*Original Research Article*

# Sickle Cell Anemia contributes to Liver Abnormality


**ABSTRACT**

**Aim**: This study sought to investigate the implication of sickle cell anemia on hepatic biomarkers.

**Methodology**: This is a case-control study which enrolled 60 sickle cell anemia patients (30 males and 30 females) in a stable state at the Obafemi Awolowo University Teaching Hospital in Ile-Ife, Osun State, Nigeria, as well as 60 healthy controls (30 males and 30 females). Each participant had five millilitres (5 mL) of venous blood taken and dispensed into a lithium heparin sample vial. Hepatic biomarkers were assessed using established procedures utilizing the blood sample.

**Results**: Hepatic indices were higher in sickle cell anemia patients than in control subjects, according to the findings of this study. It's worth noting that the effect of sickle cell anemia on liver parameters was stronger in male patients than in females. The activity of ALP in male sickle was 280 UI/L compared to the 198 UI/L in their female counterparts. Both values were significantly ($p<0.05$) higher than those found in the control subjects. This was not different in other hepatic parameters investigated in this study.

**Conclusion**: The findings of this investigation revealed that sickle cell patients had significantly higher liver indices than control persons. This could indicate that its synthesis in the liver has been increased or metabolism was reduced sequel to sickle cell disease.



**Keywords:** *Liver abnormality; sickle cell anemia, steady state*




## 1. INTRODUCTION

Sickle cell anemia (SCA) is a genetic blood disease caused by a faulty gene that creates an aberrant form of haemoglobin, the component of red blood cells that transports oxygen from the lungs to the tissues. Haemoglobin S is an aberrant hemoglobin that causes red blood cells to deform after they release oxygen into the tissues. Deformed red cells sickle and are called sickle cells [1]. Millions of people throughout the world suffer from sickle cell disease (SCD), which is especially prevalent among those whose ancestors immigrated from Sub-Saharan Africa, South America, the Caribbean, Central America, Saudi Arabia, India, and Mediterranean countries (such as Turkey, Greece, and Italy) [2]. It is a multi-systemic illness that affects around one out of every 500 African Americans. Every year, over 300,000 babies are born with it [3].

In Nigeria, sickle cell anemia is the most common genetic blood condition [4,5]. The

sickle cell anemia (SS) is a homozygous situation in which both the father and mother inherit the sickle gene. Heterozygous sickle cell disease can also arise in the presence of other haemoglobin beta chain defects [6,7].

The genetic foundation for sickle cell anemia was discovered in 1949, when it was discovered that heterogeneity in the sickle cell gene causes sickle cell trait and homogeneity causes sickle cell anemia [8]. The sickling is caused by a mutation in the haemoglobin gene (a change in the genetic material (DNA) of a cell). According to research, males have a life expectancy of 42 and females have a life expectancy of 48 [9]. Sickle cell anemia, which typically manifests in childhood, is more common in persons (or their descendants) from tropical and sub-tropical countries where malaria is or will be prevalent. Because malaria is common in Sub-Saharan Africa, one-third of all indigenous populations carry the gene [10,11]. Sickle cell anemia is nearly endemic in some parts of the world, and children aged 1 to 10 have the highest mortality rate [12].

Two-thirds of those affected by the disease are from very poor socioeconomic backgrounds. However, improving living circumstances, nutrition, and medical care have resulted in considerable reductions in mortality in certain sections of the country [13]. In vertebrates and several other animals, the liver is an important organ. It performs a variety of tasks, including detoxification, protein synthesis, and biochemical generation for digesting. The liver is required for survival, and there is currently no means to compensate for its absence [14].

Hepatic disease (Liver disease) is a well-known consequence of sickle cell anemia caused by a variety of variables including intra-hepatic sinusoidal sickling, bilirubin, gallstones, transfusion-related hepatitis infections, and excessive iron deposition [15]. Hepatomegaly is caused by the entrapment of sickle cells during passage through the hepatic sinusoids, which are consumed by phagocytes, resulting in clinical indications of hepatic dysfunction in sickle cell disease patients [16]. A number of assays are done in clinical chemistry that are important in assessing liver functioning, diagnosing, monitoring, and evaluating the prognosis of liver illnesses. The goal of this study was to see how sickle cell anemia affected liver biomarkers.

## 2. METHODOLOGY

### 2.1 Research Design

This study is a case-control study carried out in the Obafemi Awolowo University Teaching Hospital in Osun State, Nigeria, is a cross sectional research on adult SCA patients in the steady state. The study was authorized by the Hospital's Ethical and Scientific Committee. Adult Nigerians of both genders who volunteered and given informed written consent were used as research subjects. Patients who came to the hematology clinic were enrolled in the order in which they arrived. People without sickle cell anemia served as the control group.

The study included 60 SCA patients (30 males and 30 females) and 60 control volunteers (30 males and 30 females). Each participant had five millilitres (5 mL) of venous blood collected

and distributed into a lithium heparin sample container for the measurement of liver parameters.

### 2.2 Inclusion criteria for Patients

SCA patients 16 years of age and older in steady state (a period of stable clinical condition occurring at least one week before or three weeks after a VOC or three months after a haemolytic crisis requiring a blood transfusion).

### 2.3 Inclusion Criteria for Control Subjects

Healthy individuals with HbA from Ile-Ife community who were 16 years of age and older.

### 2.4 Exclusion Criteria for Controls

People who are taking any drugs as well as those who smoke or drink too much alcohol (14 units per week for females and 21 units per week for males) were excluded from the study.

### 2.5 Exclusion Criteria for Patients

- ✓ Any additional medical conditions, such as hypertension or diabetes mellitus.
- ✓ Patients with sickle cell anemia who smoke or drink excessively (14 units per week for females and 21 units per week for males). 12 pint of beer (approx. 300 mL) equals 1 unit of alcohol (8-l0 g): 25 mL distilled spirit 1 glass sherry, 1 glass wine Patients in crisis or those who have had a blood transfusion within the last three months.

### 2.7 Determination of Hepatic Indices

The activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were measured using Randox commercial enzyme kits according to Reitman and Frankel's method [17]. Babson et al. [18] used the Phenolphthalein Monophosphate technique to assess the activity of alkaline phosphatase (ALP). Henry et al. [19] described the Biuret method for determining total protein content. The bromocresol green (BCG) method established by Doumas et al. [20] was used to estimate albumin. Globulin concentration was calculated by subtracting albumin from total protein, as described by Airaodion et al. [21]. Royden and Alfred [22] described a diazo technique for determining total bilirubin concentration. The concentration of conjugated bilirubin was measured using Compernolle's technique [23]. Unconjugated bilirubin was calculated by subtracting conjugated bilirubin from total bilirubin, as described by Airaodion et al. [21].

### 2.8 Statistical Analysis

The mean and standard deviation are used to express the results. One-way Analysis of Variance (ANOVA) and Tukey's test were used to determine the degree of homogeneity among the groups. P values less than 0.05 were considered statistically significant in all analyses performed with Graph Pad Prism Software Version 8.00.

## 3. RESULTS

Hepatic indices were higher in sickle cell anemia patients than in control subjects, according to the findings of this study. It's worth noting that, as shown in Figs. 1-10, the influence of sickle

cell anemia on liver parameters was greater in male patients than in female patients.

