

Sex Hormones Secretion Pattern in Pregnant Sickle Cell Subjects in Niger Delta Region, South of Nigeria

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Abstract

Sickle cell anemia (SCA) patients are reported with infertility and low rate of pregnancies. This is associated with wide range of reproductive issues that are still relevant because of the complications and problems of the disease that still persist till date. This study was carried out to establish the secretion pattern of the sex hormones (Progesterone and Estradiol) in the three trimesters of pregnant sickle cell disease subjects in the Niger Delta Region, south of Nigeria. The study included twenty (20) pregnant sickle cell anemia subjects with average age of 27.4 years and twenty (20) apparently healthy (Hemoglobin AA) subjects with average age of 28.2 years. Their samples collection started when they registered for antenatal care at the clinics within the first trimester of pregnancy. The Enzyme Linked Immunosorbent assay (ELISA) method was used in the measurement of the hormones in the plasma of the subjects. The result showed a statistical significant reduction ($P < 0.05$) in the values of the hormones in the three trimesters obtained for the sickle cell disease (SCD) subjects when compared with apparently healthy subjects with the same age range. Statistical analysis showed a strong positive correlation ($r = 0.8151$ for Estradiol and $r = 0.8793$) for Progesterone) between the secretion of the sex hormones, in the sickle cell subjects and the control. The result is attributed to the sickle cell gene abnormality and the treatment of SCA that affects the endocrine system by inhibiting the production of gonadotropins from the pituitary gland. The SCD itself does not directly damage the reproductive system; however it can affect other systems which will eventually cause harm to the reproductive system. The study concluded that the sickle cell anemia patients require the administration of the hormone drugs during

pregnancy to prevent to a certain extent, complications arising from hormonal imbalance.

Keywords

Hormones, Progesterone, Estradiol, Sickle Cell, Secretion, Trimesters

1. Introduction

Sickle cell disease (SCD) comprises a group of diseases characterized by the presence of sickle hemoglobin (HbS) [1]. It is the most common genetic disorder in persons of African origin [2] and the disorder comprises of spectrum of syndromes that range from the most completely benign trait or carrier state (the AS genotype) to the most severe syndrome, the sickle cell anemia due to the homozygous presence of the B-hemoglobin (producing the HbSS genotype) [3].

The SCD is globally wide spread. About 150,000 to 300,000 children with the sickle cell anemia are born in Africa every year [4]. In Nigeria, the most populous country in the sub-region with about 150 million inhabitants [5], 24% of the population is carriers of the mutant gene and the prevalence of the sickle cell anemia is about 20 per 1000 births [3]. The severity of the sickling phenomenon has been observed to be more at pre-puberty, but at puberty, the level of crisis becomes fairly stable. This has been attributed to sex hormones that are responsible for development of sexual characteristics in both male and female [6]. Although menses onset is delayed in females with SCD [7] [8], menstrual bleeding patterns remain normal [9].

Sickle cell disease is caused by the paring of an inherited autosomal recessive gene (Bs-globin), which affects the red blood cells [10]. Deoxygenation of the red blood cells caused these cells to change from their normal round shape to a rod like sickle shape. These sickle shape cells adhere to the blood vessels, eventually clogging the vessels and blocking normal flow of blood and oxygen to tissues and organs. The sickling phenomenon occurs as a result of intracellular polymerization of sickle haemoglobin (HbS) which occurs upon deoxygenation of erythrocytes from patients homozygous for HbS [11] [12]. Vaso-occlusive events (e.g. pain episodes, acute chest syndrome) are a significant cause of morbidity and mortality [13]. Management includes appropriate diagnostic evaluations, adequate pain control and hydration, antibiotics when appropriate, hydroxyurea therapy when affordable and blood transfusion if indicated and available [14].

Sickle cell disease in pregnancy still poses problems to health care providers because of the wide range of reproductive issues and complication that still persist till date. In pregnancy, SCD poses problems to both mother and fetus [15]. Maternal problems can arise from chronic underlying organ dysfunction such as renal disease [16] [17] or pulmonary hypertension [18], from acute complica-

tions of SCD such as Vaso-occlusive crises [19] and chest syndrome [20]. Fetal problems include alloimmunization, growth restriction, preterm delivery and still birth opioid [21].

