**Original Communication** 

# INVESTIGATION INTO IRRITANT CONSEQUENCES OF EMBALMING CHEMICALS ON RESPIRATORY TRACTS OF EMBALMERS

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#### ABSTRACT

Exposure to toxic substances including embalming chemicals was established as health hazard depending on specific properties of contaminant, exposure situations and individual's health status. Broadly, this study was designed to make comprehensive auditing of safety and comfortability of embalming center; to demonstrate respiratory occupational health hazards amongst embalmers; and to investigate implementation of basic precautionary measures in embalming centers. Study conducted in hospitals' mortuaries and anatomic laboratories. The study was carried out on 34 embalmers in Ekiti State, Southwestern Nigeria, and 34 controls of the same gender and relatively same age group in the same institution after oral consent. A proforma designed for collection of data was categorized into 4 sections: sociodemographics, mortuary design, pre-employment assessment and respiratory function using spirometer. Data collated and analysed with statistical-significance as p<0.05. Embalmers' highest age-group (18-44years) constituted 17 (50.0%). Religion: Christianity 31 (91.2%) and Islam 3(8.8%). Education: primary 6 (17.6%), secondary 14 (41.2%) and tertiary 14 (41.2%). Respiratory rates, forced expiratory volumes within 1 second and maximum voluntary volumes were significantly deranged amongst embalmers while relative differences were observed in tidal volumes. Concordance test (p>0.05) showed inter-dependency amongst the parameters assessing respiratory function. Embalmment was dreadful on respiration of embalmers by deranging lung volumes. Approximative 6% of embalmers developed obstructive respiratory lesion equated to a disaster. Embalming centres in this locality, perhaps, Nigeria at large were rated low due to myriad of unwarranted exposure to embalming fluids. Standard and well-equipped mortuaries/anatomic laboratories were recommended in reducing to the barest minimum: dreadful effects of embalming chemicals, typically, formaldehyde.

**Keywords:** Embalming chemicals; Respiration; Health hazard; Anatomy; Prevention

#### RESUMEN

La exposición a sustancias tóxicas, incluídos los productos químicos de embalsamamiento, se estableció como un peligro para la salud dependiendo de las propiedades específicas de los contaminantes, las situaciones de exposición y el estado de salud del individuo. En términos

generales, este estudio fue diseñado para realizar una auditoría integral de la seguridad y la comodidad del centro de embalsamamiento, para demostrar riesgos de salud ocupacional respiratoria entre los embalsamadores e investigar la implementación de medidas de precaución básicas en los centros de embalsamamiento. Estudio realizado en mortuorios y laboratorios anatómicos de hospitales. El estudio se llevó a cabo en 34 embalsamadores en el estado de Ekiti, suroeste de Nigeria, y 34 controles del mismo sexo y relativamente el mismo grupo de edad en la misma institución después del consentimiento oral. Un formulario diseñado para la recopilación de datos se clasificó en 4 secciones: socio-demográficas, diseño mortuorio, evaluación previa al empleo, y function respiratoria con espirómetro. Los datos recopilados y analizados fueron significativos estadísticamente con p <0,05. El grupo de edad más alto de los embalsamadores (18-44 años) constituía 17 (50.0%). Religión: Cristianismo 31 (91,2%) e Islam 3 (8,8%). Educación: primaria 6 (17,6%), secundária 14 (41,2%) y terciaria 14 (41,2%). Las tasas respiratorias, los volúmenes espiratorios forzados dentro de 1 segundo y los volúmenes voluntaries máximos fueron estadísticamente diferentes entre los embalsamadores, mientras que se observaron diferencias relativas en los volúmenes corrientes. La prueba de concordancia (p> 0.05) mostró interdependencia entre los parámetros que evalúan la function respiratoria. EI embalsamamiento fue terrible para la respiración de los embalsamadores al alterar los volúmenes pulmonares. Aproximadamente el 6% de los embalsamadores desarrollaron una lesion respiratoria obstructive equivalente a un desastre. Los centros de embalsamamiento en esta localidad, y tal vez en general en Nigeria, recibieron una calificación baja debido a la miríada de exposición injustificada a los fluidos de embalsamamiento. Se recomendaron laboratorios de anatomía / mortuorios estándar y bien equipados para reducir al mínimo: los efectos terribles de los productos químicos de embalsamamiento, por lo general, formaldehído.

