X ± SD	P-VALUE	REMARK
Total CHOL	4.51±0.37	4.67±0. 56	0.930	NS
HDL	0.96±0.88	1.32±0.306	0.00	S
LDL	3.45±0.99	2.25±0.89	0.002	S
TRIG	1.78±0.48	1.91±0.86	0.296	NS

Key: Results are expressed as Mean ± Standard Error of Mean (SEM). P< 0.05 is considered significant, S=Significant, NS=Non-significant. Total CHOL= Total Cholesterol; HDL= High Density Lipoprotein; LDL= Low Density Lipoprotein; TRIG= Triglyceride.

Table 4.2: Comparison of the Level of Lipid peroxidation product Malondialdehyde Among Mortuary Workers and Control Group.

PARAMETERS	CONTROL n= 20 (X ± SD)	MORTUARY WORKERS (X ± SD)	P-VALUE	REMARK
MDA (µmo/L)	1.78±0.303	3.27±0.530	0.001	S

Keys: Results are expressed as Mean ± Standard Error of Mean (SEM). P< 0.05 is considered significant, S=Significant, NS=Non-significant.MDA= Malondialdehyde

Table 4.3: Effect of Duration of Exposure to Embalming Chemical on liquid profile Parameters of Mortuary Workers

PARAMETERS	CONTROL (n = 20) (X±SD)	DURATION OF EXPOSURE < 2 years (n = 3) X±SD	2-5 years (n = 10) X±SD	> 5 years (n = 7) X±SD	P-Value
T CHOL (mmo/L)	4.511±0.37	4.23±0.23	4.35±0.22	5.03±0.83	0.453
HDL (mmo/L)	0.96±0.88	1.35±0.63	1.35±0.59	1.39±0.186	0.073
LDL (mmo/L)	3.45±0.99	2.79±0.50	1.77±0.49	2.61±1.17	0.237
TRIG (mmo/L)	1.98±0.48	2.11±0.45	1.588±1.11	1.52±0.507	0.521
MDA (µmol/L)	1.78±0.303	1.84±0.42	2.08±0.53	3.78±0.604	0.010*

Key: Results are expressed as Mean ± Standard Error of Mean (SEM). P< 0.05 is considered significant. Total CHOL= Total Cholesterol; HDL= High Density Lipoprotein; LDL= Low Density Lipoprotein; TRIG= Triglyceride.

DISCUSSION

Chronic exposure to chemicals like formaldehyde, phenol, methanol, ethanol, which are the ingredients of the modern embalming solution, portend an adverse effect to humans. Studies have shown the toxicity of embalmment chemicals, especially formaldehyde to the physiology

and homeostasis of man, including carcinogenicity and other adverse health effects [6]. However, this study was designed to evaluate possible alterations in the serum lipid parameters and lipid peroxidation indices of mortuary attendants exposed to embalming chemicals.

The study results showed that the mean values of total cholesterol, high-density lipoprotein cholesterol, and triglycerides of the attendants were slightly higher than the control group. Though, there was no statistically significant (P>0.05) difference in the mean values of total cholesterol and triglycerides, while high-density lipoprotein cholesterol (HDL-C) showed a statistically significant (p<0.05) difference. The increase in high-density lipoprotein cholesterol (HDL-C) with constant moderate exposure to ethanol or formaldehyde is thought to be associated with an increase in the transport rates (TRs) of apoA-I and -II. This hypothesis is in agreement with works by Naud*et al.*[23] who reported that low, to moderate constant ethanol exposure, would be associated with reduced risk for coronary disease by raising HDL-cholesterol.

The mean value of low-density lipoprotein cholesterol (LDL-C) showed a statistically significant (P<0.05) reduction in the exposed attendants when compared with the control group. The reduction in low-density lipoprotein cholesterol (LDL-C) could be associated with genetic variations in the apolipoproteins metabolism, particularly apolipoprotein A5 polymorphism. This is consistent with a previous study by Perissinotto and his team of experts [23] who reported a reduction in low-density lipoprotein cholesterol (LDL-C) levels in formaldehyde-exposed individuals.

Formaldehyde can trigger oxidative stress by increasing the formation of Reactive Oxygen Species (ROS), and for this reason, some secondary toxic effects in cardiac cells and tissues [25]. ROS can cause oxidative damage and lipid peroxidation by interacting with biological molecules such as DNA and lipids, cum the activation of oxidases, and inhibition of scavenging protocols [26]. This present study revealed that the mean value of malondialdehyde (MDA) of the mortuary attendants understudy is significantly (p<0.05) higher than the control group. Somewhat authenticating the works of Olisah*et al.*[27]and Tasdemir*et al.*[28] who reported that MDA levels in the formaldehyde exposed group was significantly higher when compared with the unexposed (P <0.05).

Consequently, the results also revealed that the mean values of total cholesterol, high-density lipoprotein cholesterol, and triglycerides levels were statistically insignificantly ($p>0.05$) increase across the three groups of mortuary attendants, while low-density lipoprotein cholesterol showed a statistically non-significant ($p>0.05$) increase across the three groups of mortuary attendants when compared with the control group. However, the mean value of malondialdehyde (MDA) showed a statistically significant ($p<0.05$) increase in the mortuary attendants of > 5years exposure to embalming chemicals when compared with the control group. This finding is in agreement with Odiegwu and Colleagues [6] who documented that the adverse effect of exposure to formaldehyde is dependent on the duration of exposure.

CONCLUSION

The results revealed that Mortuary attendants exposed to the embalming chemicals (formaldehyde or ethanol) showed significant variations in serum lipid profile levels. The serum malondialdehyde (MDA) level was significantly higher in the formaldehyde/ethanol exposed attendants than in the unexposed group. This alteration is based on the prolonged duration of exposure.

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