The sex hormones (progesterone, estradiol) performs very important functions in the body that includes the stimulation of the growth of the uterus [22], maturation and differentiation of the endometrium [23], stimulates the decidualization required for implantation and inhibits Myometrial contractions [24].

New research reports that when compared to healthy pregnant women, pregnant women with SCD are six times more likely to die, during or following pregnancy and have an increased risk for still birth, high blood pressure or preterm delivery [25]. The need arises for health care providers to properly counsel a sickle cell patients considering pregnancy.

This is because the risk in SCD pregnancy has largely depend on health well being with respect to capital per income [25] [26]. SCD pregnant women in low income countries have the highest risk for complications since this largely depend on access to good health care.

The sex hormones, particularly estradiol and progesterone has been reported to have antioxidant properties and can be used to reduce polymerization of Sickled erythrocytes [12] where there are demonstrable deficiency of the hormones. Several studies have reported complications in pregnant women with SCD but focused only upon prenatal outcomes [21] [27] [28] [29] [30]. Because of the complications that arises in women with sickle cell disease, this study focused on establishing the levels of these hormones in the different stages of pregnancy in sickle cell disease subjects in the Niger Delta Region of Nigeria. This is with a view to establish if the SCD phenomenon has any effect on the hormones secretion pattern in pregnant SCD subjects. If there is demonstrable deficiency, the hormones can be used in preventing the increased incidence of spontaneous abortion and still birth as observed in SCD [27] [28].

This study will contribute to the knowledge required in the management of maternal and fetal complications occurring in the sickle cell disease subjects willing to get pregnant and give birth, particularly in the therapeutic measures used to control these complications in pregnancy.

2. Materials and Methods

2.1. Study Area

This study was undertaken in the teaching hospital of Niger Delta University, Bayelsa State, Braithwaite Memorial General Hospital and University teaching hospital in Port Harcourt, Rivers State, all in the Niger Delta Region of Nigeria.

2.2. Study Population

The subjects comprised of pregnant sickle cell patients followed at these hospitals. Their statuses were confirmed after genotyping using the Helena hemoglo-

bin electrophoresis machine. They included fifteen (15) SCD subjects that got pregnant by marriage and five (5) others that had unplanned pregnancy, but decides to keep the pregnancy. The twenty (20) sickle cell anaemia subjects were of age between 20 to 32 years with an average age of 27.4 years. Those that registered late for antenatal care at second and third trimesters of pregnancy were excluded from the study. Also excluded from the study were SCD subjects that had stillbirth and those that lost their lives during the period of study and could not carry the pregnancy to term. The study also included twenty (20) apparently healthy control subjects with hemoglobin genotype AA and their age ranged from 22 to 33 years with average age of 28.2 years. The control subjects were identified pregnant women within the same institutions and period of study that was from May 2004 to June 2017.

2.3. Sample Collection and Preparation

The samples for this study were collected from the subjects when they registered for maternity care within the first trimester of the pregnancy. The sample for the estradiol and progesterone assay was collected twice in every trimester until the 37th and 38th weeks of pregnancy. About 2.0 ml of blood were collected by standard venipuncture technique into a Lithium Heparin tube. This was separated and the plasma stored frozen at -20°C . The quantitation of the hormones was done within 7 days of sample collection.

2.4. Ethical Clearance

The essence and details of study were explained to the subjects (Sickle cell pregnant women and normal apparently healthy women) and consent gotten before sample collection. Institution ethical clearance was gotten before the sample collection was performed from the committee responsible for human studies.

2.5. Method

The genotypes of the subjects were confirmed using hemoglobin Electrophoresis (Helena electrophoretic machine). The Enzyme linked Immunosorbent assay (ELISA) method of hormones estimation was used in this study [31]. The Principle is based on the solid phase enzyme linked Immunosorbent assay [32] [33]. The product kit was acquired from Micro Well Laboratories USA. The components of the ELISA kit were specifically designed to analyse the sex hormones, estradiol and progesterone.

2.6. Statistical Analysis

Data are presented as descriptive statistics, including means, standard errors of the mean/deviation, and percentages. The students t-test was used to compare the mean (pair two samples for mean) 95% confidence level ($P < 0.05$) were used and considered significant.

