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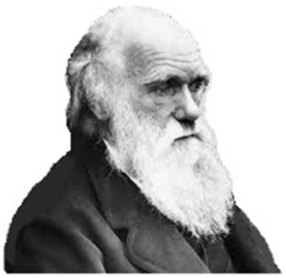

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Polymerization of Sickle Cell Disease and Methaeglobin in the Presence of Paracetamol

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ABSTRACT

This study was designed to examine the Level of Polymerization of Sickle Cell Disease and Methaeglobin in the Presence of Paracetamol, the research was carried out using standard procedures. The present study has shown the level of polymerization of sickle cell disease and methaemoglobin in the presence of paracetamol. Within the experimental time of 30-180 seconds, 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 mg/dl, there was an increase in the polymerization form 66.05 ± 0.98 to 69.42 ± 0.92. 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. The present study showed that the level of polymerization of Hemoglobin S (HbS) molecules was attenuated upon the introduction of the anti-malarial drugs in the polymerizing mixture. The percentage of methaemoglobin increases with the increase in concentration of paracetamol from 2.77 ± 0.05 to 3.30 ± 0.03 starting from 0 mg/dl to 500 mg/dl concentration.

***Keywords*:** Methaemoglobin, paracetamol, polymerization, sickle cell

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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 vaso-occlusive 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. 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]. Sickle cell disease is depicted by a fundamental abnormality in the beta-globin chain of the hemoglobin molecule within the red blood cells (RBCs). The sickle mutation is a single base change (GAT → GTT) in the sixth codon of exon-1 of the beta-globin gene on chromosome 11. This alteration steers to the synthesis of the beta-globin polypeptide of the hemoglobin molecule [3]. This mutation leads to the substitution of the normal glutamic acid with valine acid, thus leading to the development of the sickle cell hemoglobin (HbS).

This hydrophobic amino acid substitution causes the hemoglobin to take on a sickle shape when in a deoxygenated state [5]. The proficiency of these sickled cells to acclimatize to their surroundings is compromised, especially in the microvasculature. These cells hemolyze prematurely, accounting for the chronic anemia frequently encountered by patients with SCD. The paucity of sickled cells in newborns with Sickle cell disease led to the discovery that fetal hemoglobin (HgF) reduces the severity of SCD by preventing the formation of the hemoglobin S polymer [6, 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 test 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 sickle cell disease is categorized into three sub-headings

i. Sickle cell anemia: It is frequently reserved specifically for patients who are homozygous for hemoglobin S (hemoglobin SS)
ii. Sickle Cell Disorder: this include all states in which a sickle gene is inherited. This set includes all patients with a positive sickle preparation smear. The patient may or may not be symptomatic.
iii. Sickle Cell Disease: It is a disorder in which significant indisposition, such as organ failure or vaso-occlusive pain crises (VPC), results from the sickling of red blood cells [5].

Sickle cell disease is a hereditary hemoglobinopathy leading from inheritance of a mutant version of the β-globin gene (βA) on chromosome 11, this gene codes for assembly of the β-globin chains of the protein hemoglobin A. The mutant β-allele (βS) codes for the production of the alternate hemoglobin, hemoglobin S. The sickle cell gene mutation is a point mutation in the sixth codon of exon in the βA gene, replacing adenine with thymine (guanine-adenine-guanine guanine-thymine-guanine) [9].

