

Effect of Sex Hormones and Prolactin on Sickle Cell Erythrocytes Polymerization in PortHarcourt, Rivers State, Nigeria

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ABSTRACT- Sickle cell disease (SCD) is an inherited hematological disorder that causes red blood cells to break down continuously. This leads to a rigid, sickle like shape under certain conditions, causing polymerization of the sickled hemoglobin. This study was undertaken to know whether sex hormones (estradiol, progesterone, testosterone and prolactin) exert any effect on the polymerization of sickle cell erythrocytes *in vitro* and the possibility of these hormones having an effect on the sickling phenomenon. The hemoglobin polymerization test was carried out when hemoglobin S undergoes gelation after it was deprived of oxygen using 2% sodium metabisulphite as reductant. The polymerization inhibition studies were shown that estrogen, progesterone, testosterone and not prolactin had a statistical significant reduction effect ($P < 0.05$) on the polymerization of the sickle cell erythrocytes. The polymerization of the sickle cell erythrocytes was reduced to 50.90%, 62.74%, 67.56%, and 92.16% at the concentration of 50.0 pg/ml of estrogen, 5.0 ng/ml of progesterone, 6.0 ng/ml of testosterone, and 7.0 ng/ml of prolactin in the same order. This effect was achieved at a low concentration of these hormones. Higher concentrations of the hormones increased polymerization. The result suggests that using the hormones substances at low concentrations can help to ameliorate the intracellular polymerization of sickle cell hemoglobin.

Key-words- Sickle cell, Hormones, Polymerization, Progesterone, Estradiol, Testosterone, Prolactin

INTRODUCTION

Sickle cell disease is the most common genetic disorder in persons of African origin ^[1] and the disorder comprises a spectrum of syndromes that range from the most completely benign trait or carrier state (the HbAS genotype) to the most severe syndrome, the sickle cell anemia due to the homozygous presence of the Beta S (βS) globin genes (producing the HbSS genotype). Sickle cell anemia, an inheritance of mutant hemoglobin genes from both parents is globally wide spread ^[2].

Sickle cell anaemia is particularly common among people whose ancestors come from sub-Saharan Africa, India, Saudi Arabia and Mediterranean Countries and migration raised the frequency of the gene in the African Continent ^[3]. In Nigeria, the most populous country in the sub region with about 150 million inhabitants, 24% of the

populations are carriers of the mutant gene and the prevalence of the sickle cell anaemia is about 20 per 1000 births ^[4]. This means that in Nigeria alone, more than 100,000 children are born annually with sickle cell anaemia ^[5].

Sickle cell disease is caused by the pairing of an inherited autosomal recessive gene (βs -globin), which affects the red blood cells ^[6-7]. Deoxygenation of the red blood cells causes 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 organs and tissues. The sickling phenomenon occurs as a result of intracellular polymerization of sickle hemoglobin (HbS) which occurs upon deoxygenation of erythrocytes from patients homozygous for HbS ^[8].

There are two cardinal pathophysiological features of sickle cell disorder; chronic hemolytic anemia, and vas-occlusion (that results in ischaemic tissue injury ^[9]). Hemolytic anaemia may be related to repeated cycles of sickling and unsickling which interact to produce irreversible cell membrane changes, red cell dehydration and erythrocyte destruction.

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The hormones are chemical substance which travels from a special tissue where they are released into blood stream to distant responsive cells where the hormones exert their characteristics effects. The sex hormones are responsible for the development of sexual characters in men and women and have been established to play a lot of physiologic and metabolic roles in the body^[10].

The challenge at present is to improve the health of the numbers of patients with sickle cell disease worldwide. Results of many exciting studies initiated earlier in the decade have now become available to revolutionize the management of patients with sickle cell disease. This is categorized into those strategies which attempt to modify the clinical severity of the disease and strategies which attempts to cure the disease.

At present, strategies include the use of prophylactic therapy^[11], experimental treatments^[12], the RBC transfusion^[13], hydroxyurea^[14] and the bone marrow transplant with vitamin supplements among others^[15]. There are increasing data on the efficacy of newer agents including decitabine, a Gardos channel inhibitor (ICA-17043), butyrate and nitric oxide.^[12] Current data suggest that hydroxyurea therapy should be initiated early for adults with sickle cell disease. A growing body of literature supports the safety and efficacy of initiating hydroxyurea therapy in childhood. Long term treatment with hydroxyurea is safe, effective and affordable^[16].

The severity of the sickling phenomenon has been observed to be more at pre puberty, but at puberty the level of crises becomes fairly stable. This has been attributed to sex hormones that are responsible for the development of sexual characteristics in both male and female^[5]. The female attains puberty earlier and better sickling stability than the male and this might be as a result of the female sex hormone, estrogen.

