Evaluation of Some Haemostatic Parameters among Cement Loaders in Port-Harcourt, Nigeria

ABSTRACT

Exposure to cement dust has been serious public health concern with increasing industrial activities in Port Harcourt. This study was aimed at evaluating the effect of cement dust exposure on haemostatic parameters on cement loaders in Port Harcourt. A total of 200 healthy males were recruited for the study; 100 of which were cement loaders and the other 100 were individuals not exposed to cement dust. These subjects were selected randomly at different cement depot in Port Harcourt after consenting to participate in the study. Venous blood was called via venipuncture technique and 5 mls of blood was collected for the laboratory analysis of prothrombin time (PT), activated partial thromboplastin time (APTT), International normalized ratio (INR), D-Dimer, Von-Willebrand factor and fibrinogen. The result showed that there was a significant increase (p-value<0.05) in APTT, VWF, D-Dimer and fibrinogen levels in the cement exposed group but there was no significant difference in PT and INR levels in the exposed group (p-value>0.05). This study has shown that exposure to cement dust affects haemostasis among cement loaders in Port Harcourt.

1.0 INTRODUCTION

Cement can be defined as adhesive substances that are capable of uniting fragments or masses of solid matter to a compact whole. There are over ten different varieties used in building and construction, each with a different composition and suited for different purposes [1]. These include rapid-hardening cement (RHC), quick-setting cement (QSC), low-heat cement (LHC), sulphate-resistant cement (SRC), blastfurnace slag cement (BFSC), high-alumina cement (HAC), white cement (WC), coloured cement (CC), pozzolanic cement (PzC), air-entraining cement (AEC), and hydrophobic cement (HPC). Lime (CaO), Silica (SiO2), Alumina (Al_2O_3), Calcium Sulphate ($CaSO_4$), Iron Oxide (Fe_2O_3), Magnesia (MgO), Sulphur (S), Alkalies are different ingredients found in cement. Ordinary Portland cement (OPC) is the most used type of cement, and is known to have increased lime content in rapid-hardening cement [1]. It is made by crushing, grinding, and combining particular proportions of lime or calcium Oxide (CaO: from limestone, chalk, shells, shale or calcareous rock), iron (Fe_2O_3: from from clay, iron ore, scrap iron and fly ash), silica (SiO_2: from sand, old bottles, clay or argillaceous rock), alumina (Al_2O_3: from bauxite, recycled aluminum, clay), and Gypsum, $CaSO_4.2H_20$: found together with limestone [2].The chemical interactions between the cement and the water give Portland cement its strength in a process known as Hydration. This is a complicated process that is best understood by first learning about cement's chemical makeup [2]. Aluminum, Chromium, Iron (Fe^{3+}), and Calcium constitutes the main components of cement and those this study is focused on.

Haemostasis is an aggregate mechanism; a well linked procedure specialized in salvaging blood loss, thus restoring the damaged tissue rectitude [3]. Haemostasis is therefore influenced by procedures that activate the exercise in order to cease the loss of blood hence inhibiting the production of thrombus [3]. There is primary and secondary haemostasis. The primary Haemostasis is responsible for the development of a plug at a damaged exposed cell area with platelets during an injury while the secondary haemostasis forms a common pathway that sprouts

from the binding of two coagulation pathways, the intrinsic and the extrinsic pathways. These common pathways stimulate fibrinogen to fibrin through a process to effect platelet plug [4]. The haemostatic paramaters being evaluated in this study among cement loaders in Port Harcourt includes; the C-reative proteins, promthrombin, Von-Willebrand factor, fibrinogen, and D-dimer.

