



## Assessment of Methaemoglobin in Haemoglobin Variants in Selected Ethnic Groups in Bayelsa State

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### Authors' contributions

*This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.*

### Article Information

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### ABSTRACT

Methaemoglobin (Met-Hb) is a type of the oxygen-carrying metalloprotein hemoglobin. The heme group iron exists as ferric (Fe<sup>3+</sup>) iron, rather than the ferrous (Fe<sup>2+</sup>) iron of typical hemoglobin. Met-Hb is unable to perform the function of binding to oxygen like oxyhaemoglobin does. The aim of this study was to compare methaemoglobin levels between AA and AS haemoglobin variants among the Ijaw, Igbo and Yoruba ethnic groups residing in Bayelsa State, Nigeria. A total of 150 subjects were enrolled for the study. One hundred and sixteen subjects constituted the Ijaws; 21 Igbos and 13 Yorubas. For each subject, 4mls of blood sample collected in EDTA bottle was assayed for methaemoglobin using a spectrophotometric method. Results revealed there was no significant difference in the methaemoglobin mean levels between the AA and AS haemoglobin variants (P-value > 0.05) of the ethnic groups except the Igbo ethnic group (P-value < 0.05). However, comparing the methaemoglobin mean levels among the ethnic groups showed a significant mean difference of methaemoglobin (P-value < 0.05). All Post-hoc groups showed significant difference except the Igbo and Yoruba ethnic groups (P-value > 0.05). In conclusion, this study has revealed that methaemoglobin levels change significantly based on studied tribes but does not change based on studied haemoglobin variants.

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**Keywords:** Haemoglobin variant; methaemoglobin; Ijaw; Igbo; Yoruba.

## 1. INTRODUCTION

The word 'genotype' is also used to describe a gene or a set of genes that determine a particular trait of an organism. There are haemoglobin genotypes of humans which include; AA, AS, AC, SS, SC, and erythrocytes are involved in the determination of the genotype of humans. Human erythrocytes consist of haemoglobin, an iron-containing, oxygen-transport protein. Haemoglobin molecule is composed of two pairs of globin proteins and a heme part. The two pairs of globin proteins are composed of  $\alpha$ -2 and  $\beta$ -2 globin chains. Any amino acid substitutions that occur in these chains bring different types of hemoglobin. These amino acid substitutions in the globin proteins are classified under thalassemia. Haemoglobin variants change hemoglobin organization and chemical characteristics causing insignificant to severe physiological effects [1]. Haemoglobinopathies are among the most common genetic disorders globally, with sickle cell disorders and the thalassaemias being the most common, and occur in individuals of African, Asian, South European and Middle Eastern descent [2]. Methaemoglobin (Met-Hb) is a type of the oxygen-carrying metalloprotein hemoglobin. The heme group iron exists as ferric ( $\text{Fe}^{3+}$ ) iron, rather than the ferrous ( $\text{Fe}^{2+}$ ) iron of typical hemoglobin. Met-Hb is unable to perform the function of binding to oxygen like oxyhaemoglobin does. A minute concentration of Met-Hb is usually produced spontaneously in humans, but when the methaemoglobin concentration becomes so high above the normal, the blood thus turns to unusually dark bluish brown. The NADH-dependent enzyme methemoglobin reductase (diaphorase I) converts methaemoglobin back to hemoglobin. The presence of high concentration of methaemoglobin had a hereditary implication [3]. Normally, about one to two percent of an individual's hemoglobin is composed of Met-Hb. Exposure to different chemicals can bring about higher concentration of Met-Hb than normal. Factors that can predispose methaemoglobinemia include; contact to oxidant drugs or toxins, genetic modifications in erythroid methaemoglobin reductase enzyme systems [4,5], variants in the globin chain. Increased concentration of Met-Hb can also be due to genetic disorder. A high concentration above the normal level can result in a health problem called methaemoglobinemia [6]. A study of the

presence of methaemoglobin in a population could be useful especially in drug administration and certain pathological conditions. This study is intended to compare the methaemoglobin level between AA and AS haemoglobin variants in different ethnic groups.

## 2. MATERIALS AND METHODS

### 2.1 Study Area

The study was conducted among Ijaw, Igbo and Yoruba subjects of the Niger Delta region of Nigeria who resides in Yenagoa Local Government Area of Bayelsa State, Nigeria. Bayelsa state is located within Latitude  $4^{\circ} 15'$  North and Latitude  $5^{\circ}$  and  $23'$  South [7].

