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Original article:

Study of hepatic enzymes and serum homocysteine (t-Hcy) variations in sickle cell disease.

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ABSTRACT:

BACKGROUND: Serum GOT, SGPT, ALP and Sr. Homocysteine (tHcy) level have been observed increased in sickle cell disease. The biological mechanism of synthesis and regulation of the serum GOT, SGPT, ALP and Sr. Homocysteine (tHcy) was unclear. The aim of the research study is to provide an overview of the Serum GOT, SGPT, ALP and Sr. Homocysteine (tHcy) level changes and its significance in sickle cell disease.

METHODS: This study was conducted in the Department of Biochemistry, People's College of Medical Science and Research (PCMS & RC) and Centre for Scientific Research and Development (CSRD), People's University Bhopal. The study protocol will be approved by RAC, IEC and UDC committees of our institute/university. Serum GOT- SGPT was estimated by Reitman & Frankel's method, Sr. ALP pNPP kinetic method and Sr. Homocysteine (tHcy) was estimated by spectrophotometric method. All the values cases were compared with the reference values.

RESULTS: The study showed that, the significant variations in Serum GOT- SGPT, ALP and Sr. Homocysteine (tHcy) parameters. Serum GOT was found significantly increased in cases (69.98 ± 69.31) as compaired to controls (25.17 ± 5.25), calculated't' value = 6.79. SGPT was also found to be significantly increased in cases (65.28 ± 60.07) as compared to the controls (22.72 ± 5.47), the calculated' was 7.43, and $P < 0.0000$ was highly significant. Sr. ALP in cases was found significantly increased (150.78 ± 31.36) as compare to control (53.68 ± 22.34), 't' value was 26.57 and $P < 0.0000$. Sr. Homocysteine (tHcy) were significantly high (18.53 ±1.92) in Sickle Cell Disease cases when compared with the healthy controls (11.03 ±1.91) and $P < 0.0000$.

KEYWORDS: Sickle Cell Disease, SGOT, SGPT, ALP, and Sr. Homocysteine (tHcy), bio-markers for the diagnosis

INTRODUCTION:

It is a genetic disorder, characterized by the presence of the hemoglobin S (HbS), where valine is replace by glutamic acid ($\beta^{s\ 6\ Glu \rightarrow Val}$) at the beta globin chain, that has a single point mutation (GAG → GTG) at the sixth codon of the β- globin (*HBB*) gene. [1] This point mutation is responsible for the alteration in the properties of the hemoglobin tetramer, with a tendency to polymerize in the deoxygenated state altering normal, flexible, biconcave shaped red blood cells (RBCs) are changes in to stiff, rigid, sickle cell RBCs.

Sickle cell disease is a group of disorders associated with a mutation in the β globin gene, associated with multi organ damage, with various diseases like; hemoglobinopathy, causes sickling of red blood cells, resulting in vessel blockage, stroke, anemia, inflammation, and extreme pain. [2]

Homocysteine (tHcy) is a sulfur containing amino acid, found at low concentration in blood and cells and is an important intermediate molecule involved in the biosynthesis of methionine and cysteine. The high plasma concentration of tHcy is a well established risk factor for several disorders, including cardiovascular disease stroke, venous thrombosis and arteriosclerosis. [3] It (tHcy) is an important vascular risk factor, may contribute to the ischemic phenomena of sickle cell disease (SCD) has attracted some interest in plasma total homocysteine (tHcy) levels in patients with SCD. However, almost all the studies in SCD have been in children, who presumably have a lesser predisposition to hyper homocysteinemia than adults. [4]

Homocysteine, may contribute to the ischemic phenomena present in HbSS has attracted some interest in plasma total tHcy. In previous study it is showed that, the plasma concentration of tHcy among HbSS subjects was approximately 1.5-fold higher than that of healthy controls. Additionally, SCD patients have higher plasma tHcy concentration in spite of elevated plasma folate levels and vitamin B_{12} concentration similar to those observed in controls. [5] Elevations of plasma homocysteine levels have been shown to a risk factor for the endothelial cell damage and thrombosis, which are implicated in sickle cell disease (SCD)-related vaso-occusion. [6]

