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IN-VITRO CHANGES IN THE LIVER FUNCTION TEST OF SICKLE CELL ANEMIA PATIENTS BY AZOCOMPOUNDS.

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ABSTRACT

The most common and best-known type of sickle cell disease is sickle cell anemia, which is also called SS disease. SCA is an inherited blood disorder caused by single nucleotide substitution in the β-globin gene that renders their hemoglobin (HbS) much less soluble than normal hemoglobin (HbA) when deoxygenated. The polymerization of HbS upon deoxygenation is the basic pathophysiologic event leading to RBC sickling, hemolysis, vasoocclusion and ultimately to chronic organ damage. The aim of the study was to evaluate in-vitro the changes in the biochemical parameters of sickle cell anemia patients by Azocompounds. The study was conducted with 25 sickle cell anemia patient's blood samples form Nagpur area. Azocompounds like Hydroxyurea (HU) has been reported to be effective in improving survival and reducing morbidity in some SCA patients. Inspired by the effectiveness of this drug, the further study focuses on the testing of other Azocompounds on the sickle RBCs in this disorder. The p-amino benzoic acid and its derivatives poses a wide variety of pharmacological properties such as anti-bacterial, anti-fungal, anti-tuberculosis, analgesic, local anesthetic. Serial liver function tests were performed in each case. The significance of the tests in this disease is discussed and the importance of serial determinations is indicated. Elevated level of Total bilirubin level was significantly decreased by the Azocompounds. The preliminary results in the biochemical parameters are noticeable and encourage further investigations.

KEYWORDS: Sickle cell disease, Sickle cell anemia, hemoglobin S, Azocompounds, Hydroxyurea.

INTRODUCTION

Sickle cell disease is an inherited blood condition which is most common among people of African, Arabian and Indian origin. In disease of African origin, research has led to models of care which prevent serious complications, improve the quality of life, and increase survival.[1] Sickle cell anemia (SCA) is the most common monogenic, inherited disease in the world. It originated in Africa and occurs predominantly in people of African descent. The disease has spread heterogeneously in Brazil because of racial miscegenation, thereby facilitating the continuity of this type of anemia in Brazil. It is considered a serious public health problem by Brazilian scientific literature.[2] The disease is caused because of a point mutation at position 6 in the beta-globin gene that gives rise to an abnormal hemoglobin molecule called hemoglobin S (Hb S).[3] This causes physiological changes that affect the hemoglobin molecule in its deoxygenated state through the sickling of red blood cells; this triggers the formation of Hb S polymers, oxidative degradation of the Hb S molecule and the generation of oxidizing free radicals.[4] These erythrocytes have a greater adherence to the vascular endothelium, thus contributing to episodes of vaso-occlusion which is associated with the disease The SCA Patients require blood transfusions to improve oxygen transport and to improve blood volume. One of the complications of long-term transfusion therapy is iron overload. Approximately 25% of the iron in the body of a normal adult is stored in the form of ferritin (each ferritin molecule has 4500 atoms of iron) and hemosiderin. The main clinical manifestations of SCA are infections, acute chest syndrome (ACS), splenic sequestration, pain crises, renal disorders, cardiac disorders (heart failure), osteoarticular disorders, neurological disorders (stroke), ocular disorders, sores on the lower limbs and priapism. There are other studies suggesting that the main causes of liver injury in SCD patients are due to factors other than intrahepatic sickling, which was considered to be reversible, such as viral hepatitis or transfusional iron overload.[5]

The incidence of liver disease in sickle cell disorders is difficult to ascertain despite being a component of the multi organ failure that occurs in sickle cell disease,[6] Elevation of the different liver enzymes correlates with the different categories; haemolysis raises plasma aspartate transaminase (AST)[7] while plasma alanine

transaminase (ALT) levels more accurately reflects hepatocyte injury.[7,8] High levels of serum alkaline phosphatase are commonly seen in patients with sickle cell anaemia, this may be because of either cholestasis or bone disease.

Azocompounds like Hydroxyurea (HU)[6,8] administration seems to be the best available treatment option for SCA patients (HU) has been reported to be effective in improving survival and reducing morbidity in some SCA patients. HU has been shown to reduce the frequency of painful crises and acute chest syndrome in adults, and to lessen the need for blood transfusions. HU has been found to be effective in the prevention of brain injury due to cerebrovascular disease.[7,8] HU is used to reduce the complications associated with sickle cell disease in adults this drug shows many positive and adverse effects. Administration of this drug reduces the pain crises, increases concentration of hemoglobin, decreases frequency of blood transfusion. Inspired by the effectiveness of this drug, the further study focuses on the testing of other Azocompounds on the sickle RBCs in this disorder.