Generally, these constituents of cement: Aluminum (Al), Silica, Chromium, Iron (fe^{3+}), and Calcium, has no recognized critical role in biological systems and has not been found to be needed for human and/or animal development, reproduction, or survival. On the contrary, component such as Al is known as a neurotoxic that may induce neurodegeneration and has been linked to a variety of brain illnesses, including Alzheimer's, Parkinson's, and multiple sclerosis [5]. Iron (Fe), calcium (Ca), and silicon (Si) exposure in the workplace has been linked to lower lung function indicators in exposed employees. Some of the systemic effects of silica have been described as hepatosplenic silicosis and hepatic porphyria, both of which have been linked to alterations in liver function indices. Persistent aluminum exposure is able to elevate lipid peroxidation in various tissues and as a result, anaemia, neurotoxicity and renal failure ensues. Silica brings about silicosis whose symptom is cough at initial stage. Acute silicosis gives rise to chest ache, fever and also trouble breathing then unexpectedly, the production of phlegm ensues. This can be accompanied with wheezing sound right inside your lungs [6]. The pathologic processes of cement dust-induced toxicities have been identified as lipid peroxidation, oxidative damage, and immunologic pathways [7]. Chromium, one of the components of cement dust has been reported to be extremely harmful. It is a powerful oxidizing element particularly Cr (VI). There is huge amount of Cr (VI) found in cement dust and this is as a result of oxidation. The penetration of Cr (VI) to very important organs like the kidney, the respiratory system (the lungs), and the liver can bring about adverse damaging effects through the production of reactive oxygen species (free radicals) and consequential inflammatory response [8]. The presence of chromium in cement makes cement a carcinogenic agent. In studies such as epidemiological researches, the consequence of cement is questionable. A part of Koh's study was to know the link between cement dust with the prevalence of cancer [9]. Iron is one of the origins of oxidants. It can be detached from met Hb. When free iron is involved, certain reactions occur. This reaction is known as Haberweiss reaction whose occurrence can be affected by the existence of ferritin found in RBCs. This ferritin, with subscribe to the search of free iron [10].

Studies conducted by various scholars in several part of the world reveals that cement dust has the ability to 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 [8]. Inhaling cement dust has been linked to changes in serum element levels, as well as lung and liver functioning, while long-term exposure reduces peak expiratory flow rate [7]. Studies shows that exposure to cement dust brings about lungs dysfunction or damage, persistent lungs ailment, lung cancer and laryngeal cancer [11]. A lot of studies have estimated the effects that 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 these continue, might lead to fibrosis, cancer or granulomatous diseases [6]. The degree of inhaled trapped dust in the body's respiratory tract is dependent or assessed by the toxin (pollutant) acidity, shape, size and grain. Cement dust has the capacity to circulate round the respiratory tract and its diameter is about 0.05 to 5.0μm. Substances which are less than 10 μm are evacuated and then sieved in the nasal opening. The substances whose diameters are

between 0.1 to 3.0 and 3.0 to 10 μm get trapped in the alveoli and bronchi. Thus, right in the bronchi and the alveoli, DNA destruction, inflammatory responses, carcinogenesis and oxidative stress are stimulated by the presence of silica and Cr (VI) [12]. Residents around cement factory could negatively be impacted by high total Cr and blood Cr values but for an improved awareness about the impact, constant check is essential. A study published by Afaghi *et al.* [13] observed that diseases like asthma, respiratory disorders and chronic bronchitis are present in asbestos cement factory workers in Port Harcourt, a result which agrees with that found cement workers in other parts of the world. The risk of asbestos comes with symptoms of phlegm, anemia, and shortness of breath, cough, wheezing, chest pain, weight loss and loss of appetite. It was found that exposed asbestos cement workers exhibited symptom of poisoning [13]. The discoveries based on a research by Rahmani, [14] finalized that the exposure to cement components as well as inhalation leads to problems of the respiratory system and over time can not only bring about destruction in the epithelium but also can be a source of inflammation if allowed to build up. 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 inflammation by way of assessing inflammatory markers in Port Harcourt. Therefore, this study will focus on assessing the effect of cement dust on inflammatory markers among cement loaders in Port Harcourt.

2.0 MATERIALS AND METHODOLOGY

Study Area

This study was carried out in Port Harcourt, Nigeria. Port Harcourt is a metropolitan city in Rivers State and it is the center of industrial activities in the state. The cross-sectional study was conducted at different cement loading cites in the metropolis.

Study Population

This study was conducted among cement workers. A total of 100 healthy male cement workers participated in the study while a total of 100 healthy male individuals not exposed to cement dust participated; this unexposed participated constituted the control group.