Hepatic diseases are the commonly known complications of sickle cell disease due to multiple factors such as intrahepatic sinusoidal sickling, bilirubin gallstones, transfusion related hepatitis infections or excess iron deposition. There are studies suggesting that the main causes of liver injury in sickle cell patients are due to factors other than intra hepatic sickling, which was considered to be reversible, such as viral hepatitis or transfusion iron overload. [7-10]

Therefore, present study is aimed to assess the liver function enzymes and serum homocystein (tHcy) in sickle cell disease.

MATERIAL AND METHODS:

This study will be conducted in the Department of Biochemistry, People's College of Medical Science and Research (PCMS & RC) and Centre for Scientific Research and Development (CSRD), People's University Bhopal. The study protocol will be approved by the DDC, RAC, IEC and UDC committees of our institute/university.

Study Design:

This is a hospital based case control study and the sample size is done by the expert statistician.

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Study subjects:

For the study, Patients sample will be collected from Peoples hospital, Rajeev Gandhi Hospital, and Red Cross society of Bhopal, Govt. Hospitals, and The Peripheral Govt. hospitals of Bhopal, and Govt. hospital Khandwa, Madhya Pradesh.

Total 111 subjects will be enrolled after applying inclusion and exclusion criteria and written informed consent will be taken. Sickle cell disease patients will be included and excluded with the help of physician, Dept of Medicine, People's College of Medical Science and Research (PCMS & RC).

The inclusion and exclusion criteria will be follows:

Inclusion criteria	Exclusion criteria
1. Patients more than 5years age and less than 80 years age. **2.** Normal healthy individuals will be recruited for the control group.	1. Patients less than 5years and more than 80 years. 2. Individuals with any other hemoglobinopathy, and the patients having any history of blood transfusion within 3 months. 3. SCD patients under the treatment of chemotherapy will be excluded from the study.

The control group consisted of 111 healthy individuals recruited from the of the People's College of Medical Sciences & Research Centre, departmental staff, medical students posted for internship or the relatives who were healthy and accompany their IPD ward or OPD and their health condition will be detected. Rajeev Gandhi Medical College, and Govt. hospitals of Bhopal; these group was characterized by absence of hematological disorders or inflammatory conditions.

All procedures followed will be in accordance and approved by the Research Ethics Committee of the People's College of Medical Sciences & Research Centre, Bhopal.

Sample collection:

The 10 ml overnight fasting venous blood had been collected from patients and controls under aseptic conditions. 6 ml blood was collected in plain vacutainer, and remaining 4ml blood had been poured in EDTA anticoagulated vacutainer. The Sample had been centrifuged at 3000 rpm for 10 minutes; serum are separated and immediately stored in deep freezer at – 20^{o}C until further analysis.

Screening tests for the sickle cell disease subjects:

The following screening test will be carried out with the blood sample of SCD patients for the confirmation of sickle cell RBCs.

1. Sickling test with 2% metabisulphite: It is the principle of sickling test, will be based on microscopical observation of sickling of red blood cells when exposed to a low oxygen tension.

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2. Solubility test with 0.02% sodium dithionate: It is the principle of solubility method, based on turbidity created when Hb S is mixed with sodium dithionate.
3. Peripheral blood film method: Thin blood films, stained with giemsa stain were examined by light microscopy (×100).
4. Hb electrophoresis: The cellulose acetate membrane Hb electrophoresis method will be used to determine the presence of Hb-S in the sample.
5. Patient's history and blood cell counts such as; RBC, WBC, and HCT, MCH, MCV, MCHC, will also be carried out.

The complete blood count and red cell indices were measured by automated analyzer using Trans Asia Diagnostic kit. Biochemical parameters (AST, ALT, and ALP) had been estimated by Trans Asia Bio-Medicals Ltd., kit, Daman –India.

The serum homocysteine (tHcy) level had been estimated by Elisa kit Method.