3. Results

The results of the study on the secretion pattern of the sex hormones in the three trimesters of pregnancy in sickle cell anemia subjects showed that the values obtained at the different trimesters of pregnancy for the sickle cell disease patients were significantly ($P < 0.05$) reduced when compared with the normal haemoglobin AA pregnant women as presented in **Table 1**, **Table 2**, **Table 3** and **Figure 1**, **Figure 2**, **Figure 3**.

Table 1. Means \pm SEM of estradiol and progesterone in sickle cell and healthy pregnant subjects.

| Characteristic | n | Estradiol (pg/ml) | | Progesterone (ng/ml) | |
|-----------------------------|-----|---------------------------------|--|-------------------------------|--|
| | | Mean \pm SEM | | Mean \pm SEM | |
| Disease Status | | | | | |
| Sickle Cell | 60 | 431.32 \pm 27.53 ^a | | 41.92 \pm 2.98 ^a | |
| Healthy (Control) | 60 | 872.96 \pm 53.90 ^b | | 55.25 \pm 2.08 ^b | |
| Age Group (years) | | | | | |
| 21 - 25 | 23 | 675.36 \pm 63.95 | | 49.61 \pm 5.00 | |
| 26 - 30 | 85 | 673.18 \pm 46.36 | | 49.25 \pm 2.60 | |
| 30+ | 12 | 458.65 \pm 82.89 | | 41.94 \pm 6.93 | |
| Mean \pm SD | 120 | 27.80 \pm 2.53 | | 27.80 \pm 2.53 | |
| Trimester | | | | | |
| 1st Trimester (4 - 12 Wks) | 40 | 337.87 \pm 22.10 ^a | | 24.89 \pm 1.82 ^a | |
| 2nd Trimester (13 - 25 Wks) | 40 | 632.19 \pm 42.51 ^b | | 45.09 \pm 1.82 ^b | |
| 3rd Trimester (26 - 40 Wks) | 40 | 986.37 \pm 66.03 ^c | | 75.78 \pm 1.82 ^c | |

Abbreviation: SEM = Standard error of the mean. Within characteristic Means \pm SEM with different superscripts are significantly different at $p < 0.05$. Ezeiruaku *et al.*, 2018, p. 5.

Table 2. Comparison of estradiol (pg/ml) secretion between sickle cell and healthy pregnant women (control).

| Characteristic | n | Estradiol (pg/ml) | | | |
|-----------------------------|----|---------------------------------|----------------------------------|-------------|---|
| | | Sickle Cell | Healthy (Control) | % Reduction | Sickle Cell vs. Control ^β Test Statistics p-value |
| | | Mean \pm SEM | Mean \pm SEM | | |
| Age Group (years) | | | | | |
| 21 - 25 | 5 | 596.20 \pm 54.65 ^a | 960.32 \pm 178.44 | | 0.015* |
| 26 - 30 | 49 | 369.34 \pm 27.71 ^b | 896.40 \pm 60.51 | | <0.0001**** |
| 30+ | 6 | 308.52 \pm 52.50 ^b | 608.78 \pm 135.86 | | 0.066 ^{ns} |
| Trimester | | | | | |
| 1st Trimester (4 - 12 Wks) | 20 | 246.74 \pm 15.05 ^a | 429.00 \pm 30.06 ^a | 42.48 | <0.0001**** |
| 2nd Trimester (13 - 25 Wks) | 20 | 416.73 \pm 36.86 ^b | 847.64 \pm 34.27 ^b | 50.84 | <0.0001**** |
| 3rd Trimester (26 - 40 Wks) | 20 | 630.49 \pm 39.76 ^c | 1342.25 \pm 54.67 ^c | 53.02 | <0.0001**** |

Abbreviation: SEM = Standard error of the mean; ^βComparison between sickle cell and healthy pregnant women. Within characteristic Means \pm SEM with different superscripts are significantly different at $p < 0.05$. Significant Level: * = $p < 0.05$; **** = $p < 0.0001$; ns = Not significant ($p > 0.05$). Ezeiruaku *et al.*, 2018, p. 6.

