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Original article

Study of Hemoglobin Count, Iron and Total Iron Binding Capacity variation pattern in Sickle Cell Disease

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Abstract:

Background: Sickle cell disease is common hereditary hemoglobinopathy with a multi - system disease, associated with episodes of acute illness and progressive organ damage.

Material and Methods: The study was conducted in the Department of Biochemistry, S.M.B.T. Institute of Medical Sciences and Research Centre, Dhamangaon - Nashik – Maharashtra. The patients were selected by applying inclusion criteria - exclusion criteria with screening tests. Hb level was done by an automated Cell Counter, Sr. Iron and Sr.TIBC estimation was by Ferrozine / $MgCO_3$ method, by using Trans Asia diagnostic kit.

Results: The hemoglobin was (7.68 ± 1.59; 15.19 ± 0.74, $p<0.001$); Sr. Iron was found significantly decreased in cases (42.92 ± 5.09; 121.50 ± 25.95, $t=15.84$ and $p<0.001$) and Serum Total iron binding capacity (TIBC) was found increase significantly very high (457.12 ± 62.07; 347.46 ± 40.68, and $p<0.001$) in cases of SCD as compared to controls.

Conclusions: The study concluded that, Hemoglobin in blood and iron level decreases however the total iron binding capacity (TIBC) increases in Sickle cell disease patients, this could be the biochemically important tools in diagnosis of sickle cell disease.

Keywords: Hemoglobin, Total Iron Binding Capacity (TIBC), Sickle cell disease

INTRODUCTION:

Sickle cell disease (SCD) is commonly found in central region of Indian states Chhattisgarh, and in tribes of Madhya Pradesh, Rajasthan and Maharashtra. [1,2, 3] SCD is a group of inherited autosomal recessive, blood related disorders characterized by abnormal hemoglobin S molecules (HbS); which is caused due the single point mutation in the 2β-globin chain of the globin tetramer. [4, 5] In the view of molecular or genetic level, sickle-cell disease is a group of blood related inherited, genetic disorder transformed from parents, characterized by the presence of the 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. [4, 5]

Sickling of red blood cells, causes in hematological parameters which shows the higher Mean Corpuscular Hemoglobin Concentrations (MCHC), vessel blockage,

stroke, anemia, inflammation, and attacks of pain sickle cell crisis/ anemia, swelling in the hands and feet, bacterial infections, and it is also associated with multi organ damage, with various diseases like the vaso-occlusive events which leads to organ damage [6, 7, 8, 9]

The haematological and laboratory variations are: decrease in haemoglobin concentration, Red blood cells, increase in serum bilirubin level, fever, aggregated and irreversibly sickle cells, increased erythrocyte life span, and almost no change in other types of Hbs concentrations varies from the control. [10, 11] In this study, the basic biochemical markers Hemoglobin, Iron and TIBC will have been studied as promising molecules for better understanding the SCD. In SCD the sr. iron level is depleted due the sickling process which causes the damage of hemoglobin in the RBCs that shows iron deficiency and TIBC is excessesively activated to increase the iron level, and it is regulated by the serum hepcidine level in SCD. [12- 15]

MATERIAL AND METHODS:

The study was conducted in the Department of Biochemistry, S.M.B.T. Institute of Medical Sciences and Research Centre, Dhamangaon Tal: Igatpuri, Dist: Nashik –Maharashtra. The study protocol had been approved by Institutional Ethical Committee. (Reference: IEC- Research project/protocol no: SMBT/IEC/2017/Project-52; dated 02/05/2017)

This was a hospital based case control study, and the sample size had been carried out by the expert statistician.

Study subjects:

For this study, the patients sample was collected from Civil Hospital Nashik, and SMBT Hopital Dhamangaon, Nashik – Maharashtra.

Total 30 subjects had been enrolled after applying inclusion and exclusion criteria and written informed consent was taken before starting the study.