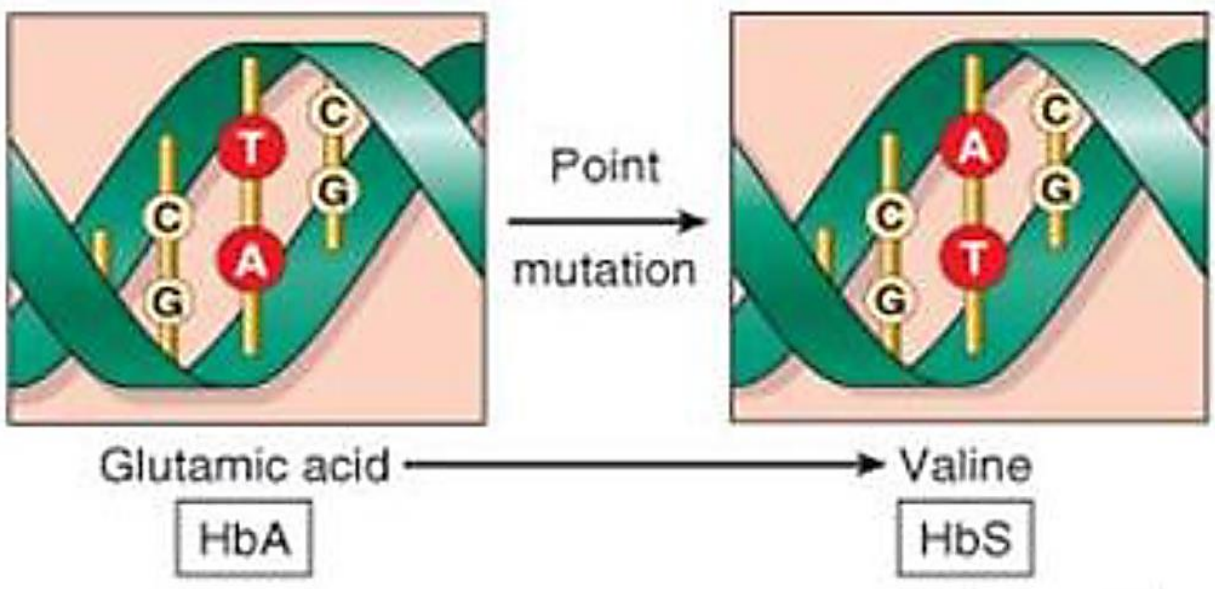

Figure 1. Representing replacing adenine with thymine

Homozygosity for the sickle mutation (i.e., HbSS disease) is accountable for the furthermost common and most austere variant of SCD. Numerous other genetic modifications of SCD result from the communication of dissimilar mutations of the human b-globin genes. Sickle-cell disease represents all genotypes containing at least one sickle gene, in which HbS makes up at least half the haemoglobin present [10]. In relation and additively to the homozygoticHbSS disease (sickle-cell anaemia), five other major sickle genotypes are connected to the disease. Production of HbS is a monogenic occurrence, which determine the polymerisation of the deoxygenated haemoglobin. The procedure is an essential however lacking determinant of phenotype. By divergence, the phenotype of sickle-cell frailty is multigenic [11].

Different qualities, unlinked to the β-globin locus, endorse in significant neurotic occasions (e.g., fast demolition of sickle cells, thick cell arrangement and attachment to endothelium) that are controlled by numerous qualities, known as pleiotropic or optional effector genes. Harshness and the seriousness of sickle cell weakness among various people changes since there are distinction in patients having indistinguishable pleiotropic qualities. A portion of the transporters have transformed qualities that can either enhance or intensify the phenotype [12]. The significant transmission factor of this Sickle cell sickliness is essentially starting with one age then onto the next which is from guardians to posterity, attributable to the accomplishment of two anomalous qualities from every one of the guardians. It creates when a heterozygote (AS) (transporter) weds whichever a kindred transporter (AS) or a homozygote (SS) (victim) Sickle cell paleness advances [9].

1) The conjugal between two carriers has been establish to have 25% possibility of having sickler, 25% normal and 50% carrier.
2) Marriage amongst normal person and Carrier, there is 50% chance of giving birth to normal children and 50% carriers
3) The marriage between a normal person (AA) and homozygote (SS) are seen as having 100% chance of having carriers.
4) The conjugal amongst heterozygote (AS) and homozygote (SS) has been shown to have 50% chance of having children that are sickler and 50% carrier.
5) The marriage between sicklers is very infrequent. If it happens then it will eventually have 100% chance of having sicklers [3, 13].

The specific aim of this research work is to determine the level of sickle cell disease and methamoglobin in the presence of paracetamol.

The polymerization of sickle cell hemoglobin (HbS) is the key event in the pathophysiology of sickle cell anemia. The polymerization has been assigned through a double-nucleation mechanism, an embellishment of the nucleation-and-growth scenario typical of first-order phase transitions, conferring to which homogeneous nucleation of single fibers is surveyed by their growth and branching by heterogeneous nucleation of new fibers on top of existing ones [14].