### 2.2 Study Population and Sample Size Description

The study population consisted of apparently healthy male and female Ijaws, Igbos and Yorubas who resides in Yenagoa Local Government Area, Bayelsa State of Nigeria. A total of 150 subjects were enrolled for the study. 116 subjects constituted the Ijaws; the remaining 34 comprised two tribes among the three major ethnic groups of Nigeria; 21 Igbos and 13 Yorubas. All subjects were aged between 16 and 48 years in the general population and included both males and females.

### 2.3 Subjects Eligibility Criteria

All the subjects utilized for the study were apparently healthy as portrayed by the research clinician. Subjects with history of diabetes mellitus and other chronic diseases were excluded from the study. More so, subjects that were not origins of Ijaw, Igbo and Yorubas were not included in this study.

### 2.4 Specimen Collection

Four milliliters of blood samples were collected from the subjects utilizing standardized phlebotomy venepuncture method [8,9,10]. Blood was withdrawn into EDTA for methaemoglobin evaluation.

### 2.5 Sample Analysis

MetHaemoglobin analysis was performed using quantitative spectrophotometric method [11].

Two milliliters of plasma were added to iso-osmotic phosphate buffer of pH 7.4. The mixture was centrifuged for 30 minutes at 1200 – 1500g and its absorbance measured spectrophotometrically at 630nm wavelength. About 5mg of solid sodium dithionite was added to the diluted plasma, the tube was shaken gently to dissolve the dithionite and allowed to stand for 5 minutes for complete reduction of the methaemalbumin. The absorbance was remeasured and the difference between the two readings was taken as the absorbance due to methaemalbumin.

## 2.6 Statistical Analysis

Data were analyzed with Statistical Package for Social Sciences (SPSS) version 20, and Microsoft excel. Student *t*-test was used for comparing methaemoglobin levels between AA and AS haemoglobin variants in each of the ethnic groups. ANOVA and Post-hoc analysis were used for comparing methaemoglobin levels

among the ethnic groups and  $p \leq 0.05$  was considered significant.

## 3. RESULTS

When MetHb was compared, a mean value of  $12.01 \pm 1.90$ g/dl for AA and  $12.19 \pm 1.69$ g/dl for AS as shown in Table 1. No significant difference was observed between groups for all parameters,  $p > 0.05$  using independent *t*-test.

Significant difference was observed among the groups for MetHemoglobin,  $p < 0.0001$ . Igbo showed a mean MetHb concentration of  $13.08 \pm 1.39$  (g/dl), Ijaw,  $11.78 \pm 1.88$  (g/dl), and Yorubas,  $12.86 \pm 0.91$ (g/dl).

MetHb was compared with a mean value of  $11.80 \pm 2.06$ g/dl for AA and  $11.76 \pm 1.52$ g/dl for AS as shown on Table 3. No significant difference was observed between groups,  $p > .05$ , using independent *t*-test.

**Table 1. Comparing methaemoglobin levels based on haemoglobin electrophoretic patterns**

Parameters	AA(n=99)	AS(n=51)	p-value
MetHb(g/dl)	$12.01 \pm 1.90$	$12.19 \pm 1.69$	0.54 NS

Key: MetHb = Methaemoglobin.

**Table 2. Comparing methaemoglobin levels based on ethnic groups**

Ethnic Groups	MetHb(g/dl)
Igbo(A)(N = 21)	$13.08 \pm 1.39$
Ijaw(B) (N= 116)	$11.78 \pm 1.88$
Yoruba(C) (N = 13)	$12.86 \pm 0.91$
<i>p</i> -value	<0.0001
A vs B	S(0.0007)
A vs C	NS(0.59)
B vs C	S(0.002)

Key: MetHb= Methaemoglobin; N = No. of subjects; S = Significant; NS= Not Significant; Post hoc testing was done using Games-Howell

**Table 3. Comparing methaemoglobin between haemoglobin variants amongst the Ijaws**

Parameters	AA(n=75)	AS(n=41)	p-value
MetHb(g/dl)	$11.80 \pm 2.06$	$11.76 \pm 1.52$	0.92 NS

Key: MetHb = Methaemoglobin; NS = Not Significant.