All the selected and enrolled patients had been confirmed with their stability state, without a single blood transfusion of four months prior to blood drawn; and the patients were included in the study did't show any infections, hospitalization or vaso-occlusive event, and were not under the antibiotics therapy and corticosteroids or hyroxyurea (HU) treatments. The control group volunteers consisted of 30 healthy individuals. All procedures followed were being in accordance and approved by the Research Ethics Committee of the SMBT Institute of Medical Sciences & Research Center, Dhamangaon, Nashik and also with the Helsinki Declaration of 1975 and its revisions. Informed consent forms will be obtained from all patients as well as from the control group volunteers.

Inclusion criteria for normal healthy subjects:

Normal healthy person were comprised of departmental staff, medical students and the relatives who were healthy and accompany their OPD or IPD wards. And their health condition was being detected.

Inclusion criteria:

A) Patients more than 5years age and less than 80 years of age.

B) Normal healthy individuals was recruited for the control group

Exclusion criteria:

A) Patients less than 5years and more than 80 years.

B) Individuals with any other hemoglobinopathy and the patients having any history of blood transfusion within 3 months.

C) SCD patients under chemotherapy treatment were excluded from the study.

Screening tests for the sickle cell disease subjects:

The following screening tests had been carried out with SCD patients for the confirmation of sickle cell RBCs.

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1. **Sickling test with 2% meta-bisulphite:** It is the principle of sickling test, was based on microscopical observation of sickling of red blood cells when exposed to a low oxygen tension.
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 had been 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, was being carried out.

To confirm the diagnosis, blood sample was examined under a microscope to check for the number of sickle cells, patient's history and blood cell counts with RBC, WBC, and HCT, MCH, MCV, MCHC, counts was also carried out.

Sample collection:

The 10 ml overnight fasting venous blood had been collected under aseptic conditions from patients and controls. About 6 ml blood was collected in plain vacutainer, and remaining 4ml blood was being poured in EDTA anticoagulated vacutainer. The sample was centrifuged at 3000 rpm for 10 minutes for the separation of serum sample and then the separated sample was immediately stored in deep freezer at – 20°C until further analysis. The sample collection had been started from the year 2018-19 and all work was finished in June -2020.

METHODOLOGY:

Hb and Complete blood count (CBC) had been carried out by an automated Cell Counter and Sr. Iron and Sr.TIBC was estimated by Ferrozine / $MgCO_3$ method, using Trans Asia diagnostic kits; Manufactured by TRANSASIA BIO- MEDICALS LTD., B-11, and OIDC. RINGANWADA, DAMAN-396210- INDIA.

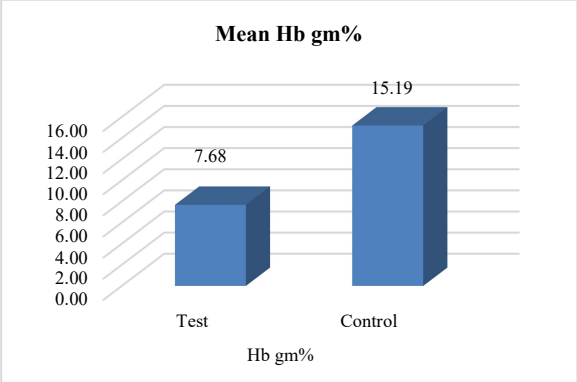
RESULTS:

Table no: 1

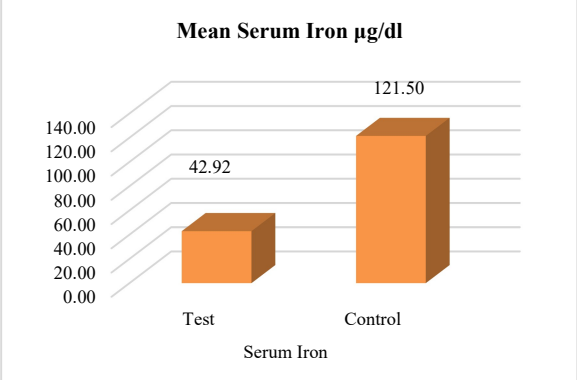
Variable	Group	Sample n=30	Mean ± SD	t statistics	P value
Hb gm%	Test	30	7.68 ± 1.59	24.77	< 0.001
	Control	30	15.19 ± 0.74		
Serum Iron µg/dl	Test	30	42.92 ± 5.09	15.84	< 0.001
	Control	30	121.50 ± 25.95		
TIBC µg/dl	Test	30	457.12 ± 62.07	8.25	< 0.001
	Control	30	347.46 ± 40.68		

* Significant p<0.005; Table no: 1 representing the mean ± standard comparisons of Hb, Sr.Iron and TIBC from the SCD cases and healthy controls.

