Original Research Article

Evaluation of Some Inflammatory, Cancer Markers and Total Antioxidant Status Among Cement Loaders in Port-Harcourt

ABSTRACT

The effect of cement dust has been linked to many diseases but very few studies have been able to study the impact of cement on oxidative stress, inflammation and carcinogenesis in Port Harcourt. Therefore, this study was focus on assessing the effect of cement dust on total antioxidant status (TAS), inflammatory markers and cancer markers among cement loaders in Port Harcourt. The study was conducted in Port Harcourt among 100 cement loaders and 100 non-cement exposed individuals who made up the control group. Upon consenting to participate in the study, subjects were randomly selected for recruitment into the study. Five milliliters of blood was collected from each subject and assayed for total antioxidant status (TAS), IL-10, IL-1β, Neuron specific Enolase (NSE), C-Reactive protein (CRP), Vascular Endothelial Growth Factor A (VEGF-A) using ELISA technique. Results compared TAS, IL-10, IL-1β, NSE, CRP and VEGF-A levels between cement exposed group and control group. TAS result revealed that there was a significant decrease between in the exposed group (P-value=0.0011). CRP, result revealed that there was no significant difference between both groups (P-value=0.1103). IL-10, result revealed that there was a significant decrease in the exposed group (P-value=0.0030). IL-1β, result revealed that there was a significant increase in the exposed group (P-value=0.0012). VEGF-A result revealed that there was no significant difference between both groups (P-value=0.653). NSE, result revealed that there was a significant increase in the exposed groups (P-value<0.0001). This study has shown that human exposure to cement dust increases oxidative stress, affects inflammatory responses and may increase the risk of cancer among cement workers in Port Harcourt.

Comment [S1]: Delete it

Comment [S2]: Please change it to technique and total antioxidant status (TAS) using enzymatic colorimetric method.

Keywords: *Cement dust, inflammatory markers, cancer markers, oxidative stress, ELISA*

Comment [S3]: Arrange the keywords in alphabetical order

1.0 INTRODUCTION

Cement is a fine particulate powder which arises from the crushing and grinding of limestone with quartz or other sources of silica, iron ore and other additives (Dunuweera and Rajapakse, 2018). The raw materials (silica, iron ore, and other additives undergoes series of chemical reactions at very high temperature (~1450°C) to arrive at the finished product (cement). Different types of cement with varying compositions are available for different construction purposes. Molecules of principal value in cement essentially include 60-67% calcium oxide, 17-25 silicon oxide (SiO2), 3-5% aluminium (Al) oxide, with some amount of iron oxide, chromium (Cr), potassium, sodium, sulphur and magnesium oxide (Kakooei *et al.,* 2012; Gbadebo and Bankole, 2007).The production process of cement generates large quantity of dust during quarrying, grinding and when the finished cement is blended, packed and shipped (Fell *et al.,* 2016). The cement industry is considered as a major pollution problem because of dust and particulate matter emitted at various steps of cement production (Friday *et al.,* 2016). The cement dust generated and emitted in the production, packaging, shipping, and the use of cement has led to various health challenges (Rahmani *et al.,* 2018). The individual components of cement has been shown by studies to exert different health effects on those exposed to cement dusts. Aluminum for instace has been linked to a variety of brain illnesses, including Alzheimer's, Parkinson's, and multiple sclerosis (Inan-Eroglu and Ayaz, 2018). A research carried out by

Comment [S4]: All references in the Paper must be numbered in the order that they appear in the text. In the text, citations should be indicated by the reference number in brackets

Comment [S5]: Please change it to [1]

Comment [S6]: Please change it to additives)

