Impact of Hours of Exposure to Cement on Haemostatic Parameters among Cement Workers

Jonathan Nyebuchi a*, Jeremiah Owubokiri Ngowari b, Collins Ohwonigho Adjekuko c, Adams Matthew Okur d and Mieiwari Ibifubara Jumbo a

a Department of Medical Laboratory Science, Rivers State University, Port Harcourt, Nigeria. 
b Department of Haematology and Blood Transfusion Science, University of Port Harcourt Teaching Hospital, Port Harcourt, Nigeria. 
c Department of Biological Sciences, University of Delta, Agbor, Nigeria. 
d Shehu Idris Institute of Allied Health Sciences, Kaduna State University-Makarfi Campus, Kaduna State, Nigeria.

ABSTRACT

Inhaling cement dust has been linked to changes in the health integrity of vulnerable groups. This study was focused at evaluating the impact of hourly exposure to cement dust on haemostatic markers in cement workers in Port Harcourt. One hundred subjected working at cement depot and sites were recruited for the study and were divided into three study groups based on hours of exposure to cement dust during work. Group 1 was 27 subjects with 1-5hourly exposure, Group 2 was 62 subjects with 6-10hourly exposure and the last group was Group 3 containing 11 subjects with over 10hours of exposure. Subjects were selected by convenient sampling technique. Blood samples were drawn using venipuncture method and samples were collected into trisodium citrate and plain bottles for PT, APTT, fibrinogen and Von Willebrand factor and D-dimer respectively. PT and APTT were assayed using manual methods but other studied parameters were assayed using ELISA. ANOVA result showed that the mean values of PT level among the classes were 15.58 ±2.11; 16.29 ±4.07 and 16.17 ±2.07 and were not significantly different (p=0.6775). Mean values for
APTT level among the classes were 35.09 ±10.06; 32.62 ±9.61 and 33.00 ±12.74 but not significantly different (p=0.5695). VWF level among the classes were 69.62 ±36.89; 64.23 ±31.32 and 71.92 ±16.17 but not significantly different (p=0.6396). D-Dimer level among the classes were 496.60 ±490.00; 457.60 ±409.80 and 519.20 ±357.70 but statistically non-significant (p=0.8661).

Fibrinogen level among the classes were 4.74 ±4.63; 5.33 ±4.67 and 6.53 ±6.09 but statistically not significant (p=0.6604). There was no significant difference in haemostatic markers levels among the groups (P-value>0.05). This study has shown that hourly exposure to cement dust does not have any key effect on haemostatic markers of cement workers in Port Harcourt.

Keywords: Prothrombin time (PT); Activated partial thromboplastin time (APTT); Von Willebrand factor (VWF); fibrinogen.

1. INTRODUCTION

The rate at which the environment is compromised due human activities such as cement production is alarming. Increased industrial activities have given rise to the production of harmful or toxic substances that are detrimental effect on human health [1,2]. The toxic substances in heavy metal and when living component interact with the environment they get exposed and bioaccumulate these toxicants which eventually cause harmful effect on them especially over a long time [3,4,5]. Cement is manufactured when limestone, quartz and/or other sources of silica, iron ore and other additives are mixed and grounded together [6]. The mixture is poured into a rotating kiln alongside burning fuels, comprising of natural gas, coal oil and/or alternative fuel source (eg, household waste, car tyres) and the heat is raised to ~1450°C [7]. The blend undergoes series of chemical reactions causing the materials to bond and form grey nodules which is then combined with other additives and gypsum to arrive at a final product, a powdery particulate substance called cement. Cement is therefore, a very fine substance possessing adhesive and cohesive features which serves as glue for the individual elements. There are more than ten varieties of cement. Cements are used mainly for construction purposes. Cement is composed mainly of Aluminum (Al), Silicon, Calcium (Ca), Chromium (Chr), and Iron (Fe³⁺) in varying percentages [6].

The process of manufacturing cement has been shown to generate and emit large amount of dust, which has become a major cause of pollution with health defects to those exposed to the dust [8]. From the production process to the finished cement, packaging and shipping, cement has been known to emit large quantity of dust due to the nature of the materials used in the process [9]. It has also been reported that chronic exposures to aluminium, an ingredient in cement could increase lipid peroxidation in different tissues resulting in anaemia, neurotoxicity and renal failure [10]. Chromium (Cr), one of the constituents of cement via scrapes of refractory lining of kiln and steel balls used in the mills [11]. It has been proven to be highly toxic and a strong oxidising agent, particularly Cr (VI) which is vastly found in cement as a result of oxidation. Consequently, toxicity is the exposure effect of Cr (VI) and it affects organs like kidney, lungs and liver [12]. This is accomplished through production of free radicals along with resultant inflammatory reactions. Hence, oxidative stress is an issue of concern. Concentrations of particular respiratory quartz leads to elevated fibrinogen and this is a notice of cardiovascular ailment amongst individuals exposed to dust [13]. 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 [14]. Carcinogens attributed to the lungs range from silica to hydrocarbon, heavy metals, polycyclic aromatic, radon etc [15]. The rest of the components of cement poses one health risk or the other to those exposed to the dust.