Eligibility Criteria

Only healthy males working as cement loaders in cement depot were recruited. They were also selected based on their age, such that only loaders between the ages of 20-60 years were selected provided they provided written consent to participate in the study. Workers not involved in loading cement were not recruited. Other non-cement workers who exposed to cement dust due to residential proximities were excluded from the control group. Those without regular job engagement (fix job) with the cement company were excluded

Ethical Clearance and Consent

Approval for the commencement of the research work was obtained from the Ethics Committee, Rivers State Ministry of Health. Consent letter was submitted by participant for study participation

Selection Method

Via randomly sampling technique, subjects were recruited in the study. Using a system of numbers between "0" and "1", all participants who picked "1" were selected while those who picked "0" were not selected.

Sample Collection Method

In the study, blood sample (4ml) was taken into a vacutainer sample bottle of 0.5 ml of 32.0 g/L trisodium citrate concentration, and appropriately mixed up to prevent clotting and was used to analyze prothrombin time and activated partial thromboplastin time. Samples for D-Dimer, VWF and fibrinogen were also collected via venipuncture technique.

Determination of PT (Prothrombin Time)

Prothrombin time assay was performed with PT reagent using coagulation analyzer.

Procedure

The PT for every sample was to be determined at least twice. The procedure had to do with either manual or semi-automated coagulation structure.

Manual Technique for PT

The reagent vials were tenderly swirled prior to use and were not shaken. For instant use, adequate reconstituted PT reagent was dispensed from the vial into a dry-neat test tube.

For 10 minutes at 37^0C the dispensed PT reagent was pre-warmed. At 37^0C 100µL of plasma was put into test cuvette and for 3 minutes was incubated. 200µL pre-warmed PT reagent was then put into the test cuvette effectively. Concurrently, the timer was started and the clotting time was finally documented in seconds.

Determination of Activated Partial Prothromboplastin Time

This assay was carried out with BioCelin reagent using coagulation analyzer.

Procedure

The reagents were tenderly swirled and properly mixed before use. In a clean-dry test tube, sufficient reagent from the reagent vial was aspirated. The reagents were brought to room temperature before they were pre-warmed at 37^0C for testing process. Prior to use, the calcium chloride solution was brought to room temperature. 0.1ml test plasma and 0.1ml BioCelin were added to 12×75mm test tubes. Test tubes were briefly shaking to properly mix up the reagent and plasma and tubes were then placed for 3 minutes at 37^0C. 0.1ml pre-warmed calcium chloride was quickly added and the stop watch was instantly started. The tubes were shaken in order to mix the content properly and then kept for 20 minutes at 37^0C. After incubation for 20 seconds, the tubes were removed, tilted gently, back and forth until gel clot formed then the watch was stopped and the specific time was recorded. A repeat was done considering a duplicated test with the use of same test plasma. The averaged was then sought from the test values duplicated. This is what is known as the patient plasma Activated Partial Thromboplastin Time.

Determination of Human D-Dimer

Assay for Human D-Dimer was performed with the use of ELISA Kits

Assay Procedure for Human D-Dimer

All reagent samples and standards were put in order based on the instruction. At room temperature, the assay was performed as every reagent was brought to room temperature prior to use. The amount of stipes needed for assay was verified and determined and were inserted in the frames prior to use. To the standard well, 50µl of standard was added. To the sample well, 40µl sample was added then 10µl of the anti-D2D antibody was added to the sample wells, followed by 50µl of strptavidin-HRP to the sample wells and standard wells respectively. They were properly mixed and with a sealer, the plate was covered. It then was incubated at 37^0C FOR 60 minutes. The sealer was taken away and with wash buffer for 5 minutes. The wells were saturated for 30 minutes to 1 minute for with 0.35ml wash buffer for every wash. 50µl substrate solution A was added to every well and 50µl substrate solution B was then added to each well. The plate was covered with a fresh sealer and incubated in the dark at 37^0C for 10 minutes. 50µl solution was then added to every well and the blue colour instantly changed and turned yellow. With the use of a microplate reader which was set to 450 nm in 10 minutes after putting stop solution, the optical density (OD) was established.

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.

Standard Range for D-Dimer

The standard curve range for D-dimer is 20pg/ml to 4000 pg/ml.