Comment [S7]: Please change it to SiO_2

Comment [S8]: Please change it to instance

UNDER PEER REVIEW

Giorgianni shows that Aluminum alters cognitive responses, with the effects being more noticeable in complex processes (Giorgianni, 2014). A study conducted by Meo (2004) revealed that cement dust penetrate the systemic circulation and so reach almost all of the body's organs, impacting various tissues such as the heart, liver, spleen, bone, muscles, and hairs, eventually changing their microstructure and physiological function (Meo, 2004). Exposure to cement Aluminum (Al), iron (Fe), calcium (Ca), and silicon (Si) in the workplace has been linked to lower lung function indicators in exposed employees. Many studies have estimated the effects the exposure of cement has posed with the help of spirometric and radiologic methods. The process of inflammatory response of work place hazard to silica has been said to be found in some human body organs like skin, liver and lungs. When this is not checked, it might result to fibrosis, cancer or granulomatous diseases (Ogunbileje & Akinosun, 2011). So many researchers have examined silica in staff of dust exposed production factories and lung cancer risk has been accounted for in some of these workers not all (Malvezzi, 2016).

Merenu and his team disovered that exposure to emissions and cement dust particles have been noted to have caused damage to the health of many. Their study was focused on Sheep within or close to cement exposed area in Sokoto, Nigeria (Merenu *et al.* 2015). A proven theory from a research has verified that cement dust contact is capable of bringing along problems in peak expiratory flow (PEF) values in cement factory staff, exposed to cement dust (Omidianidost *et al.,* 2019). Going by the studies conducted on this subject in other countries of the world and some part of the nation, some male subjects in Port Harcourt were evaluated for inflammatory, total antioxidant status and cancer makers among individuals exposed to cement dust, based on the number of hours they work.

Comment [S9]: Please change it to discovered

The effect of cement dust has been linked to many diseases but very few studies have been able to study the impact of cement on oxidative stress, inflammation and carcinogenesis in Port Harcourt. Therefore, this study was focus on assessing the effect of cement dust on total antioxidant status, inflammatory markers and cancer markers among cement loaders in Port Harcourt.

2.0 MATERIALS AND METHOD

2.1 Study Design

A cross sectional study design was employed for this study. A convenient sampling size of 200 subjects was used. This research work was carried out on 200 male subjects; of which 100 were cement exposed healthy subjects working in one cement depot and eight loading sites and 100 were apparently healthy non exposed male subjects. The exposed subjects were selected randomly. The medical history as well as the bio-data, socio demographics, environmental and lifestyle of the study subjects were obtained with the use of a well structured questionnaire.

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.

UNDER PEER REVIEW

2.3 Study Population

This study involved 100 cement loaders who worked in cement depots and shops, and another 100 control subjects who were not involved in the use of cement occupationally. The biodata of the subjects were obtained using questionnaire. Individuals involved in any occupation that involves the use of cement, were not recruited as control subjects. Informed consent was also obtained from the subjects. All subjects were apparently healthy.

2.4 Eligibility Criteria

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.
The unexposed control subjects were apparently healthy male subjects who reside outside the vicinity of the cement exposed areas. Subjects with no history or signs suggestive of respiratory, haematologic, bone or liver diseases were considered eligible and selected into both the exposed and unexposed groups.

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.

2.5 Informed Consent and Ethical Clearance

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 individuals before recruitment into the study.

2.6 Sampling Technique

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

2.7 Sample Collection

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, (2010). In the study, blood sample (4ml) of the venous

Comment [S10]: Or five milliliters as you mentioned in abstract

UNDER PEER REVIEW

blood was drawn into plain vacutainer bottles for the evaluation of Neuron specific Enolase, C-Reactive protein, Vascular Endothelial Growth Factor A, IL-1β, IL-10 and Total antioxidative stress. The blood collected allowed to clot and then spun at 4000rpm for 5 minutes to obtain the serum.

2.8 Laboratory Analysis

Comment [S11]: Dear author as the same method used for VEGF-A, IL-1β, IL-10, NSE and CRP, I preferred to write the method used for their determination under the same title

Determination of Human VEGF-A (Vascular Endothelial Cell Growth Factor A)

Assay for human VEGF-A (Vascular endothelial cell growth factor A) was performed ELISA kit.

Comment [S12]: Please change it to performed by

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 minuites at 37^0C. 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 awhile 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 37^0C. 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 minuites at 37^0C. The solution was decanted from each well; the washing process was repeated for 5 minuites 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 37^0C for 15 minuites. 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 minuites. Prior to the OD measure, the Microplate reader was preheated for 15 minuites. 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.