Table 3. Comparison of progesterone (ng/ml) secretion between sickle cell and healthy pregnant women (control).

| Characteristic | n | Progesterone (ng/ml) | | | Sickle Cell vs. Control Test Statistics p-value |
|-----------------------------|----|---------------------------|---------------------------------|-------------|--|
| | | Sickle Cell Mean ± SEM | Healthy (Control) Mean ± SEM | % Reduction | |
| Age Group (years) | | | | | |
| 21 - 25 | 18 | 48.42 ± 4.83 | 53.90 ± 11.41 | | 0.652 ^{ns} |
| 26 - 30 | 36 | 39.38 ± 3.41 | 56.49 ± 3.64 | | 0.001 ^{***} |
| 30+ | 6 | 37.67 ± 8.36 | 46.21 ± 10.41 | | 0.515 ^{ns} |
| Trimester | | | | | |
| 1st Trimester (4 - 12 Wks) | 20 | 20.73 ± 0.88 ^a | 29.04 ± 0.93 ^a | 28.62 | <0.0001 ^{****} |
| 2nd Trimester (13 - 25 Wks) | 20 | 39.24 ± 1.55 ^b | 50.93 ± 2.45 ^b | 22.95 | 0.0003 ^{***} |
| 3rd Trimester (26 - 40 Wks) | 20 | 65.80 ± 2.93 ^c | 85.77 ± 2.47 ^c | 23.28 | <0.0001 ^{****} |

Abbreviation: SEM = Standard error of the mean; [#]Comparison between sickle cell and health pregnant women. Within characteristic Means ± SEM with different superscripts are significantly different at p < 0.05. Significant Level: * = p < 0.05; *** = p < 0.001; **** = p < 0.0001; ns = Not significant (p > 0.05). Ezeiruaku *et al.*, p. 7.

