Effect of Hours of Exposure to Cement Dust on Cancers Markers on Cement Workers in Port Harcourt

Jonathan Nyebuchi [a*], Mieiwari Ibifubara Jumbo [a],
Collins Ohwonigho Adjekuko [b], Adams Matthew Okur [c]
and Arit Okechukwu Nwogu [a]

[a] *Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria.*
[b] *Department of Biological Sciences, University of Delta, Agbor, Nigeria.*
[c] *Shehu Idris Institute of Allied Health Sciences, Kaduna State University, Makarfi Campus, Kaduna State, Nigeria.*

Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Original Research Article

ABSTRACT

The study was aimed at evaluating the effect of cement dust exposure on cement loaders in Port Harcourt. The study was a cross sectional study which used convenient sampling size of 100 healthy male cement workers recruited in one cement depot and eight cement loading sites in Port Harcourt using simple random technique. Subjects were classified into three groups based on daily hour cement exposure; group 1 (1-5 hrs), group 2 (6-10 hrs) and group 3 (>10 hrs). Group 1 had 27 subjects, group 2 had 62 subjects and group 3 had 11 subjects. 4 ml of the venous blood was drawn into plain vacutainer bottles for the evaluation of Neuron specific Enolase, Vascular Endothelial Growth Factor A, and Total Antioxidant Status. ELISA method was used for the laboratory determination of VEGF-A and NSE while a colorimetric method was used for TAS estimation. Results showed TAS level among the classes was not significantly different (p=0.3304) and has the mean value of 2.02±0.40; 2.16±0.41 and 2.1 ±0.41 in groups 1, 2 and 3 respectively. VEGF-A level among the classes was not significantly different (p=0.7123) with mean value of 406.00 ±234.80; 439.60 ±369.40 and 361.00 ±171.00 in groups 1, 2 and 3 respectively. The mean

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*Corresponding author E-mail: tamaranyebuchi@gmail.com;

value for NSE level among the groups (1, 2, and 3) were 3.78±1.49; 4.17±2.91 and 3.42±0.98 but there was no significantly difference (p=0.5551). This has shown that hourly exposure to cement dust among healthy cement workers does not have significant impact on cancer markers (VEGF-A, NSE and TAS). A higher timeframe assessment may provide a better picture of the effect of cement dust exposure since many diseases associated with cement dust exposure are chronic.

1. INTRODUCTION

The growing concern of environmental pollution and its effect in human lives is a local, national and international issue requiring urgent attention. Modernization which is birthed by industrialization in associated with release of harmful industrial wastes which eventually become harmful to man [1-2]. These industrial wastes could contain heavy metals and eventually pose serious health threat to lives, workers and residents within the area. Heavy metals are known to have toxic effects in human health especially due to increased bioaccumation from the environment [3-5]. Cement is the product of a mixture of limestone with quartz or other sources of silica, iron ore and other additives [6]. It arises from the grinding, crushing, and mixing of these materials together at a very high heat (as high as 1450ºC) which the end product is a fine particulate substance, which possesses the ability to bind other solid materials together into a compact whole. Cements are generally useful in the construction of concrete materials, bridges, houses, culverts, and other structures [7]. Four key ingredients are required in cement production and they include: Calcium, Silicon, Aluminum, and iron. The main element, Calcium, can be derived from limestone, whereas Silicon can be obtained from sand. Bauxite and Iron Ore can be used to extract Aluminum and Iron, and just a little amount is required [8]. There are over ten different varieties of cement used in building, each with a different constituent and made for specific purposes [6]. Cement production processing which involves quarrying and grinding generates large amounts of dust when the finished cement is blended, packed and shipped [9]. Therefore, the cement industry is considered as a major pollution challenge because of dust and particulate matter emitted at various steps of cement production [10].