Comment [S13]: Please change it to minutes

Standard Range for Human Vascular Endothelial Cell Growth Factor

31.25pg/mL to 2000pg/mL: This is the standard curve range for Human Vascular Endothelial Cell Growth Factor

Calculation of results

The dublicate readings for each standard and samples were averaged and the average zero standard optical density then was subtracted. A four parameter logistic curve on log-log graph paper was plotted with standard concentration on the x-axis and OD values on the y-axis. The calculated concentration multiplied by the dilution factor was the test concentration.

Determination of Human IL-1β (Interleukin 1 Beta) Test

UNDER PEER REVIEW

Assay for Human IL-1β (Interleukin 1 Beta) was determined ELISA Kits (Engvall & Perlmann, 1971).

Comment [S14]: Determined by

Procedure for Human IL-1β (Interleukin 1 Beta) 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 37^0C. The solutions were then added to the bottom of the micro plate well. Contact with the inner wall was strictly avoided as that could result to foaming. From each well, the liquid was decanted. Washing was delayed for awhile and 100μL of Biotynylated detection Ab working solution was immediately added to each well. The plate was the covered with a new sealer and incubated for 1 hour at 37^0C. 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. Immediately, the test strips were used after the wash step. The wells were not allowed to dry up and 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 37^0C. The solution was decanted from each well; the washing process was repeated for 5 minutes as it was done in step 3 and after that, 90μL of the substrate reagent was added to each well. The plate was covered with a new sealer and incubated at 37^0C for 15 minutes. Protecting the plate from light was vital 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 then preheated for 15 minutes. To each well, the addition of stop solution was done in the same order that the substrate solution was and straight away, with a micro-plate reader set to 450nm, the optical density (OD value) of each well was determined.

Standard Range for Human IL-1β (Interleukin 1 Beta)

7.81pg/ml to 500 pg/ml: This is the standard curve range for Human IL-1β (Interleukin 1 Beta).

Calculation of results

The duplicate readings for each standard and samples were averaged and the average zero standard optical density then was subtracted. A four parameter logistic curve on log-log graph paper was plotted with standard concentration on the x-axis and OD values on the y-axis. The calculated concentration multiplied by the dilution factor was the test concentration.

Determination of Human IL-10 (Interleukin 10) Test

Assay for Human IL-10 (Interleukin 10) was determined with the use ELISA kit

Procedure for Human IL-10 (Interleukin 10) Test

The ELISA wells for human IL-10 test 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 37^0C. The solutions were rightly added to the bottom of the micro plate well. Tampering with the inner wall was strictly avoided as that could result to foaming. From each well, the liquid was decanted. Washing was delayed for awhile and 100μL of Biotynylated

UNDER PEER REVIEW

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 37^0C. 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.At once, the test strips were used after the wash step. The wells were not allowed to get dry and 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 37^0C. The solution was decanted from each well; the washing process was repeated for 5 minutes as it was done in step 3. At this point, 90µL of the substrate reagent was added to each well. The plate was covered with a new sealer and incubated at 37^0C for 15 minutes. Guarding the plate from light was very imperative 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 minuites.
To each well, the adding up of stop solution was done in the same order that the substrate solution was. Instantaneously, with a micro-plate reader set to 450nm, the optical density (OD value) of each well was determined.

Standard Range for Human IL-10 (Interleukin 10)

7.81pg/ml to 500pg/ml: This is the standard curve range for Human IL-10 (Interleukin 10).

Calculation of results
The dublicate readings for each standard and samples were averaged and the average zero standard optical density then was subtracted. A four parameter logistic curve on log-log graph paper was plotted with standard concentration on the x-axis and OD values on the y-axis. The calculated concentration multiplied by the dilution factor was the test concentration.