Determination of Fibrinogen Using Human Fibrinogen ELISA Kit

Procedure of Human Fibrinogen Test

Based on manufacturer's instructions, every reagent, standard solutions and samples used were prepared as instructed. Every used reagent was brought to room temperature prior to analysis. 50µl of standard was added to the standard micro well without adding any antibody to the standard microwell because the standard solution include biotinylated antibody already. After which 40µl of sample was put into the sample wells and 10µl anti-fibrinogen antibody was added to the sample wells then 50µl of streptavidin-HRP was eventually added to the sample wells and standard wells without adding to the blank control well. The plate was covered with a sealer after the mixtures were missed up. At 37 °C the plate was incubated for 60 minutes and thereafter, the sealer was removed and the plate was washed five times with the wash buffer (For each wash, the wells were soaked with 0.35 ml wash buffer for 30 seconds to 1 minute). When washing was done, the plate was blotted with the use of absorbent tissue paper. 50µl substrate solution A was then added to each well and then 50µl substrate solution B was also added to each well. The plate was covered with a new sealer and then incubated for 10 minutes at 37 °C in the dark. After the second incubation, 50µl of the stop solution was added to each well, and the blue colour changed into yellow immediately. The optical density (OD value) of each well was finally determined immediately by the use of a microplate reader that was set at 450 nm within 10 minutes after the stop solution was added.

Standard Range for Fibrinogen Factor

0.05 mg/ml to 15 mg/ml: This is the standard curve range for human fibrinogen.

Determination of von Willebrand Factor Using ELISA Kit

Procedure of von Willebrand Factor Test

Based on manufacturer's instructions, every reagent, standard solutions and samples used were prepared as instructed. Every used reagent was brought to room temperature prior to analysis. 50µl of standard was added to the standard micro well without adding any antibody to the standard microwell because the standard solution includes biotinylated antibody already. After which 40µl of sample was put into the sample wells and 10µl of anti-vWF antibody was added to the sample wells then 50µl of streptavidin-HRP was eventually added to the sample wells and standard wells without adding to the blank control well. The plate was covered with a sealer after the mixtures were missed up. At 37 °C the plate was incubated for 60 minutes and therefter, the sealer was removed and the plate was washed five times with the wash buffer (For each wash, the wells were soaked with 0.35 ml wash buffer for 30 seconds to 1 minute). When washing was done, the plate was blotted with the use of absorbent tissue paper. 50µl substrate solution A was then added to each well and then 50µl substrate solution B was also added to each well. The plate was covered with a new sealer and then incubated for 10 minutes at 37 °C in the dark. After the second incubation, 50µl of the stop solution was added to each well, and the blue colour changed into yellow immediately. The optical density (OD value) of each well was finally determined immediately by the use of a microplate reader that was set at 450 nm within 10 minutes after the stop solution was added.

Standard Range for Von Willebrand Factor

0.5ng/ml to 200ng/ml: This is the standard curve range for human Von Willebrand.

Statistical Analysis

The data collected were entered in Ms Excel and were analyzed for descriptive analysis and inferential analysis (T-test) using SPSS version 23. Significance of the test was set at p-value<0.05.

RESULTS

Comparison of Haemostatic Parameters of Control and Exposed Subjects

In **Table** 1, PT, APTT, INR, VWF, D-Dimer and fibrinogen levels were compared between Control and Exposed groups. In PT, result revealed that there was no significant difference between both groups (15.71±11.68; 16.08±3.45; *p*-value>0.05). In APTT, result revealed that there was significant difference between both groups (27.20±26.36; 33.33±10.05; *p*-value<0.05). In INR, result revealed that there was no significant difference between both groups (1.40±1.23; 1.31±0.35; *p*-value>0.05). In VWF, result revealed that there was significant difference between both groups (54.94±18.23; 66.53±31.58; *p*-value<0.05). In D-Dimer, result revealed that there was significant difference between both groups (266.50±51.40; 589.90±95.86; *p*-value<0.05). In fibrinogen, result revealed that there was significant difference between both groups (1.71±0.21; 5.30±0.54; *p*-value<0.05), respectively.

