

Research Article

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Effect of occupational exposure hazards on methaemoglobin level of abattoir workers

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Abstract

The aim of this research was to evaluate the effect of occupational exposure of smoke inhalation on levels of methaemoglobin among Abattoir Workers involve in roasting of animals in Port Harcourt metropolis. A total of 40 (forty) persons all male were enrolled, which included these workers and Control Subjects that were eligible. 2ml of blood was collected and dispense into a container, containing 1.2 mg/ml of K₃EDTA and transported to a diagnostic laboratory for testing in a thermocool container without ice. Spectrophotometric method was used in determining the percentage of methaemoglobin. Significant difference in methaemoglobin level (P = 0.004) was observed between Abattoir Workers (10.52 ± 14.38) and those of control group (2.34 ± 1.19). It is therefore necessary to take into account variation in methaemoglobin level among different individual relative to occupational in a healthy population.

Introduction

Not all individuals are able to get a “white collar job” due to their level of education or sometimes inability to secure it. In order to meet with the demanding financial needs of our time, it is therefore imperative that some of these individual must keep their lives going, rather than stealing they sometimes decide to work in places they would not have loved to. One of such places is the Abattoir.

An abattoir is a slaughter house where animals are killed for the purpose of human consumption or any other accepted purpose. Both the educated and uneducated can be found in this less fancy but critical occupation and as such face with the hazards that are associated with it.

Occupational hazards are risks or dangers that are associated with a particular work or occupation. One of the hazards that Abattoir Workers are faced with is the daily inhalation of the thick black smoke coming from the burning materials like condemned motor tyres, charcoal, firewood, etc that are used in roasting the head of cow, the goat and also the cow skin. The Roasters actually are the ones that are most affected while

the Butchers are less affected because they are not involve in roasting.

Methaemoglobin is a reversible oxidation product of haemoglobin and can be present in excess amount either because of rapid oxidation of haemoglobin by drugs or toxic chemicals or because of a hereditary defect in the methaemoglobin reducing systems (Beutler, 2006).

According to Ramnik (2003), methaemoglobin which is also known as ferrihaemoglobin is a derivative of normal haemoglobin (ferrohaemoglobin) in which the iron of the haem complex has been oxidized from the ferrous to the ferric form. Toxic compounds in the form of nitrates and nitrites have been implicated as the major cause of abnormality in methaemoglobin levels of humans. The nitrates and nitrites contaminate water supplies (Beutler, 2006). It is therefore suspected that since most water source where these Abattoirs are located is highly contaminated with nitrates and nitrites, both could serve as a source of causing abnormality in the methaemoglobin level of the Abattoir Workers when they use

it and together with the smoke coming from the burnt materials and roasted animals. Well water sometimes contains high concentration of nitrates (Hoffbrand *et al.*, 2006). If such water is used by the Abattoir Workers, the risk involve is also high. When the methaemoglobin level of Abattoir Workers becomes higher than normal, they could suffer from methaemoglobinaemia.

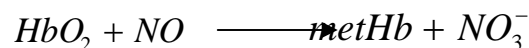
According to Ramnik, (2003), methaemoglobinaemia is used to describe the excess accumulation of methaemoglobin in the Red Blood Cell. This happens to be the major clinical condition that is likely to affect Workers in the Abattoir that are expose to the smoke that comes from tyres, burnt charcoal or firewood that is used in roasting animals, although other conditions like cyanosis (which is a blush discolouration of the skin and mucous membranes, and is usually due to a change in the colour of haemoglobin), has been observed. Cyanosis has been recognized since antiquity as a manifestation of lung or heart disease.

It is important to note at this point that Abattoir Workers are inevitable since cow meat, goat meat, and pork meat are usually used in preparing most delicacies in Africa. As a matter of fact their health and well being are very important in order for the teeming populace that enjoys their products to continually have enough to eat, hence improve on their dietary requirement. Therefore with this research, so much information that will be beneficial to them and even to the consuming population and the Scientific Community shall be revealed.