**Palabras clave**: productos químicos de embalsamamiento; Respiración; Peligro para la salud; Anatomía; Prevención

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Received: 14 September, 2019. Revised: 14 October, 2019. Accepted: 12 November, 2019.

# INTRODUCTION

Occupational health hazards are perils of exposure to pollution, noise and vibrations in the working environment and exposure limits are promoted by the International Labour Organization (ILO) according to Glossary of environmental statistics (1997). A hazard is any source of potential damage, harm or adverse health effects on something or someone while a harm is the physical injury or damage to health. Equally a risk having both qualitative and quantitative factors is the chance or probability that a person will be harmed or experience an adverse health effect if exposed to a hazard. In terms of occupational health and safety, a Public Health hazard category which is an agency for Substances and Disease Registry Toxic (ATSDR) has established standardized categories for health hazards depending on the specific properties of the contaminant, the exposure situations, and the health status of individuals (Breeding, 2011). The diverse embalming centres with different embalming techniques and chemicals as a distinctly existing integral part of health sector and medical sciences have somewhat a share in occupational health hazard and safety.

The embalming components are basically classified as: preservatives, germicides, buffers, wetting agents, anticoagulants, dyes, vehicles (diluents) and perfumes. On top of the preservatives and germicides are the aldehydes in which formaldehyde (the most implicated embalming chemical) is popularly utilized. Information from toxic substances portal described formaldehyde as a near-colourless, pungent, irritating, flammable gas based on the facts from toxic substances (2014). Acute exposure occurs by inhalation or contact with skin and eye, and readily absorbed from the lungs thereby causing airway irritation. bronchospasm and pulmonary oedema. Some research works within and outside Nigeria had testified to acute irritating ophthalmic and respiratory symptoms of formaldehyde as being very frequent (Onyije and Avwioro, 2012; Biasutto et al., 2018). Chronic exposure as applied in embalming (hot zone) may lead to reactive airway dysfunction syndrome (RADS) otherwise called chemically or irritant-induced type of asthma in sensitized individuals. By and large, having searched the web and literature, there is presently no antidote for formaldehyde: management of toxicity is basically supportive. Utility of alcohol in embalming dated to the ancient Egypt and up till today methanol, the simplest form equally has both preservative and germicidal properties apart from being used as a

vehicle, and its exposure in embalming is subdosed (Lobert, 2000). Phenol is a highly toxic, reactive and corrosive chemical in solid, powder, liquid or gaseous form and highly irritant causing damages to cell proteins very quickly with its numerous hazards on many organ systems (Moller, 2014). Chronic exposure of phenol can cause lung irritation with bronchitis. Both workplace controls and personal protective equipment as recommended by the WHO are very germane to handling of phenol and management of its toxicity as attested by World Health Organization (1994). Glycerine is a chemical used as wetting agent and to an extent a vehicle in embalming in which itsinhalation exposureis unlikely because of the low volatility at room temperature.

Other embalming compounds include Sodium Citrate Solution (USP), represent anticoagulant and buffer in embalming with no expectation of inhalation hazard and has no known health effect on chronic exposure, thereby requires no personal respiratory protection needed apart from basic precautionary measures towards chemicals. Dyes are used more for anatomic than funeral embalmment to ease dissection, prosection, exhibition, diagnosis, therapeutics, research and other teaching purposes. Most dyes have no known documented health hazards on inhalation (Sabnis, 2010). Perfumes are added to embalming fluid and, occasionally, as environmental spray to suppress the loathsome odour of formalin in embalming arena and till burial disposal of the dead. Improper use of perfumes may cause respiratory allergy in susceptible individuals (Vigan, 2010; Kolassa, 2013).

Efforts to improve occupational health are just one subset of employee health as a basic element towards sustainable growth of manpower in any organization. The disparity between the embalmers and other workforces called for attention by nature of the profession distinguishably referred to as 'Embalmment' in assessing its revival from crude ancient civilization modern scientific creativity. to Embalmers work as a team in contributing to environmental sanitization and integration of medical education for needed cadavers in anatomic laboratories. Health hazards may come either as occupational diseases or be aggravated by existing ill-health of non-occupational origins. In developing sovereignties, with advancement in technology science and to increasingly mechanized efforts, workers are still being treated as tools to deliver on productivity, thereby putting their health and lives at risk (Levy and Wegman, 1998). In a nutshell, hazard is absence of safety. Absolute safety can rarely be achieved in medical or science laboratories in view of the nature of activities performed in them but a relative safety is manageable if involved individuals are equipped with enabling environment and materials as precautionary measures towards injurious effects of work hazards (Avwioro, 2014). Embalmers are not left out of all these occupational hazards and in a quest to understand the aetiologies of hazards, preventive modalities and management of emergencies when occurred, an intuition was gear up to investigate the health hazards from embalming practices amongst the fraction of embalmers licensed to practice in Nigeria. The specific objectives were: to make comprehensive auditing of safety and comfortability of embalming center, to demonstrate respiratory occupational health hazards amongst embalmers and to investigate implementation of basic precautionary measures in embalming centers.

