

Original Research Article

EVALUATION OF REPRODUCTIVE HORMONE, ELECTROLYTES AND SERUM PROTEIN IN PREGNANT WOMEN WITH DIFFERENT GENOTYPES

ABSTRACT

Introduction : Genotype is an inward expression of gene or the combination of alleles, situated on corresponding chromosomes that determines a specific trait of an individual . Sickle Cell Disease is associated with higher clinical and obstetric complications compared to the general population.

Aim: This study sought to investigate how different genotypes might affect the levels of hormones, electrolytes and serum proteins in pregnancy.

Study design: A case-Control Study

Methodology: Participants were 120 pregnant women recruited from hospitals in Oyo State and Ekiti State. They were grouped into 40 each according to their Genotypes: AA, AS and SS. Blood samples were collected and analytes quantified by standard laboratory methods. One way analysis of variance (ANOVA) and Duncan multiple range test were used to compare variables across the three groups

Results: There was a significant reduction in the levels of Estrogen, and Prolactin in Sickle Cell group when compared to AA and AS group, while the levels of progesterone were not statistically different in the three groups. Also, there was a significant reduction in the levels of Na, K , Total Protein and Albumin in Sickle Cell Group when compared to Group 1 and 2. However, the levels of Ca, Mg, Zn, Cl and Fe, C-reactive protein were not statistically different in the three groups.

Conclusion: Low levels of reproductive hormones, total protein and electrolytes imbalance are associated with pregnancy in Sickle Cell Disease.

Keywords: Genotype, Sickle Cell Disease, Electrolytes, Pregnancy

1. INTRODUCTION

“Genotype is an inward expression of gene or the combination of alleles, situated on corresponding chromosomes that determines a specific trait of an individual. A single copy of

gene is composed of two alleles which can either be homogenous (AA) or Heterogeneous (AS). Literally both alleles are gotten from maternal and paternal parent" [1].

"In West Africa, there are six predominant classes of Hb Genotype namely: Type AA, Type AS, Type AC, Type S, Type SC, Type CC. In genotype AA, the amino acid at the sixth pocket of the polypeptide chain of β -globin is glutamate, whereas in genotype SS, the glutamate is replaced with another amino acid known as Valine. In genotype CC, glutamate is replaced with another amino acid known as Lysine" [1]. "Point mutations in the DNA sequence that lead to single amino acid substitutions in the globin moiety, results in the production of the hemoglobin variants. Abnormal hemoglobin genotypes among Nigerians include AS, AC, SC, and SS" [2]. Also, World Health Organization reported that an average of 150,000 infants are born with sickle cell disease in Nigeria.

"Nigeria ranks first as the sickle cell endemic country in the world with an annual infant death of 100,000 – representing 8% of infant mortality in the country" [3]. Many reports have documented a considerable maternal risk of morbidity and mortality and high perinatal adverse outcomes in pregnant women with sickle cell. Studies have shown that women with SCD have an increased risk of preeclampsia and maternal death, stillbirths, preterm deliveries, and small-for-gestational-age newborns. Sickle cell anemia (SCA) is caused by a homozygous mutation (hemoglobin S) and presents as chronic anemia accompanied by painful episodes caused by impaired microcirculation due to sickling of erythrocytes. Despite previous researches and insights, pregnancy in SCD is still associated with higher clinical and obstetric complications compared to the general population" [3].

"Prolactin, a very important hormone in metabolism, regulation of the immune system also acts in cell cycle-related functions as a growth- differentiating and anti-apoptotic factor, it influences hematopoiesis and angiogenesis" [4]. "Other actions include contributing to pulmonary surfactant synthesis of the fetal lungs at the end of the pregnancy and immune tolerance of the fetus by the maternal organism during pregnancy. Prolactin promotes neurogenesis in maternal and fetal brains" [5].

"Progesterone (P4)) performs very important functions in the body that includes the stimulation of the growth of the uterus [6], maturation and differentiation of the endometrium" [7], "stimulates the decidualization required for implantation and inhibits myometrial contractions" [8].

"Progesterone has been reported to have antioxidant properties and can be used to reduce polymerization of sickled erythrocytes where there is demonstrable deficiency of the hormones as seen in sickle cell disease" [9].

"Electrolytes such as sodium (Na⁺), potassium (K⁺), and chloride (Cl⁻) play various vital roles in the body and are required for optimal functioning of cells and organs. Sodium is one of the major cations and functions in regulating the total amount of water in the body" [10]. "It also plays a vital role in electrical communication in many systems especially the nervous and muscular systems. However, potassium is responsible for regulating heartbeat and muscle function and is important for the overall functioning of the cell" [11]. "Chloride helps maintain a normal balance of body fluids" [12]. Alteration of these electrolytes could, thus, lead to detrimental effects.