The study was therefore designed to establish any possible effect (beneficial or otherwise) of these hormones on the sickling phenomenon. This is with a view to adding to the knowledge required in sorting out various approaches to health maintenance and complications of patients with sickle cell disease that persist till date.

MATERIALS AND METHODS

Study Population- Blood samples were collected from subjects and volunteers (sickle cell patients) of age between four and above years at the University Teaching Hospital Port Harcourt and the General Hospital, also in Port Harcourt, Rivers State, Nigeria. Patient's samples were identified after counselling and genotyping to determine their genotype group and some already known sickle cell patients that attended the clinics and sickle cell centers for routine medical check. The sample collection and study was done between May 2007 and July 2010.

Sample collection and preparation- The sample for the polymerisation experiment was collected by standard venepuncture technique into an EDTA Centrifuge

tube. The upper level of the blood was marked on the tube with a pen.

After centrifugation for 15minutes at 1100 x g, the plasma was removed with the aid of a Pasteur pipette. Isotonic saline (0.9% NaCl) was added to the mark in the Centrifuge tube and the erythrocytes were suspended in the saline by repeated inversion of tube. The suspended erythrocyte was then frozen and subsequently thawed out to produce a hemolysate.

Hemoglobin polymerization inhibition test- The hemoglobin polymerization (gelation) test was based on the method earlier described in 1985^[17]. The underlying principle is that hemoglobin-S (HbSS) undergoes gelation (polymerization) when deprived of oxygen using 2% sodium metabisulphite as a reductant. Polymerization was assessed by measuring the turbidity at 700nm in a spectrophotometer by using 2% sodium metabisulphite solution^[18-19]. The rate of hemoglobin polymerization was inhibited by addition of these compounds.

Reagents

1. 2% sodium metabisulphite solution
2. Sodium Chloride (Normal Saline)
3. Estrogene, Progesterone, Testosterone and Prolactin Standard were obtained from stigma company, USA

Procedure- The 4.8 ml of 2% sodium metabisulphite, and 0.1ml of HbSS hemolysate were quickly mixed in a cuvette and the optical density (absorbance) readings taken at 700nm and at 2 minutes interval in a spectrophotometer. This served as control experiment for the assay. 4.8ml of 2% sodium metabisulphite, 0.1ml of Hbss hemolysate and 0.05ml of test compound (hormones) were mixed in a cuvette and the absorbance readings taken at 700nm and at 2 minutes interval for 20minutes. Different concentrations of the various hormones standard were used (Table 1 to Table 4). The percentage rate of inhibition of polymerization was calculated with respect to the control experiment without the test compounds. Each procedure with the hormone substance at different concentrations was repeated seven times and the average was taken.

RESULTS

The result of the study showed that the sex hormones (estradiol, progesterone, testosterone) had a statistical significant reduction ($P < 0.05$) on the polymerization of the sickle cell erythrocytes *in vitro*. The hormone, prolactin had no statistical significant effect on the polymerization process at this 95% confidence level. The table below show the *in vitro* effect of the varied concentrations of the hormones on the erythrocytes polymerization.

Table 1: *In vitro* effect of varied concentration of prolactin on hemoglobin SS erythrocyte polymerization

Concentration (ng/ml)	Control rate of polymerization (%)	Average percentage effect on polymerization (n= 7)
0.1	100	98.74
0.5	100	98.74
3	100	97.92
7	100	92.16
15	100	103.22
25	100	436.52
30	100	719.59

The prolactin at a concentration of 7.0ng/ml reduced polymerization of the erythrocytes to 92.16%. This was not statistically significant when compared with the controls.

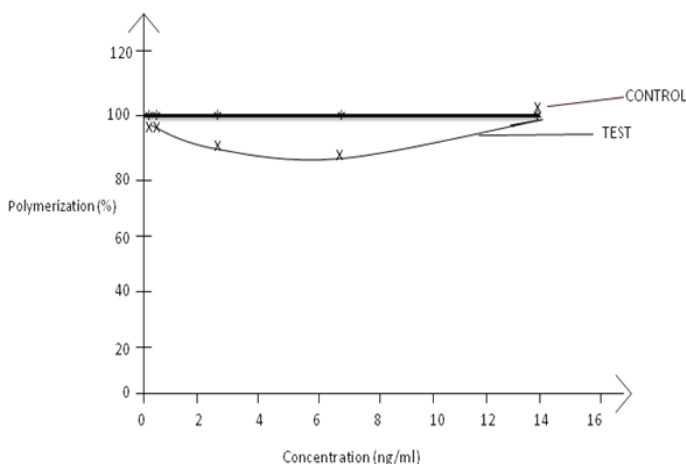


Fig 1: *In vitro* effect of varied concentration of prolactin on hemoglobin SS erythrocytes polymerization

Table 2 shows, the effect of estradiol at various concentrations on the hemoglobin SS erythrocyte polymerization. Estradiol at 50pg/ml concentration reduced the polymerization to 50.90% which was statistically significant at 95% (P<0.05) confidence level.

