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DETERMINATION OF LEVELS OF METHAEMOGLOBIN IN SICKLE CELL PATIENTS IN ABAKALIKI, EBONYI STATE, NIGERIA.

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ABSTRACT

The levels of methaemoglobin were studied in twenty confirmed sickle cell patients (12 females and 8 males) from Ebonyi State University Teaching Hospital, Abakaliki and from private laboratories. Another twenty (20) apparently healthy individuals (12 females and 8 males) served as controls. The age ranges of subjects were 1 -20 years (14 subjects) and 21 – 40 years (6 subjects). Spectrophotometric analysis showed that the mean methHb levels of the test was 4.78% compared to mean of control 1.70%, which was significant (P<0.05). Spectrophotometric analysis showed that the mean MetHb level of subjects aged 1-20 years was 5.53% compared to mean of subject aged 21-40 years 4.23% which was not significant (P>0.05). Also, spectrophotometric analysis showed that the mean metHb levels of females was 4.71% compared to the mean of males 4.84%, which was not significant (P>0.05). This therefore suggests that antioxidant activity in sickle cell anaemia was some how impaired or that the rate of production of oxidized haemoglobin overwhelms the antioxidant activity.

Keywords: Methaemoglobin, Spectrophotometric analysis, Ebonyi State, Abakaliki and Sickle cell patients.

INTRODUCTION

Normally, methaemoglobin levels are 1-2% using spectrophotometric method according to Dacie *et al.,* (2006) in healthy humans. Elevated levels of methaemoglobin in the blood are caused when the mechanism that defend against oxidative stress within the red blood cells are overwhelmed and the oxygen carrying ferrous ion (Fe^{2+}) of the haem group of the haemoglobin molecule is oxidized to the ferric state (Fe^{3+}) according to Barker *et al*., (2001).

Methaemoglobin results from oxidation of the iron moieties in haemoglobin from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state according to Hoffman (1995). Normal oxygenation of haemoglobin causes a partial transfer of an electron from the iron to the bound oxygen. Iron in this state thus resembles superoxides (O_2). Deoxygenation returns the electrons to the Iron, with release of oxygen. Methaemoglobin levels in human are in fact maintained at ≤ 1% by the methaemoglobin reductase enzyme system (nicotinamide adenine dinucleotide [NADH] – dehydratase, NADH – diaphorase, erythrocyte cytochrome b5 reductase). This enzyme reduces haemoglobin iron by transfer of an electron from NADH to oxidise cytochrome b5; cytochrome b5 then convers ferric to ferrous iron by direct interaction with haemoglobin. The generation of NADH depends on the glycotic pathway (Serjeant, 1992).

A second reducing enzyme, nicotinamide adenine dinucleotide phosphate (NADPH) – dependent methamoglobin reductase, does not normally function in erythrocytes because no electron carrier is available in erythrocytes to interact with NADPH. Exogenous electron carries, such as methylene blue, can therefore serve as pharmacologic agents for the treatment of methaemoglobinemia. Reduced glutathione and ascorbic acid reduce methaemoglobin directly, but these nonenzymatic reactions are considerably slower than the reductase pathways (Brewer and Prassad, 1993, Scott *et al,* 1971 and Gibson, 1948). Meanwhile, sickle cell disease is a generic term for a group of generic disorders characterized by the predominance of the Hbs. These disorders include sickle cell anaemia, sickle cell β – thalassemia syndrome and haemoglobinopathies in which haemoglobins occurs in association with another abnormal haemoglobin.

These disease is found in people of African, Mediterranean, Indian, and Middle East heritage. In united State of America, it is observed in blacks and people of Hispanic origin (Cheesbrough, 2000).

For the purpose of this research, the work will be restricted to the determination of levels of methaemoglobin in sickle cell anaemia which is the commonest form of sickle cell disease found within the area of this research.