Also, it has been reported that hormonal and metabolic changes during pregnancy result in alteration of different endocrine function even in normal pregnancy. However, information on the effects of hemoglobin genotype on these physiologically important biochemical parameters are few in Nigeria. Hence this study sought to investigate how different

genotypes might affect the levels of these parameters in pregnancy and hence provide information that might be instrumental in the management of pregnancy complications associated with genotype.

2. MATERIAL AND METHODS

2.1 Subject

This is a Case –Control Study. Subjects were 120 pregnant women gotten at various private hospitals in Oyo State and Ekiti

State. They were divided into groups based on their genotype namely:

Group one: 40 pregnant women with AA

Group two: 40 pregnant women with AS

Group three: 40 pregnant women with SS

2.2 Anthropometric Measurement

Height (in meters) was measured without shoes with a wall-mounted ruler. Weight (in kilogram) in light clothing was measured with a weighing balance (Zhongshan Camry Electronic Co. Ltd, Guandong, China).

2.3 Collection of samples

Whole blood was collected in a covered test tube. After collection of the whole blood, the blood was allowed to clot by leaving it undisturbed at room temperature. This usually takes 15–30 minutes. The clot was removed by centrifuging at 1,000–2,000rpm for 10 minutes in a centrifuge. Following centrifugation, the liquid component (serum) was immediately transferred into a clean polypropylene tube using a Pasteur pipette. The samples were maintained at 2–8°C while handling. The serum was apportioned into 0.5 ml aliquots, stored, and transported at –20°C or lower. Freeze-thaw cycles was avoided because this is detrimental to many serum components.

2.4 Biochemical Assays

Prolactin, progesterone, oestrogen and luteinizing hormone (LH) were determined using DRG Enzyme Linked immunosorbent assay.(ELISA) [13].

Plasma concentration of Sodium, Potassium, Bicarbonate, Phosphorus were determined using flame Photometry method as described by [14].

Serum Calcium was estimated by Arsenazo III Colorimetric method as described by [15].

Magnesium ion was estimated by colorimetric xylidyl blue complex method as described by [16].

Total protein was evaluated by Biuret method on automated chemistry platform (LW C100 plus) as described by [17].

Albumin was determined by dye binding method known as Bromocresol Green (BCG) method as described by [18].

C reactive protein was determined using Enzyme Linked immunosorbent assay.(ELISA) as described by [19].

2.5 Data Analysis

The data was analyzed using one way analysis of variance (ANOVA) and Duncan multiple range test to compare the data obtained [20]. The results obtained were grouped and expressed as mean \pm Standard Error(SE).

3. RESULTS AND DISCUSSION

Table 1: Anthropometric Measurement of Pregnant Women With Respect to their Genotype

| Parameters | Group 1 (AA) | Group 2 (AS) | Group 3 (SS) |
|----------------------------|-------------------------------|-------------------------------|-------------------------------|
| AGE (Yrs) | 33.01 \pm 0.10 ^a | 32.29 \pm 0.21 ^a | 29.40 \pm 0.10 ^a |
| WEIGHT (Kg) | 69.23 \pm 0.20 ^a | 67.79 \pm 0.10 ^a | 58.23 \pm 0.20 ^b |
| B.M.I (kg/m ²) | 22.00 \pm 0.04 ^a | 21.70 \pm 0.45 ^a | 18.80 \pm 0.11 ^b |

Note: Values that are of the same superscript within the same column are not significantly different ($P < .05$) while values with different superscripts are significantly different ($P < .05$).

Table 1 shows that there was no significant difference between the Body mass index between Group 1,2 and 3. There was a significant reduction in the weight and BMI of Group 3 when compared with Group 1 and 2.

Table 2: Biochemical parameters of pregnant women with respect to their genotype

| Parameters | Group 1 (AA) | Group 2 (AS) | Group 3 (SS) |
|----------------------|-------------------------|-------------------------|-------------------------|
| Estrogen (pmol/L) | 89.01±0.3 ^a | 82.88±0.29 ^a | 78.8±0.30 ^b |
| Progesterone(pmol/L) | 11.99± 0.2 ^a | 10.23±0.22 ^a | 10.21±0.29 ^a |
| Prolactin (pmol/L) | 70.5±0.39 ^a | 66.05±0.21 ^a | 63.01±0.30 ^b |

Note: Values that are of the same superscript within the same column are not significantly different ($P < .05$) while values with different superscripts are significantly different ($P < .05$).

Table 2 shows a significant reduction in the levels of Estrogen, and Prolactin in Group 3 when compared to Group 1 and 2. However, the levels of progesterone was not statistically different in the three groups.

