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Polymerization of Sickle Cell Disease and Methaeglobin in the Presence of Paracetamol

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Abstract: This study was designed to examine the level of polymerization of sickle cell disease and methaeglobin i n the presence of paracetamol using standard procedures. The present study has shown the level of polymerization o f sickle cell disease and methaemoglobin in the presence of paracetamol. Within the experimental time of 30-180s, t he relative polymerizations range between the following; 70.43 ± 0.87 to 72.10 ± 0.37 at the control (0 mg/dL), at 50 mg/dL, there was an increase from 65.78 ± 0.89 to 69.47±1.00, at 100 mg/dL there was an increase in the polymeriz ation from 68.96 ± 0.99 to 72.33 ±1.02, at 200 mg/dL there was an increase in the polymerization from 65.96 ± 69.2 6 ± 1.00 and at 500 mg/dL, there was an increase in the polymerization form 66.05 ± 0.98 to 69.42 ± 0.92. This incr ease in polymerization can be said to be due to the increase in the absorbance of paracetamol. However, the absorba nce of the polymerization mixture in the presence of the malarial drug was not significantly different ($p<0.05$) from t he control sample at the 30second. The present study showed that the level of polymerization of HbS molecules was attenuated upon the introduction of the anti-malarial drugs in the polymerizing mixture. The percentage of methaem oglobin increases with the increase in concentration of paracetamol from 2.77 ± 0.05 to 3.30 ± 0.03 starting from 0m g/dL to 500mg/dL concentration.

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1. Introduction

Sickle cell disease (SCD) is an extremely pulverizing condition brought about by an autosomal latent acquired haemoglobinopathy. This disease influences great people comprehensively which brings about genuine intricacies because of vasoocclusive marvel and haemolysis. This hereditary irregularity is because of replacement of amino corrosive valine for the glutamic corrosive at the 6th situation of beta chain of hemoglobin. This ailment was depicted around one multi year prior. The hemoglobin S (hbS) delivered as consequence of this imperfection is inadequately dissolvable and polymerized when deoxygenated. Manifestations of sickle cell illness are because of incessant frailty, torment full emergencies, intense chest disorder, stroke and powerlessness to bacterial disease [1]. As of late estimates like pre-birth screening, better clinical consideration, parent instruction, vaccination and penicillin prophylaxis have effectively decreased dreariness and mortality and have expanded massively future of influenced people. Three head current therapeutics modalities accessible for youth SCD are blood bonding, Hydroxy urea and bone marrow transplantation. Hereditary advising, proceeded with clinical training for wellbeing experts about sickle cell infection, its entanglements and the executives is essential. World wellbeing association has effectively advanced a few national screening programms with double objectives of illuminating regenerative decision and consequently lessening the quantity of seriously influenced children. Sickle cell disease (SCD) is the most conventional inherited blood disorder in the U.S., affecting about 7,500 Americans [2]. The most common of this disease is sickle cell anemia and this disease is the most common hereditary disease among African-Americans and affects about one out of every 500 newborns [3]. People of other tribes are also affected by Sickle cell disease, with a rate of one of every 1,000 to 1,400 Hispanic-American births. A meaningful prevalence of the mutation which geared for sickle cell has been reported among other ethnic groups such as those native to Turkey, Italy, Greece, China, Pakistan, Saudi Arabia, India, Bangladesh, and Cyprus [4-6]. There has been an ongoing blast of enthusiasm among scientists to grow new therapies for SCD, as shown by the enormous number of dynamic