Determination of Neuron Specific Enolase
Assay for Neuron Specific Enolase was performed with the use of ELISA kit

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 37^0C. 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 37^0C. 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 37^0C. 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 37^0C for 15 minutes. Protecting the plate from

UNDER PEER REVIEW

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 minuites. Prior to the OD measure, the Microplate reader was preheated for 15 minuites. 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.
Standard Range for Human Neuron Specific Enolase
2.34ng/ml to 150ng/ml: This is the standard curve range for Neuron Specific Enolase

Calculation of results
The dublicate readings for each standard and samples were averaged and the average zero standard optical density then was subtracted. A four parameter logistic curve on log-log graph paper was plotted with standard concentration on the x-axis and OD values on the y-axis. The calculated concentration multiplied by the dilution factor was the test concentration.

Determination of Human C - reactive protein (CRP)
Assay for C-Reactive Protein (CRP) was performed with the use of ELISA

Assay Procedure for C - reactive protein 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 37^0C. The solutions were addedto 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 awhile 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 37^0C. 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 minuites at 37^0C. The solution was decanted from each well; the washing process was repeated for 5 minuites as it was done in step 3 then 90μL of the substrate reagent was added to each well. The plate was covered with a new sealer and incubated at 37^0C for 15 minuites. 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 minuites. Prior to the OD measure, the Microplate reader was preheated for 15 minuites. 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.
Standard Range for Human C - reactive protein
0.39ng/ml to 25ng/ml: This is the standard curve range for Human C - reactive protein

Calculation of results
The dublicate readings for each standard and samples were averaged and the average zero standard optical density then was subtracted. A four parameter logistic curve on log-log graph

UNDER PEER REVIEW

paper was plotted with standard concentration on the x-axis and OD values on the y-axis. The calculated concentration multiplied by the dilution factor was the test concentration.

Determination of Total Antioxidant Status

This assay was performed using enzymatic colorimetric Method.

Comment [S15]: Please mention the reference of method used

Total Antioxidant Status Test

ABTS (1, 2'- Azino-di-(3-ethylbenzthiazoline sulphonate) is incubated with a peroxidase) (metmyoglobin) and H_2O_2 to produce the radical cation $ABTS^{+\cdot}$ 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.

Calculation of Absorbances

A2-A1 = ΔAbsorbance sample/Calibrator/Blank

Results: ΔAbs Sample/ΔAbs Calibrator × Calibrator concentration

A growing number of studies reveal that oxidative stress is related to cancer development. However, there are few reports assessing the relationships between oxidative stress and carcinogenesis (Małgorzata, Kiełczykowska and Bartłomiej, 2019). Enzymatic colorimetric method was used to measure Total Anti Oxidant Status (TAS) in this study.

Comment [S16]: Delete it

Standard Range for Total Antioxidant Status

1.3mmol/l-2.3mmol/l: This is the standard curve range for Total Antioxidant Status**.**

2.9 Statistical Analysis

Data obtained were collated in Ms Excel and data was analyzed using SPSS version 23.0 for descriptive analysis (mean and standard deviation) and inferential statistics (T-test). Test significance was set at *p*-valve<0.05.

3.0 RESULTS

Comparison of Total Antioxidant Status, Inflammatory and Cancers Parameters of Control and Exposed Subjects

Table 1 shows the comparison of TAS, inflammatory and cancer markers in exposed and control groups. TAS result revealed that there was a significant decrease between in the exposed group (P-value=0.0011). CRP, result revealed that there was no significant difference between both groups (P-value=0.1103). IL-10, result revealed that there was a significant decrease in the exposed group (P-value=0.0030). IL-1β, result revealed that there was a significant increase in the exposed group (P-value=0.0012). VEGF-A result revealed that there was no significant difference between both groups (P-value=0.653). NSE, result revealed that there was a significant increase in the exposed groups (P-value<0.0001).