### MATERIALS AND METHODS

The study was carried out amongst 34 embalmers as subjects in Ekiti State, Southwestern Nigeria with secondary and tertiary government, and private health institutions running mortuary system/anatomic laboratory, and 34 controls of same gender and relatively same age group in same institution after oral consent with exclusion of smokers. A proforma designed for collection of data was categorized into 4 sections: socio-demographics (age, gender, religion, education, marital status and including year of service), design of the mortuary, pre-employment assessment and respiratory function. The design of each centre encompassed: location, needs, disposition, apparatus, preparation room, changing room, postembalming room (mourner's room), waiting room doorway. Pre-employment respiratory and function, chest radiography, likelihood of any derangement and treatment offered were amassed for each participant. Apart from respiratory rate, the investigation on respiratory function with spirometer was carried out: tidal volume (TV), timed vital capacity (FEV<sub>1</sub>) and maximal voluntary volume (MVV). Mortuaries and anatomic laboratories across the state were visited as a team of investigators administering the proforma on each centre and individual embalmer. Equal number of controls, with similar religious beliefs as running outdoors employees, were evenly drawn from other junior workers outside the embalming/anatomic departments of the same institutions. Data were collated and entered into IBM Statistical Package for Social Scientists (IBM SPSS version-25) software for analysis. Statistical-significance was taken as p < 0.05.

Limitations: Formalin was second to water in percentage composition but exact proportions varied and not disclosed. All the centres run dry (open) embalming system as the cooling circuits could not be maintained because of general epileptic power supply in the country: the ambient temperature could not be taken seriously. Use of gas mask not popular in this locality and the only face mask peradventure available was not appropriate in the real sense of it. Assessment of other useful matters like thyroid function text required collection of blood samples as minimally-invasive study with separate ethical clearance: controversially, public collection of blood for any test is linked to ritual for so many veritable socio-cultural heritages.

Item	No respiratory derangement	Assessment not disclosed
Subject (within respiratory	13(44.8%)	21(53.8%)
derangement)		
Control (within respiratory	16(55.2%)	18(46.2%)
derangement)		
Total	29	39

**Table 1**: Comparative analysis of pre-employment respiration (P=0.462). Since p>0.05, null hypothesis (H<sub>0</sub>) is hereby accepted while alternate hypothesis (H<sub>1</sub>) is rejected: no difference between the respiratory assessment values of the subject and the control before taking up the job. The failure to disclose was equally as saying no derangement.

Item	No chest radiographic	Assessment not disclose			
	derangement				
Subject (within radiological	14(46.7%)	20(52.6%)			
derangement)					
Control (within radiological	16(53.3%)	18(47.4%)			
derangement)					
Total	30	38			

**Table 2:** Comparative analysis of pre-employment chest radiographs (P=0.625). P>0.05, there is no need to reject null hypothesis: the findings on pre-employment chest radiographic assessment were similar.

Parameter	Study	Minimum	Maximum	Mode	Mean	SD	SEM
	group						
RR	Subject	12	24	18	18.47	2.24	0.385
( <b>p=0.036</b> )	Control	16	20	16	17.24	1.21	0.207
TV	Subject	400	480	480	470	6.00	1.038
( <b>p=0.151</b> )	Control	400	570	520	570	4.30	0.746
FEV1	Subject	1.7	2.8	2.6	2.59	1.09	0.187
( <b>p=0.030</b> )	Control	2.3	3.5	3.5	3.48	1.88	0.151
MVV	Subject	118	140	130	136.15	42.29	7.252
( <b>p=0.001</b> )	Control	128	164	162	154.32	33.17	5.688

**Table 3:** Assessment of function with control. RR: Respiratory Rate (cycle/min). TV: Tidal Volume (ml/inspiration or expiration). FEV1: Forced Expiratory Volume in first second (L/s). MVV: Maximum Voluntary Volume (L/min). Note that most of the measured values amongst the subjects were reduced or deranged compared with those from controls.

