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Assessment of Methaemoglobin and Carboxyhaemoglobin Levels among Pregnant Women Infected with Hepatitis B Virus

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Authors' contributions

This work was carried out in collaboration between all authors. Author ADA designed and coordinated the study. Authors ADA and BDA performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors FKA and AMJO managed the analyses of the study. Author KIE assessed the participants clinically. Author FKA coordinated sample collection while authors KIE and ADA managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The goal of this research is to determine plasma levels of MetHb and COHb in pregnant women with hepatitis B, which might enhance oxidative stress and hypoxemic condition of this state if it is not ameliorated on time.
Study Design: Prospective case-control study.
Place and Duration of Study: Antenatal clinic at Primary Health Centres, Sagamu, Ogun State, Nigeria between February, 2015 and August, 2015.
Methodology: Blood levels of MetHb, COHb and bilirubin were determined in ninety four (94) participants (aged 18-40 years), divided into three groups: 33 pregnant women infected with hepatitis B virus, 30 apparently healthy pregnant women and 31 age matched non pregnant women

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apparently healthy controls. Blood levels of MetHb, COHb and bilirubin were determined using standard spectrophotometric method.
Results: There was progressive increase and decrease in mean blood levels of (TBil, and MetHb) and mean blood levels of COHb respectively from controls through pregnant subjects with HBV. PCV and DBil had no specific pattern of differences across the groups.
Conclusion: This study showed a slight increase in blood levels of MetHb in pregnant women with hepatitis B and apparently healthy pregnant women compared to non-pregnant controls, which might enhance oxidative stress and hypoxemic condition of this state. It would also be helpful to incorporate MetHb screening as routine tests for better management of pregnant women especially with HBV.

Keywords: Methaemoglobin; carboxyhaemoglobin; spectrophotometer; pregnant women and hepatitis B.

1. INTRODUCTION

Methemoglobinemia is a pathological condition in which iron in hemoglobin is in its trivalent oxidation state (Fe^{3+}) rather than its divalent one (Fe^{2+}); this results in the formation of a hemoglobin subform that is unfit for transporting oxygen [1,2]. Methemoglobin in blood can originate from in vivo exposure to oxidants [3], which gives rise to a variably serious picture of tissue hypoxia. Concisely, methemoglobin is formed when ferrous iron (Fe^{2+}) of deoxyhemoglobin is converted to the ferric iron (Fe^{3+}) state on exposure of erythrocytes to oxidizing agents and oxygen free radicals [2]. Studies have shown that methemoglobin is formed continuously in plasma but rarely exceeds 1.5% of total plasma haemoglobin [2,4]. Basically two enzymes, Diaphorase I and diaphorase II, in synergy with red blood cell non–enzymatiic antioxidants, ascorbic acid, glutathione, and other sulfydryl derivatives serve to minimize erythrocyte methemoglobin level [2,5]. Cyanotic presentation is typically observed at methemoglobin concentration greater than 15% and is often one of the earliest clinical evident features of methemoglobinemia [6,7].

Carboxyhaemoglobin (COHb) on the other hand is formed by the preferential binding of carbon monoxide (CO) instead of oxygen (O_2) to haemoglobin. The affinity of CO to haemoglobin is 200-250 times more than that of oxygen. It is then unstable for oxygen transport. When one molecule of CO binds to one monomer of the Hb molecules, it increases the affinity of others to oxygen; so that the O_2 bound to these monomers are not released. This would further decrease the availability of oxygen to the tissues [4].

Conditions which cause haemolysis and lead to free haemoglobin (Hb) appear to contribute to disease pathogenesis and often severe clinical manifestations, such as renal impairment, vascular disease or inflammations [7] and pregnancy had been established to be an inflammatory state [8,7]. One proposed mechanism is the liberation of haem from Hb with its pro-inflammatory effects. The proposed chain of events start with the lysis of red blood cells (RBC) and the production of free haemoglobin (Hb) which is oxidized to methaemoglobin (MetHb), liberating the haem group. Haem oxygenase (HO-1) degrades haem, producing biliverdin, iron and carbon monoxide (CO) and subsequently carboxyhaemoglobin (COHb) [7]. Certainly, COHb levels may also be influenced by many environmental factors, such as smoke exposure or air-pollution, making them less suitable as a marker of haemolysis [9].

Free haem is not limited to infections and has also been associated with non-infectious haemolytic conditions, such as sickle cell disease (SCD), malaria etc [7]. Patients with overt haemolysis as in sickle cell crisis often have noticeably increased MetHb and COHb levels [10,7]. Three studies in malaria reported different MetHb levels as compared to controls, although the magnitude of the difference as well as the observed values varied several-fold between studies [11,12]. Slight increase in the level of methaemoglobin and COHb in the sickle cell patients in comparison with healthy person had been established [13,3].