* Graph: 1 representing the mean ± standard comparisons of Hb, Sr.Iron from the SCD cases and healthy controls.



* Graph: 2 representing the mean ± standard comparisons of Sr.Iron from the SCD cases and healthy controls.



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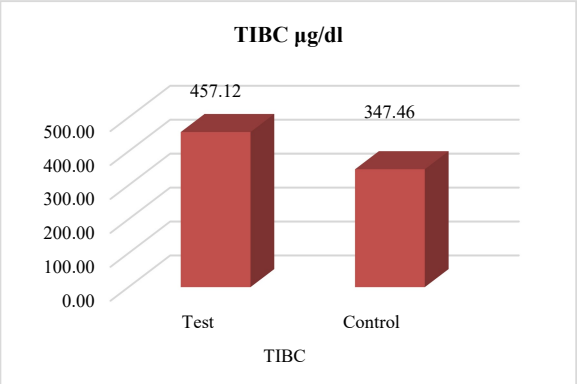
* Significant p<0.005; representing the mean ± standard comparisons of Sr. TIBC from the SCD cases and healthy controls.

* Graph: 3 representing the mean ± standard comparisons of Sr. TIBC from the SCD cases and healthy controls.



DISCUSSION:

In the above observation (table no: 1) Hemoglobin was found significantly decreased below the normal in cases as compared to control (7.68 ± 1.59; 15.19 ± 0.74); student 't' test was found 24.77and p<0.001. Sr. Iron level was also found significantly decreased in cases (42.92 ± 5.09; 121.50 ± 25.95) compared to controls, however Sr. TIBC was found increased significantly as compared to control (457.12 ± 62.07; 347.46 ± 40.68); the student 't' test was 8.25 and p was <0.001 found (highly significant) in SCD cases.

Our findings are correlated with different articles that are discussed here under: Akinsegun Akinbami et al., (2012) they stated that, the mean cell volume, mean cell hemoglobin, and mean cell hemoglobin concentrations were reduced in anemia of chronic disease. Expectedly, the mean hemoglobin concentration, packed cell volume, mean cell volume, and mean cell hemoglobin of the controls were higher than SCD cases, The overall mean Hb conc.^{n} for cases was 7.93 ± 1.47 g/dl, packed cell volume 24.44 ± 4.68%, (MCV- 81.52 ± 7.89 fl,) and (MCH-26.50 ± 3.20 pg.) While for controls, mean Hb conc.^{n} was 13.83 ± 1.32 g/dl, PCV 43.07 ± 3.95%, MCV-86.90 ± 4.69 fl, and MCH-28.50 ± 1.34 pg. [16]

Abdul Rahman H Majrashi et al., (2016) noted that, the mean of WBC in the patients was found 15.4 ± 7.3 × 10^9/L while in controls it was 7.25 ± 3.75 × 10^9/L that were considered highly significant (P<0.0001). Similarly, the mean of platelet in the female patients was 370.23 ± 177.38 while in control it was 300 ± 150, considered significant (P<0.0628).

All other CBC indices like RBC, Hb, Hct, MCV, MCH, and RDW were showed highly significant difference (P<0.0001) in the cases of sickle cell anemia. [17]

In the study, Sr. Iron was found decreased significantly low in cases (57.22 ± 25.42; 106.33 ± 26.62) as compared to controls. However, the Sr.Total Iron Binding Capacity was found increased very significantly high (344.57 ± 25.42; 289.85 ± 33.40) in cases of SCD.