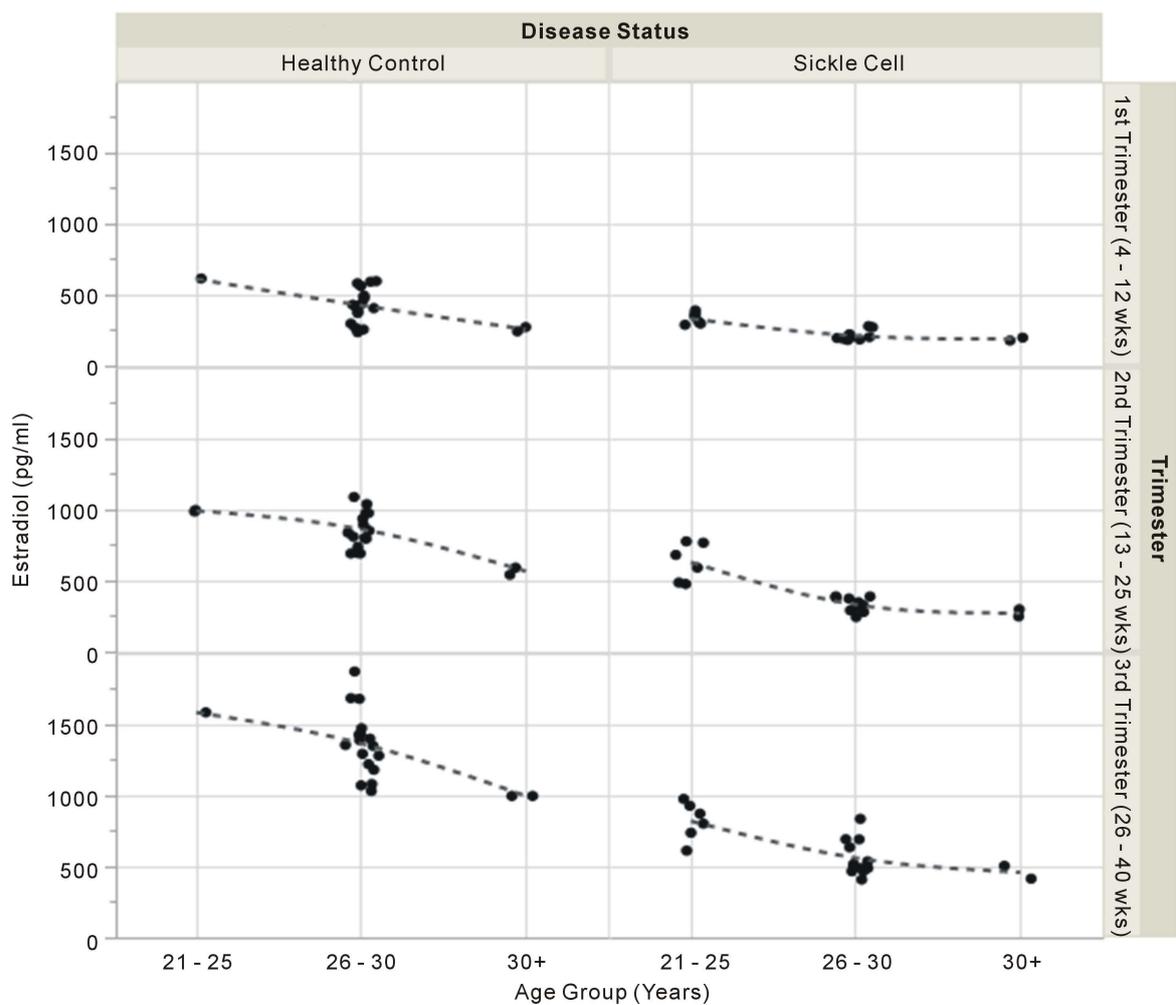


Figure 1. Estradiol levels in sickle cell and healthy pregnant women by age group and trimester. Ezeiruaku *et al.*, 2018, p. 8.