Sickle cell disease is an inherited multisystem disorder. Its cardinal features, chronic haemolytic anaemia and recurrent painful episodes, relates to the presence of mutant sickle cell haemoglobin S within red blood cell. The illness affects most organ systems and the psychosocial adjustment of these affected. Traditional concepts of sickle cell pathpphysiology ascribe all features of the disease to the change from sequential affects of the A – T nucleotide substitution in the sixth position of the β- globin gene, resulting in substitution of valine for glutamic acid on the outer surface of the HbS molecule, reduced solubility, sickling and occlusion by sickle red cells microvasculature (Hoffbrand *et al*, 2001).

In addition to its abnormal electrophoretic mobility and solubility, HbS is unstable. Consequences of HbS instability are the increased generation of methaemoglobin and release of haem, processes that contribute to the increased generation of oxidative radical by sickle red cells. In addition, the normal physiological process of haemoglobin oxygenation and deoxygeneration generate methaemoglobins and oxidative stress within sickle red cells

that has major pathophysiologic importance to metabolism, membrane lipids, membrane proteins, and the integrity of the HbS molecule itself (Bender *et al,* 1981, Adachi *et al*, 1980, Asakura *et al*, 1973 and Macdonald and Charache, 1982).

Oxidative stress on red cell metabolism is particularly important to sickle cell pathophysiology. Impaired reductive defense mechanisms potentiate other sources of free haeme within sickle red cells inhibits the activity of several enzymes needed to generate NADH and NADPH for protection against oxidation. Sickle cells have been found to have decreased NADH redox potential, hexosemonophorphate shunt activity and GSH content (Zerez *et al*, 1988, Schrader *et al*, 1993 and Lachant, 1983). Because of the damaging effect of high levels of methaemoglobin on the red cell patient, it is necessary to determine the levels of methaemoglobin in the patients and take adequate treatment instantly to avoid the attendant consequences and that call for this research for the improved life and longevity of the affected patients.

AIMS AND OBJECTIVES

The aims and objectives of this study include;

a. To determine the levels of methaemoglobin in sickle cell patients
b. To compare the level obtained with that of normal individuals.

MATERIAL AND METHODS

SUBJECTS

Twenty (20) confirmed sickle cell subjects from different places including Ebonyi state University Teaching Hospital, Abakaliki, Federal Medical centre, Abakaliki, Unversity of Nigeria Teaching Hospital, Ituku Ozara, Enugu, Enugu state, and so many other places were selected. Twelve (12) subjects were females while eight (8) were males. Fourteen (14) subjects were aged 1-20 years while six (6) were aged 21-40 years. Veinous blood for analysis was obtained from subjects after informed consent using materials and standard methods at JEM Diagnostic laboratory, Ogaja Road, Abakaliki, Ebonyi state. Blood was also collected from 20 apparently healthy subjects (12 female and 8 males) and was used as the control.

STANDARD METHODS FOR BLOOD COLLECTION AND PRECAUTION FOR ACCURATE DETERMINATION OF LEVELS OF METHAEMOGLOBIN

1. A sterile, dry plastic syringe was used.
2. A tourniquet was applied in the upper arm of the subject and was asked to make a fist to enable the veins to be seen and felt.
3. The puncture area was then cleaned with methylated spirit and then allowed to dry
4. Using the index finger, the antecubital vein was searched for and felt.
5. He venepunture made with the bevel of the needle directed upwards in the line of the vein, with the thumb of the left and holding down the skin below the puncture site.
6. The planger of the syringe was the steadily withdrawn allowing the blood from the vein to fill the syringe
7. 2ml of blood were added to clean EDTA containers.

8. The blood and anticoagulant were mixed thoroughly by gentle inversion several times.

The control samples were similarly treated. Thereafter, the samples were ready for testing.

PRECAUSION FOR ACCURATE DETERMINATION OF METHAEMOGLOBIN ACCORDING TO DACIE *ET AL* .,(2006)

The test should be carried out within 1 hour of collection of the blood. After dilution, the buffered lysate can be stored for up to 24 hours at 2 – 4^{o}C without significant autoxidation of Hb to Hi.