clinical protocols [23-27]. The intrigue and progress in finding new medicines have been energized by different elements. With a proper kin coordinate, the disease can be healed in the both children and grown-ups by stem cell transplantation [28]. Moreover, SCD is a proving ground for the energizing developing techniques for quality treatment and gene editing [28]. Although these are likely treatments for patients, however, neither one of the will be accessible for a very long for a huge number of patients in sub-Saharan Africa and elsewhere. Therefore, what is expected to treat by far most of patients is a moderate medication that can be taken orally. [7] find out the capacity of aqueous concentrate of N. tabacumto alters with polymerization of deoxygenated sickle cell hemoglobin (deoxyHbS) atoms in vitro. Spectrophotometric method was utilized to quantify level of sodium metabisulphite induced polymerization of deoxyHbS atom brooded in fluid concentrate of N. tabacumfor 180 s. The polymerization profile of deoxyHbS particles of control and tests demonstrated expanding level of polymerization with movement of trial time. The investigation demonstrated that fluid concentrate of N. tabacumexacerbated polymerization of deoxyHbS atoms in a fixation and time subordinate way. [8] Reported that drugs extracts cause alterations on the shape and physiology of erythrocytes. The two test centralizations of P. guajava and T. catappa ensured the erythrocytes against osmotic worry, as confirm by diminishes in the estimations of MCF contrasted and the control test ($P < 0.05$). In any case, 800 mg/dL of A. occidentale advanced critical ($P < 0.05$) distabilization of sickle erythrocytes. End: Whereas the two test centralizations of fluid concentrates of P. guajava and T. catappa settled erythrocyte layer, higher fixation (800 mg/dL) of A. occidentale displayed no film defensive impact. The specific aim of this research work is to determine the level of sickle cell disease and methamoglobin in the presence of paracetamol. Acetaminophen and paracetamol are two official names of similar chemical compound which is a derivative from its chemical name: N-acetyl-para-aminophenol and N-acetyl-para-aminophenol. This medicine has an extensive record and, as it often happens with significant findings, it was found by chance [17-20]. Paracetamol is commonly innocuous at recommended doses. Paracetamol is classified as a mild analgesic which is commonly used in many homes and hospital places for treating mild headache, fever, Osteoarthritis, Postoperative pain, low back pains etc. It does not have significant anti-inflammatory activity and how it works is not entirely clear. Paracetamol was discovered in 1877 [21].

2. Experimental

Study Design

Collection of Acetylsalicylic Acid

Acetylsalicylic acid was gotten from a chemical store around Wetheral road, Owerri, Imo State.

Preparation of Acetylsalicylic Acid

Collection of sample

Five milliliters (5.0 ml) of human venous blood of HbSS genotype was collected by venipuncture and was stored in the EDTA anticoagulant tube. The blood sample was obtained from a male volunteer (71kg) in the age of 29 years attending clinic at the St. David Hospital Owerri. The erythrocytes was washed by centrifugation methods as described by [21], within two hours of collection of blood sample, portion of 1.0 ml of the sample was introduced inside centrifuge test tube containing 3.0 ml of buffer solution pH = 7.4: 250 MM tris (hydroxyl methyl) amino ethane– HCl (Tris–HCl) 140 mm NaCl (1.0 mm) MgCl (10 mm glucose). Erythrocyte was spited from plasma by centrifugation at 1200g for 10 minutes and washed three times by the same centrifugation method and the buffer solution. The erythrocyte was finally re-suspended in 1.0 ml of this buffer and stored at $4^{O}C$. The washed erythrocytes were lysed by freezing as described by [22]. The erythrocyte hemolysate was used for the polymerization analyses

Biochemical Assay

Polymerization Studies

0.1ml (milliliter) of Hbss hemolysate was introduced into a test tube. 0.5ml of the phosphate buffered saline solution was introduced into the same test tube and also 1ml of water. The mixture was transferred in a cuvette and 3.4ml of 2g% aqueous solution of $Na_2SO_2O_5$ was added. The absorbance of the assay mixture was recorded with a spectrophotometer (Uv-2600 MODEL, Craic technologies) at every 30 seconds for 180 seconds at max = 700nm (control sample). This procedure was repeated by substituting the distilled water with 1.0ml of the corresponding four increasing concentrations of acetylsalicylic acid.

The % polymerization was calculated as expressed in (1)

% polymerization= At / c .100/Ac180th sec (1)

Where: At/c =Absorbance of test/ control assay at time = ts

Ac180th s =Absorbance of control assay at the 180s

Methemoglobin Concentration Assay

Control: In a test tube containing 5.0ml of distilled water, 0.02ml of whole blood was added. The mixture was allowed to stand for 60 minutes at room temperature and the absorbance was read at two different wave lengths maximum, 540nm and 630nm, using a spectrophotometer.