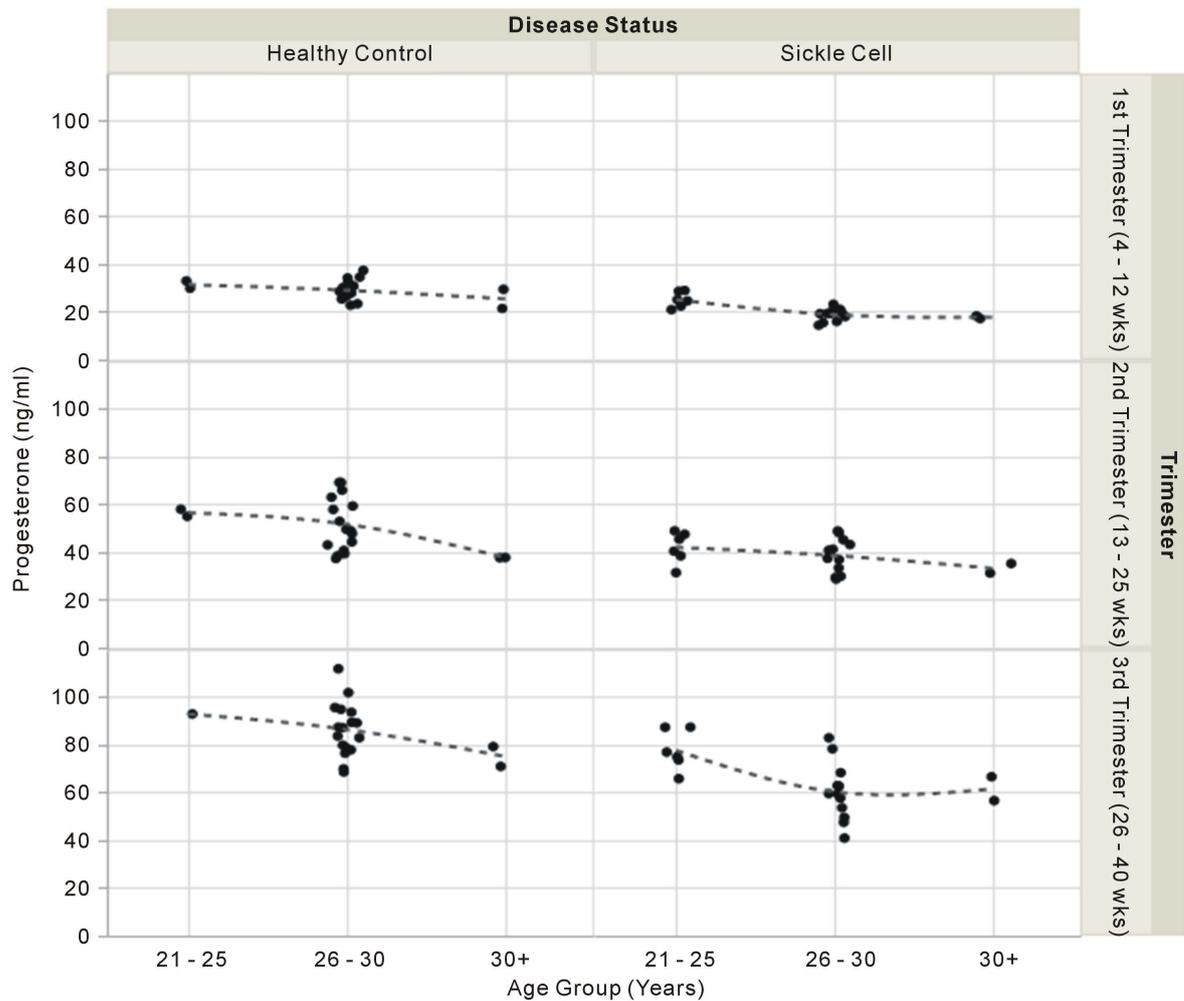
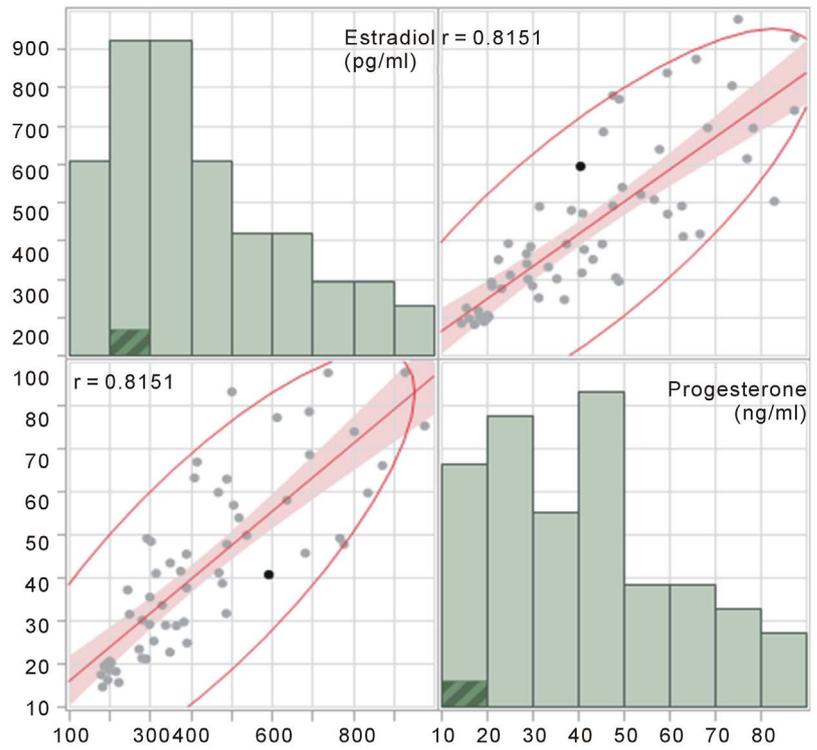


Figure 2. Progesterone levels in sickle cell and healthy pregnant women by age group and trimester. Ezeiruaku *et al.*, p. 9.