UNDER PEER REVIEW

Table 1: Comparison of Total Antioxidant Status, Inflammatory and Cancers Parameters of Control and Exposed Subjects

Subjects	Parameters					
	TAS (mmol/L)	CRP (ng/mL)	IL-10 (pg/mL)	IL-1β (pg/mL)	VEGF-A (pg/mL)	NSE (ng/mL)
Control (n=100)	2.18±0.76	7.90±5.87	18.98±16.24	3.20±0.88	364.34±237.60	2.79±0.85
Exposed (n=100)	1.84±0.46	6.62±5.47	13.13±10.73	3.71±1.26	350.83±182.86	3.98±2.44
T-value	4.605	1.604	3.004	3.290	0.451	4.602
P value	<0.001	0.1103	0.0030	0.0012	0.653	<0.0001
Remark	S	NS	S	S	NS	S

Key: TAS- total antioxidant status, CRP- C-reactive protein, IL-10- interleukin 10, IL-1β- interleukin 1 beta, VEGF-A- vascular endothelial factor-A, NSE- neuron specific enolase, n-significant at $p<0.05$ and ns-not significant at $p>0.05$.

Comment [S17]: Please change it to S

4.0 DISCUSSION

Comment [S18]: Dear author you don't discuss the result of CRP

This study evaluated some inflammatory, total antioxidant status and cancer makers among individuals exposed to cement dust. In this study, 100 subjects between the ages of 20 and 60 years were selected from the population of interest (cement loaders) and 100 subjects were recruited from non-cement loaders population to form the control group.

The results from this study indicated that the exposed subjects had significantly lower TAS levels and IL-10 levels but significantly higher IL-1β levels and NSE levels. The levels of CRP and VEGF-A did not defer between the exposed and control subjects. The results from this study showed that the exposed subjects had significantly lower TAS than the control subjects. This observation indicates oxidative stress. Cement dust inhalation may have resulted to oxidative stress leading to significant decrease in TAS level among cement loaders in Port Harcourt. Ogunbileje at al. (2010) and Obaji-Ogar et al. (2020) stated that increased state of oxidative stress is associated with individuals exposed to cement dust and chronic exposure could lead to the depletion of antioxidants. This finding also agrees with the work of Salhen (2014) who reported a decrease in antioxidation status parameters among Libyan cement factory workers. According to Bagchi et al. (2001), 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

UNDER PEER REVIEW

attributed to high oxidative stress. IL-10 is a cytokine that exerts immunoregulatory effects. These immunoregulatory effects are broad and lead to attenuation of the expression of pro-inflammatory cytokine. In other words, IL-10 is predominantly anti-inflammatory cytokine (Steen *et al.,* 2020). Therefore, significantly reduced level of IL-10 in this study could be an indication of reduced anti-inflammatory condition of the exposed subjects. This finding agrees with the work of Fell *et al.* (2011) who reported a similar finding in Norwegian cement production workers. IL-10 exhibits a circulation rhythm wherein its levels rise in the day time (Lange *et al.,* 2010). In consonance with this study, Fell et al. (2011) observed low levels of IL-10 which they attributed to be due to inflammatory response or probably an indication of exposure to injurious substances. Although, this study expressed a reduction in the levels of VEGF-A among the cement dust exposed subjects compared to the non exposed control group, it was not statistically significant. Soe et al. (2000) stated in a study that in several malignancies, high VEGF expression has been linked to the occurrence of metastases and a poor prognosis. So in this study, exposure to cement may not have significantly impacted tendencies of carcinogenesis since VEGF-A did not significantly vary from the control group.

The exposed subjects in this study have significantly higher levels of IL-1β and NSE. Lopex – Castejon and Brough, (2011) and Villeneuve *et al.,* (2018), IL-1β is a cytokine that mediates inflammatory response. The raised levels of IL-Iβ may be due to inflammation, which resulted in the production of inflammatory markers including IL- Iβ. The Neuron-specific enolase (NSE) is an isoenzyme of enolase that is known to be expressed in neurons and neuroendocrine cells (El-Maraghi *et al.,* 2013). Inhalation of silica, which is also found in cement, can lead to silicosis, which is a type of chronic pulmonary fibrosis (Huang *et al.,* 2021) under normal physiological conditions, NSE is released into the extracellular space, but it is released and possibly upregulated when there is a damage or injury to the cell (Bezek *et al.,* 2020). Therefore, the raised levels of the NSE in this study are probably due to injury to the lung cells due to the inhalation of cement dust that may have occurred among the exposed subjects.