In conclusion, this study evaluated the effect of working hours on some haemostatic parameters among cement loaders in Port Harcourt was conducted and it was found that despite the all the defect imposed by cement dust, there was no significant differences in the parameter.

2. MATERIALS AND METHODS

2.1 Study Design

A cross sectional study designed was employed for this study. A convenient sampling size of 100 subjects was used. All subjects were healthy males working in cement depot and sites.
Subjects were classified into three groups based on hours of exposure to cement dust: group 1 (1-5 hours) which was composed of 27 subjects, group 2 (6-10 hours) which was composed of 62 subjects and group 3 (>10 hours) which had 11 subjects.

2.2 Study Area

The study was conducted 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. 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.

2.3.2 Exclusion criteria

Subjects with previous exposure to any occupational agents other than cement dust 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

Blood samples were drawn using venipuncture technique by means of vacutainer sample containers. This is in accordance to the description given by Cheesbrough, [16].

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 fibrinogen, prothrombin time and activated partial thromboplastin time.

4ml of the venous blood was lastly drawn into plain vacutainer bottles for the evaluation of Von Willebrand factor, and D-dimer, making it a round of 11.0ml of blood sample drawn from every participant.

The blood sample drawn into the trisodium citrate bottles were all for 5 minutes centrifuged at 2500rpm to get citrated plasma. This was separated, using pipettes into a sterile blood sample container for the evaluation of fibrinogen, prothrombin time (PT) as well as activated partial thromboplastin time (APTT).

The venous blood samples that were drawn into plain non-anticoagulated sample bottles were let 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 D-dimer. To the point of analysis, all drawn samples were conveyed via cold chain (ice packs/crushed ice in air tight and sealed thermo-container).

2.5 Sampling Technique

A simple random sampling method was adopted for subject recruitment to give everyone an uncompromised chances of been recruited into the study to rule out bias.

2.6 Sample Analyses

The blood samples for prothrombin time as well as activated partial thromboplastin time were assayed with the use of manual technique just as described by Quimica Clinica Applicada, South Africa, (2018). The samples for fibrinogen, D-
2.6.1 Determination of PT (Prothrombin Time) using QCA [Quimica Clinica Aplicada, SA]

Prothrombin time assay was performed with PT reagent using coagulation analyzer. The kit was manufactured by Agappe Diagnostics Switzerland.

2.6.1.1 Principle

Prothrombin time also known as Quick time is the time described in seconds following citrated plasma clotting at 37°C in the lead of adding external coagulation factor called thromboplastin and calcium ion. The test signifies in general the effectiveness of the extrinsic pathway. Unusual prolonged time measured in seconds compared to the time in seconds gotten for healthy subjects signifies paucity in some components of blood coagulation system. In the presence of ca++, activation of the extrinsic pathway of human blood coagulation cascade takes place by tissue thromboplastin. Activation time is proportional to the concentration of individual clotting factors taking part in the coagulation cascade. This assists in estimating cause and extent of haemorrhagic disorder. When thromboplastin reagent is added to citrated plasma, clotting cascade is initiated forming gel clot. The time required for clot formation would be prolonged if there is deficiency of factor(s) activity in the pathway of the coagulation cycle.

2.6.1.2 Procedure

The PT for every sample was to be determined at least twice.

2.7 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°C the dispensed PT reagent was pre-warmed. At 37°C 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.

2.7.1 Determination of activated partial prothromboplastin time using QCA [Quimica Clinica Aplicada, SA]

This assay was carried out with BioCelin reagent using coagulation analyzer. This reagent was manufactured by NS BIOTEC Medical Equipments, Egypt.

2.7.1.1 Principle

Cephaloplastin activates the coagulation factors of the intrinsic pathway of the coagulation mechanism in the presence of calcium ions. APTT is prolonged by deficiency of one or more clotting factors of the intrinsic pathway and in the presence of coagulation inhibitors like heparin.

2.7.1.2 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 prewarmed at 37°C 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°C. 0.1ml prewarmed 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°C. 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.

2.7.2 Determination of human D-Dimer

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

2.7.2.1 Principle of D-dimmer test

The ELISA plate used had already been layered with Human D2D antibody that was human précised. This D2D here was added and binds to antibodies layered on the wells. The Human
specific biotinylated D2D Antibody was then attached and fastened to D2D in the sample. Afterward, Streptavidin-HRP was put in and fastened to the Biotinylated D2D antibody. Soon after incubation, unbound Streptavidin-HRP was washed away at the stage of a washing process. After that, the Substrate solution was added and this brought about color change which progressed in proportion to the quantity of Human D2D. The D-Dimer absorbance was measured at 450nm as the reaction was established through the addition of acidic stop solution.