Moreover, a pronounced physiological decrease in packed cell volume (PCV) occurs as the pregnancy proceeds [14] and this would thus increase free haemoglobin levels during this condition. Consequently, one would expect that the presence of free Hb and haem, as well as the increase of HO-1 should be associated with altered MetHb and COHb levels in pregnancy

and possibly more worsening in pregnant women with hepatitis infections. The goal of this research is to determine plasma levels of MetHb and COHb in pregnant women with hepatitis B, which might enhance oxidative stress and hypoxemic condition of this state if it is not ameliorated on time.

2. MATERIALS AND METHODS

2.1 Subjects

After obtaining an approval from the Sagamu Local Government Ethics Review Committee (SLG.633/II/218) and written informed consent (approved by the SLG Ethics committee) from each subject, a total of ninety four (94) participants (aged 18-40 years) were recruited into this study, divided into three groups: 33 pregnant women already infected with hepatitis B virus (HBV), 30 apparently healthy pregnant women without HBV and 31 age and sex matched apparently healthy non-pregnant controls. The pregnant subjects were recruited from the antenatal clinic at Primary Health Centres, Sagamu, Ogun State, Nigeria.

2.2 Inclusion Criteria

The participating subjects were pregnant women with or without hepatitis B and apparently healthy non-pregnant controls, aged between 18 and 40 years.

2.3 Exclusion Criteria

The subjects with established complications such as HbSS, symptoms of malaria, drug or blood transfusion history, human immunodeficiency virus (HIV) and cancer were excluded from the study.

2.4 Ethical Consideration

This study was approved by the Sagamu Local Government Ethics Review Committee (SLG.633/II/218) and also, written informed consent (approved by the SLG Ethics committee) was obtained from each subject.

2.5 Collection and Preparation of Sample(s) for Analysis

Blood Samples: Five (5) millilitres (mls) of venous blood was aseptically obtained from the antecubital fossa vein with minimal stasis using pyrogen-free disposable needles and syringes into ethylene diamine tetraacetic acid (EDTA) bottle.

Once blood is collected from the patient, blood MetHb levels increase with time. Owing to the instability of the MetHb in the drawn blood sample, the usual clinical recommendation is to carry out the assay as rapidly as possible using fresh sample [15,16]. The spectroscopic method is the analytical method of choice for determination of MetHb as co-oximetry and pulse-oximetry illustrates false elevation after methylene blue therapy [17]. Thus, a very simple new method had been established which described stabilization of MetHb for the determination of MetHb levels [16]. A stabilized MetHb blood samples can be stored and stable for 9 days at 4-8℃. Similarly, a stabilized COHb blood samples could also be stored and stable indefinitely when kept refrigerated [18]. In regards to this, MetHb and CoHb in blood samples were first stabilized within 10 mins of blood collection and analyzed later within five hours of stabilization technique. Plasma was appropriately obtained and stored at −20℃ until bilirubin analyses were done.

2.6 Assay Methodology

Blood levels of MetHb [1,19], COHb [20,21], and Bilirubin [22] were determined using standard spectrophotometric method. PCV was determined as described by Cheesbrough, [23]. Hepatitis B virus and human immunodeficiency virus (HIV) were done using rapid test kits (RKT) [24].

2.7 Statistical Analysis

A statistical package for social scientist (SPSS) 17.0 was used for the analysis of the data. The distribution of the data was assessed using histogram with normal curve. Results are presented as mean ± standard deviation for gausian distributed data. Analysis of Variance (ANOVA) was used to compare all the three groups while differences between two groups were determined using independent Student's t-test as appropriate. Pearson's correlation coefficient was used to determine the relationship between all the parameters in each group. The level of significance was taken at 95% confidence interval and P-values less than 0.05 were considered significant.

3. RESULTS AND DISCUSSION

3.1 Results

A total number of 94 female subjects comprising 33 pregnant subjects with HBV mean age (29.94±5.38) years, 30 pregnant subjects without HBV mean age (29.0±4.88) years and 31 non-pregnant subjects (control) with mean age (28.26±5.92) years were studied. Table 1 shows biochemical parameters in pregnant subjects (with and without HBV) and non-pregnant controls using One way analysis of variance (ANOVA), all the components of the biochemical parameters between the three groups (pregnant subjects with or without HBV and controls) were significantly different. There was progressive increase and decrease in (TBil, and MetHb) and COHb respectively from controls through pregnant subjects with HBV. PCV and DBil had no specific pattern of differences.