Decrease in Sr. Iron levels was due to rapid sickling and hemolysis, (releases the iron) of the RBCs in sickle cell disease. But the total iron binding capacity level was found increased because that iron level is regulated by the negative feedback mechanism, and that is only because of the rapid sickling mechanism.

In support of the above biochemical variations, scientists Raoet al.,(1983) Rao KRP et al., (1983) and Haddy TB et al., (1982) reported that, the iron deficiency may be associated with a marked reduction in number of sickled erythrocytes in blood smears and decrease in levels of Sr. Indirect bilirubin and lactate dehydrogenase in the sickle cell disease. [18, 19, 20]

In our study findings, graph-3 indicating that, the increased levels of the Serum LDH and total iron binding capacity (TIBC) in cases as compared to the control. But the serum iron level is showing decreased in the above graph in SCD cases.

The above variations are correlated and supported by the various articles: Erlandson M. E et al., (1962) stated that in sickle cell anaemic children with chronic haemolysis, that results in increased availability of iron directly from the broken red cells and also from increased absorption of iron from the gastrointestinal tract. [21] in such conditions the high load of iron is provided through the multiple blood transfusions. [12, 13]

Jeyakumar LH et al. (1987) stated that, the Sr. iron concentration is a balance between intakes on the one hand and excretion on the other hand; as well as increased utilization of the other functions, since there is no empirical reason to believe that HbSS subjects had lower dietary intake of iron. [14]

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O'Brien RT.et al, (1978) after his observation he declared that, in children with sickle cell anemia and chronic haemolysis results in increased availability of iron directly from the broken red cells and also from increased absorption of iron from the gastrointestinal tract. [12]

Vichinsky E., et al., (1981) However, reported that; the reduced frequency of transfusion implies a reduction in sources of iron level and, therefore, increased vulnerability to iron deficiency anemia. This had been tressed by a study in the USA which suggested that iron deficiency was commoner than expected in untransfused patients with sickle cell anemia. [22]

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Ballas S. K et al., (2001) stated that, the high load of iron provided by multiple blood transfusions in SCD and Thallassemia conditions. [13]

Stuart MJ and Najel RL (2004) states that, sickle cell patients have iron overload due to chronic blood transfusions in the treatment or prevention of sever sickle cell related complications such as stroke. [15]

Our findings are also strongly correlated and supported to the above articles with same hypothesis.

CONCLUSION:

The study concluded that, Hemoglobin, and Sr.Iron levels were found decreased; but on the other side Sr.TIBC was found increased in the SCD cases as compare to healthy controls. These biochemical variables could be the diagnostically important tool in the assessment of sickle cell disease.

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REFERENCES:

1. Ojodu J, Hulihan MM, Pope SN, Grant AM; Centers for disease Control and Preventation (CDC), Incidence of sickle cell trait – United States, 2010. MMWR Morb Mortal Wkly Rep, 2014; 63:1155 – 1158. [PMID:25503918]
2. Lehmann H. and Cut bush M., Sickle-cell Trait in Southern India " Br Med J. 1952; 1(4755):404–405.doi: 10.1136/bmj.1.4755.404PMID: 14896162.
3. Vikas K. Gupta, Nilima Singh, S.W. Masram, S. K. B. Patil; Status of some Hematological and Antioxidant

Parameters in SCA patients of Chhattisgarh Region, Journal of Medical Science and Clinical Research, 7, July 2015. Vol.03. [www.google scholar]