Methemoglobin is a procedure of the oxygen-carrying metalloprotein hemoglobin, in which the iron in the heme group is in the Fe^{3+} (ferric) state, not the Fe^{2+} (ferrous) of normal hemoglobin. Methemoglobin cannot bind oxygen, contrasting oxyhemoglobin [15]. It is bluish chocolate-brown in color and in human blood, a trace amount of methemoglobin is usually produced instinctively, but when present in excess the blood becomes abnormally dark bluish brown. Methemoglobinemia is seen as a condition considered by the occurrence of a higher

than normal level of methemoglobin (metHb, i.e., ferric [Fe^{3+}] rather than ferrous [Fe^{2+}] haemoglobin) in the blood [15].

The drug treatments of different diseases follows different disease-dependent therapeutic strategies, such as substituting certain systemic deficiencies as also in insulin management of type I diabetes mellitus, prophylactic managements and medicines as also in the use of pyrimethamine in preventing malaria, modulating receptor collaboration always involve dysfunctional biochemical mechanisms either in the host or in the offending pathogen [16]. A number of diseases are into the following category, type II diabetes mellitus, Creutzfeldt–Jakob disease, Alzheimer's disease, Parkinson's diseases, and sickle cell disease. Their clinical accounts can often be traced to mutational changes in amino acid sequence, which frequently instigate abnormal folding and aggregation behavior of the concerned protein. In sickle cell disease (SCD), a point mutation which involves the replacement of glutamic acid at position 6 of the β-globin chain of hemoglobin to valine leads to the polymerization of hemoglobin.

Recent reviews expressed different treatment modalities and works to develop new drugs targeting Sickle Cell Disease. Number of research endeavors have been made to design interventions aimed at modulating the structural properties, aggregation tendencies, and defective oxygen gas (O_2) transport properties of sickle hemoglobin. For instance, covalent modifiers and allosteric modulators of HbS that stabilize the non-polymer developing R-state Hb conformation have been reported by researchers and include the recently food and drug administration (FDA) approved voxelotor (GBT 440) and derivatives of vanillin [17-19].

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 [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 [21-24].

The aim of this research work is to determine the polymerization of sickle cell disease and methaeglobin in the presence of paracetamol. The conceptual framework is a set of coherent ideas and concepts organized in a manner that makes them easy to communicate.

2. EXPERIMENTAL

Study Design

Collection of Acetylsalicylic Acid

Acetylsalicylic Acid was gotten from a chemical store around wetheral road. The acetylsalicylic acid was identified by Dr. Ikpa C. B. C. in the Department of Chemistry, Imo State University 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 (71 kg) 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) $MgCl_2$ (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 °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

Aim: To determine the level of polymerization and methaemoglobin concentration in the presence of Acetylsalicylic Acid using a spectrophotometer

Principle: Hbss molecules under gelation when deprived of oxygen, $Na_2S_2O_3$ was used as a reductant. The level of polymerization was monitored by recording increasing absorbance of the assay mixture with time

Procedure:

0.1 ml (milliliter) of Hbsshemolysate was introduced into a test tube. 0.5 ml 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 3.4 ml 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 = 700 nm (control sample). This procedure was repeated by substituting the distilled water with 1.0 ml of the corresponding four increasing concentrations of acetylsalicylic acid.

Calculation:

% 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

Aim: To determine the methemoglobin concentration in the presence of acetylsalicylic acid using a digital spectrophotometer

Principle: This determination is based on the fact that hemoglobin and methemoglobin absorb light at different wavelengths, at 540 nm and 630 nm as their respective peak absorbance. This approach employed the establishment procedure of lysing whole blood in distilled water.

Procedure:

Control: In a test tube containing 5.0 ml of distilled water, 0.02 ml 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, 540 nm and 630 nm, using a spectrophotometer. Test: The effect of acetylsalicylic acid on plasma methemoglobin concentration was carried out by introducing 0.02 ml 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 540 nm and 630 nm using a digital spectrophotometer. The percentage plasma methemoglobin was obtained with the formula:

Calculation:

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

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

3. RESULTS AND DISCUSSION

Table 1. Percentage polymerization.