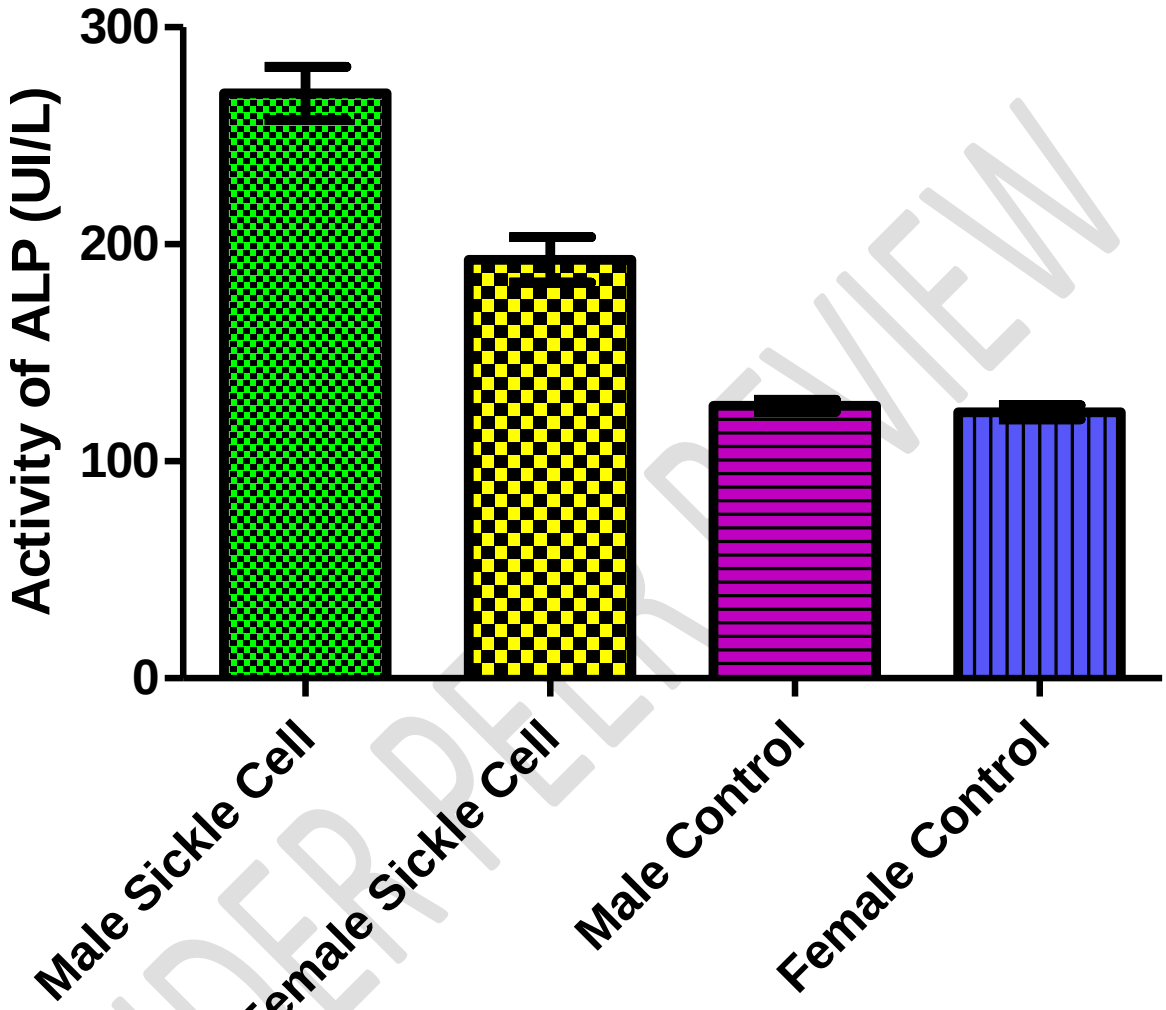




**Fig. 1. Effect of Sickle Cell Disease on the Activity of Alkaline Phosphatase (ALP) in Male and Female Patients**

*Results are presented in mean±standard deviation with n = 30*

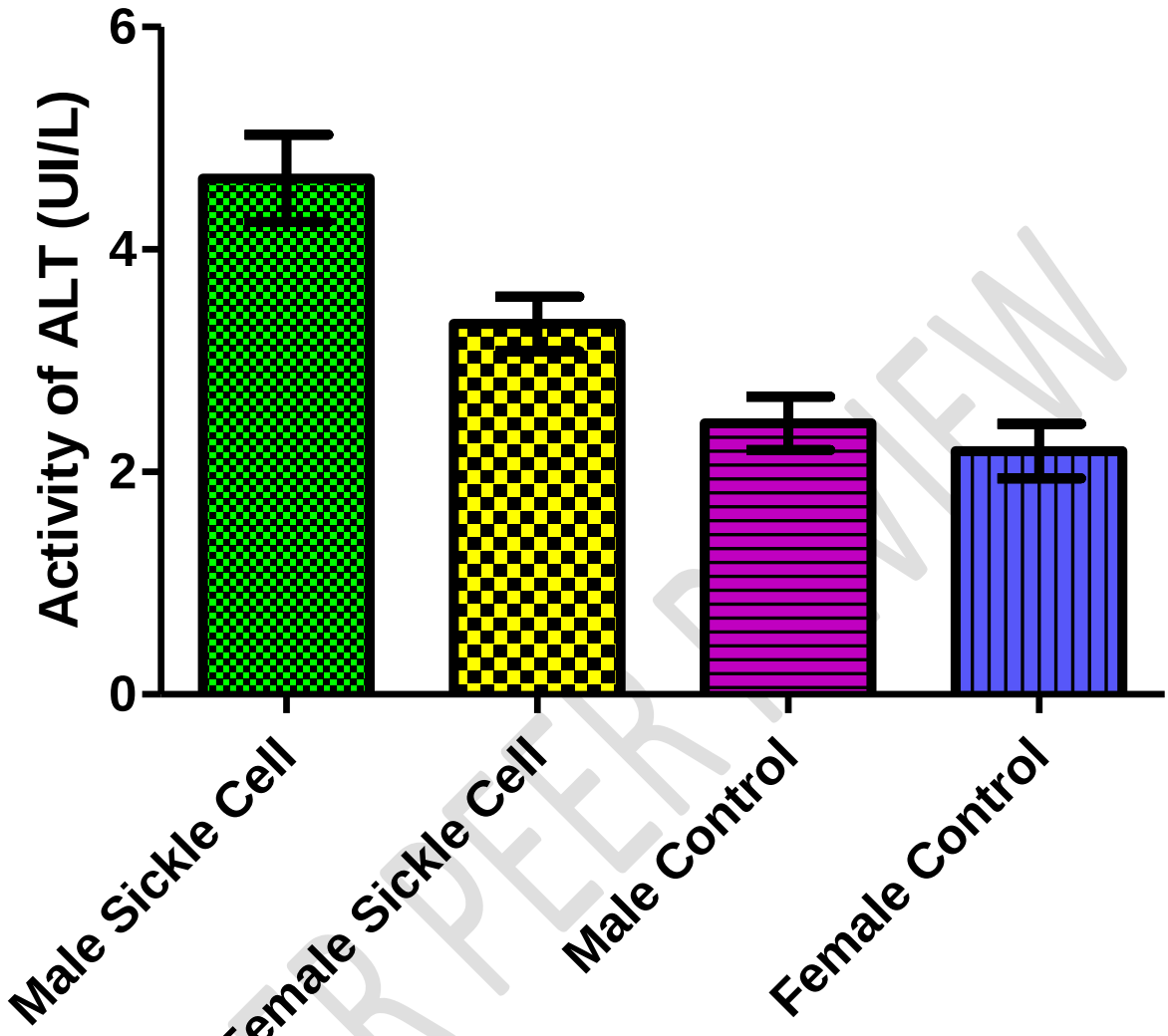




**Fig. 2. Effect of Sickle Cell Disease on the Activity of Alanine Aminotransferase (ALT) in Male and Female Patients**

*Results are presented in mean±standard deviation with n = 30*

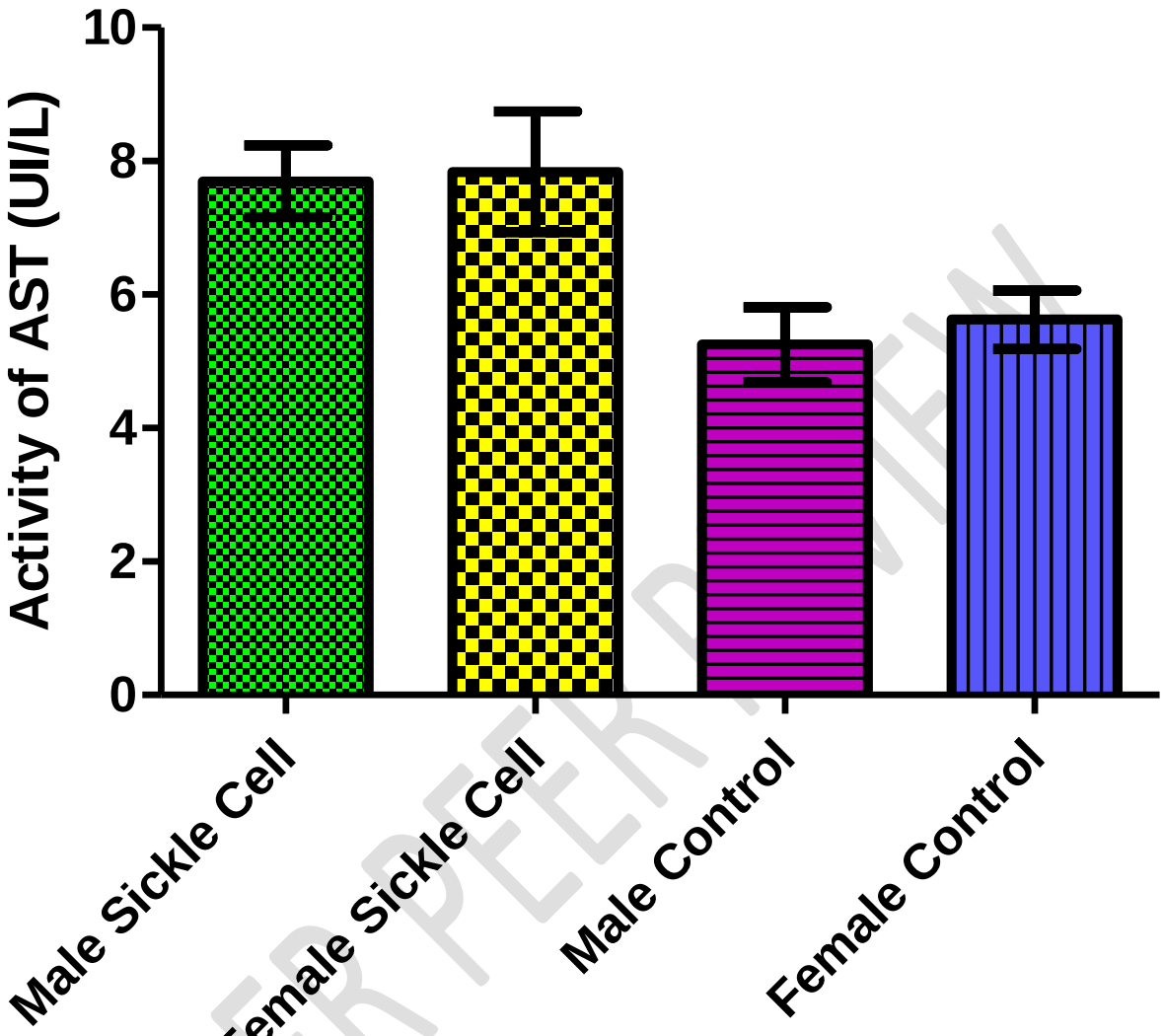




**Fig. 3. Effect of Sickle Cell Disease on the Activity of Aspartate Aminotransferase (AST) in Male and Female Patients**