In Table 2, the mean biochemical parameters among pregnant subjects were compared according to trimester of pregnancy. There was no statistical difference between the three groups, even though there were slight differences among the mean.

In Table 3, PCV, TBil, DBil and MetHb were significantly higher while COHb was significantly lower in pregnant subjects with HBV compared with the pregnant subjects without HBV. Similarly, TBil, DBil and MetHb were significantly higher while PCV and COHb were significantly lower in pregnant subjects with HBV compared with non-pregnant controls. However, PCV was significantly lower while other parameters showed no statistical significant in pregnant subjects without HBV compared with non-pregnant controls.

To find out if there is any interaction between rate of haemolysis and (MetHb and CoHb), pregnant subjects with HBV were classified into two groups based on the plasma levels of total bilirubin into >1.0 mg/dl and ≤1.0 mg/dl groups. As shown in Table 4, the two groups exhibited a similar pattern and thus there was no statistical difference between the two groups. In Table 5, plasma levels of total bilirubin had insignificant inverse correlation with PCV and COHb among pregnant subjects with HBV and COHb among pregnant without HBV.

3.2 Discussion

Cyanotic presentation is typically observed at methemoglobin concentration greater than 15% and is often one of the earliest clinical evident features of methemoglobinemia [6,7]. One proposed mechanism is the liberation of haem from Hb with its pro-inflammatory effects. The proposed chain of events start with the lysis of red blood cells (RBC) and the production of free haemoglobin (Hb) which is oxidized to methaemoglobin (MetHb), liberating the haem group. Assessment of oxyhemoglobin saturation in patients with haemolytic condition is vital for prompt recognition of hypoxemia [13].

The central chain of events in the haemolysis/MetHb/haem hypothesis [25,7] is based on the idea that levels of all relevant biochemical parameters, which include free Hb, MetHb, liberated haem, HO-1 expression, CO and COHb are all expected to be increased in haemolytic conditions. Eventhough studies have shown that methaemoglobin is formed continuously in plasma but rarely exceeds 1.5% of total plasma haemoglobin [2,4], yet patients with overt haemolysis as in sickle cell crisis [13] and malaria [12,7] often have noticeably increased MetHb and COHb levels. Consequently, one would expect that the presence of free Hb and haem, as well as the

Table 1. Biochemical parameters in pregnant subjects (with and without HBV) and non-pregnant controls

	Preg with HBV (n=33)	Preg without HBV (n=30)	Non-preg control (n=31)	F-value
PCV (%)	33.23±4.25	30.67±3.17	35.29±3.69	0.000*
TBil (mg/dl)	0.96±0.55	0.48±0.17	0.46±0.20	0.000*
DBil (mg/dl)	0.32±0.21	0.20±0.08	0.24±0.12	0.006*
MetHb (%)	2.64±1.11	1.92±0.72	1.82±0.78	0.001*
COHb (%)	0.19±0.36	0.51±0.31	0.58±0.32	0.000*

**significant at p<0.05;*

Key: n = sample size, PCV = packed cell volume, TBil = Total bilirubin, DBil = direct bilirubin, MetHb = methaemoglobin, COHb = carboxyhaemoglobin, preg with HBV = pregnant subjects with hepatitis B virus, Preg without HBV = pregnant subjects without hepatitis B virus, Non-preg controls = non-pregnant sugjects

Table 2. Comparison of mean biochemical parameters among pregnant subjects according to trimester of pregnancy

	1st trimester (n=04)	2nd trimester (n=33)	3rd trimester (n=26)	F-value
PCV (%)	30.25±3.59	32.33±4.49	31.88±3.30	0.603
TBil (mg/dl)	0.94±0.91	0.67±0.36	0.77±0.54	0.486
DBil (mg/dl)	0.15±0.05	0.25±0.17	0.28±0.18	0.346
MetHb (%)	2.60±1.27	2.10±0.74	2.50±1.22	0.262
COHb (%)	0.22±0.24	0.42±0.34	0.26±0.40	0.213

**significant at p<0.05*

Table 3. Biochemical parameters in pregnant subjects (with and without HBV) and non-pregnant controls

	Non-preg control (n=31)	Preg with HBV (n=33)	Preg without HBV (n=30)	P-value		
				a	b	c
PCV (%)	35.29±3.69	33.23±4.25	30.67±3.17	0.009*	0.044*	0.000*
TBil (mg/dl)	0.46±0.20	0.96±0.55	0.48±0.17	0.000*	0.000*	0.637
DBil (mg/dl)	0.24±0.12	0.32±0.21	0.20±0.08	0.004*	0.069	0.126
MetHb (%)	1.82±0.78	2.64±1.11	1.92±0.72	0.004*	0.001*	0.591
COHb (%)	0.58±0.32	0.19±0.36	0.51±0.31	0.000*	0.000*	0.338