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 $P \leq 0.05$

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 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. 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 500 mg/dL concentration.

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 30second. These values were indications that polymerization of HbS molecules occurred in the control sample and in the presence of the malarial drug (Figure 2). 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.

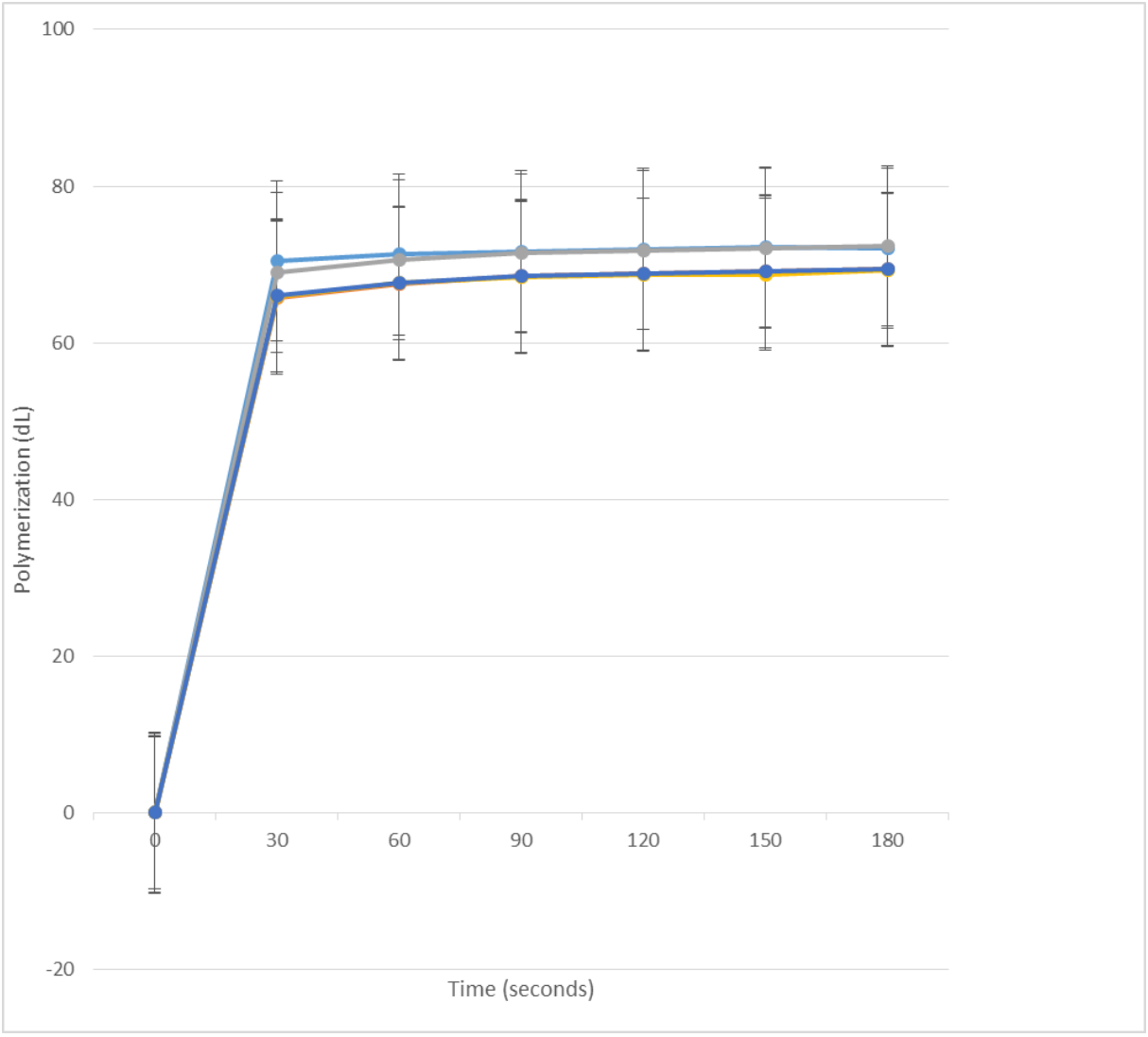

Figure 2. Plot of polymerization against time.