Comment [S19]: Please change it to IL-1β is a cytokine that mediates inflammatory response [references number].

CONCLUSION

This study has revealed that exposure to cement dust can lead to increased oxidative stress which are the background causes for many chronic diseases and cancer. Also, the study has also shown that cement dust exposure triggers inflammatory responses. Although only one cancer marker levels was significant, key attention should be paid because of increased oxidative stress coupled with increase in NSE level can pose as risk for carcinogenesis.

RECOMMENDATION

The cement loaders should not only be educated on the importance of using adequate personal protective equipment (PPE), they should be provided with one considering that sometimes their wage may not be enough to cater for the PPE. Updated scientific educational program delivered through the media to raise public health awareness of the effects of cement on those who are exposed to it is required.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is

UNDER PEER REVIEW

absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors

REFERENCES

Comment [S20]: Please rewrite the reference according to journal style

Amadi, A. I & Dimkpa, K (2020). Towards waste minimization in wet Trades: metrological based model of cement demand in Port Harcourt metropolis. *The Environmental Studies Journal*, 3(1), 61-76.

Bagchi, D., Bagchi, M., & Stohs, S. J. (2001). Chromium (VI)-induced oxidative stress, apoptotic cell death and modulation of p53 tumor suppressor gene. *Molecular and cellular biochemistry*, *222*, 1-2, 149-158.

Bezek, S., Biberthaler, P., Martineze-Espina, I., Bogner-Flatiz, V. (2020). Pathophysiology and clinical implementation of traumatic brain injury biomarkers: neuron-specific enplase. In WU, A.H.B. & Peacock, W.F. (Eds). Biomarkers for Traumatic Brain Injury (pp. 169-182). Cambridge: Elsevier.

Cheesbrough, M. (2010). District laboratory practice in tropical countries, 2nd edition, Part 2. Cambridge: Cambridge University Press.

Dunuweera, S.P. & Rajapakse, R. M. G. (2018). Cement Types, Composition, Uses and Advantages of Nanocement, Environmental Impact on Cement Production, and Possible Solutions. *Advances in Materials Science and Engineering,* 2(1), 1-11.

El-Maraghi, S., Yehia H., Hossan, H., Hazen, Y, Yehia, A. & Mowafy, H. (2013). The prognostic value of neuron specific enolase in head injury. *The Egyptian Journal of Critical Care Medicine*, 1 (1), 25-32.

Fell, A. K., Møller, A. & Karl, C. N. (2016). Association between exposure in the cement production industry and non-malignant respiratory effects: A systematic review. *British Medical Journal,* 7, 4012 - 4028.

Fell, A. K., Notø, H. & Skogstad, M. (2011). A cross-shift study of lung function, exhaled nitric-oxide and inflammatory markers in blood in Norwegian cement production workers. *Journal of Occupational and Environmental Medicine,* 68, 799–805.

Friday, E. T., Alabi, O. J. & Akpa, M. (2016). Investigation Effect of Free Radical Generated from Cement Dust on Antioxidant Enzymes Activities on Cement Factory Workers. *International Journal of Biochemistry & Physiology*, 1(1), 1 – 5.

Gbadebo, A.M. & Bankole, O.D. (2007). Analysis of potentially toxic metals in airborne cement dust around Sagamu, Southwestern Nigeria. *Journal of Applied Science,* 7, 35-40.

Giorgianni, C. M., D'Arrigo, G., Brecciaroli, R., Abbate, A., Spatari, G., Tringali, M. A., Gangemi, S., & De Luca, A. (2014). Neurocognitive effects in welders exposed to aluminium. *Toxicology and Industrial Health*, *30*(4), 347–356.

UNDER PEER REVIEW

Haung, H., Huang, J., Xu, X., Huang, K., Lin, Y., Lin, J. & Zhuang, X. (2021). Serum, neuron-specific enolase: A promising biomarker of silicosis. World Journal of Clinical Cases, 9 (5), 1016-1025.