The American cancer society (ACS) defined cancer as a collection of different diseases structured by abnormal cell development and spread without. When it is not properly managed, could lead to death [11] (NIH, 2020). Every organism has an innate and inherent way of response, one of which is, to inhibit the immunity of anti-tumor. As soon as the organism's response turns to be the organism's pathophysiological term, the consequences lead to cancer emergence [12]. According to the study done by Christopher & Charles [13], it was concluded that cancer is the cause of huge mortality rate at the University of Port Harcourt Teaching Hospital and it was recommended that there should be provision to create awareness for timely cancer screening, basic cancer education and cancer precautionary ways of life and that in females as well as males, cancer of the lungs takes a lead role in the existence of cancer mortality worldwide. The case of cancer seems to be little considering the high frequency experienced in developing countries. This might be as a result of inadequate statistic records or lack of interest in medical checkups [14].

The parameters covered in this study are the total antioxidant status (TAS), inflammatory (VEGF-A) and cancer markers (NSE) respectively. Free radical development by silica activities provokes oxidative stress. The silica gets to an alveolar macrophage and phagocytosis then takes place by the macrophage. This can bring about the destruction of the membrane of lysosomes. It is all as a result of communication with hydrogen ion and crystalline silica right in the cell membranes [15]. Vascular endothelial growth factor-A (VEGF-A) is a tumor border ELISA and a biomarker for malignant diseases. From studies, it has been established that VEGF-A functions in immune-inflammatory reactions and has also been concluded that dust mite pollens can trigger cells of the airway to elevate the secretion of VEGF. This could play a vital function in the intonation of eosinophilic inflammation. The discharge of VEGF-A differs amid subjects with diverse atopy medical expressions and this could be based on the gravity and severity of inflammatory response

[16]. Neuron Specific Enolase is a tumor marker that is associated with lung cancer. This biomarker uses blood and the tests aid in the analysis of disease and determines the response or reaction to treatment. Hence, elevated levels of Neuron-specific enolase (NSE) have been accounted for in non-small cell lung cancer (NSCLC). It has been concluded that NSE is essential in the analysis, prediction and checks of small cell lung cancer (SCLC) [17]. There limited knowledge on the effect of cement on carcinogenic tendency in Port Harcourt. Therefore, this study is targeted at assessing carcinogenic tendencies in cement loaders exposed to cement dust on working hours basis in Port Harcourt.

2. MATERIALS AND METHODS

2.1 Study Design

A cross sectional study design was employed for this study. A convenient sampling size of 100 subjects was used. This research work was carried out on 100 male subjects who were cement exposed healthy subjects working in one cement depot and eight loading sites. The exposed subjects were selected randomly. The subjects were divided into three groups based on hourly exposure. Group 1 contained subjects exposed to cement dust between 1-5 hours. Group 2 contained subjects exposed to cement dust between 6-10 hours and Group 3 contained subjects exposed to cement dust above 10hours daily for at least 3 months.

2.2 Study Area

The study was carried out in Port Harcourt metropolis, Rivers State, Nigeria. Port Harcourt is the capital and biggest city of Rivers State, Nigeria with its geographic coordinates as latitude: 4°46′38″N, longitude: 7°00′48″E and elevation above sea level: 16 m = 52 ft. It lies along the Bonny Stream and is situated in the Niger Delta.

2.3 Eligibility

2.3.1 Inclusion criteria

The cement loaders involved in the study were those that had been exposed to cement dust for a minimum period of three months, who gave their consent to participate in this study and are adults between the ages of 20 to 60 years of age.

2.3.2 Exclusion criteria

Subjects with previous exposure to any occupational agents other than cement silica etc. were excluded from the study. Also, those with history or diagnosed case of asthma or any respiratory diseases or other diseases like diabetes mellitus, pulmonary tuberculosis, having history of acute or chronic infection or recent case of hospitalization, and those with these chronic illnesses were exempted from the study.

Those who had worked for less than three months as well as those who did not consent were excluded from this study.

With the aid of questionnaire and interview, all participating cement loaders were interviewed by trained interviewers. All participants went through medical assessment to rule out the presence of diseases like asthma, diabetes, hypertension, anemia, cancer, infections or those who have recently had blood transfusion, thyroid and heart problems. Participants with diseases, drug therapy and alcohol, antioxidants, exposure to deadly substances or radiation therapy were not included in the study.