#### RESULTS

Sixty eight (68) individuals participated in the study with 34 each for the subject (embalmer) and the control. All the embalmers were males, hence, the controls. Embalmers in government institution were 28: anatomic laboratory/mortuary

3, two teaching hospitals 9, specialist hospitals 6 and general hospitals 10. Embalmers in private setting were 6 with 2 from each of the three centres. Age group of embalmers: 18-44years was 17 (50.0%); 45-64years was 15 (44.1%) and  $\geq$ 65years was 2 (5.9%). Marital status of embalmers: single 6 (17.6%) and married 28 (82.4%). Religion of embalmers: Christianity 31 (91.2%) and Islam 3 (8.8%). Education of embalmers: primary 6 (17.6%); secondary 14 (41.2%) and tertiary 14 (41.2%). Year of service for both subject and control ranged from 5-20 years. Out of the 68 individuals, 29 (42.6%) was sure of having no respiratory derangement at the

pre-employment screening while 39 (57.4%) knew nothing about the result of the screening. Similarly, 30 (44.1%) was told of having no significant radiological findings and 38 (55.9%) was never affirmed of the result. Other results on measured parameters were contained in tables 1 to 4.

Pre-employment assessment (KCC=0.987)								
Parameter	Pre-employme	ent P	re-employme	ent	Pre-employment		Pre-employment	
	respiration		radiograph		respiratory		radiograph	
					derangemen	ıt	derangement	
Mean Ratio	1.50		1.51		3.51		3.48	
Respiratory function (KCC=0.977)								
Parameter	Respiratory	Tidal	volume	Forced expiratory		М	Maximum voluntary	
	rate			vo	volume is first		volume	
					second			
Mean Ratio	2.00	1	.00		3.14		5.00	

**Table 4:** Test of agreement on pre-employment assessment and respiratory function. The insignificant values of kendall's coefficient of concordance (KCC 0.987 and 0.977 respectively) brought about acceptance of null hypothesis: the measured tools were synonymously explaining the various outcomes

# DISCUSSION

Preservation of the body or embalming is a sociocultural issue calling for proper evaluation in different communities as a way of preventing or minimizing the potential health hazards. The health of the embalmers then necessitates action from time to time as an exercise in continuity in order to reduce to the barest minimum the occupational health hazard from chemical exposure, most crucial, respiratory system while a large number of embalmers had earlier been investigated for cancer and other ailments (Walrath and Franmeni, 1984). Albeit, the embalmers in this study were drawn across governmental and private health centres in the state, individuals at the youthful age-group of 18-44 years constituted the majority as likewise documented from the past study with equivalent range of 27-37 years in the US Department of Health (1980). All the embalmers were males to

prove the fact that business of embalming was really for male factors regardless of the fact that there are now fewer females (about 5%) coming on board as reported in the afore-mentioned American study. In this present investigation, 28 (82.4%) were married to prove the level of family responsibility amongst the embalmers. The fact that Christians constituted majority 31 (91.2%) which was amounted to 9/10<sup>th</sup> might not be surprising since embalming, most especially funeral category which constituted major embalming practices across the globe is never made popular in Islamic injunction. With this, the 1/10<sup>th</sup> Islam might be amongst the embalmers carrying out anatomic category of embalming which is majorly performed in teaching and or research setting of tertiary institutions. In the past, mortuary and embalming affairs were practiced in this part of the world by lower educational status individuals but with the advent of improvement in anatomic and medical

sciences, individuals with secondary and tertiary educational standards are now getting more involved. This assertion outrightly proved the higher educational background of individuals who participated in this study.