**Table 4. Comparing methaemoglobin between haemoglobin variants amongst the Igbos**

Parameters	AA(n=15)	AS(n=6)	p-value
MetHb(g/dl)	$12.66 \pm 1.21$	$14.12 \pm 1.33$	0.03 S

Key: AA = Haemoglobin AA phenotype; AS = haemoglobin AS phenotype; MetHb = Methaemoglobin; NS= Not Significant; S= Significant

**Table 5. Comparing methaemoglobin between haemoglobin variants amongst the Yorubas**

Parameters	AA(n=9)	AS(n=4)	p-value
MetHb(g/dl)	12.64±0.88	13.35±0.90	0.21NS

Key: n= no.of subjects; AA = Haemoglobin AA phenotype; AS= haemoglobin AS phenotype; MetHb = Methaemoglobin; NS= Not Significant.

MetHb mean concentration was  $12.66 \pm 1.21$ (g/dl) and  $14.12 \pm 1.33$  (g/dl) for AA and AS respectively with  $P = .03$  which means that a significant difference was seen between groups using independent t-test.

MetHb mean concentration was  $12.64 \pm 0.88$ (g/dl) and  $13.35 \pm 0.90$  (g/dl) for AA and AS respectively with  $P = 0.21$  which means that no significant difference was observed between groups using independent t-test.

#### 4. DISCUSSION

Out of the 150 subjects that participated in this study, 99 (66%) were HbAA while 51(34%) were HbAS genotypes. These were the common haemoglobin variants encountered in this study. This was consistent with the findings of a researcher who reported that hemoglobin genotype AA and AS were the common haemoglobin variants among Nigerians [2]. Evaluation of the concentration of methaemoglobin (MetHb) among the two common haemoglobin variants (HbAA and HbAS) among the study subjects showed no significant difference. On the other hand, subjects of Igbo extraction had significantly higher MetHb levels than Ijaw and Yorubas. This may be probably due to exposure of Igbos to certain oxidant chemicals and substances that causes oxidation of ferrous iron ( $Fe^{2+}$ ) to ferric iron ( $Fe^{3+}$ ). Post-hoc evaluation of MetHb concentration among the three ethnic groups also reveal a significant difference ( $p= 0.0007$ ) between Igbo and Ijaw, but no significant difference ( $P= 0.59$ ) between Igbo and Yorubas, and a significant difference ( $p=0.002$ ) between Ijaw and Yorubas. MetHb were also evaluated among the two commonly found Haemoglobin variants (HbAA, HbAS) among the Ijaws. There was no statistically significant difference ( $p> 0.05$ ) observed among the studied parameters for the two variants. The mean methaemoglobin concentration, expressed as percentage (MetHb %) of total haemoglobin concentration of two genotypes (HbAA, HbAS) variants was also evaluated among the Ijaws. There was no significant difference ( $p> 0.05$ ) observed. It is therefore in consonance with a study that also

reported no significant difference ( $p>0.05$ ) in methaemoglobin concentrations between HbAA and HbAS erythrocyte of non-malarious human subjects/volunteers [12]. Chikezie, in 2011 also reported no significant difference in methaemoglobin concentration and NADH-methaemoglobin reductase activity between HbAA and HbAS erythrocytes [13].

The studied parameter among the Igbo Ethnic group showing a significant difference ( $p<0.05$ ) was observed for MetHb between the two common variants among the Igbos with significantly higher levels in HbAS than HbAA. The mean MetHb was also significantly higher in HbAS than in HbAA among the Igbos and thus do not agree with the findings of similar studies [12,13] both of which observed no significant difference between HbAA and HbAS erythrocytes. The raised Met haemoglobin concentration observed among the Igbos may probably be due to the simple oxidation of haem which converts it from its normal  $Fe^{2+}$  (ferrous) state to the ( $Fe^{3+}$ ) (ferric) form and the corresponding conversion of haemoglobin A (HbA) to methaemoglobin (Met-HbA). This is a very important mechanism for the red cell and conversion of Met-HbA back to HbA requires the generation of NADH from glycolysis. MetHb was also evaluated among the Yorubas and the mean metHb concentration (g/dl) was  $12.64\pm 0.88$ g/dl and  $13.35\pm 0.90$ g/dl for HbAA and HbAS genotype respectively and agrees with Chikezie and his team who also observed no significant difference in MetHb concentration between HbAA and HbAS genotypes in his two separate studies [12,13].

#### 5. CONCLUSION

This study has shown that methaemoglobin levels may change significantly based on differences in ethnic groups but this change may not be significant based on haemoglobin variants. More studies are encouraged with larger sample size for stronger inferential claims.

#### CONSENT

Informed consent was also obtained from each subject after been educated on the study.

## ETHICAL APPROVAL

The experimental protocol was approved by the Ethics Committee of the Bayelsa State Ministry of Health.

## COMPETING INTERESTS

Authors have declared that no competing interests exist.

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