2.4 Sample Collection, Transportation, Processing and Preservation

After seeking consent and giving explanations, venous blood samples were drawn from the antecubital fossa of this study subjects using vacutainer sample containers. This is in accordance to the description given by Cheesbrough, [18].

4 ml of the venous blood was lastly drawn into plain vacutainer bottles for the evaluation of Neuron specific Enolase, Vascular Endothelial Growth Factor A, and Total Antioxidative Stress. The venous blood samples were allowed to clot and via separation after centrifuging, the serum was obtained and transferred into a new sterile plain sample bottle and stored at freezing temperature prior to the analysis of Neuron specific Enolase, Vascular Endothelial Growth Factor A, and Total antioxidative stress. All drawn samples were conveyed via cold chain (ice packs/crushed ice in air tight and sealed thermo-container).

2.5 Sampling Technique

Simple random sampling technique was used for recruitment to give everyone equal chances of been recruited into the study to rule out bias.

2.6 Sample Analyses

The samples for Neuron Specific Enolase, Vascular Endothelial Growth Factor A were assayed with the use of ELISA machine (Labtech auto ELISA plate reader, 13485 2003 CE & amp; WHO GMP compliance Co.). The assay for Total antioxidant status was performed using Enzymatic Colorimetric Method. The Total antioxidant status liquid stable reagent kit was manufactured by Fortress Diagnostics Limited, unit 2C Antrim Technology Park, Antrim, BT41 1QS, United Kingdom.

2.6.1 Determination of human VEGF-A and NSE

Assay for human VEGF-A (Vascular endothelial cell growth factor A) was performed with the use of Elabscience kits manufactured by Elabscience Biotechnology. Inc., USA.

Principle:

The ELISA kits used the sandwich-ELISA principle. The micro ELISA plate provided in this kit has been pre-coated with an antibody specific to Human. Samples (or Standards) are added to the micro ELISA plate wells and combined with the specific antibody. Then a biotinylated detection antibody specific for Human VEGF-A (Vascular endothelial cell growth factor A) and avidin-Horseradish Peroxidase (HRP) conjugate are added successively to each micro plate well and incubated. Free components are washed away. The substrate solution is added to each well. Only those wells that contain Human VEGF-A (Vascular endothelial cell growth factor A), biotinylated detection antibody and Avidin-HRP conjugate appeared blue in color. The enzyme-substrate reaction was terminated by the addition of stop solution and the color turned yellow. The optical density (OD) was measured spectrophotometrically at a wavelength of 450±nm. The OD value was proportional to the concentration. The concentration of Human VEGF-A could be calculated in the samples by comparing the OD of the samples to the standard curve.

2.6.2 Assay procedure for human VEGF-A (Vascular endothelial cell growth factor A)

The ELISA wells were determined for diluted standard, blank and sample into the appropriate wells (It was recommended that all samples and standards be assayed in duplicate). The plate

was covered with the sealer provided in the kit. It was incubated for 90 minutes at 37ºC. The solutions were added to the bottom of the micro plate well. Laying a hand on the inner wall was strictly avoided as that could result to foaming. From each well, the liquid was decanted. Washing was delayed for a while and 100µL of Biotynylated detection Antibody working solution was immediately added to each well. The plate was then covered with a new sealer and incubated for 1 hour at 37ºC. From each well, the solution was decanted, 350µL of wash buffer was added to each well, soaked for an hour and the solution was aspirated or decanted from each well and was patted dry on an absorbent paper. These wash steps were repeated 3 times. To achieve these steps, a microplate washer could be used. Instantly, the test strips were used after the wash step. The wells were not allowed to get dry then 100µL of HRP Conjugate working solution was added to each well; the plate was covered with a brand new sealer and incubated for 30 minutes at 37ºC. The solution was decanted from each well; the washing process was repeated for 5 minutes as it was done in step 3and 90µL of the substrate reagent was added to each well. The plate was covered with a new sealer and incubated at 37ºC for 15 minutes. Protecting the plate from light was very essential as the reaction period could be reduced or extended based on color change. This should not have been for more than 30 minutes. Prior to the OD measure, the Microplate reader was preheated for 15 minutes. To each well, the addition of stop solution was done exactly in the same order that the substrate solution was. Instantly, with a micro-plate reader set to 450nm, the optical density (OD value) of each well was determined.