DETERMINATION OF METHAMOGLOBIN

PRINCIPLE: 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 Hb in the sample is then measured after complete conversion will measure oxyhaemoglobin and Hi but not SHb. Thus, the presence of a large amount SHb will result in an erroneously low measurement of total Hb. Turbidity of the haemolysate can be overcome by the addition of a non-ionic detergent according to Dacie *et al*., (2006).

RESULTS

N = number of subjects

MetHb = methaemoglobin

SD = standard deviation

P – value = level of significance

Subjects (n)	Mean of MetHb ± SD%	P – Value
Test (20)	4.78% ± 2.25	Significant
Control (20)	1.70% ± 0.40	

Table 1:MEAN VALUE OF METHAEMOGLOBIN FOR TEST AND CONTROL

Age (n)	Mean of MetHb ±SD%	P- value
1-20 years (14)	4.53%± 0.35	Not significant
21 – 40 years (6)	4.23% ± 3.5	

Table 2: MEAN VALUES OF METHAEMOGLOBIN FOR THE AGE RANGES

N = number of subjects

MetHb = methaemoglobin

SD = standard deviation

P – value = level of significance

Sex (n)	Mean of MetHb ± SD%	P – value
Female (12)	4.71%±3.2	Not significant
Male (8)	4.84%±1.3	

Table 3: MEAN VALUES OF METHB FOR THE SEXES

N = number of subjects

MetHb = methaemoglobin

SD = Standard deviation

P – value = level of significance

DISCUSSION, CONCLUSION AND RECOMMENDATION

DISCUSSION

Methaemoglobin is a conversion product of haemoglobin molecule. The levels are stringently controlled by various biochemical processes to ensure homeostasis.

The result of methaemoglobin determination showed significant difference with the control ($P < 0.05$). This implies that sickle cell anaemia adversely affected haemoglobin metabolism to produce methaemoglobin metabolism and methaemoglobinemia. The levels obtained in the subjects are not higher than the findings elsewhere, it agrees with other people's finding as there were no symptoms observed which is in accordance with http://www.emedicine.com/med/topic1466.htm which opines that healthy people may not have many symptoms with methaemoglobin level (< 15%),

however patients with co-morbidities such as anaemia, cardiovascular disease, lung disease, sepsis or presence of other abnormal haemoglobin species such as sickle haemoglobin may experience moderate to symptoms (as low as 5-8%). The results obtained are slightly higher than that of healthy persons which according to Winterbourn and Carrel (1995) opined that about 1-3% of haemoglobin is converted to methaemoglobin on daily basis and without an effective reducing system, the erythrocyte becomes non- functional. The impaired MetHb reduction and absence of MetHb from sickle erythrocytes, despite increased generation of methaemoglobin (Zerez *et al.*,1990) suggest rapid conversion of MetHb to another form which promotes recognition and removal by macrophages which ensure that the level is still normal despite increased generation. This slight increase observed may be as a result of increase in body temperature as they experience fever due to frequent crisis, source of drinking water and bacteria activity on the nitrate in the gastrointestinal tract (GIT) as well as the use of some household equipment such as shoe polish which contains nitrobenzene.

This can be corroborated by the fact that the subjects studied were not suffering from methaemoglobin when age (1-20 years and 21-40 years) and sex were used in comparism of test and control groups, the same pattern of result was obtained but it is slightly higher in younger subjects and males.

CONCLUSION

There is slight increase in the level of methaemoglobin in the sickle cell patients studied in comparism with healthy person. This could be due to haemolytic crisis, bacterial activity, body temperature increase, nutrition, self medication, unstable sickle cell, occupation and environmental temperature.

RECOMMENDATION

It is therefore recommended that

a. Levels of methaemoglobin should be studied in different geographical areas.
b. Other conditions not related to congenital methaemoglobinemia like source of drinking water, shoe polish, furniture, drug history, nutrition, temperature changes, e.t.c should be borne in the mind when carrying out the research to avoid conflicting of results.

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