Table 2: *In vitro* effect of varied concentration of Estradiol on hemoglobin SS Erythrocyte Polymerization

Concentration (pg/ml)	Control rate of polymerization (%)	Average percentage effect on polymerization (n=7)
0.1	100	97.35
5	100	77.55
10	100	70.15

25	100	61.24
50	100	50.90
75	100	575.30
100	100	968.40

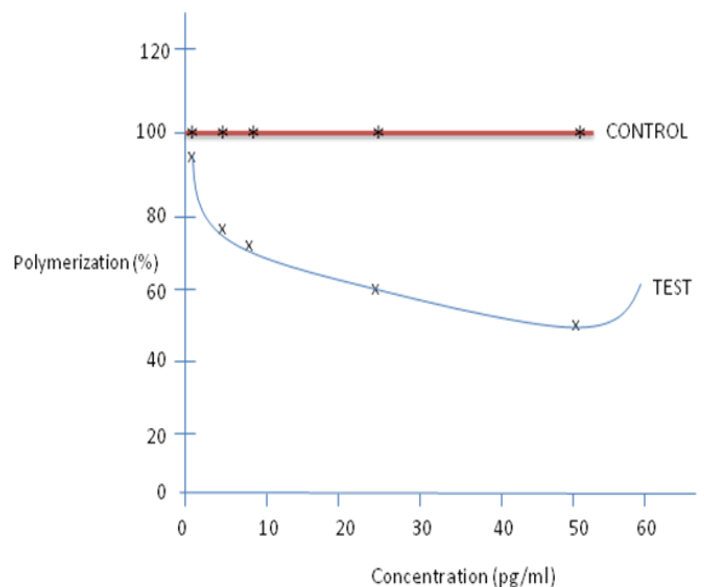


Fig 2: *In vitro* effect of varied concentration of Estradiol on hemoglobin SS Erythrocyte Polymerization

Table 3 showed the effect of progesterone at various concentrations on the hemoglobin SS erythrocyte polymerization. Progesterone at 5ng/ml concentration reduced the polymerization to 62.74% which was statistically significant at 95% (P<0.05) confidence level.

Table 3: *In vitro* effect of varied concentration of progesterone on hemoglobin SS Erythrocyte polymerization

Concentration in ng/ml	Control rate of polymerization (%)	Average percentage effect on polymerization (n=7)
0.1	100	98.16
0.5	100	90.10
1	100	73.60
2	100	69.50
5	100	62.74
10	100	219.22
25	100	622.70

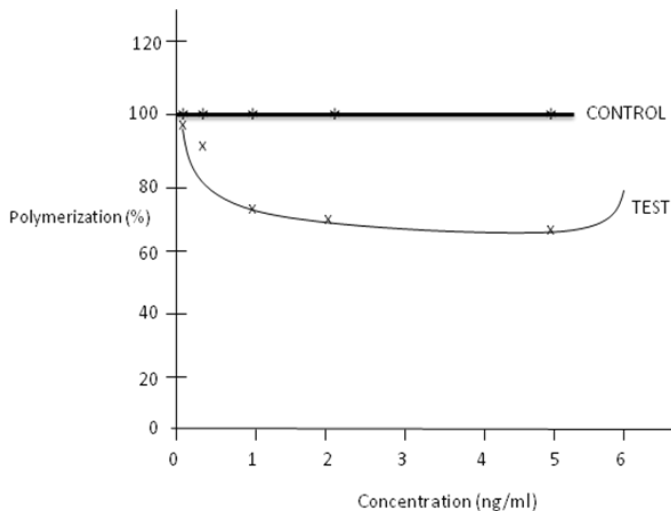


Fig 3: *In vitro* effect of varied concentration of progesterone on hemoglobin SS erythrocyte polymerization

Table 4 shows testosterone at concentration of 6ng/ml reduced hemoglobin SS erythrocyte polymerization to 67.56%. This was significant at the 95% confidence level (P<0.05) Considered.