*Results are presented in mean±standard deviation with n = 30*

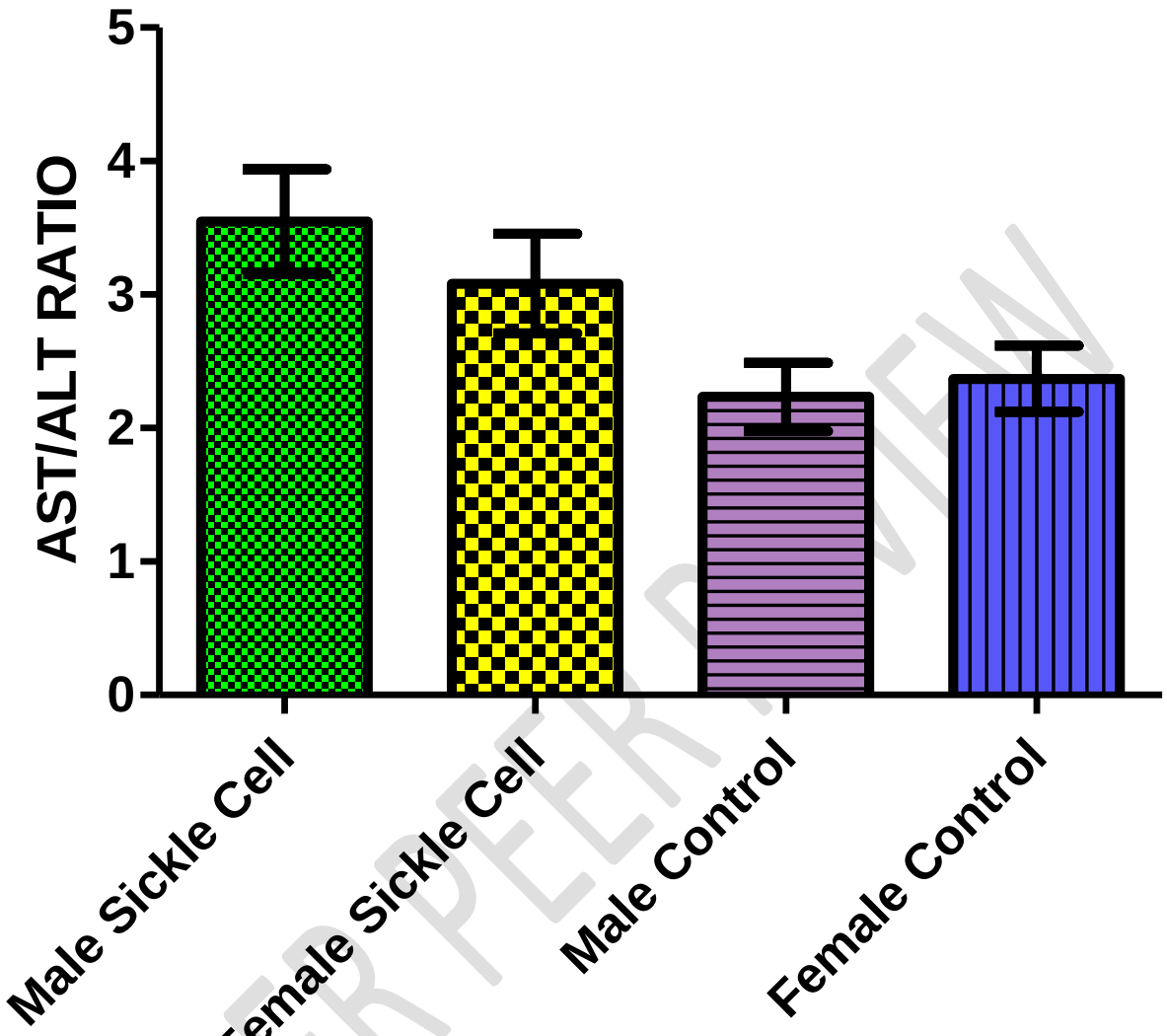




**Fig. 4. Effect of Sickle Cell Disease on the Aspartate Aminotransferase/Alanine Aminotransferase (AST/ALT) Ratio in Male and Female Patients**