RESULTS:

The data obtained from the different biochemical parameters were analyzed by using the Statistical Package for Social Sciences (SPSS) software. Student t- test was used to compare means of variables between SCD cases and controls. The $P < 0.05$ was considered as statistically significant.

The study showed that, the significant variations in Serum GOT, SGPT, ALP and Sr. Homocysteine (tHcy) levels. Serum GOT was found significantly increased very high in cases (69.98 ± 69.31) as compaired to controls (25.17 ± 5.25), calculated't' value = 6.79. SGPT was also found to be significantly increased very high in cases (65.28 ± 60.07) as compared to the controls (22.72 ± 5.47), the calculated't' was 7.43, and $P < 0.0000$ was highly significant. Sr. ALP in cases was increased very high and shown very highly significant (150.78 ± 31.36) as compare to controls (53.68 ± 22.34),'t' value was 26.57 and $P < 0.0000$.

The graphical representation of SGOT, SGPT and Serum Alkaline Phosphatase (ALP) is shown as below column graph diagram.

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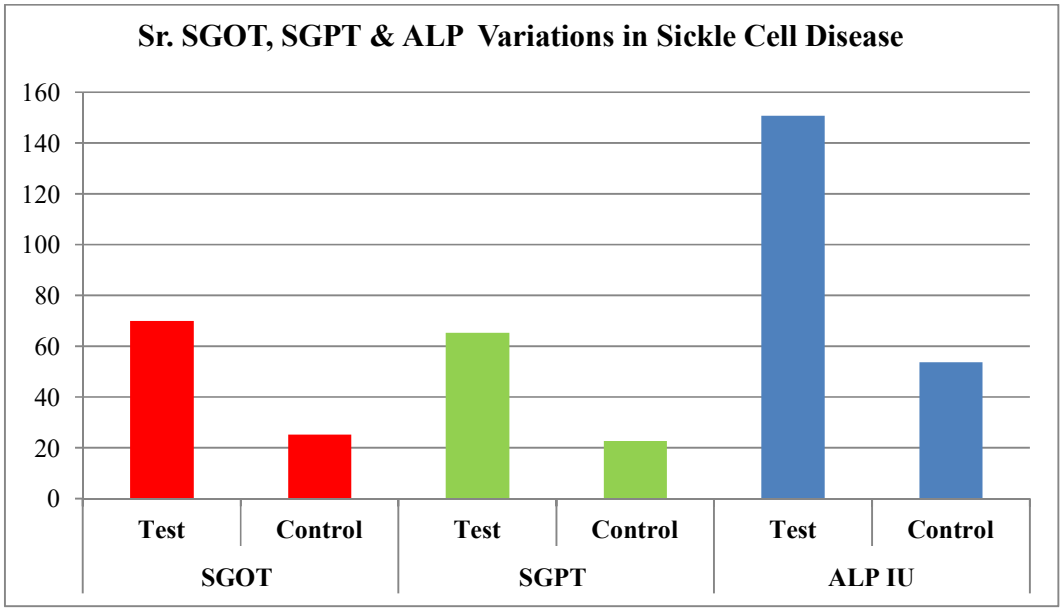
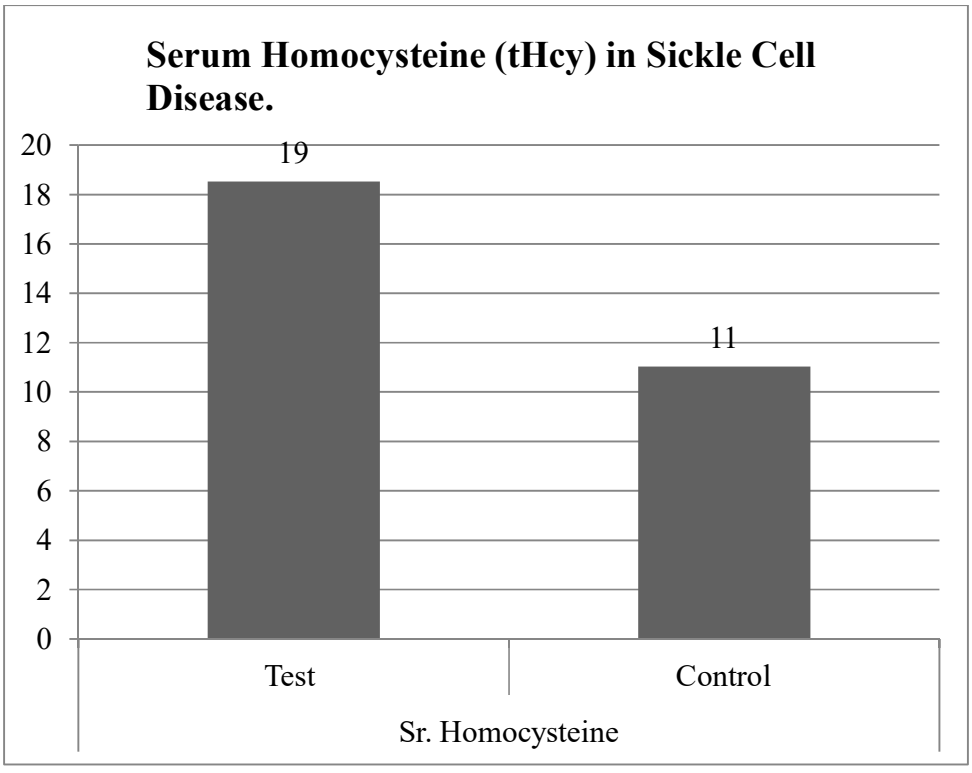