**significant at p<0.05*

Key: a=Preg with HBV vs Preg without HBV; b=Preg with HBV vs Non-preg control; c=Preg without HBV vs Non-preg control

Table 4. Pattern of biochemical parameters among pregnant subjects with HBV based on plasma levels of total bilirubin

	> 1.0 mg/dl (n=14)	≤ 1.0 mg/dl (n=19)	P-value
PCV (%)	32.57±3.23	33.74±4.90	0.445
MetHb (%)	2.64±1.24	2.64±1.03	0.998
COHb (%)	0.08±0.09	0.28±0.45	0.110

**significant at p<0.05*

Table 5. Correlation of plasma levels of total bilirubin with PCV, MetHb and COHb in pregnant subjects

Parameters	Preg with HBV (n=33)		Preg without HBV (n=30)	
	r-value	p-value	r-value	p-value
PCV (%)	-0.081	0.653	0.251	0.182
MetHb (%)	0.167	0.368	0.172	0.365
COHb (%)	-0.280	0.115	-0.201	0.287

increase of HO-1 should be associated with altered MetHb and COHb levels in pregnancy and possibly more worsening in pregnant women with hepatitis infections.

This study shows progressive increase in blood levels of total bilirubin and MetHb from controls through pregnant subjects with HBV. This was in agreement with a slight increase in the level of methaemoglobin in the sickle cell patients in comparison with healthy subjects which had been established [13,3] and also MetHb which was significantly different between healthy controls and children with malaria in Africa [7]. The result documented from this study still appears to be consistent with the central chain of events in the haemolysis/MetHb/haem hypothesis, and thus it does not provide evidence to refute this hypothesis. The increased blood levels of MetHb which was significantly higher in pregnant subjects with HBV compared with the pregnant subjects without HBV obviously corroborated the notion.

The observed value of COHb was significantly lower in controls through pregnant subjects with HBV. Our observation contradicts the earlier report that had documented the highest COHb levels (6.6%) in sickle cell disease [26,13] and also in malaria [7]. It thus appears doubtful if the

measurement of COHb is a reliable way to assess haemolysis and disease severity because it did not follow the pattern of haemolysis as expected. In Hepatitis, as in other haemolytic diseases, a by-product of haemoglobin catabolism is carbon monoxide which binds to haemoglobin, forming COHb [10,13], and it is ought to be slightly higher. Decrease in levels of COHb as reported in this study might be as a result of asymptomatic stage of the condition.

Moreover, according to report of this study, there was no statistical difference between mean TBil, MetHb and COHb among pregnant subjects when compared according to trimester of pregnancy, even though there were slight differences among the mean. This might be an indication that gestational age has no effects on these parameters once the haemolysis is not yet set in. Similarly, this study showed no statistical difference between the two groups based on the plasma levels of total bilirubin (>1.0 mg/dl and ≤1.0 mg/dl groups) among pregnant subjects with HBV. This is attributed to asymptomatic state of the subjects which was in accordance with Tietz, [4], opines that healthy people may not have many symptoms with MetHb level less than 15%. This is further corroborated in Table 5, when plasma levels of total bilirubin had inverse correlation with PCV and COHb among pregnant subjects with HBV, although insignificant statistically.

Not only do patients with haemolytic diseases have decreased oxygen carrying capacity due to chronic anemia, they have elevated MetHb, which are unable to transport oxygen [13]. Methemoglobinemia is a pathological condition in which iron in hemoglobin is in its tervalent oxidation state (Fe^{3+}) rather than its divalent one (Fe^{2+}) [2]. These results in the formation of a hemoglobin subform that is unfit for transporting oxygen, which will give rise to a variably serious picture of tissue hypoxia.

4. CONCLUSION

In conclusion, this study showed a slight increase in plasma levels of MetHb in pregnant women with hepatitis B and apparently healthy pregnant women compared to non-pregnant controls, which might enhance oxidative stress and hypoxemic condition of this state. It would also be helpful to incorporate MetHb screening as routine tests for better management of pregnant women especially with HBV. This may improve risk of developing hypoxia and assist in clinical decision making to ameliorate this condition promptly.

CONSENT

All authors declare that written informed consent was obtained from each subject before being enrolled into the study.

ETHICAL APPROVAL

This study was approved by Sagamu Local Government Ethics Review Committee (SLG.633/II/218) and also, written informed consent (approved by the SLG Ethics committee) was obtained from each subject.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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