*Results are presented in mean±standard deviation with n = 30*

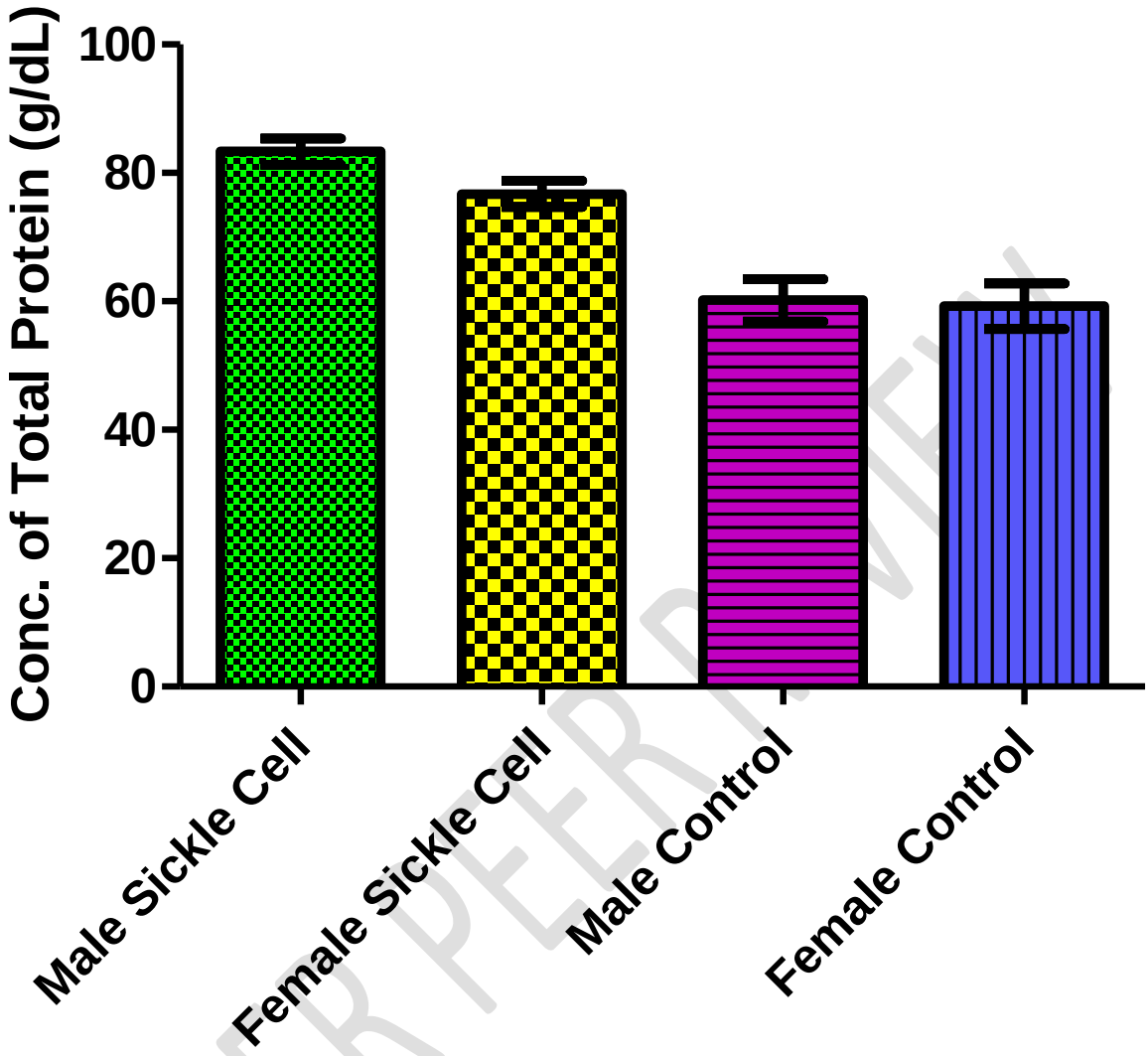


**Fig. 5. Effect of Sickle Cell Disease on the Concentration of Total Protein in Male and Female Patients**

*Results are presented in mean±standard deviation with n = 30*

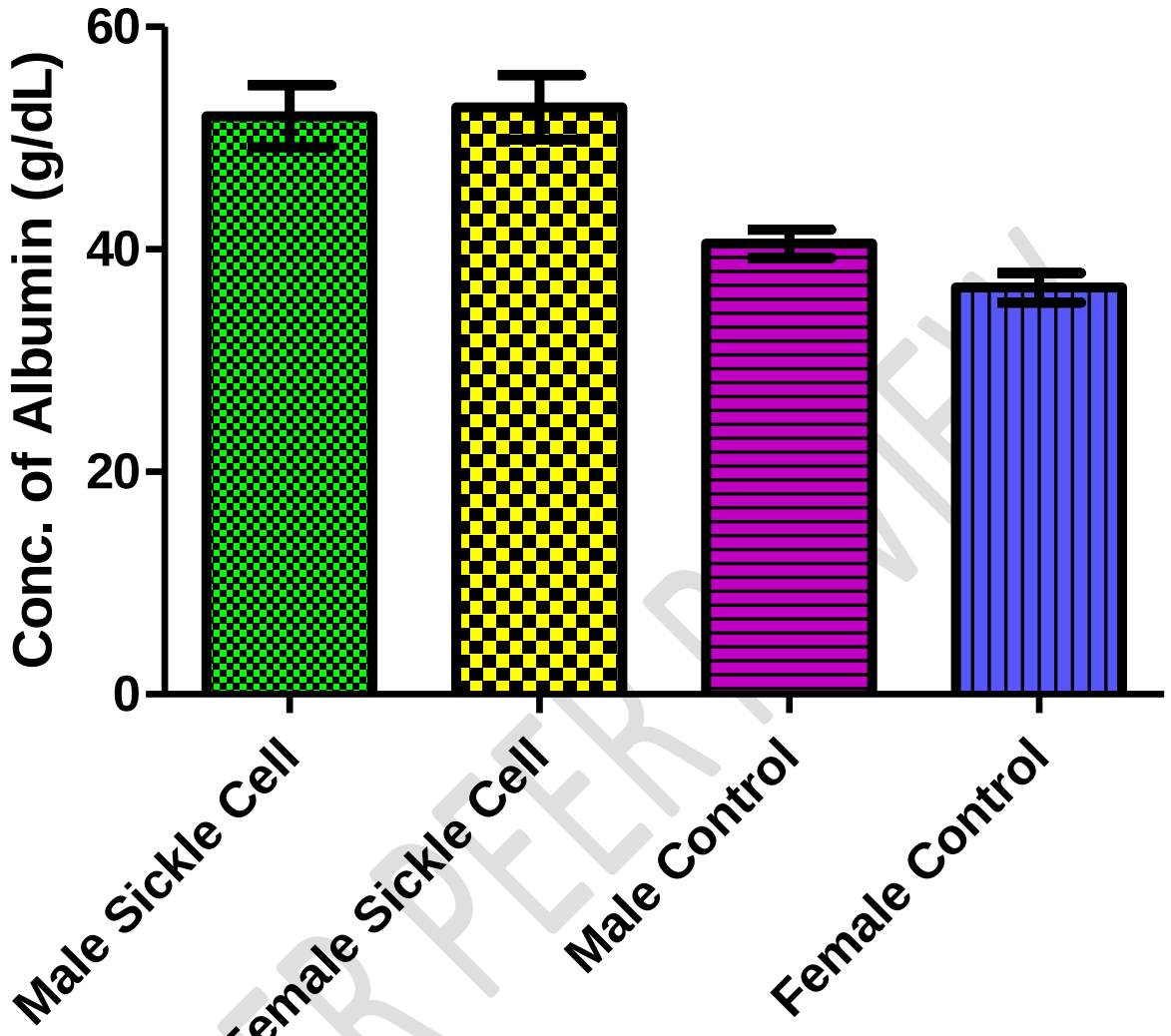




**Fig. 6. Effect of Sickle Cell Disease on the Concentration of Albumin in Male and Female Patients**

*Results are presented in mean±standard deviation with n = 30*

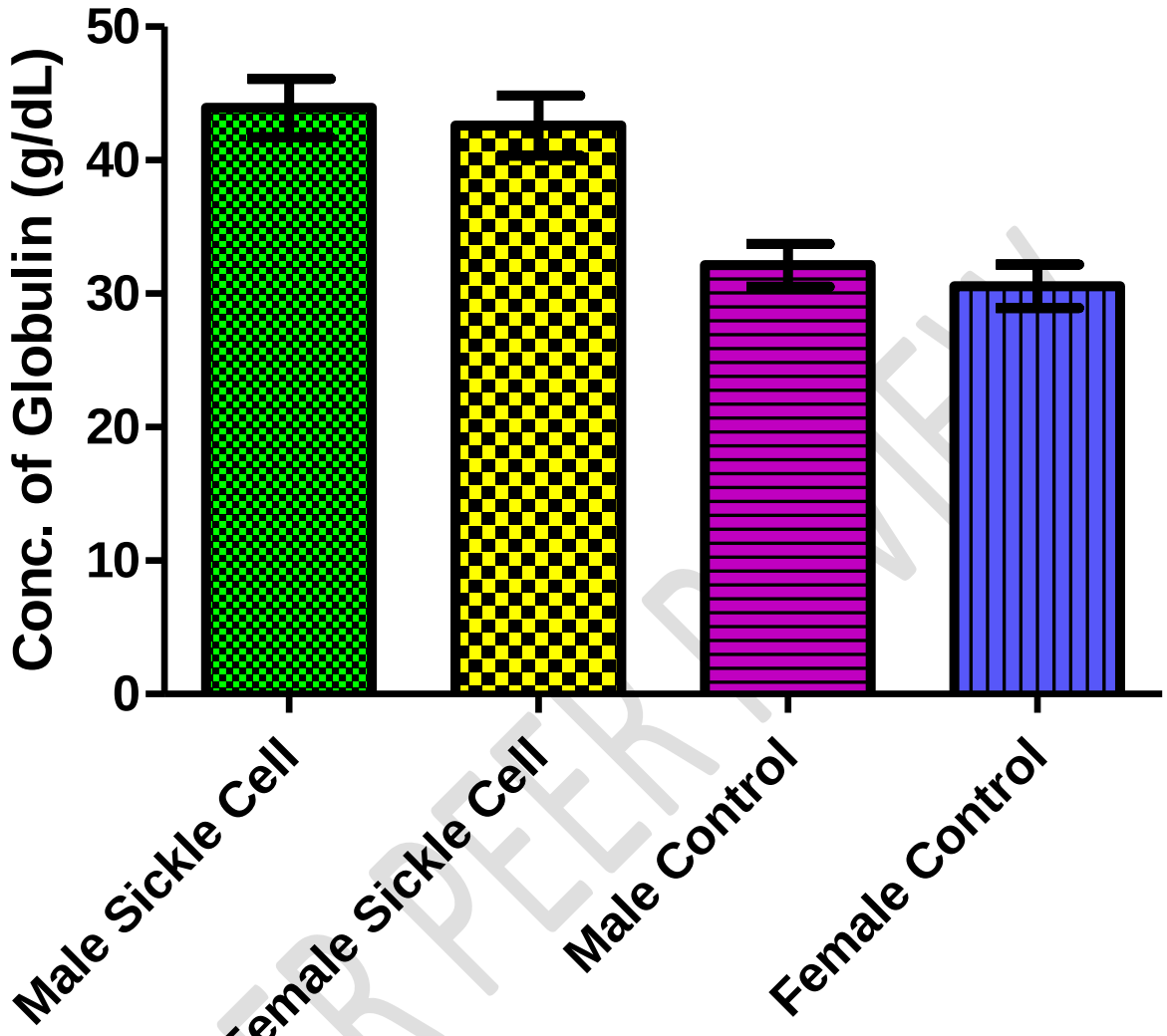




**Fig. 7. Effect of Sickle Cell Disease on the Concentration of Globulin in Male and Female Patients**

*Results are presented in mean±standard deviation with n = 30*

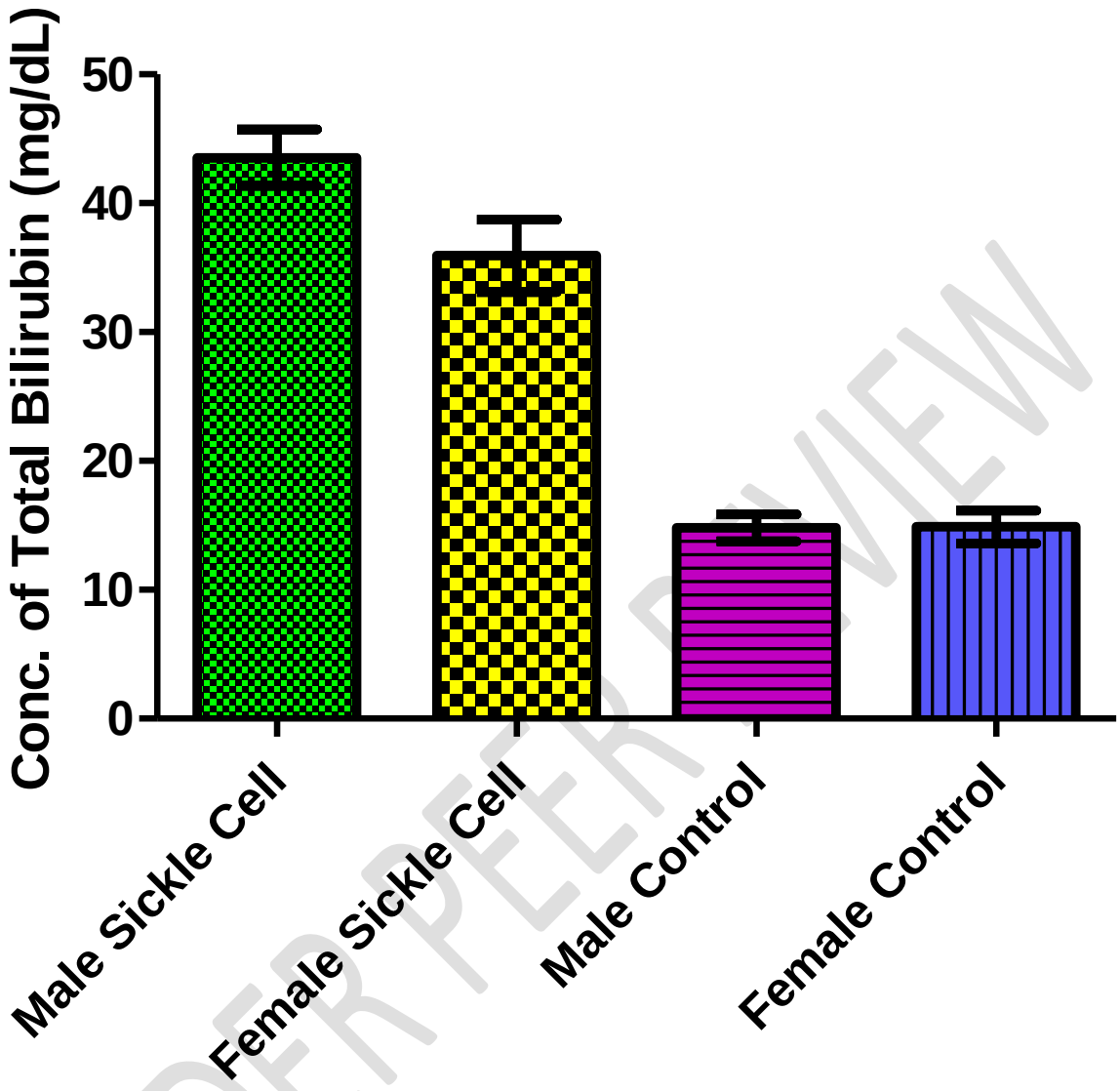




**Fig. 8. Effect of Sickle Cell Disease on the Concentration of Total Bilirubin in Male and Female Patients**

*Results are presented in mean±standard deviation with n = 30*

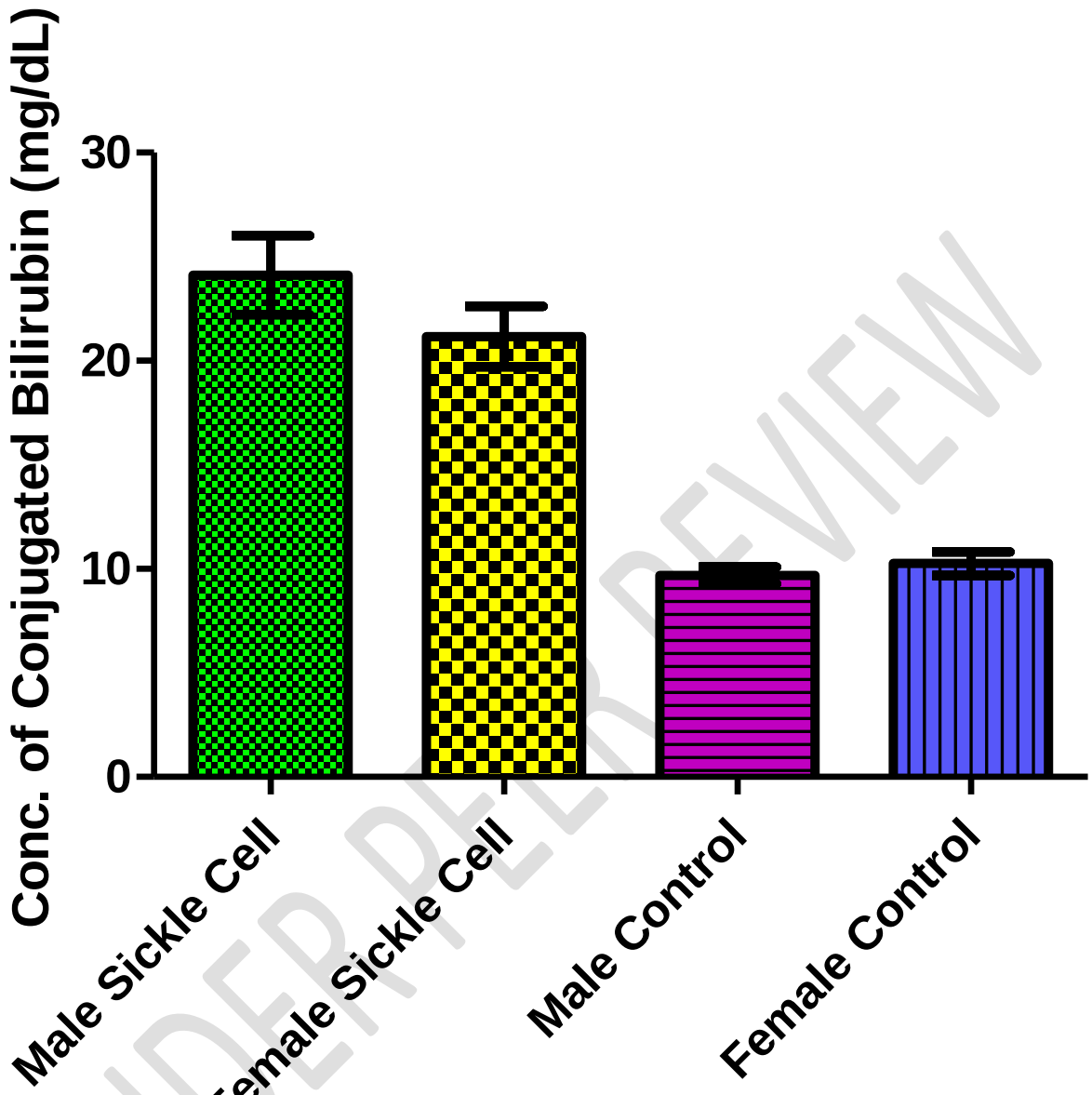