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. Methemoglobin is a technique of the oxygen-conveying metalloprotein hemoglobin, in which the iron in the heme bunch is in the Fe^{3+} (ferric) state, not the Fe^{2+} (ferrous) of ordinary hemoglobin. Methemoglobin can't tie oxygen, differentiating oxyhemoglobin. Methemoglobin is a methodology of the oxygen-conveying metalloprotein hemoglobin, in which the iron in the heme bunch is in the Fe^{3+} (ferric) state, not the Fe^{2+} (ferrous) of ordinary hemoglobin. Methemoglobin can't tie oxygen, differentiating oxyhemoglobin [16]. Methemoglobinemia is viewed as a condition considered by the event of

a higher than typical degree of methemoglobin (metHb, i.e., ferric [Fe^{3+}] instead of ferrous [Fe^{2+}] hemoglobin) in the blood.

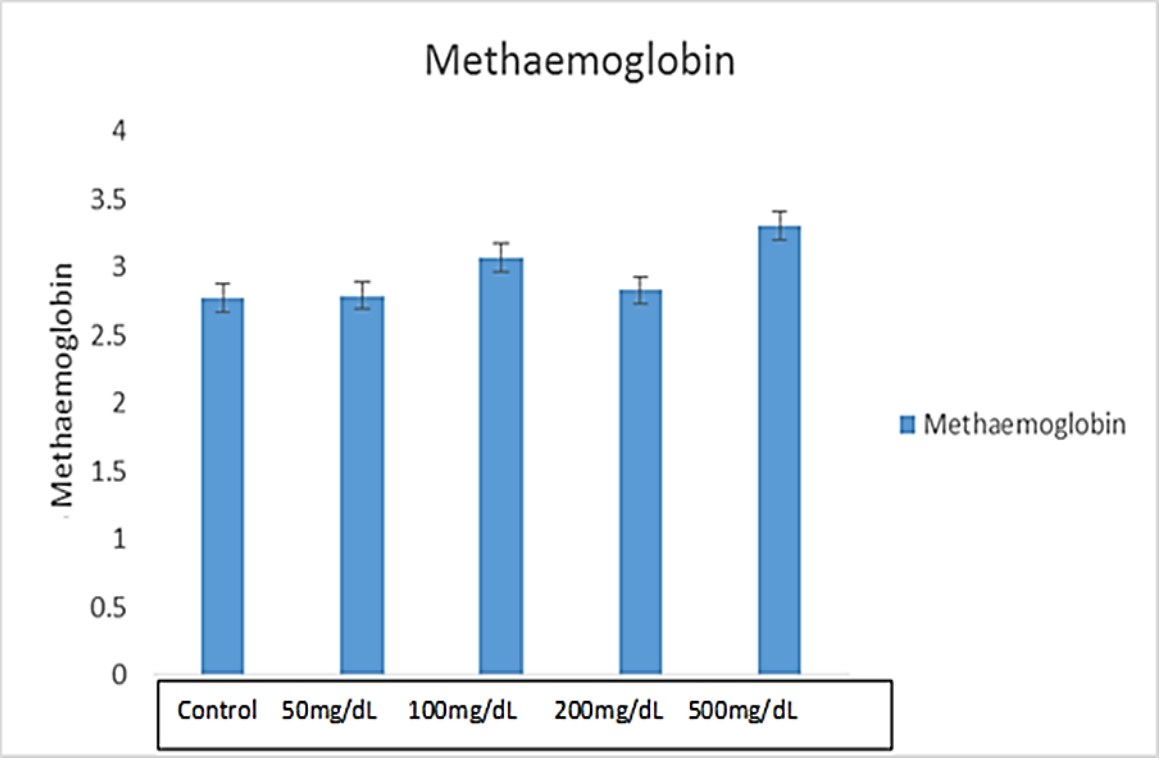

Figure 3. Bar chart with standard error bars showing the level of Methaemoglobin

4. CONCLUSIONS

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.

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