Azo compounds are compounds bearing the functional group R-N=N-R', in which R and R' can be either aryl or alkyl. IUPAC defines azo compounds as: "Derivatives of diazene, HN=NH, wherein both hydrogens are substituted by hydrocarbyl groups the more stable derivatives contain two aryl groups. The N=N group is called an azo group. The p-amino benzoic acid and its derivatives poses a wide variety of pharmacological properties such as anti-bacterial, anti-fungal, anti-tuberculosis, analgesic, local anaesthetics and many more with the indicator property for-base titrations. The literature survey reveals that when p-amino benzoic acid undergoes complexation, it shows a wide variety of pharmacological properties. Some aryl Azocompounds was synthesized under mild conditions and used for further observations and investigations.[9]

MATERIALS AND METHODS

The study was conducted on 25 sickle cell anemia patients with (SCA Hbss) both the genders (Male and Female) were included for the study with the age group between 19 to 45 years old from Nagpur Maharashtra, India over three months period (April 2017 to September 2017). All the patients had been taking folic acid as a treatment and some patients were undergoing blood transfusion therapy. The blood is the vital fluid that transports gases and nutrients to the tissues of the body. The biochemistry and functional capacity of the blood is directly linked to the status of the blood components. Freshly collected blood samples were taken and immediately processed for further analysis. Two Azocompounds were taken for the study. Both the Azocompounds were dissolved in Distilled water and their effect on sickle cell anemia patient's blood samples were observed separately. All the biochemical investigations of LFT were done by RMS BCA-20 auto-analyser using Standard kits. This study is approved by the Institutional Ethical Committee, Nagpur, Maharashtra, India. All the Patients gave their informed consent. This study is approved by the Institutional Ethical Committee, Nagpur, Maharashtra, India.

Inclusion Criteria and Exclusion criteria

Inclusion Criteria: Patients with Sickle cell anemia disease were included for the study.

Exclusion criteria: Patients were excluded from the study if they were smokers consumed alcoholic drinks and patients who were pregnant were also excluded from the study.

RESULT AND DISCUSSION

The mean age of the sickle cell anemia (HbSS) patients was 37.44 (21-44).56% were female and 44% were male. The blood samples were collected from the sickle cell anemia patients and liver function test of the patients was carried out before and after addition of Azocompounds Azo-1 and Azo-2 and parameters taken for the study were Total Bilirubin, Direct bilirubin, Indirect bilirubin, aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatase(ALP) levels in SCA patients.

Bilirubin: - Bilirubin is made by the liver and is excreted in the bile. Elevated levels of bilirubin may indicate an obstruction of bile flow or a problem in the processing of bile by the liver. Initial laboratory evaluation revealed a total bilirubin level.[10] The mean concentration of Total bilirubin in sickle cell anemia patients was elevated. The mean concentration of Total bilirubin (mg /dL) was (1.316±0.429), After addition of Compound Azo-1 and Azo-2 mean concentration of total bilirubin was (0.896±0.314**) and (2.248±0.935***) this result showed that the concentration of Total bilirubin in Azo-1 was significantly decreased while Azo-2 showed increase concentration of total bilirubin. The mean concentration of Direct bilirubin in sickle cell anemia patients was (0.612±0.0815) and After addition of Azo-1 and Azo-2 mean concentration of Direct bilirubin (mg /dL) was found to be (0.512±0.165***) and (1.38±0.255***). Azo-1 showed slightly decrease in concentration but non-significantly and Azo-2 showed the non-significant increase in concentration of direct bilirubin. The mean concentration of Indirect bilirubin (mg /dL) in SCA patients was (0.712±0.415) which is decreased significantly after addition of Azo-1 (0.384±0.182**) and increased non-significantly in Azo-2 (0.908±0.879***).

Table 1: Mean Level of Bilirubin in SCA patients and SCA patients with Azo-1 and Azo-2.

Sr. No.	LFT Parameters	Mean ±SD	Mean ±SD	Mean ±SD
		SCA Patients	SCA patients with Azo-1	SCA patients with Azo-2
1)	Bilirubin			
a)	Total Bilirubin	1.316±0.429	0.896±0.314**	2.248±0.935***
b)	Direct Bilirubin	0.612±0.0815	0.512±0.165***	1.38±0.255***
c)	Indirect Bilirubin	0.712±0.415	0.384±0.182**	0.908±0.879

Values represent the Mean ± SD. *P<0.0001, **p<0.001, ***p>0.05.