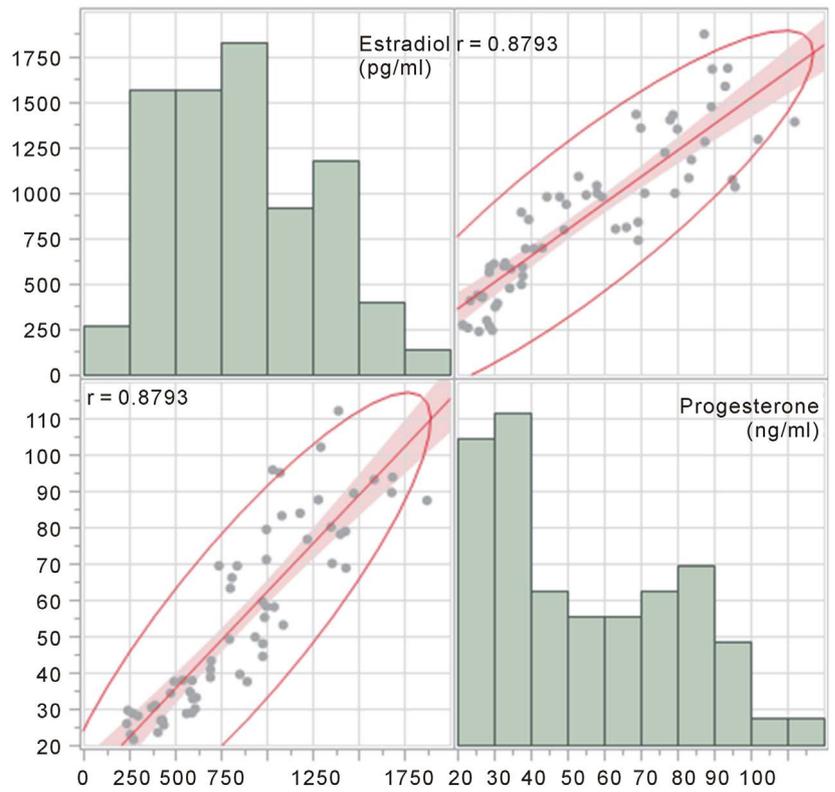
4. Discussion

This study shows that sickle cell disease itself does not directly damage the reproductive system of the subjects in question. It did show that the disease, as a result of the gene abnormality and its management can affect other system which eventually cause harm to the reproductive system. Published report [34] has shown that the treatment for SCA affects the endocrine system by inhibiting the production of gonadotropins from the pituitary gland. This means that sex hormones as shown in **Table 1** are not readily released.

The result of the study has shown that when compared with apparently healthy subjects, the sex hormones (estradiol, progesterone) secretion pattern in pregnant sickle cell anemia patients were significantly reduced. As shown in **Table 2** and **Table 3**, the sex hormones secretion were reduced to 42.48%, 50.84% and 53.02% for plasma estradiol, 28.62%, 22.95%, and 23.28% for plasma progesterone respectively for 1st, 2nd and 3rd trimester of pregnancy in SCD subjects. Statistical analysis showed a strong positive correlation, $r = 0.8151$ for estradiol and $r = 0.8793$ for progesterone (**Figure 3**) when the secretion pattern in sickle



(a)



(b)

Figure 3. Correlation between estradiol (pg/ml) and progesterone (ng/ml) secretion in sickle cell and healthy pregnant women. (a) Sickle Cell Pregnant Women; (b) Healthy Pregnant Women. Ezeiruaku *et al.*, 2018, p. 9.

cell disease patients is compared with the apparently health pregnant women. Further analysis of data obtained from the study, has shown that the estradiol and progesterone secretion decreases (**Figure 2** and **Figure 3**) with aging of the sickle cell pregnant women when compared with the apparently healthy pregnant subjects.

The sickle cell disease crises and the complications that arise as a result of pregnancy are as a result of many factors [35] [36] [37]. Studies [25] has it that how affluent the environment is goes a long way to predict the SCA patients health well being. Maintenance and proper management of the disease helps to reduce the risk of crises and complications in pregnancy.

When pregnancy rates of patients with SCD and healthy controls have been compared, the lower number of pregnancies in women with SCD has been used to infer that fertility is reduced in women with SCD [9]. Presently, we understand that many factors other than infertility may have influenced the number of pregnancies per patient. The level of sex hormone secretion in pregnancy might as well be a contributing factor in pregnancy success rates, its sustainability and increase of risk of stillbirth and complications.

5. Conclusion

Little is known about fertility in women with Sickle Cell Disease (SCD) because few studies have really addressed the fertility issue particularly as it affects the hormone secretions in pregnancy. This study focused on the sex hormone (estradiol, progesterone) secretion pattern in pregnant sickle cell disease subjects. Estimation of the hormones was carried out using the Enzyme Linked immunosorbent Assay (ELISA) and the result from the study has shown a reduction that is statistically significant ($P < 0.05$) in the three trimesters of pregnancy in the sickle cell anemia subjects. This is attributed to the treatment and management of the disease that affect the gonadal function that eventually cause harm to the reproductive system. The sickle cell gene abnormality has effects on endocrine functions. Hence follow up on pregnant SCD patients are advocated in the management of endocrine dysfunctions.

Conflicts of Interest

There are no conflicts of interest.

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