2.6.3 Procedure for neuron specific enolase test

The ELISA wells were determined for diluted standard, blank and sample into the appropriate wells (It was recommended that all samples and standards be assayed in duplicate). The plate was covered with the sealer provided in the kit. It was incubated for 90 minutes at 37ºC. The solutions were added to the bottom of the micro plate well. Touching the inner wall was strictly avoided as that could result to foaming. From each well, the liquid was decanted. Washing was delayed for a while and 100µL of Biotynylated detection Antibody working solution was immediately added to each well. The plate was then covered with a new sealer and incubated for

1 hour at 37^{0}C. From each well, the solution was decanted, 350µL of wash buffer was added to each well, soaked for an hour and the solution was aspirated or decanted from each well and was patted dry on an absorbent paper. These wash steps were repeated 3 times. To achieve these steps, a microplate washer could be used. Instantly, the test strips were used after the wash step. The wells were not allowed to get dry. 100µL of HRP Conjugate working solution was added to each well, the plate was covered with a brand new sealer and incubated for 30 minutes at 37ºC. The solution was decanted from each well; the washing process was repeated for 5 minutes as it was done in step 3. Thereafter, 90µL of the substrate reagent was added to each well. The plate was covered with a new sealer and incubated at 37ºC for 15 minutes. Protecting the plate from light was very important as the reaction period could be reduced or extended based on color change. This should not have been for more than 30 minutes. Prior to the OD measure, the Microplate reader was preheated for 15 minutes. To each well, the addition of stop solution was done in the same order that the substrate solution was. Instantly, with a micro-plate reader set to 450nm, the optical density (OD value) of each well was determined.

2.6.4 Determination of total antioxidant status

This assay was performed using enzymatic colorimetric Method. The Total Antioxidant Status liquid stable reagent kit was manufactured by Fortress Diagnostics Limited, United Kingdom. Cat No: BCX0554A, BXCO553C.

2.6.5 Total antioxidant status test principle

ABTS (1, 2'- Azino-di-(3-ethylbenzthiazoline sulphonate) is incubated with a peroxidase) (metmyoglobin) and H_2O_2 to produce the radical cation ABTS$^{+.}$ This has a stable blue green colour which is measured at 600-660nm. Antioxidants in the sample, suppress the formation of this colour, to a degree which is proportional to their concentration.

2.7 Statistical Analysis

Collected data were analyzed using SPSS version 23. Results were presented as Mean± standard deviation. Statistical significance was defined as $p<0.05$.

3. RESULTS

In Table 1, TAS, VEGF-A and NSE levels were compared among working hours classifications:

1-5hrs; 6-10hrs; >10hrs. TAS level among the classes was not significantly different (p=0.3304) but has the mean value of 2.02 ±0.40; 2.16 ±0.41 and 2.11±0.41 respectively. VEGF-A level among the classes was not significantly different (p=0.7123) with mean value of 406.00 ±234.80; 439.60 ±369.40 and 361.00 ±171.00. The mean value for NSE level among the classes were 3.78 ±1.49; 4.17 ±2.91 and 3.42 ±0.98 but was not significantly different (p=0.5551).

4. DISCUSSION

This study evaluated some inflammatory, total antioxidant status and cancer makers among individuals exposed to cement dust. Chromium, which is one of the components of cement has been reported to be a potent oxidizing agent extremely harmful capable of causing oxidative stress and resultant organ damage. The penetration of Cr (VI) to very important organs like the kidney, the respiratory system (the lungs), and the liver can bring about harmful effects through the production of reactive oxygen species (free radicals) and resultant inflammatory response. Thus posit that high level of dust exposure has deleterious effects on blood and tissues which is attributed to high oxidative stress [19]. Increased state of oxidative stress has been reported in individuals exposed to cement dust [20]. And chronic exposure leads to the depletion of antioxidants [21]. This finding agrees with the work of Salhen [22] who reported a decrease in antioxidation status parameters among Libyan cement factory workers. This study showed that changes in duration of hourly cement dust exposure did not significantly impact changes in antioxidant status among cement workers. P-value>0.05.