**Fig. 9. Effect of Sickle Cell Disease on the Concentration of Conjugated Bilirubin in Male and Female Patients**

*Results are presented in mean±standard deviation with n = 30*

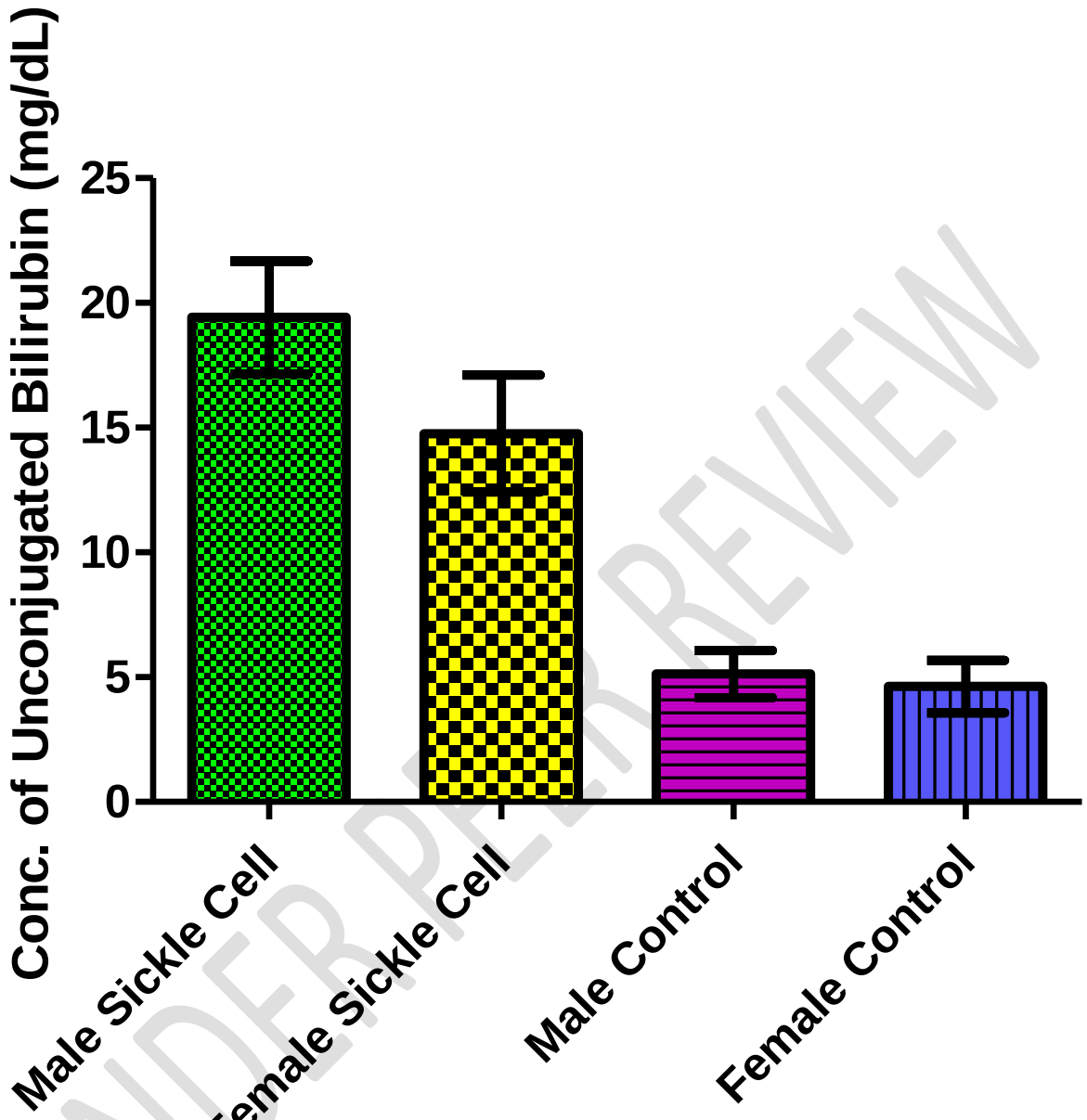




**Fig. 10. Effect of Sickle Cell Disease on the Concentration of Unconjugated Bilirubin in Male and Female Patients**

*Results are presented in mean±standard deviation with n = 30*

## 4. DISCUSSION

The use of hepatic biochemical parameters such as enzymes (aspartate transaminase, alanine transaminase and alkaline phosphatase) and metabolites (total proteins, albumin, globulin and bilirubin) to assess the functional integrity of the liver during subacute exposure to chemical substances or natural products/plant extracts is very useful [24]. Transaminases (ALT and AST) are enzymes that help with carbohydrate and amino acid metabolism, whereas alkaline phosphatase (ALP) helps with phosphate bond hydrolysis. They are frequently utilized to check the liver, plasma