Although other research work on the effect of occupational hazards on haemoglobin level and other haematological parameters has been carried out, none particularly on methaemoglobin level of Abattoir workers in Nigeria and Port Harcourt to be precise has been seen or come across, hence this research will produce new data for medical laboratory Scientist, Doctors or Clinicians and other health professionals. Methaemoglobin is a derivative of normal haemoglobin in which the iron of the haem complex has been oxidized from the ferrous to the ferric form (Ramnik, 2003). Methaemoglobin can be also be defined as a reversible oxidation product of haemoglobin and can be present in excess amount either because of a hereditary defect in the methemoglobin-reductase system (Beutler, 2006).

Methaemoglobin does not combine with oxygen and thus does not take part in oxygen transport. In normal red cells, methaemoglobin is continually being formed by the auto-oxidation of haemoglobin, but it is reduced as soon as it is formed; thus the concentration of methaemoglobin in the red blood cells under normal conditions is less than one percent of total haemoglobin (Ramnik, 2003). Methaemoglobin has an increased affinity for oxygen and a left shifted oxygen dissociation curve (Vassilliou and Green, 2005).

According to Beutler, (2006), when NO is bound by haemoglobin, the hemoglobin is oxidized to methaemoglobin in the reaction:



Removal of NO by haemoglobin may play an important physiologic role and account for the oesophageal pain sometimes encountered in paroxysmal nocturnal haemoglobinuria and for the hypertension occurring after infusion of some experimental haemoglobin solution.

The reduction of methaemoglobin is accomplished by the enzyme NADH Methaemoglobin reductase (NADH – Cytochrome b₅ reductase) in the presence of glycolysis (Ramnik, 2003).

Methaemoglobin also result from its reaction with certain drugs such as phenacetin or sulphonamides (Ochei and Kolhatkar, 2000). Methaemoglobin is formed intracellularly and is not found in plasma except when their formation is associated with intravascular haemolysis (Lewis and Roper, 2006).

According to Ganong (2005), methaemoglobin is dark cloured, and when it is present in large quantities in the circulation, it causes a dusky discolouration of the skin resembling cyanosis. Some oxidation of haemoglobin to methaemoglobin occurs normally, but an enzyme system in the blood cell, the NADH – Methaemoglobin reductase system converts methaemoglobin back to haemoglobin.

Methaemoglobin levels vary but may be as high as 40% of the total haemoglobin (Wild and Bain, 2006). The haematological value for normal adults expressed as a mean $\pm 2SD$ (95% range) is less than 2% (Lewis, 2006). Levels of methaemoglobin exceeding 60 to 70% of the total pigment may be associated with vascular collapse, coma and death, but recovery was documented in one patient with a level as high as 81.5% of the total pigment (Beutler, 2006). Cyanosis occurs when methaemoglobin constitute about 15% of the total pigment. When the concentration of methaemoglobin reaches 30-40% anoxic symptoms commonly develop. In case of acute poisoning, the concentration may exceed 60 to 70% (Ramnik, 2003).

According to Lewis and Roper, (2006), methaemoglobin is present in small amounts in normal blood and constitute 1 to 2% of the total haemoglobin. Its concentration is very slightly higher in infants, especially in premature infants, than in older children and adults. About 10% of methaemoglobin which is equivalent to 15g/l can lead to cyanosis.

The term methaemoglobinaemia is used to describe the excess accumulation of methaemoglobin in the red blood cell. Methaemoglobin lacks the capacity to carry oxygen, and methaemoglobinaemia causes symptoms and signs of hypoxia. The great majority of cases of methaemoglobinaemia are due to the action of chemical agents that increase the rate of auto oxidation of haemoglobin in the red blood cells (Ramnik, 2003).

According to Hoffbrand *et al.* (2006), methaemoglobinaemia is a clinical state in which circulating haemoglobin is present with iron in the oxidized (Fe^{+3}) instead of the usual Fe^{+2} state. It may arise because of a hereditary deficiency of reduced nicotinamide adenine dinucleotide (NADH) diaphorase or inheritance of structurally abnormal haemoglobin (HbM). These contain an amino acid substitution affecting the haem pocket of the globin chain.

According to Vassilliou and Green (2005), methaemoglobin has an increased affinity for oxygen and a left-shifted oxygen dissociation curve. Pathological acquired methaemoglobinaemia can result from exposure to strong oxidants (e.g. dapsone, paraquat, benzocaine) and can be life-threatening when severe but is rarely sufficiently long-lived to give rise to polycythaemia.