Vascular endothelial growth factor-A being a main angiogenesis umpire through tyrosine kinase receptor class IV group of VEGF Receptors (VEGFRs), acts as an indicator. Its ligands connect to VEGFR1 and VEGFR2 and signal through VEGFR2 to bring about endothelial cell production [23]. It is a tumor border ELISA and a biomarker for malignant diseases. Increased VEGF expression has been linked to lymphangiogenesis, colon cancer, lung cancer and various other cancers. The presence of VEGFA in the supernatant fluid was linked to malignant and bloody effusion. VEGFA in the supernatant fluid should be investigated further in the clinical environment as a tumor marker to distinguish benign from malignant effusions [24]. Although, a study expressed a reduction in the

Table 1. Effect of working hours on total antioxidant status, inflammatory and cancers parameters of exposed subjects

Parameters	Working Hours			F value	P value	Remarks
	1-5 hours (n=27)	6-10 hours (n=62)	>10 hours (n=11)			
TAS (mmol/L)	2.02 ±0.40	2.16 ±0.41	2.11 ±0.41	1.12	0.33	NS
VEGF-A (pg/mL)	406.00 ±234.80	439.60 ±369.40	361.00 ±171.00	0.34	0.71	NS
NSE(ng/mL)	3.78 ±1.49	4.17 ±2.91	3.42 ±0.98	0.5921	0.5551	NS

Key: TAS- total antioxidant status, CRP- C-reactive protein, VEGF-A- vascular endothelial factor-A and NSE- neuron specific enolase and ns= not significant at p>0.05

levels of VEGF-A among the cement dust exposed subjects compared to the non-exposed control group, it was stated in a study that in several malignancies, high VEGF expression has been linked to the occurrence of metastases and a poor prognosis [25]. This study did not find a significant reduction or increase VEGF values among groups of hourly cement exposed subjects. P-value > 0.05.

NSE marker is of importance in the diagnosis associated with neuro endocrine tumors, neuroblastoma, ischaemic stroke, etc. A high level in its serum level is indicated to be linked to melanoma and Merkel cell tumor which are both skin disorders [17]. Elevated levels of Neuron-specific enolase (NSE) have been accounted for in non-small cell lung cancer (NSCLC). It has been concluded that NSE is essential in the analysis, prediction and checks of small cell lung cancer (SCLC) [17]. At the stage of pathophysiological silicosis growth, lung tissue is constantly excited by the constituents of silica dust, leading to area based limited damage triggering repair machinery of the body and during the course of lung damage and restoration the pulmonary neuroendocrine cells are stimulated as well [26]. Largely the meta-relative risks estimated across 26 published studies failed to provide indication of elevated risks for cancer relative to cement exposure [27]. In agreement, this research work, hourly cement dust exposure on cement workers did not have any significant effect on NSE levels among studied groups. P-value >0.05.

However, the incidence of cancer and mortality among employees exposed to cement is an age-long public health concern. This is seen in the asbestos cement industry. The insignificant effect of hourly cement exposure on cement worker in causing changes in cancer markers may be due to hourly exposure timeframe comparison. A higher timeframe assessment may provide a better picture of the effect of cement dust.

5. CONCLUSION

This study evaluated effect of working hours per day on some cancer markers among cement loaders in Port Harcourt, Nigeria. The study shown that exposure hour to cement dust on healthy cement workers in Port Harcourt does not have significant effect on carcinogenic tendency or development.

CONSENT AND ETHICAL APPROVAL

Ethical approval for this research was obtained from the Rivers State Health Research Ethics Committee. Permission was also gotten from the authorities of cement loading sites/shops and Dangote cement depot, RIVOC, Trans-Amadi, Port Harcourt. Informed consent was given by subjects before recruitment into the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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