Test: The effect of acetylsalicylic acid on plasma methemoglobin concentration was carried out by introducing 0.02ml of the specified concentrations (50 - 500 mg/dL) of the Acetylsalicylic acid solution into separate test tubes. This was followed by the addition of 5ml of distilled and 0.02 ml of the whole blood sample. The mixture was allowed to stand for 60 minutes at room temperature, after which, the absorbance was read at 540nm and 630nm using a digital spectrophotometer. The percentage plasma methemoglobin was obtained with the formula (2)

$$\% \text{ methemoglobin} = (A630)2/(A540)2+(A630)2 \times 100 \quad (2)$$

Where A540 and A630 was absorbance at maximum wavelength of 540nm and 630nm respectively

Data analysis

The data were calculated for their percntage. The statistical analysis were carried out using Microsoft excel 2013. The test statistics was used to test for differences between means both at 5 % level of significance.

3. Results and Discussion

This present study has shown the level of polymerization of sickle cell disease and methaemoglobin in the presence of paracetamol. The change in absorbance of the control test of the samples and the corresponding percentage polymerizetion are presented in Table 1 above and in Figure 3. Within the experimental time of 30-180s, the relative polymerizations range between the following; 70.43 ± 0.87 to 72.10 ± 0.37 at the control (0 mg/dL), at 50 mg/dL, there was an increase from 65.78 ± 0.89 to 69.47±1.00, at 100 mg/dL there was an increase in the polymerization from 68.96 ± 0.99 to 72.33 ±1.02, at 200 mg/dL there was an increase in the polymerization from 65.96 ± 69.26 ± 1.00 and at 500 sec mg/dL, there was an increase in the polymerization form 66.05 ± 0.98 to 69.42 ± 0.92.

Table 1: Percentage inhibition of polymerization

Conc. (mg/dL)	30 sec	60 sec	90 sec	120 sec	150 sec	180 sec
0	70.43 ±0.87^{b}	71.26±0.89^{b}	71.68±1.06^{b}	71.99±0.93^{b}	72.21±1.11^{b}	72.1±0.37^{b}
50	65.78±0.89^{a}	67.50±1.00^{a}	68.52±1.00^{a}	68.76±0.99^{a}	69.17±1.03^{a}	69.47±1.00^{a}
100	68.96±0.99^{b}	70.6±1.05^{b}	71.43±0.76^{b}	71.82±1.01^{b}	72.07±1.07^{b}	72.33±1.02^{b}
200	65.96±0.95^{a}	67.66±0.94^{a}	68.32±0.93^{a}	68.67±1.02^{a}	68.74±0.64^{a}	69.26±1.00^{a}
500	66.05±0.98^{a}	67.59±0.97^{a}	68.47±0.97^{a}	68.77±0.97^{a}	69.06±0.58^{a}	69.42±0.92^{a}

Above values are mean ± standard deviation. The means in the same column with different superscript are statistically significantly different at $P \leq 0.05$

Generally, there was a declining capacity of the anti-malaria's to inhibit HbS polymerization as the experimental time approached the 180s. The present study showed that the level of polymerization of HbS molecules was attenuated upon the introduction of the anti-malarial drugs in the polymerizing mixture. The pattern by which these drugs effected this inhibitory action was similar to phenylalanine [14]. From the result obtained from table 2 and figure 2, the percentage of methaemoglobin increases with the increase in concentration of paracetamol from 2.77 ± 0.05 to 3.30 ± 0.03 starting from 0mg/dL to 500mg/dL concentration.

Table 2: percentage Methaemoglobin

S/N	mg/dL	Methaemoglobin (g/dL)
1.	0	2.77± 0.05^{a}
2.	50	2.79±0.07^{ab}
3.	100	3.07 ± 0.24^{ab}
4.	200	2.83 ±0.34^{ab}
5.	500	3.30±0.03^{a}

Values of the table above are mean ± standard deviation and mean with different superscripts are statistically significantly different at P = 0.05 in the same column only not row.