4. Awasthy N., Aggarwal K.C., Goyal P.C., Prasad M.S., Saluja S, Sharma M. "Sickle cell disease: Experience of a tertiary care center in a non endemic area". Annals of Tropical Medicine and Public Health. 2008; 1 (1): 1–4. [www.google scholar]
5. Ashley-Koch A., Yang Q., Olney R.S. Sickle hemoglobin (HbS) allele and sickle cell disease: a HuGE review. Am J Epidemiol. 1, May 2000; 151(9):839-45. https://www.ncbi.nlm.nih.gov/pubmed/10791557
6. "What Are the Signs and Symptoms of Sickle Cell Disease?" National Heart, Lung, and Blood Institute. June 12, 2015. Archived from the original on 9 March 2016. Retrieved 8 March 2016. https://www.nhlbi.nih.gov/health/health topics// sca/signs.
7. Brianna Lutz, B.S., Steffen E. Meiler, Yuan-Xiang Tao et. al, Transl Perioper Pain Med. 26,July 2015; 2(2): 8–17. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4542088/
8. Steiner, C.; Miller, J. HCUP Statistical Brief 21. Rockville, MD: Agency for Healthcare Research and Quality; 2014. Sickle Cell Disease Patients in U.S. Hospitals, 2006. https://www.hcup-us.ahrq.gov/reports/statbriefs/sb21.pdf
9. Kanter J, Kruse-Jarres R. Management of sickle cell disease from childhood through adulthood. Blood Reviews. 2013; 27:279–87. https://www.ncbi.nlm.nih.gov/pubmed/24094945; doi:10.1016/j.blre.2013.09.001.
10. Steinberg, M.H., 2009. Genetic etiologies for phenotypic diversity in sickle cell anemia. Scientific World Journal (Special Issue: Hemoglobinopathies), 46-47. https://www.ncbi.nlm.nih.gov/pubmed/19151898;
doi: 10.1100/tsw.2009.10
11. Harrick JB Peculiar elongated and sickle shaped corpuscles in case of severe anemia. Yale J Biol Med. 2001 May-Jun; 74(3): 179–184. PMCID: PMC2588723 PMID: 11501714
12. O'Brien R. T., "Iron burden in sickle cell anaemia," Journal of Pediatrics, 1978; (92): 579–582 doi: 10.1016/s0022-3476(78)80291-1.
13. Ballas S. K, "Iron overload is a determinant of morbidity and mortality in adult patients with sickle cell disease," Seminars in Hematology, 2001; (38):1: 30–36. https://pubmed.ncbi.nlm.nih.gov/11206959/ PMID: 11206959 DOI: 10.1016/s0037-1963(01)90058-7
14. Jeyakumar L. H., Akpanyung E. O., Akinyemi A. A., and Emerole G. O., "An investigation into the iron status of children with sickle-cell disease in Western Nigeria," Journal of Tropical Pediatrics, 1987; 33: (6) 326–328.
15. Stuart MJ, Nagel RL, Gautam R, Daniel F, Wallace, Nathan V, et al. Sickle-cell disease. Lancet. 2004; 364:1343-60. https://www.ncbi.nlm.nih.gov/pubmed/15474138
16. Akinsegun Akinbami, Adedoyin Dosunmu, Adewumi Adediran, Olajumoke Oshinaike, Phillip Adebola, et al; Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeri , BMC Res Notes. 2012; (5): 396. doi: 10.1186/1756-0500-5-396 PMCID: PMC3423074 PMID: 22849350
17. Abdul Rahman H Majrashi, Abbas H Alsaeed, Mohammed Abbas Alsaeed, Abjal Pasha Shaik., Correlation of haemoglobin-f levels with biochemical parameters in pediatric patients with sickle

cell anemia from Jazan, Saudi Arabia. Biomedical Research 2016; 27 (3): 702-709.

18. Rao K R, Patel A R, Honig G R, Vida L N, McGinnis P R. Iron deficiency anemia and sickle cell anemia. Arch Intern Med. 1983 May; 143(5):1030-2. PMID: 6679216.
19. Haddy TB, Castro O. Overt iron deficiency in sickle cell disease. Arch Intern Med 1982; 142:1621–1624.
20. Rao KRP, Ray VH, Patel AR. Serum ferritin and sequestered stores of body iron. Am J Clin Pathol 1983; 80:743–745.
21. Erlandson M. E., Walden B., Stern G., Hilgartner M. W., Wehman J., and Smith C. H., "Studies on congenital hemolytic syndromes, IV. Gastrointestinal absorption of iron," Blood, 1962; 19:359–378.
22. Vichinsky E., Kleman K., Embury S., and Lubin B., "The diagnosis of iron deficiency anemia in sickle cell disease," Blood, 1981;58:(5), 963–968. PMID: 7296005

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