According to Bradberry *et al.* (1994), methaemoglobinaemia has occurred as a result of accidental contamination of drinking water with sodium nitrite.

Toxic methaemoglobinaemia occurs when a drug or other toxic substance oxidizes haemoglobin and the patient is likely to show cyanosis (Hoffbrand, 2006). Occupational cause of toxic methaemoglobinaemia in industries is most commonly due to absorption of nitro and amino aromatic derivatives. The substances are usually absorbed through the respiratory tract or skin, and the disorder is most often seen in workers in chemical factories and explosive plants. Well water sometimes contains high concentration of nitrates, and in country areas the use of this water to prepare milk mixtures for infants has resulted in methaemoglobinaemia (Ramnik, 2003).

It can be infer that the source of water used by abattoir workers in Port Harcourt is contaminated with nitrogenous matter and therefore polluted with nitrates and nitrites, as this water body or steam is a prerequisite for slaughter house or abattoir citing. Excreta of the cows and goats are usually washed down by erosion into the stream or water body.

According to Beutler, (2006), nitrates, and nitrites contaminating water supplies or used as preservatives in foods are also common offending agents that can provoke toxic methaemoglobinaemia.

According to Gordon-Smith *et al.* (2005), nitrites in water or vegetable juices may cause methaemoglobinaemin in infants who have a physiological impairment of the reducing systems. Well water that comes from land with an excess of nitrites and which is used to reconstitute artificial feeds has produced cyanosis in infants. Cases have also been reported and described following the enthusiastic feeding of juice from carrots grown on artificially or organically fertilized land and of spinach juice.

AIM

The aim of this research is to evaluate and determine the effect of smoke produced during roasting of slaughtered animals on methaemoglobin levels of Abattoir Workers.

OBJECTIVES

The objectives of this work are:

To assess levels of methaemoglobin among Abattoir Workers and control subjects.

To provide information to the Scientific Community.

Materials and Method

EXPERIMENTAL DESIGN

The research is a case control study which is primarily aimed at evaluating and assessing the methaemoglobin levels.

SUBJECTS/SETTINGS

A total population of forty (40) subjects which consists of twenty (20) Abattoir Workers and twenty (20) control subjects were recruited for the study between with ages between 18 and 55 years.

For Abattoir Workers, twelve (12) of them were source from mile 3 Slaughter in Port Harcourt and eight (8) of them were source from Eastern-By-pass Slaughter in Ogbunabali, Port Harcourt. For the control subjects, eleven(11) of them were source from Chinwo Orowere Street, Ogbunabali, Port Harcourt and nine (9) of them were source from Okija Electrical Spare Part Market, Port Harcourt, they were apparently healthy and not on drugs.

CONSENT

Informed consent was obtained from individuals recruited for the study.

COLLECTION OF SAMPLES

Blood sample was collected between 10.00 – 11.30 am by venipuncture, 3ml of blood sample was introduced into an EDTA container and was transported in a thermocol container without ice to Tropmel (Tropical Medical Laboratories), 32 Emekuku Street, D/line, Port Harcourt. Where the samples were analyzed.

HAEMATOLOGICAL ANALYSIS

Manual methods for methaemoglobin measurement in blood, by Evelyn and Malloy were carried out.

PRINCIPLES FOR DETERMINATION OF METHAEMOGLOBIN

Methaemoglobin (Hi) has a maximum absorption at 630nm. When cyanide is added, this absorption band disappears and the resulting change in absorbance is directly proportional to the concentration of Hi. Total Haemoglobin in the sample is then measured after complete conversion to HiCN by the addition of ferricyanide – cyanide, reagent. The conversion will measure oxyhaemoglobin and methaemoglobin but not sulphaemoglobin. Thus, the presence of a large amount of sulphaemoglobin will result in an erroneously low

measurement of total Haemoglobin. Turbidity of the haemolysate can be overcome by the addition of a nonionic detergent.