Table 4: *In vitro* effect of varied concentration of testosterone on hemoglobin SS Erythrocyte Polymerization

Concentration (ng/ml)	Control rate of polymerization (%)	Average percentage effect on polymerization (n=7)
0.5	100	97.91
1	100	94.05
2	100	84.41
3	100	79.22
6	100	67.56
10	100	273.62
16	100	504.10

DISCUSSION

The sickling phenomenon in sickle cell disease occurs as a result of the intracellular polymerization/gelling of sickle hemoglobin (HbS) which occurs upon de-oxygenation of erythrocytes. This brings up several issues about the treatment of sickle cell disease. The medicine, hydroxyurea does not cure the disease, but it can reduce the number of crisis which normally results in hospitalization. There is no specific therapy for crisis; treatment is pain management, hydration and treatment of complications, if present. The recent hydroxyurea can raise fetal hemoglobin, which carries oxygen at the infant age, red blood cell with its hemoglobin content need to be maintained in its flexibility shape to enable oxygen transport; this establishes the need to reduce polymerization or gelling, particularly during crises as a result of de-oxygenation.

The study tried to give a broad view of hormones, which also have antioxidant properties to the classic antioxidants [20]. The polymerization inhibition studies showed that estrogen, progesterone and testosterone, but not prolactin had a statistical significant (P<0.05) effect on the polymerization of sickle cell erythrocytes *in vitro*. The polymerization of the sickle cell erythrocytes was shown to be reduced to 67.56% by testosterone, 62.74% by progesterone, 50.9% by estrogen and 92.16% by prolactin respectively. This effect was achieved at an optimal concentration of these hormones. Higher concentrations of the hormones increased polymerization. Hormones useful effects are best exhibited at low concentration [21]. This could be explained by the powerful effects exhibited by these hormones when they are used at high doses, which goes to explain why hormones should be used where there was a demonstrable deficiency, in physiological doses and monitored. The mechanism of the hormones effects on polymerization might not be clear, but might have acted as an antioxidant, thereby reducing the effect of the oxidants in the polymerization process. Reduced antioxidant in SCD increases the phenomenon of hemolysis [22]. The hormones most probably protected the RBCs against hydrogen peroxide that might have induced haemlysis.

For decades, it has been appreciated [23] that increasing the amount of circulating, non gelling hemoglobin should ameliorate some or all of the manifestation of the sickle cell anemia. The sex hormones studies have some of the properties required in reducing gelation and thus can increase the level of circulating, non gelling hemoglobin in sickle cell patients. This study showed that this could be achieved in small doses of these hormones since increased amount of the hormones substances caused polymerization. Several authors in the past [8,24] have used different non-covalent inhibitors (Urea, Amino acids) and covalent reagents (anti coagulant agents, Glyceraldehydes, nitrogen mustards) for inhibition of HbS sickling. They believe that agents that are capable of having intra cellular interaction will reverse sickling by maintaining the integrity of the sickle cell membranes.

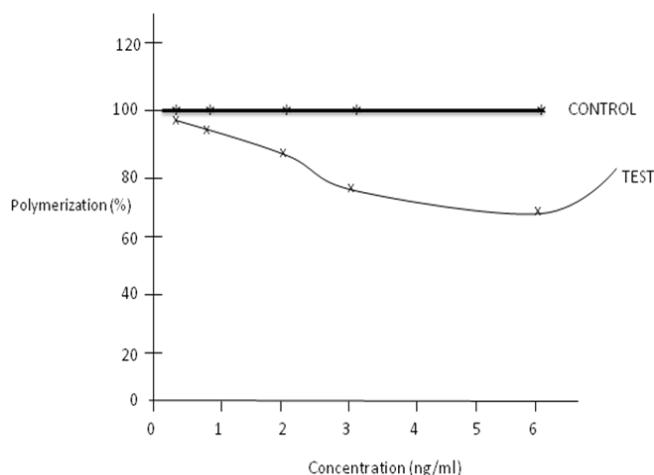


Fig. 4: *In vitro* effect of varied concentration of testosterone on hemoglobin SS erythrocyte polymerization

CONCLUSIONS

The study was carried out to establish the effect of sex hormones and prolactin on the polymerization of sickle cell erythrocytes *in vitro*. The 2% sodium metabisulphite was used as the reductant compound in the polymerization experiment. The result showed that estradiol, progesterone and testosterone had a statistical significant effect in reversing polymerization of sickled erythrocytes at low concentrations. Higher concentrations of the hormones increased polymerization. The hormone prolactin had no statistical effect on the polymerization of the sickled erythrocytes. The study showed that the sex hormones have antioxidant properties that can be used to reduce polymerization *in vitro* and can therefore be used where there is a demonstrable deficiency at low concentrations in the management of sickle cell complications.