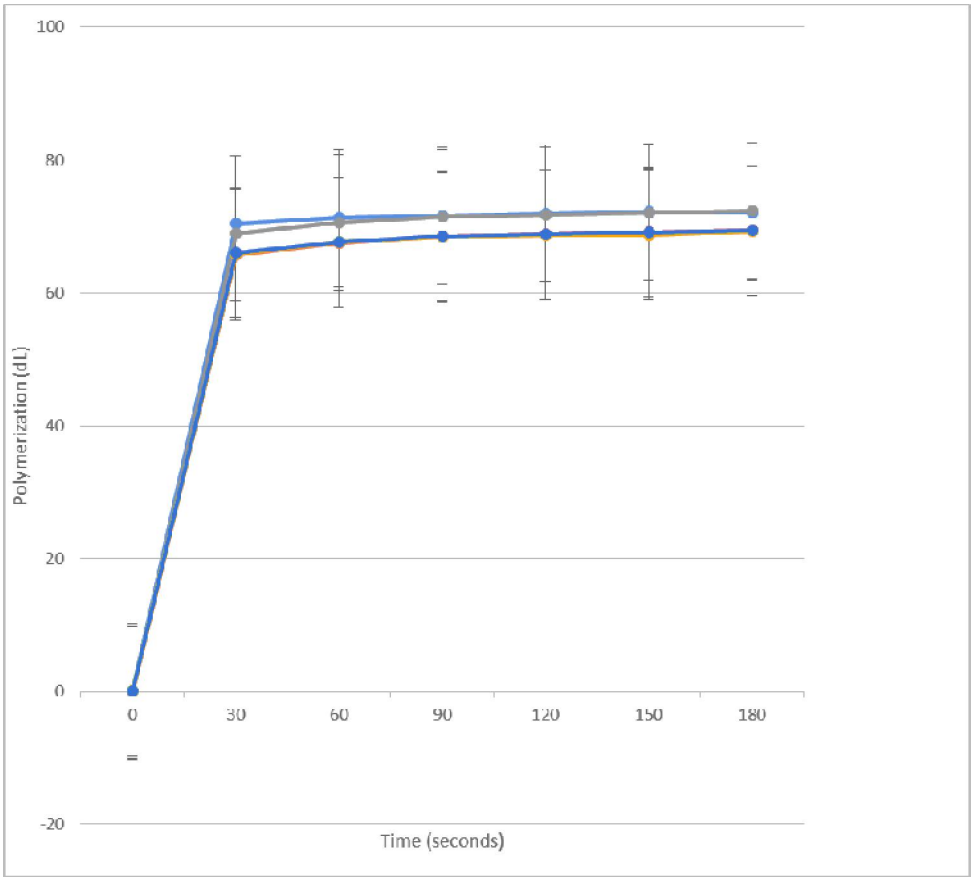

Figure 2: Plot of polymerization against time

This increase in polymerization can be said to be due to the increase in the absorbance of paracetamol. However, the absorbance of the polymerization mixture in the presence of the malarial drug was not significantly different ($p<0.05$) from the control sample at the 30 second. These values were indications that polymerization of HbS molecules occurred in the control sample and in the presence of the malarial drug (Figure 2), this result was slihtly differentt from the result obtained from [16]. There are sensational advances in relieving sickle cell ailment by hematopoetic undifferentiated cell transplantation, with quality treatment fixes in the close future. However, these medicines are costly and require propelled clinical offices and are in this way not accessible to by far most of patients on the planet experiencing sickle cell ailment and may not be for quite a long time. Thusly, what is direly required presently is an economical anti - sickling pill. Treatment won't require a medication that totally hinders sickling, however one that just expands the defer time to permit more cells to get away from the microcirculation before strands structure. There is in this way cause for good faith, as there are a few systems for expanding defer times other than by expanding fetal hemoglobin synthesis [15]. They incorporate expanding cell volume to diminish HbS focus, restricting a medication to the R adaptation, in this way moving the T - R harmony toward the non - polymerizing R compliance, diminishing intracellular 2,3 - diphosphoglycerate (2,3 - DPG) to destabilize the fiber and moving the quaternary balance toward R, expanding intracellular pH (2,3 - DPG additionally increments intracellular pH), and destabilizing the fiber by restricting a medication to square intermolecular contact.