Table 3 Serum levels of some Minerals in pregnant women with respect to their genotypes

| Minerals (mmol/L) | Group 1 (AA) | Group 2 (AS) | Group 3 (SS) |
|-------------------|-------------------------|-------------------------|-------------------------|
| Na | 142.3±0.41 ^a | 130.1±0.31 ^a | 111.0±0.40 ^b |
| K | 4.59±0.30 ^a | 4.24±0.41 ^a | 2.99±0.33 ^b |
| Ca | 3.45±0.36 ^a | 3.44±0.29 ^a | 3.12±0.31 ^a |
| Mg | 0.70±0.40 ^a | 0.62±0.31 ^a | 0.60±0.25 ^a |
| Zn | 0.020±0.35 ^a | 0.014±1.30 ^a | 0.007±0.40 ^b |
| Cl | 90.0±0.41 ^a | 84.9±0.40 ^a | 85.1±0.39 ^a |
| Fe | 0.04±0.40 ^a | 0.03±0.41 ^a | 0.02±0.50 ^a |

Note: Values that are of the same superscript within the same column are not significantly different ($P < .05$) while values with different superscripts are significantly different ($P < .05$).

Table 3 shows that there was a significant reduction in the levels of Na and K in Group 3 when compared to Group 1 and 2. However, the levels of Ca, Mg, Zn, Cl and Fe were not statistically different in the three groups.

Table 4: Levels of Total Protein, Albumin and C-reactive Protein of pregnant women with respect to their genotype

| Parameters | Group 1 (AA) | Group 2 (AS) | Group 3 (SS) |
|----------------------|-------------------------|------------------------|------------------------|
| Total protein (g/dL) | 7.52±0.27 ^a | 6.94±0.20 ^a | 5.79±0.25 ^b |
| Albumin (g/dL) | 4.91± 0.41 ^a | 3.99±0.31 ^a | 3.01±0.40 ^b |
| CRP (mg/L) | 2.01±0.33 ^a | 2.41±0.32 ^a | 2.63±0.31 ^b |

Note: Values that are of the same superscript within the same column are not significantly different ($P < .05$) while values with different superscripts are significantly different ($P < .05$).

Table 4 shows that there was a significant reduction in the levels of Total Protein and Albumin in Group 3 when compared to Group 1 and 2. However, the levels of C-reactive Protein was not statistically different in the three groups.

Discussion

There was a significant reduction in the weight and BMI of SS pregnant women when compared with AA and AS genotype in Table 1, this is in accordance with the work of [21]. Homozygous sickle cell disease is associated with a risk of low birth weight 4 times higher than in the general population [22]. The cause is chronic hypoxia of the fetal-placental unit, which itself is the consequence of anemia and rheological anomalies in the placental level [22].

A significant reduction in the levels of Na and K of SS pregnant women when compared with AA and AS genotype in Table 3, which was also observed by [23]. In most cases, the deoxygenation of sickle cell promotes cation permeability of electrolytes [23]. These processes may consequently lead to increased cell dehydration in the sickle cell patients, with aggravated clinical complications. The sodium-potassium ATPase activity has been shown to be more active in sickle cell and contributes to cell dehydration [24]. This may consequently lead to frequent crises and associated painful episodes due to vaso-occlusion of the sickle erythrocytes, as observed in sickle cell patients. This is mainly as a result of the

sustained and rapid polymerization of the Haemoglobin S molecule, where intracellular concentrations (of the HbS molecules) are increased in sickle cell patients [25].

Also, the observed reduced level of progesterone in SS pregnant women in Table 2, is similar to a report by [26]. The level of sex hormone secretion in pregnancy is a contributing factor in pregnancy success rates. Any alteration in the levels of these sex hormone as seen in sickle cell, increases the risk of stillbirth and results in maternal complications [26].

There was a significant reduction in the levels of Total Protein and Albumin of SS pregnant women when compared with AA and AS genotype in table 4, which is in agreement with those reported by [27,28]. Low levels of total protein and albumin in patients with sickle cell anemia could be as a result of the quick fall in the concentration of short-lived hepatic proteins and loss of albumin into ascetic fluid respectively[29].Albuminuria,the most common clinical manifestation of glomerular damage, is highly prevalent in patients with SCD. The fall in protein concentration seen during pregnancy most likely is a result of the dilution of the plasma, since total protein concentration is inversely related to plasma water concentration [30]. The total amount of albumin is apparently unchanged; decreases in concentration are thought to be the result of an increase in plasma volume [28].

4. CONCLUSION

Low levels of reproductive hormones, total protein and electrolytes imbalance are associated with pregnancy in sickle cell Disease. These hormonal and metabolic changes can result in complications like low fetal birth, preterm labour and fetal death. Therefore, expectant mothers with sickle cell anemia should be given adequate perinatal care, healthy dietary intake, adequate rest and vitamin supplements to ensure overall wellbeing of the mother and fetus.

CONSENT

Written informed consent was duly signed by all participants.

ETHICAL APPROVAL

A letter of identification from the Department of Biochemistry, Ekiti State University, Ado-Ekiti was used in pre-survey visits to hospitals to obtain permission from the hospital authorities. An ethical clearance was also obtained from Ekiti State University Teaching Hospital, Ado-Ekiti and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

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