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REFERENCES

- [1] Huisman JHJ. Sickle cell anaemia as a syndrome. A review of diagnostic features. *American journal of Haematol.*, 1981; 6: 173-77.
- [2] World Health Organization. World Health Organization report on Sickle cell anaemia. Geneva, 2005.
- [3] Wick T. New hope of patients with sickle cell anaemia. Sickle cell information centre, Georgio Institue of Technology Geogia USA, 1998; pp. 5-15.
- [4] National Institutes of Health (NIH). Fact about sickle cell anaemia, 2006.
- [5] Oredugba PA, Savage KO. Anthropometric findings in Nigeria Children with sickle cell disease. *Paediatric Dentist.*, 2002; 24(4): 321-25.
- [6] Jennifer KL, Kathryn MC. Progesterone only contraceptive use among women with sickle anaemia: A systematic review. *Contraception*, 2005; 73: 195-204
- [7] Ashley-Koch A, Yang Q, Olney RS. Sickle haemoglobin (HbS) allele and sickle cell disease. *Review J. Epidemiol.*, 2000; 151: 839-45.
- [8] William NP. Non covalent inhibitor of sickle haemoglobin gelation. Effects of aliphatic alcohol, amides and ureas. *Biochem.*, 1980; 19: 3194-99.
- [9] Quinn CT, Ahmed H. Prognostic Significance of Early Vaso Occulsive Complications in Children with sickle cell anaemia. *Blood*, 2007; 109: 40-49.
- [10] Goldfiel A. The Gonadal Hormones and inhibitors. In *basic and claimed pharmacology*, (Katzung B Gred) Appleton Harge, 1998; pp: 653-80.
- [11] Wang WC. Role of nutritional supplement in sickle cell disease. *J. Paediatric Haematol. Oncol.*, 1999; 21: 176-78.
- [12] Zakari YA, Ashaunta RT, Gregory JK. Current therapy of sickle cell disease. *Haematologica*, 2008; 91(1): 7-10.
- [13] Wanko SO and Telen, MJ. Transfusion management in sickle cell disease. *Haematol. Oncol. Clinical North Am.*, 2005; 19: 803-26.
- [14] Charache S, Terrin ML, Moore RD, Dover GJ, Barton FB, et al. Effect of Hydroxyurea on the frequency of painful crises in sickle cell anaemia. Investigators of the multicenter study of hydroxyurea in sickle cell Anaemia. *New England J. Med.*, 1995; 332: 1317-22.
- [15] Steinberg MH. Sickle cell disease and hydroxyl urea, the good, the bad and the future. *Blood*, 2005; 105: 441-52
- [16] Barbara PY, George RB, Araba NA, et al. Management of sickle cell disease. Summary of the 2014 evidence based report by expert panel members. *J. Am. Med. Assoc.*, 2014; 32(10): 1033-48.
- [17] Noguchi CT and Schechter AN. Sickle cell polimerisation in solution and in cells. *Ann. Review Biophysical Chem.*, 1985; 14: 239-46.
- [18] Iwu MM, Igboko AO, Onwubiko H, Ndu UE. Effect of cajaminose from *cajanus cajan* on gelation of oxygen affinity of sickle cell haemoglobin; *J. Etnopharmacol.*, 1988; 23: 99-104.
- [19] Uwakwe AA and Akhidue NP. The effect of quinine on human haemoglobin -S-(HbS) Erhthrocyte sickle and Hbs gelation rate. *Journal of applied science Environ. Management*, 2000; 4(2): 75-77.
- [20] Mancini A, Festa R, Didoma V, Leore E, Litteru G, et al. Hormones and antioxidant systems. Role of pituitary and pituitary dependent axis. *J. Endocrinol. Investigation*, 2010; 33(6): 422-33.
- [21] Myhill S. Steroid hormones secretion and transport 2004; <http://www.lib.mcg.edu/eshuphysio/prog/section5>, Retrieved Oct., 2005.
- [22] Ren H, Ghebremeskel K, Okpala I, Lee A, Ibegbulam O, et al. Patients with sickle cell disease have reduced blood antioxidant protection. *Int. J. Vitamin Nutrition Res.*, 2008; 78 (3): 139-47.
- [23] Dean J, Schechter A. Sickle cell anaemia: The molecular basis of therapeutic approaches, 1978; 299: 801-11.
- [24] Micheal LT, Gerald W, Gorman BD, Word CR. Sickle cell haemoglobin gelation inhibition by tri (hydroxymethyl amino methane and sugars. *Biochem. Pharmacol.*, 1973; 22: 667-74.

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