Table 1 : Comparison of Haemostatic Parameters of Control and Exposed Subject

Subjects	Parameters					
	PT (secs)	APTT (secs)	INR	VWF (ng/mL)	D-DIMER (pg/mL)	FIBRINOGEN (mg/mL)
Control (n=100)	15.71±11.68	27.20±26.36	1.40±1.23	54.94±18.23	266.50±51.40	1.71±0.21
Exposed (n=100)	16.08±3.45	33.33±10.05	1.31±0.35	66.53±31.58	589.90±95.86	5.30±0.54
T-value	0.3102	2.174	0.6447	3.178	2.974	6.169
P value	0.7567	0.0309	0.5198	0.0017	0.0033	<0.0001
Remark	NS	S	NS	S	S	S

Key: PT-prothrombin time, APPT- activated partial prothrombin time, INR- international normalized ratio and VWF- von Willebrand factor, S-significant at $p<0.05$ and NS-not significant at $p>0,05$.

DISCUSSION

This study evaluated some haemostatic parameters among individuals exposed to cement dust. 100 subjects between the ages of 20 and 60 years were selected from the population of interest (cement loaders) and another 100 subjects were recruited from non-cement loaders population to form the control group for this research. The subjects were all males as the society views this occupation as fit for only males. The data from this study showed that the exposed subjects had significantly higher levels of APTT, VWF, D-dimer and fibrinogen ($p < 0.0001$) than control subjects.

According to Akiibinu et al. [15], the constituents of cement can be inhaled or ingested into the body; some can diffuse through the lungs, and enter the circulation while others, especially larger particles, deposit in the alveolar of the lungs and cause irritation and inflammation. Also, Margetic, [16] stated that there was a link between inflammation and haemostasis, such that the activation of one leads to the activation of the other. The increase in markers of haemostasis may be due suspected inflammation arising as a result of cement dust exposure. According to Zeleke et al. [17] these cement particles can cause the activation of macrophages, mesothelial cells and fibroblasts in the lungs, which then lead to generation of free radicals with the consequent production of inflammatory cytokines like TNF - α and NFKB in individuals exposed to cement dust.

Von willebrand factor is a glycoprotein that facilitates clot formation by binding to factor VIII, platelets as well as connective tissues. In essence, it stabilizes factor VIII [18]. The increase in VWF and fibrinogen levels among cement workers may be due to inflammation. This is in

consonance with the reports from Meiring et al. [19] and Rahmani et al. [14] who reported an up-regulation of VWF and fibrinogen in the presence of inflammation.

According to Olson, [20], D-dimer is a product of the degradation of fibrin. In this study, there is a notable elevation in the levels of D-dimer among the cement dust exposed grouped compared to the non-exposed control subjects which may be due to inflammatory response as supported by the study by Kaya et al. [21] who stated that degradation of fibrin is commonly seen in inflammation.

Fibrinogen is a protein that is produced in the liver and enters the circulation. It is cleaved by thrombin and then undergoes polymerization to form clot [22]. From the study, it was observed that there was increase in fibrinogen level among the exposed cement dust subjects compared to the control group, which could imply a threat of deep venous thrombosis or pulmonary embolism. This was in agreement with the work by Klovaite et al. [23] who linked increase in plasma fibrinogen to increased risk of venous thromboembolism such as deep venous thrombosis (DVT) and/or pulmonary embolism (PE). Also, this finding was consistent with the study of Westberg *et al.* [24] and Hilt *et al.,* [25] who reported elevated fibrinogen levels in increased and reduced inhalable dust exposed subjects. Westberg *et al.* [24] study on pulp and paper industry showed a notable response for fibrinogen and many other measures with dust exposure inclusive. Numerous studies have concluded and recorded elevated levels of fibrinogen in relation to cement dust exposure of increased concentration [26, 24, 25].

Conclusion

This study has shown that human exposure, particularly, male exposure to cement ducts affects haemostatic regulation such that most haemostatic parameters are up-regulated. Haemostatic assessment may be a key indication of exposure to cement dust more especially among cement workers. Although this study did not focus on non-cement workers who were passively exposed to cement dust by way of residential proximities, one cannot underestimate the effect it may their haemostatic regulation.

Recommendation

The cement loaders should not only be educated on the importance of using adequate PPE, but the use of the appropriate PPE should be made part of the criteria for all loaders. Regular medical check-up including haemostatic assessment among loaders should also be encouraged.

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