membrane, and endoplasmic reticulum for functional integrity [25]. The enzyme activities of serum alkaline phosphatase (ALP), transaminases (Alanine Transaminase and Aspartate Transaminase), levels of total protein, albumin, globulin, total bilirubin, conjugated bilirubin, and unconjugated bilirubin were statistically significant ($p<0.05$) in patients with sickle cell disease when compared to control subjects, as shown in Figs. 1-10. This study's findings are consistent with those of Doumas, et al. [26], Britenham, et al. [27], McGlynn, et al. [28], Hamatz, et al. [29], Cage [30], and Obi et al. [31].

When compared to control people, the activities of the liver enzymes ALP, ALT, and AST were generally greater in sickle cell patients. It is possible that sickle cell disease boosted the expression of genes involved in glucose absorption, glycolysis, and lipogenesis. Glucose inhibits the synthesis of cyclic Adenosine monophosphate (cAMP), a nucleotide necessary for the beginning of transcription in a vast number of inducible enzyme systems, including the Lac operon, repressing the induction of inducible operons [32]. Cyclic AMP (cAMP) is required to activate the allosteric protein catabolite activator protein (CAP), which binds to the promoter CAP site and stimulates the binding of ribonucleic acid (RNA) polymerase to the promoter for transcription initiation. However, cAMP must be available to bind to CAP, which binds to deoxyribonucleic acid (DNA) to facilitate transcription. Adenylase cyclase (AC) activity is inhibited in the presence of glucose. AC is necessary for the production of cAMP from Adenosine Triphosphate (ATP) [33]. As a result, when cAMP levels are low, CAP is inactive and transcription is not possible. Consequently, glucose suppresses these inducible enzymes by lowering the quantity of cyclic AMP. Patients with sickle cell disease may have higher cAMP levels, resulting in a considerable rise in these inducible enzymes.