The Serum Homocysteine (tHcy) were found significantly very high (18.53 ±1.92) in Sickle Cell Disease cases when compared with that of the healthy controls (11.03 ±1.91) and P < 0.0000.

The graphical representations of Sr. Homocysteine (tHcy) in cases were also found significantly increased in cases as compared to the controls is shown in below column graph diagram.



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DISCUSSION:

The prevalence of sickle cell disease in Africa and Jamaica is very high as compared to the Indian population. [11] Hepatic diseases are the commonly known complications of sickle cell disease due to multiple factors such as intrahepatic sinusoidal sickling, bilirubin gallstones, transfusion related hepatitis infections or excess iron deposition. [7-10]

The transaminase enzymes Serum GOT, SGPT and ALP were very significantly increased in sickle cell disease. Akuyam et al., in 2007 also reported that, these enzymes (Sr. GOT, SGPT and ALP) were found very high and significantly increased along with the significant elevations of Sr. Total bilirubin found in sickle cell disease. [12]

S. Pandey, et al., in 2012. and T. Kotila et al ., in 2005. The significant higher levels of plasma of hepatic enzymes seen in Sickle cell anemia subjects in that study support earlier observations and had been related to sickle cell hepatopathy. [13, 14] These two articles are similar to this article which shows the levels of SGPT which was found to be significantly increased very high in cases (65.28 ± 60.07) as compared to the controls (22.72 ± 5.47), and $P < 0.0000$ was highly significant. Sr. ALP in cases was increased by (150.78 ± 31.36) as compare to controls (53.68 ± 22.34),'t' value was 26.57 and $P < 0.0000$.

Mohamad AO et al., (1993) studied that the, Elevations of serum alkaline phosphatase (ALP) is commonly seen in patients with Sickle cell anemia during the pain crisis. [15] The finding of the present study is also supported by the previous study of elevation of AST (94.4%), ALT (2.8%), ALP (31%), which had been found in SCD patients by Akuyam AS et al in 2007 ,T. Kotila et al in 2005, Mohamad AO et al., (1993) and Brody JIet al., in 1975. [12, 14, 15, 16] Norris WE, (2004) reported that, the acute sickling process selectively affect the liver in 10% of the patients, causing to liver crisis with abdominal pain, nausea, fever, jaundice and transaminases elevations. [17] Sheehy TW, in 1977. Schubert TT, in 1986. Stephan JL., in 1995 and Johnson CS, in 1985 they were stated that, the liver enzymes transaminases are significantly increased in sickle cell hepatopathy, enzyme ALP was markedly elevated in the sickle cell disease, but the ratio of AST and ALT enzymes was not altered significantly. The enzyme AST was mostly found elevated excessively due to increased rate of hemolysis RBCs in sickle cell disease, significantly increased level of serum bilirubin was found also due to the on-going increased rate of hymolysis, intra hepatic cholestatis, and renal impairments encountered in sickle cell hepatopathy in comparison with remaining diseases. [18] [19] [20] [21]