Calculation:

$$\text{Methaemoglobin Hi(\%)} = \frac{D_1 - D_2}{D_3 - D_4} \times 100$$

REAGENTS

- Phosphate buffer: 0.1mol/L, P^H 6.8
- Potassium cyanide: 50g/L
- Potassium ferricyanide: 50g/L
- Non Ionic Detergent: 10g/L

LABORATORY PROCEDURE

Lyse 0.2ml (200ml) of blood in a solution containing 4ml of buffer and 6ml of detergent solution. Divide the Lysate into 2 equal volumes (A and B). Measure the absorbance of A with a spectrophotometer at 630nm wavelength (D₁). Add I drop of potassium cyanide solution and measure the absorbance again, after mixing (D₂). Add I drop of potassium ferricyanide solution to B, and after 5 minutes, measure the absorbance at the same wavelength (D₃). Then add I drop of potassium cyanide solution to B and after mixing make a final reading (D₄). N/B: All the measurements are made against a blank containing buffer and detergent in the same proportion as present in the sample.

DATA REPORTING

Results of Methaemoglobin level were expressed in percentage.

STATISTICAL ANALYSIS

Data were analyzed by using SPSS 17.0 for windows statistical package. The test distributions were tested to be

normal using one sample kolmogorov–smirnov test. The sample populations were grouped into Abattoir Workers and Control Subjects distribution. Student T. Test was used to test for difference between Abattoir Workers and Control Subjects. An error probability (P = 0.004) was considered significant.

Results

DEMOGRAPHIC DETAILS OF PARTICIPANTS

A total of forty (40) subjects were enrolled into this study, twenty (20) Abattoir Workers and twenty (20) Control Subjects that were apparently healthy, all males. The age range was between eighteen (18) to fifty-five (55) years. These details are listed in table 1.

2 COMPARISON OF METHAEMOGLOBIN LEVEL BETWEEN ABATTOIR WORKERS AND CONTROL SUBJECTS

The values of methaemoglobin level were compared for the mean and standard deviation between Abattoir Workers and Control Subjects. Table 2 is a detail list of the statistics.

3 COMPARISON OF BODY MASS INDEX (BMI) OF ABATTOIR WORKERS IN RELATION TO THEIR METHAEMOGLOBIN LEVELS

The body mass indexes (BMI) of Abattoir Workers were grouped and the mean methaemoglobin level for each groups were calculated. These details are listed in table 3.

4 COMPARISON OF ABATTOIR WORKERS NUMBERS OF YEARS EXPOSURE TO SMOKE IN RELATION TO THEIR METHAEMOGLOBIN LEVELS

The numbers of years the Abattoir Workers have worked in the abattoir and expose to the smoke were grouped and the mean methaemoglobin levels for each group based on years of exposures were calculated. Details of these are listed in table 4.

Table 1: Demographic details of Participants

Parameter	Number of Persons/Age
Total Number of subjects	40
Total Number of Abattoir Workers	20
Total Number of Control Subjects	20
Age range	16 to 55 years
Weight range (Kg)	54 – 98
Height range (m)	1.50 – 1.80
BMI	20.20 – 33.71

Table 2 Comparison between Methaemoglobin Levels of Abattoir Workers and Control Subjects.

Parameter	Methaemoglobin (%) Mean ± SD
Abattoir workers	10.52 ± 14.38*
Control Subjects	2.34 ± 1.19

* Significant difference observe (P = 0.004) using students t-test.

Table 3: Comparison of Body Mass Index (BMI) of Abattoir Workers in Relation to their Methaemoglobin Levels.

BMI – Ranges	Mean Methaemoglobin (%)
20.00 – 21.99	18.53
22.00 – 23.99	10.08
24.00 – 25.99	5.64
26.00 – 27.99	4.94
28.00 – 29.99	12.11

BMI has no significant role to play in maintaining methaemoglobin levels of Abattoir Workers within limits of normal.

Table 4: Comparison of Abattoir Workers Numbers of Years Exposure to Smoke in Relation to their Methaemoglobin Levels

Number of Year of Exposure	Mean Methaemoglobin Level (%)
1 – 5	9.59
6 – 10	12.69
11 – 15	14.69
16 and above	4.56

Number of years of exposure has no significant role in keeping methaemoglobin levels of Abattoir Workers within limits of normal.