Medical report and pre-employment screening exercises are jeered towards improvement of occupational health as one subset of employee health accounting for a basic element towards sustainable growth of workforce in any organization. With this, workers deserved to knowing the state of their health before, during and after retirement to avoid unfounded litigation between employees and employers. In this study, majority of the workers 57.4% and 55.9% knew nothing about the results of their pre-employment respiration function and chest radiographs respectively which were germane to health evaluation of embalmers due to perceived inhalational chemically-induced airway lesions in which Respiratory Airway Dysfunction Syndrome (RADS) was significant (US Department of Health, 1980; Walrath and Franmeni, 1984). The screening exercise for physical and medical fitness should not be perceived as fun of it rather purposely to promoting occupational health of the citizenry: employees officially should know the status of their health from time to time to assist International Labour Organization (ILO) in advancing exposure limit's program which has been on for more than two decades according to glossary of environmental statistics (1997). With pre-employment and regular screening, and revealing of health status to the concerned Homo sapiens loquens, acute irritating symptoms of common embalming chemicals being very frequent as earlier alluded by Onyije and Avwioro (2012) and Biasutto et al (2018) would be better progressing tackled before to chronic manifestations with attendant obstructive or restrictive features.

At rest, the physiological range of 12-15 cycles/minute was taken for normal respiration (Ganong, 2005; Hall, 2006). This study (Table 3) depicted a non-statistically significant 12-24 cycles/minute for the subject while the control had 16-20 cycles/minute as a template for normal. With this relative difference, deduction could be made that there was no embalmer with either brachypnea, but tachypnea which might have been from possible irritant effects of chemicals (formaldehvde embalming in particular) on respiratory system (Lobert, 2000). It was possible the pump (chest wall, respiratory muscles and nerves connecting muscles with respiratory centres in the brain) might being aroused by the embalming chemicals following established account from Toxic Substances (2014).

Broadly speaking, measured parameters were found to be statistically-significant lowered amongst the subjects than the control as previously demonstrated in similar study conducted in India (Saravanan et al., 2015). Despite the fact that, the tidal volume (TV) as a measure of normal respiratory function to evaluate lung volume was generally lower amongst the subjects than the control, the nonstatistically-significant value of p=0.151 (Table 3) was a measure of similar volume changes in both subject and control. The effect of chronic exposure to embalming chemicals on the lung parenchyma might then be considered relativebased on the limited investigative tools at disposal in this study. The forced expiratory volume in first second (FEV1) assessed respiratory function along with accessory muscles of respiration and was statisticallysignificantly lower amongst the embalmers. This could be likened to some levels of obstructive airway lesions developing amongst embalmers due to irritating effects of chemicals in which respiration was being supported by accessory muscles. It was of note that one of the embalmers was confirmed to have developed what seemed to be Reactive Airway Dysfunction Syndrome (RADS) diagnosed 4 years after engaging in embalming business while another embalmer from a different centre was incidentally (by this team of investigators) diagnosed of similar airway lesion. The affectation of these 2 embalmers out of 34 was approximated to 6% which was comparatively synonymous to a disaster. A limitation of this study was on the infeasibility of measuring the room evaporative concentration of the various embalming chemicals, mainly, formaldehyde and phenol in which the exposure levels were not determined for better quantitative judgement. Besides, estimating period of exposure was equally not feasible due to imperfect work schedule in this locality, meaning that embalmers should take solace in self-protection as one of the ways out of hazards from embalming chemicals (Muller, 2014). The study was not designed ab initio as a prospective one, however, the year of services amongst the embalmers ranged from 5-20 years in which upper limit might be enough for exposure in developing chronic lesions. The lower limit of 5 years might be for transformation of instantly acute to chronic irritating features, more importantly, in vulnerable individuals. The game of safety in embalming services should be played between the employer's and employee's obligations. It was the employer's duty to ensure the safety of the employees was observed while at work. However, since the employees were still ever agreed to work under these hash conditions, the obligation to file complaints with Occupational Safety and Health Administration (OSHA) when they felt like their legal rights and personal health were being neglected should be exercised to address some of the limitations un-wrapped in this present study according to Funeral News (2017). This should not be seen as instigating gesture, rather, to bring out innovative or restructuring measures towards sustainable auditing of embalming safety.

Maximal voluntary volume (MVV) is the largest volume of gas that can be moved into and out of the lunas in 1 minute by wilful effort: involving accessorv muscles of respiration with physiological value ranging from 125-170 L/min (Ganong, 2005). The minimum value discovered amongst the embalmers was 118 L/min as against minimum of 128 L/min of control. This statistically-significant discovery (Table 3) further proved that some embalmers might either not be able to utilize their accessory muscles of respiration adequately or hyperactivity of the said muscles resisted to yield desired positive result following obstructive respiratory lesions to meet up to standard when urgently needed compared to the control. In all the embalming chemicals. formaldehyde had been highly implicated as the most offensive airway irritant according to the US Department of Health (1980). By extension, equally highly toxic but less phenol is evaporative, unlike alcohol (methanol) which nearly all of the available information on toxicity in humans relates to the consequences of acute rather than chronic exposures thereby exempting embalmers from hazards of chronic exposure in a way (Brenner, 2014). Findings from previous discoveries on formaldehyde could be likened to those ones in this study, even though, the period and concentration of exposure were not evaluated, a call for actions by occupational therapist may go a long way to establish the exact offending embalming chemical(s) in this locality and the likes.