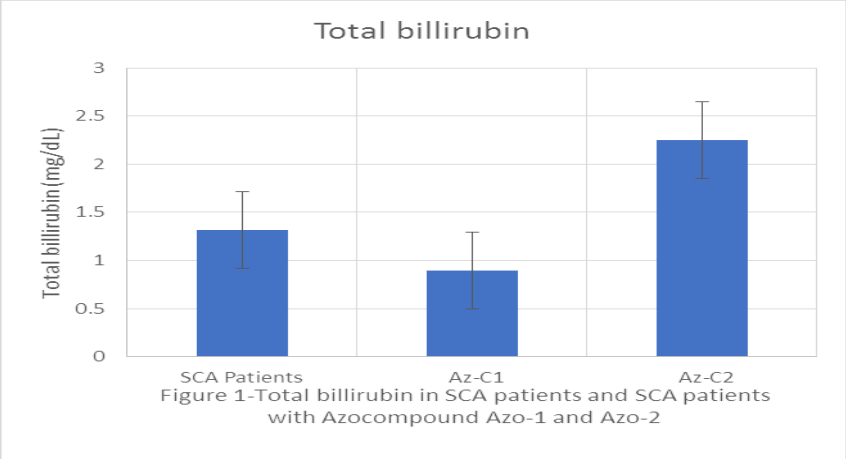

Figure 1-Total billirubin in SCA patients and SCA patients with Azocompound Azo-1 and Azo-2

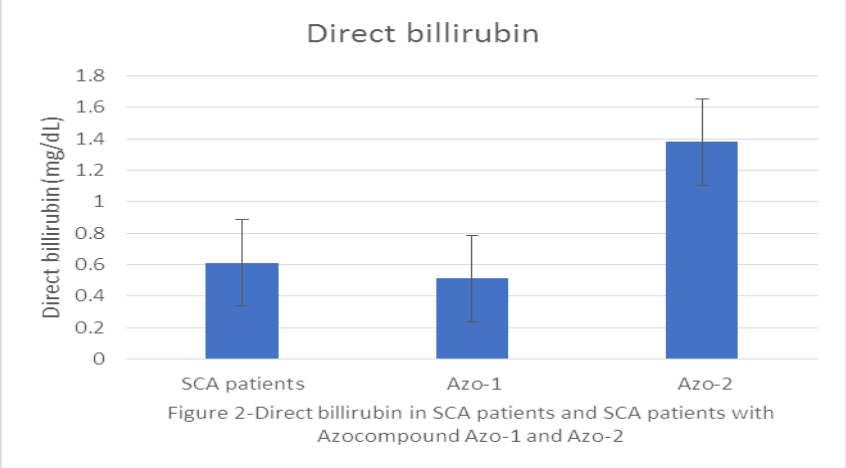

Figure 2-Direct billirubin in SCA patients and SCA patients with Azocompound Azo-1 and Azo-2

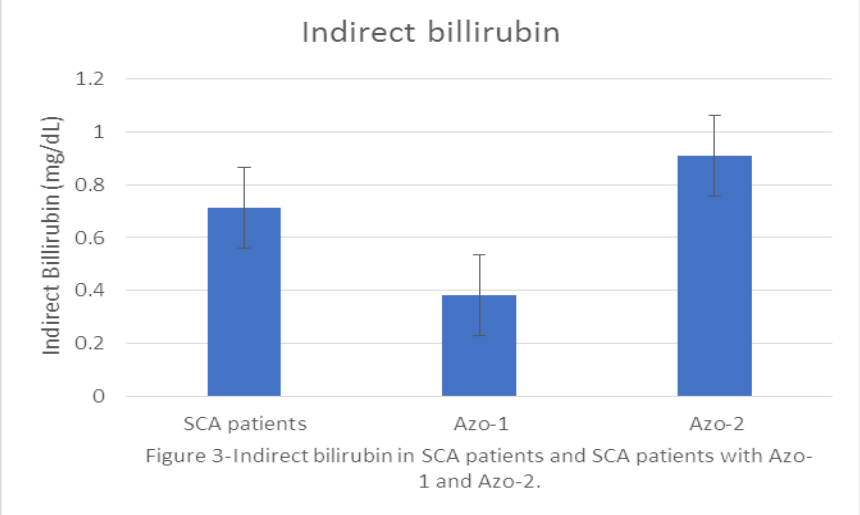

Figure 3-Indirect bilirubin in SCA patients and SCA patients with Azo-1 and Azo-2.

Aspartate transaminase (AST): - - In SCA patients, aspartate transaminase (AST) is released via intravascular hemolysis[7]. The mean level of (AST) (mg /dL) for SCA patients was (23.232±21.5807084) and level of AST in Compound Azo-1 and Azo-2 was (16.44±16.4123856***) and (19.6±19.5632308***) respectively. There was a nonsignificant decrease in level of both the Compounds Azo-1and Azo-2.