2.7.2.2 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 strips 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 streptavidin-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°C FOR 60 minutes. The sealer was taken away and with wash buffer for 5 minutes. The wells were saturated for 30 minutes to 1 minutes 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°C 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) value was established.

2.7.3 Determination of fibrinogen using human fibrinogen ELISA kit, bioassay

Technology Laboratory, Shanghai Korain Biotech Co., Ltd, Shanghai, China. Lot No: 2497051290

2.7.3.1 Principle of fibrinogen test

Fibrinogen analysis was done using Enzyme-Linked Immunosorbent Assay [ELISA] technique. The plate had been precoated with human fibrinogen antibody. Human fibrinogen which is present in the sample is added and it binds to antibodies that are encrusted on the wells. Thereafter, biotinylated human fibrinogen antibody is added and it binds to fibrinogen in the sample. Streptavidin-HRP is then added and binds to the Biotinylated fibrinogen antibody. In the lead of incubation, the Streptavidin-HRP that is not bound is washed off at the time of washing step. The substrate solution is then added and colour develops in proportion to the amount of human fibrinogen. The reaction is put to a stop by adding an acidic stop solution. With the use of an ELISA microplate reader, the absorbance is calculated at 450 nm wavelength.

2.7.3.2 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.
Willebrand factor antibody. Von Willebrand factor which is there in the sample is put in and thus binds to antibodies that are coated on the wells. Thereafter, biotinylated human von Willebrand factor antibody is added and it binds to von Willebrand factor in the sample. Streptavidin-HRP is then added and binds to the Biotinylated von Willebrand factor antibody. In the lead of incubation, the boundless Streptavidin-HRP is washed out during the washing step. The substrate solution is added and colour is produced based on the quantity or amount of human von Willebrand factor. The reaction is put to a stop by adding acidic stop solution. The absorbance is measured at 450 nm wavelength using an ELISA microplate reader.

2.7.4.2 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. 50 μl 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 50 μl 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 mixed up. At 37 °C the plate was incubated for 60 minutes and there after, 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.

2.8 Statistical Analysis

Data gathered from this study as subjected to statistical evaluation using SPSS version 23 for descriptive analysis (mean and standard deviation) and ANOVA. P-value was set at ≤ 0.05.

3. RESULTS

3.1 Effect of Working Hours on Haemostatic Parameters of Exposed Subjects

In Table 1, PT, APTT, INR, VWF, D-Dimer and fibrinogen levels were compared among working hours classifications: 1-5hrs; 6-10hrs; >10hrs. There was no significant difference (P-value>0.05) in PT, APTT, INR, VWF, D-Dimer and fibrinogen levels among the groups of working hours.

4. DISCUSSION

This study assessed the effect of working hours on some haemostatic parameters among cement workers. Haemostasis is an aggregate mechanism; a well linked procedure specialized in salvaging blood loss, thus restoring the damaged tissue rectitude [17]. Blood clot activator, encourages clotting with either silica particles or glass. The study according to Mojiminiyi et al. [18] concluded that dust exposure from cement distresses the roles and activities of haemopoiesis. A case study according to Ali et al. [19] was analysed and stated that shortness of breath began the day before for a 47-year-old male construction worker. He claimed that the day before he had spent the entire day mixing concrete and was constantly engulfed in a cloud of concrete dust. In this study, there was no significant difference in hemostatic parameters among various degrees of cement hourly exposure. Invariably, duration of exposure may not have any effect on hemostatic parameters.

This finding is not in agreement with many other studies conducted. A study demonstrated a positive relationship between fibrinogen values and dust exposure among construction staff that were exposed to dust [20]. The concentration of fibrinogen was reported to be elevated in smoker and non-smoker workers exposed to cement dust [21]. According to their study, it was concluded that there was a constructive correlation in the fibrinogen values. Again, there was elevated fibrinogen level seen in increased and reduced inhalable dust exposed subjects in the study of [13]. Numerous studies have concluded and recorded elevated levels of
Table 1. Effect of working hours on haemostatic parameters of exposed subjects

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Parameters</th>
</tr>
</thead>
<tbody>
<tr>
<td>Working hours (hr)</td>
<td>PT (secs)</td>
</tr>
<tr>
<td>1-5 (n=27)</td>
<td>15.58 ±2.11</td>
</tr>
<tr>
<td>6-10 (n=62)</td>
<td>16.29 ±4.07</td>
</tr>
<tr>
<td>&gt;10 (n=11)</td>
<td>16.17 ±2.07</td>
</tr>
<tr>
<td>F value</td>
<td>0.6778</td>
</tr>
<tr>
<td>P value</td>
<td>0.6778</td>
</tr>
<tr>
<td>Remark</td>
<td>NS</td>
</tr>
</tbody>
</table>

Key: PT-Prothrombin time, APTT- activated partial prothrombin time, INR- international normalized ratio and VWF- von Willebrand factor and ns= not significant at p>0.05
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 individuals before recruitment into the study.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

7. Azah N, Antai AB, Peters EJ, Osim EE. Effect of exposure to dust generated from...