The statistic test of agreement in pre-employment screening evaluations for both subject and control (p>0.05) using Kendall's Coefficient of Concordance (KCC) in table 4 statistically proved that the various screening modalities were interchangeable in assessing the physical and medical fitness before taking up a job as enshrined in working force of Nigeria at large. This study was then in a state of proper readiness using these measures in evaluating occupational health hazards of respiratory time around, amongst system. this the embalmers handling human remains for funerals and Anatomy as a sacred discipline. In the like manner, the test of agreement for pulmonary function for both case and control confirmed

similar notion on the importance of those functional tests in evaluating the various lesions being faced by a sector of people in the society while communing in Anatomy as the origin of medical and other allied sciences.

At this juncture, reference to the source of unwarranted exposure to toxic effect of embalming chemicals in this locality should be brought up. In all, locations of embalming centres were relatively qualitatively in order. Appropriate statistics could not be collated on this matter due so many issues in embalming room. to preparation room. changing room. postembalming room and waiting room that needed overhauling. Unfortunately, some of the centres did not have all these essential segregations of embalming system while a very few that had were just a mixed-up. Some embalming rooms lacked proper draining channels, running water and sluice. Besides, some centres had no central perforation of the embalming tables to collecting system: this further causing excessive exposure to embalming fluid and chemicals. Appropriate instrumentation to guicken dissection and other process of embalming was grossly inadequate in most of the centres. Embalming centres in most of the secondary healthcare institutions either had no waiting room or the waiting room had been converted to the so-called 'body store' for unclaimed bodies before legal/ethical disposal bureaucracy. All these inadequacies prolonged the rate of unwarrantable exposure to embalming chemicals apart from those inhaled or contacted during normal embalming, dissection, prosection, exhibition and other purposes. Inquiry into the establishment of various embalming facility complexes will be a kind of auditing affairs with assistance of 'Inspector of Anatomy' (if commissioned) as part of ways forwards in each region or zone of the country (Pillay et al., 2017). In view of the unforeseen limitations of this study, investigation of toxic effects of embalming chemicals on respiratory tract of embalmers posed many issues that needed review and amendments. Comprehensive auditing showed the practice of embalmment was dreadful on respiratory system of embalmers by deranging lung volumes and pulmonary capacities. Seeing the non-embalmers as a mirror for a better auditing measure using an index called Relative's Perspective Scale of Embalmment (RPSE) will be of an activity contributing to the fulfilment of a need or furtherance of an effort towards body preservation in an enabling environment for best global practices. (Popoola, 2018). Occupational hazard of obstructive lesion in respiratory system involving up to 6% of embalmers being synonymous to a disaster called for the basic precautionary measures. Embalming centres in this locality were rated low due to myriad of sources of unwarranted exposure to embalming fluids. Nevertheless, standard and well-equipped embalming centres should be the watch-word of each locality to reduce occupational health hazard of embalming chemicals, most especially, the dreadful effects of formaldehyde to the barest Besides, respiratory minimum. protection: self-contained positive pressure breathing apparatus (SCBA) should be made available if need arises. With all the limitations, government and non-governmental sectors should see to operations of embalming services as a verv important integral part of prevention which is better than cure in health institutions.

### **Conflict of interests**

None.

### Funding

None.

# **Ethical Approval**

Approved by ethical and clearance committee of the institution.

# **Informed Consent**

Consent was verbal.

### Contributions

SOP originated and designed this research, and played the central role in all other aspects of the research. OOA contributed in design, data collection and discussion. OOE participated in data collection and discussion. OAF participated in data collection and discussion. SAD contributed in analysis and discussion.

# ACKNOWLEDGMENT

The authors appreciate all the embalmers and other workers that consented to the study. Similarly, we thank the heads of all the institutions where the study took place for their kind permission.

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