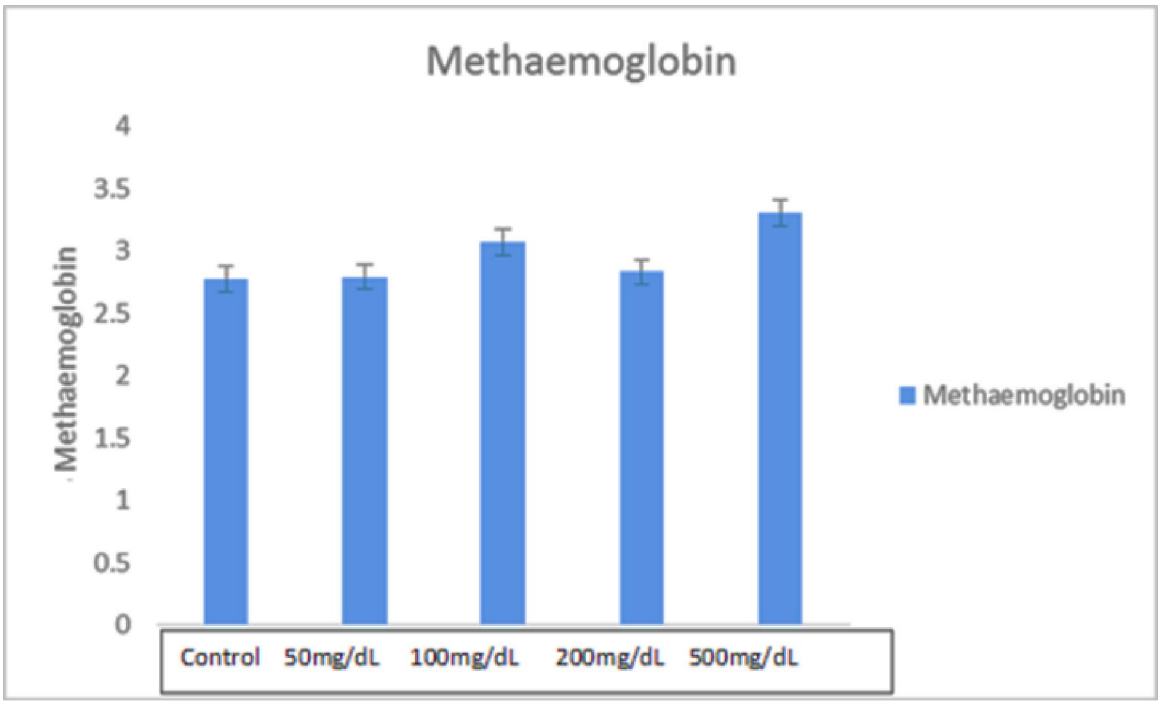

Figure 3: Bar chart with standard error bars showing the level of Methaemoglobin

Methemoglobin is a technique of the oxygen-conveying metalloprotein hemoglobin, in which the iron in the heme bunch is in the Fe3+ (ferric) state, not the Fe2+ (ferrous) of ordinary hemoglobin. Methemoglobin can't tie oxygen, differentiating oxyhemoglobin. The bar chart above on figure 3, shows the level of methaemoglobin using error bars. The highest level of methaemoglobin is on 500 mg/dL, followed by 100 mg/dL and the least seen at 50 mg/dL, its in line with the reult obtained from [26].

4. Conclusion

Sickle cell disease is a chronic, debilitating disorder with a myriad of symptoms that make disease treatment challenging. While there is a need for new treatment for sickle cell disease, especially for disease modifying agents, there is also a need to explore new approaches for improving treatment with existing modalities. Preventive measures particularly in disease endemic area must be taken such as pre-marital genetic counseling and screening. Future research must be focused on decreasing the number of crises and blood transfusion through new remedies having easy availability, less cost and minimum side effects. The management of sickle cell disease and methaemoglobin have remained a matter of concern in both developed and developing countries. A greater awareness and understanding of the communities and health care personnels about sickle cell disease and methaemoglobin and its detection has been found to be beneficial in the management of the disease. Several studies have clearly shown that genetic counseling is considered as one of the best ways of controlling the disease. The preventive measures include continued community education programmes for areas with high prevalence of the disease by creating and strengthening the national sickle-cell disease control programmes. Setting up sickle-cell screening and genetic counselling programmes. The disease should be identified during the prenatal period or at birth as part of a routine screening programme. Use of prophylactic drugs namely chloroquine and penicillin. Ongoing basic and clinical research. Provision of primary health care (access of sickle cell children to health centers). Improved standard of living and better feeding for patients with SCD.

Conflicts of interest

All the authors declared no conflict of interest.

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