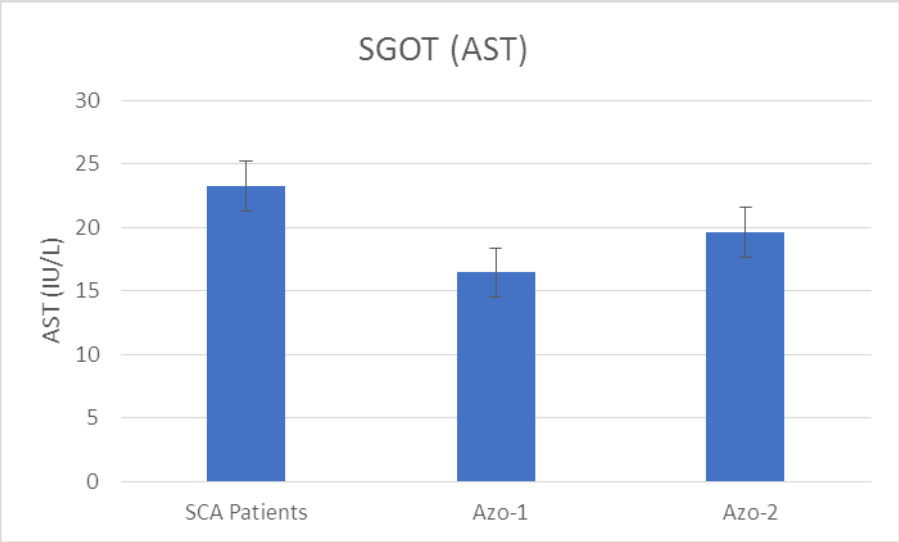

Figure 4: Aspartate transaminase levels in SCA patients and SCA patients with Azo-1 and Azo-2.

Alanine transaminase (ALT): The mean concentration of ALT was (23.833±22.182) and After addition of Compound Azo-1 and Azo-2 mean concentration of ALT was found to be non-significantly increased (28.839±17.102***) and (36.383±15.577***).

Alkaline phosphatase (ALP): -The level of alkaline phosphatase indicates severity of bone damage and is a useful guide of progress in the management of bone pains in sickle cell anaemia.[10-13] the serum alkaline phosphatase test is of limited value as an aid in differentiating jaundice of hepatic from post hepatic origin. The level of the heat-labile alkaline phosphatase indicates severity of bone damage and is a useful guide of progress in the management of bone pains in sickle cell anaemia.[14,15] The mean concentration of Alkaline phosphatase (ALP) (U/L) was found to be (137.44±18.66) and compounds Azo-1 and Azo-2 were nonsignificant (78.04±14.34289***) and (110.8±16.42681***).

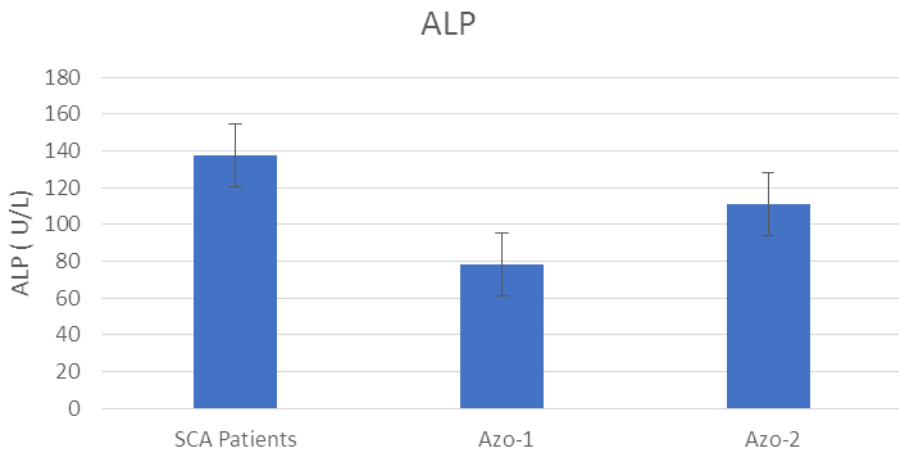

Figure 5-Alkaline Phosphatase level (U/L) in SCA patients and SCA patients with Azo-1 and Azo-2

CONCLUSION

It was observed that in-vitro changes in the liver function test of SCA patients showed elevated levels of Total bilirubin, direct bilirubin, indirect bilirubin, AST and ALP. After adding Azocompounds separately the significant change in Total bilirubin level was observed(Fig-1) Changes in direct bilirubin was found to be slightly decreased that might be the less effectiveness

of Azo-1 and increased in Azo-2 but non-significantly(Fig-2). Indirect bilirubin was significantly decreased in Azo-1 but increased in Azo-2 non-significantly(Fig-3). AST(Fig-4). ALT and ALP were nonsignificant for Azo-1 and Azo-2 but AST (Fig-4) and ALP (Fig-5) showed decreased level in sickle cell anemia patients. The results were promising and significant to some extent. Azocompounds can be a novel, effective and inexpensive modality in treating patients with sickle cell anemia. The preliminary results in the biochemical parameters are noticeable and encourage further investigations.

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Conflict of interest: There are no conficts of interest regarding this study.

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