The fact that SCA patients had significantly increased ALP activity (Fig. 1) could be attributable to cholestasis and long-term vascular lockage. A prior study discovered that bone ALP is the main enzyme fraction that increases during sickle cell crises, and that there is a link between the severity of the crises, serum ALP activity, and isoenzyme patterns [34]. It was claimed in another study that elevated ALP activities in SCA patients may be observed even when the participants were asymptomatic [35], which is similar with our result of raised ALP despite the fact that the SCA patients recruited in this study were in a stable condition. In their study, Kotila et al. [34] observed that 74 percent of SCA patients had

high serum ALP levels, but no significant link between it and liver size. As a result, liver pathology may not be the primary cause of this ALP rise.

In the steady state, Kotila et al. [34] in Ibadan, South Western Nigeria, found jaundice, a small increase in liver size, and slightly higher levels of ALT, AST, and ALP activities among adult SCA patients. According to Traina et al. [36] from Brazil's State University of Campinas, there are various degrees of liver disorders. Because ALT is confined to the liver, unlike AST, which is plentiful in other body organs such as the kidneys, brain, and hearts, it is regarded the most trustworthy marker of hepatocellular injury [37,38]. The activities of ALP, ALT, and the AST/ALT (De Ritis) ratio were substantially higher in male sickle cell patients than in female sickle cell patients. This is in line with a previous study by Uche and Akinola [39], who found that females had an advantage when it comes to the influence of sickle cell disease on biochemical markers. High AST/ALT (De Ritis) ratio is an indication of hepatotoxicity, according to Airaodion et al. [21].

It has been noted that liver dysfunction is common in children with SCA, as part of the multi-organ failure that occurs in this condition [40]. However, because of the intricacies of SCA, the pathogenesis of liver illness remains unknown. Furthermore, enlargement of the liver does not always indicate disease, and a healthy liver can be larger in size [41]. As a result, the aberrations in liver function tests (LFTs) revealed in this study could not be attributed to a single component, but rather to a mix of factors.

Total protein, albumin, and globulin concentrations in sickle cell patients were found to be considerably greater than those in control persons in this investigation (Figs. 5-7). This is consistent with the findings of Akuyam et al. [42], who examined biochemical liver function tests in steady-state sickle cell anemia patients in relation to age. By interfering with the equilibrium in the rate of synthesis and destruction, elimination or clearance of total protein and albumin from the system, SCA may have enhanced the functional activity of the liver [43]. However, an increase in total protein may cause dehydration, which is harmful to cellular equilibrium [44]. This will have a negative impact on the liver's metabolic processes and, as a result, the patients' health. Metal ions, bilirubin, and medicines are bound and transported by albumin. Its level is utilized to evaluate the liver's synthetic function. The presence of a significant increase in these indicators could indicate that its synthesis in the liver has been increased. Serum protein levels are regulated by liver synthesis, and their levels indicate the liver's ability to synthesize.

When SCA patients were compared to control subjects, there was a significant rise ($p<0.05$) in total bilirubin (Fig. 8), conjugated (direct) bilirubin (Fig. 9) and unconjugated bilirubin (Fig. 10) concentrations. Akuyam et al. [42] came to similar conclusions. Other hemoproteins include cytochromes, catalase, peroxidase, tryptophan pyrrolase, and a small pool of free heme. Bilirubin is the breakdown result of the heme moiety of hemoglobin. Hyperbilirubinemia is caused by an increase in the concentration of direct reactive bilirubin in the blood, which is

hazardous under some circumstances, causing jaundice, hyperbilirubinemia-induced auditory impairment, and neurotoxicity culminating in brain damage [37]. Modest unconjugated hyperbilirubinemia, on the other hand, acts as a mild antioxidant and may protect against cardiovascular disease and tumor formation [24]. According to a recent study, modest concentrations of direct responding bilirubin cause stroke in the body and can also cause cardiac difficulties. Under a variety of clinical situations, serum bilirubin levels are frequently elevated. Bilirubin is attached to serum albumin in the circulation of blood, which prevents the potential toxicity of free bilirubin [38]. Despite its high affinity for albumin, bilirubin is promptly and selectively absorbed by the liver, bio transformed with glucuronate, and released into bile [24]. In the liver, bilirubin is transformed to bilirubin glucuronic acid, which is expelled with bile.

According to Maher and Mansour [45], the clinical spectrum of SCD extends from moderate LFT abnormalities in asymptomatic patients to substantial hepatic abnormalities with considerable hyperbilirubinemia. Some investigations have found that LFT abnormalities are more severe during vaso-occlusive episodes [46], fever, and leukocytosis [35]. These were not investigated in our patients because they were all in a stable state.

Liver abnormality in SCA can be caused by a variety of factors, including sickle cell obstruction of sinusoids, which can lead to hepatic infarction during vaso-occlusive episodes, red cell sequestration, cholelithiasis, and cardiac failure. Ischemia, transfusion-related viral hepatitis, iron overload (hemosiderosis), and gallstones are all potential contributors to the pathogenesis of liver illness in SCD. According to Coiner et al. [41], the most common causes of liver abnormalities in SCD are hemosiderosis and viral hepatitis, both of which are linked to recurrent blood transfusions. In their investigation, all sickle cell participants with chronically increased LFTs had hemosiderosis and erythrophagocytosis in their liver biopsies. Traina et al. [36] and Mills [47] both observed comparable findings. However, because our investigation excluded SCA patients with hepatitis or who had recently received a blood transfusion, these conditions are unlikely to be the cause of aberrant LFTs in these patients. The presence of hemosiderosis and heart failure in the SCA patients in this investigation was also not proven, therefore their relevance in the pathogenesis of the observed aberrant LFTs could not be determined. Hemolysis and red cell sequestration, with resulting hyperbilirubinemia, are the most plausible explanations of changed LFTs in the SCA patients in our investigation. Hyperbilirubinemia may have resulted in bilirubin toxicity, with the excess bilirubin causing biliary tract irritation and gallstone formation/cholelithiasis, leading to an increase in serum ALP.

Benerjee et al. [48] proposed that the hepatic sequelae of SCDs, including SCA, can be divided into disorders linked to hemolysis, anemia and subsequent transfusion management, the effects of sickling and vaso-occlusion, and defects unrelated to SCD. A complex etiology for liver disease in SCD

patients has also been proposed by Maher and Mansour [45], which includes variables linked with chronic hemolytic anemia (cholelithiasis), numerous transfusions (viral hepatitis and iron overload), vascular damage, and the sickling process itself. According to Charlotte et al. [49], evidence shows the importance of vascular alterations and the major precipitation of the sickling process in most individuals. In a small number of cases of SCA, a distinct clinical presentation of sickle cell intrahepatic cholestasis, as well as a syndrome characterized by increasing cholestasis in the absence of cirrhosis, has been observed [50]. Right upper quadrant discomfort, excessive bilirubin elevation, stunning ALP rise, varied transaminase elevations, and histological characteristics such as intracanalicular cholestasis, sinusoidal dilatation, kupffer cells hyperplasia, and erythrophagocytosis characterize these cases [42].

## 5. CONCLUSION

The findings of this investigation revealed that sickle cell patients had significantly higher liver indices than control persons. This could indicate that its synthesis in the liver has been increased or metabolism was reduced sequel to sickle cell disease.

**Ethical Approval and Consent :**

Before being enrolled in the study, all individuals gave their informed consent. Before beginning the study, the Ethical and Research Committee of OAUTHC, Ile-Ife, was consulted and ethical approval was granted.

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