In our article another biochemical variable, serum homocysteine (tHcy) were found significantly very high (18.53 ±1.92) in Sickle Cell Disease cases when compared with that of the healthy controls (11.03 ±1.91) and $P < 0.0000$.

But, Ajayi et al., (2013), is not supporting to our hypothesis, they reported that, there were no significant differences found between the SCD cases and controls in homocysteine or MMA, but a significantly higher folate concentration were observed in the SCD patients. [22] And the report of Ali et al, clarified that, the elevation of tHcy is not commonly found in sickle cell trait; but if found elevated, then that may indicate meningeal vessel thrombosis. [23]

Wendell Vilas-Boas et al.,(2015). Describe that (Lower-ACS), the serum tHcy among SCA subjects was 1.5-fold higher than that of the controls. And, sickle cell disease (SCD) patients have higher plasma concentrations of tHcy in spite of higher plasma folate levels and vitamin B12 concentrations compared with those observed in healthy individuals. [24]

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Highly significant studies- in these articles, Kamal E.A. Abdelsalam-2016, it was reported that, tHcy level was found to be significantly higher in patients with sickle cell trait, when compared to control group. [25] Cattaneo M., (2001), Saeed S Faramarz, (2006), and Virdis A (2002) reported that, In the thromboembolism and cardiovascular disease, the total Homocysteine level was increased in 40% of patients with sickle cell anemia. There was a significant increased mean tHcy level in sickle cell patients by 95% compared to the control group. [26, 27, 28,]

In article, Nnodim Johnkennedy et al, Hb-SS and Hb-SS cris study, the mean level of sr. tHcy was significantly increased in sickle cell anaemia ($p<0.05$), as compared to the control (HbAA). And the mean level of sr. tHcy was significantly higher in sickle cell crisis than the sickle cell anaemia. The result concluded that the sickle cell anemia is linked with rise in sr. homocysteine level, which could lead to increased risk thromboembolic and cardiovascular disease in sickle cell anaemia. [29] In the article**,** Wendell Vilas-Boas et al- 2015. (Medi.-ACS), sr. tHcy among SCA subjects was 1.5-fold higher than that of controls.[24] S. Pandey et al 2012, studied, (40) sickle cell anemia patients the plasma homocysteine levels was surperisingly elevated. [13] Patricia E Houston MS , et al- 1997.(mild SCD strok), reported that, the serum homocysteine levels for the stroke group were significantly higher than those in patients without stroke, High serum homocysteine levels may be a risk factor for development of stroke in SCD patients**.** [30]

S.Ozdem et al., (2008) reported that the serum homocysteine levels were significantly increased in subjects of sickle cell disease. [31] Meekoo Dhar et al. 2004. also stated that, the plasma total homocysteine (tHcy) was significantly higher in the SCD subjects than the controls. [31, 32]

The overall studies [13, 24, 25, 26, 27, 28, 29, 30, 31, 32] supporting our hypothesis which showing serum homocysteine tHcy level is statistically (increased) and very highly significant in the Sickle Cell Disease.

Conclusion:

In Liver function tests, Sr. Bilirubin, Sr. SGOT- SGPT, (hyperbilirubinemia) Sr. Alkaline phosphatase, was significantly increased in cases, and that could be used in diagnosis of Sickle Cell Disease.

The Serum homocysteine level in SCD patients is observed statistically increased and extremely significant. So, the serum homocysteine (tHcy) will also be the clinical bio-marker for the diagnosis of sickle cell disease.

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