Inan-Eroglu, E., & Ayaz, A. (2018). Is aluminum exposure a risk factor for neurological disorders? *The Official Journal of Isfahan University of Medical Sciences*, 23, 51 - 59.

Kakooei, H., Gholami, A., Ghasemkhani, M., Hosseini, M., Panahi, D. & Pouryaghoub, G. (2012). Dust Exposure and Respiratory Health Effects in Cement Production. *Acta Medica Iranica*, *50*(2), 122-126.

Lange, T., Dimitrov, S. & Born, J. (2010). Effects of sleep and circadian rhythm on the human immune system. Annals of the New York Academy of Science, 1198, 48-59.

Lopez – Castejon, G. & Brough, D. (2011). Understanding the Mechanism of IL-Iβ secretion. *Cytokine & Growth Factor Review*, 22(4), 189-195.

Malvezzi, M., Negri, E., Vecchia, C. L. & Boffetta, P. (2016). Risk factors for lung cancer worldwide. *European Respiratory Journal,* 48, 889-902.

Meo, S. A. (2004). Health hazards of cement dust. *Saudi Medical Journal*, 25(9), 1153-1159.

Merenu, I. A., Omokhodion, F., Mojiminiyi, F.B. O., Ibrahim, M.T.O. (2015). Effect of cement dust exposure on lung function among residents of Kalambaina Community in Sokoto State, Nigeria. *Journal of Medical Sciences and Clinical Research,* 3, 8, 2455.

Mojekwu, J. N., Ademola, I. & Sode, O (2013). Analysis of the contribution of imported and locally manufactured cement to the growth of gross domestic production of Nigeria. *African Journal of Business Management*, 7(5), 360-371.

Obaji-Ogar, L. T., Nsonwu-Anyanwu, A. C. H. & Odum, F. A. (2020). Oxidative DNA Damage and Pro-inflammatory Response in Chronic Exposure to Cement Dust. *Asia Pacific Journal Medical Toxicology*, 9(1), 3 - 10.

Ogunbileje, J. O. & Akinosun, O. M. (2011). Biochemical and haematological profile in nigeria cement factor/workers. *Research Journal of Environmental Toxicology,* 5, 133-144.

Ogunbileje, J.O., Akinosun, P. A., Akinduti, L.A., Nwobi, O.M. & Ejilude, O.A. (2010). Serum levels of some trace metals and leucocyte differential counts in Nigeria cement factory workers: Possible toxicity implications. *Florida International Journal*, 2, 53-58.

Omidianidost, A., Gharavandi, S., Azari, M., Hashemiam, A., Ghasemkhani, M., Rajati, E. & Jabari, M. (2019). Occupational exposure to respirable dust, crystalline silica and it's pulmonary effects among workers of a cement factory in Kermanshali, Iran. *Tanaffos,* 18(2), 157-162.

Rahmani, A. H., Almatroudi, A., Babiker, A.Y., Khan, A. A. & Alsahly, M.A. (2018). Effect of exposure to cement dust among the workers: An evaluation of health related complications. *Journal of Medical Science*, 6(6), 1159-1162.

UNDER PEER REVIEW

Salhen, K.S.A. (2004). Assessment of oxidative stress, heamatological, kidney and liver functions parameters of Libyan cement factory workers. *Journal of American Science*, 10 (5), 58-65.

Seo, Y., Baba, H., Fukuda, T., Takashima, M. & Sugimachi, K. (2000). High expression of vascular endothelial growth factor is associated with liver metastasis and a poor prognosis for patients with ductal pancreatic adenocarcinoma. *Cancer*, *88*(10), 2239–2245.

Steen, E.H., Wang, X., Balaji, S. Buttle, M.J., Bollyky, P.L. & Keswani, S.G (2020). The role of the anti-inflammatory cytokine interleukin-10 in tissue inflammation. Advances in wound care, 9 (4), 184-198.

Wekpe, V. O. & Fiberesima, D. (2020). Noise mapping around the host communities of the university of port harcourt, Nigeria. *Arts & Humanities Open Access Journal*, 4 (2), 43–48.

UNDER PEER REVIEW