Discussion

Actually research on methaemoglobin levels among Abattoir Workers in Port Harcourt have not been documented, but effect of other occupational hazards on methaemoglobin levels of Industrial Workers have been carried out and documented.

This research aims at assessing the levels of methaemoglobin in Abattoir Workers and comparing the value obtained with that of Control Subjects that were apparently healthy. From the results obtained in this research work there was a significant increase (P = 0.004) in the percentage methaemoglobin level in Abattoir Workers than Control Subjects. There was an increase from the level of (2.34 ± 1.19) in normal Control Subjects to a level of (10.52 ± 14.38) in Abattoir Workers as indicated in Table 2.

The result of methaemoglobin level in Abattoir workers in this research work (10.52 ± 14.38) agrees with the observation made by Ramnik (2003), in his book in which he observed in a case of acute poisoning as a result of industrial exposure to fumes, levels that reaches 60% in workers of such industries where they are exposed to hydrocarbon fumes, and absorption of nitro and amino aromatic derivatives through their respiratory tract or skin, resulting in Methaemoglobinaemia and also cyanosis when in much higher percentages. This in turn affects oxygen delivery to tissues and body cells as methaemoglobin has a stronger affinity to oxygen than the normal haemoglobin.

It was stated by some Abattoir Workers who were seriously involved in the roasting of slaughtered animals that they normally have complained respiratory difficulties and some times, lungs or heart problems while carrying out this research work.

A higher percentage level of 60 percent was noticed in one of the Abattoir Workers who both smokes heavily and has also spend about 15 (fifteen) years working in the Abattoir.

The Body Mass Index of Abattoir Workers do not have enough role to play in decreasing methaemoglobin levels of Abattoir Workers, as almost all them have a higher than normal methaemoglobin levels as seen in Table 3, when there mean methaemoglobin levels were compared to a grouped body mass index.

It was also observed that there is no much significant difference when the mean methaemoglobin levels of Abattoir Workers were considered based on the number of years they have worked, and become exposed to smoke that emanate in cause of burning or roasting slaughtered animals (Table 4). Those who have worked for up to a year still have higher values when compared to those who have worked for about ten years! Based on this, one can infer that number of years does not necessarily increase the level of methaemoglobin but the amount that is inhaled into the system at a particular time is what causes the increase in methaemoglobin level. Therefore the result obtained in this research work agrees with that written in the book of Ramnik (2003), and that of Wild and Bain (2006).

According to Vassiliou and Green, (2005), the increase affinity of methaemoglobin for oxygen could lead to the displacement of oxygen from haemoglobin and this in turn lowers the oxygen carrying capacity of blood which can result to suffocation and an eventual death that may likely occur.

Limitations in the course of this research includes: difficulties in getting more Abattoir Workers who work in the different Abattoir to submit themselves for testing because of their ignorance and low level of education, and superstitious beliefs; which explains our inability to get up to 30 (thirty) willing Abattoir Workers to submit themselves for the research.

Getting the reagents or kits for testing mathaemoglobin level was a little bit difficult as these kits are not commercially and easily available since the test is not a routine test in government owned or private laboratories.

However, this research work successfully analyzed the levels of methaemoglobin in Abattoir Workers which when compared with that of Control Subjects, there was a significant high increase in methaemoglobin level, and this was further proved when our own levels were tested before we carried out the research, and then we exposed ourselves to the smoke that emanated from the burnt tyres, firewood and animals, subsequently took our own blood samples immediately for analysis, which was also found to be higher than before we were exposed to the smoke!

Conclusion

Significant difference in methaemoglobin level ($P = 0.004$) was observed between Abattoir Workers (10.52 ± 14.38) and those of control group (2.34 ± 1.19). It is therefore necessary to take into account variation in methaemoglobin level among different individual relative to occupational in a healthy population. Animal roasting with motor tyre should be stop and strictly condemn while fire wood should serve as the burning material. Government at all levels should see to the enforcement of laws prohibiting the use of motor tyre in roasting animals. Abattoir Workers should use Nose mask while roasting so as to reduce the amount of smoke they inhale into their system. Sanitation should be carried out regularly on weekly basis and enforced by government agencies so as to reduce or avoid contamination of water used by the Abattoir Workers with nitrates and nitrites.

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