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Study of Serum Endothelin – 1 Variation in Sickle Cell Disease

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Abstract: ***Background: Hb level decreased and Serum Endothelin-1 (ET-) level have been observed increased in sickle cell disease. The biological mechanism of synthesis and regulation of the Endothelin-1 (ET-) will be clear. The aim of the research study is to provide an overview of the Hb, CBC count, and Sr. Endothelin-1 level changes and its significance in sickle cell disease. Methods: Hematological values Complete blood count (CBC) was measured on fully Automatic blood cell counter hematology analyzer (Coulter LH 780), and HmX Hematology Analyzer with Autoloader. But in this study, we have concentrated on the variables like, Haemoglobin - (Hb), WBC, RBC, PCV and MCV count. All the values were compared to the reference values. The ET-1 was determined by using enzyme-immunoassay (ELISA kit) method, specifically designed for direct measurement of plasma ET-1. The sensitivity of the assay is 0.5pg/ml, and the cross-sensitivity of the antibodies used is reported to be < 1% with big ET-1. Results: The study showed that, the significant variations in CBC parameters were observed. The White Blood Cells counts (WBC), and Sr. ET-1levels were significantly increased. But the other indices like, Red Blood Cells (RBCs), PCV, and MCV were found significantly decreased with the controls in SCD. P < 0.05was considered to be statistically significant. Conclusion: The study concluded that, there is an extremely significant difference between mean Serum Hb, CBC and Endothelin-1 (ET-) levels in cases and healthy controls. So the Hb, CBC count and Serum Endothelin-1 level can be used as a clinical bio-marker for the diagnosis of Sickle Cell Disease.***

Keywords: Sickle Cell Disease, blood Hb, CBC count, Sr. Endothelin-1, bio-marker for the diagnosis

1. Introduction

Sickle cell disease (SCD) is more commonly occurs in African populations. In India, it was first discovered by Lehmann and Cut bush about 50 years ago, among the tribal's of Nilgiri hills in southern India, but it commonly found in central part of India. i. e. in various parts of Chhattisgarth and tribles of Madhya Pradesh. [1]

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. 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]

In red blood cells, two β globins combine with two α globins to form the oxygen carrying molecule hemoglobin, upon deoxygenating, sickle β globin polymerizes, which causes the blood cell to take on a half-moon shape, adhere to blood vessels, and hinder blood flow. These vaso-occlusive events lead to organ damage, extreme pain, stroke, and a shortened lifespan. Sickled red blood cells also die faster than normal red blood cells, resulting in hemolytic anemia. [3, 4]

In this condition, the hematological and laboratory variation are : decrease in hemoglobin concentration, lower mean corpuscular volume (MCV), lower reticulocyte count, elevated HbA2, increase in serum bilirubin level, fever, aggregated and irreversibly sickle cells, increased erythrocyte life span, and almost no change in Hb- F concentration. [5]

The routine hematological and biochemical parameters; Hb, Hct, Iron, TIBC, MCV, MCHC, RET (Reticulocyte), RED, WBC, PLT (Platelet counts) were showed the variation in the sickle cell disease from the control. [6]

The new biomarker ET-1 will have been studied with its importance, as promising molecule for better understanding the Sickle cell disease.

Endothelin – 1 (ET-1) is a 21-amino acid bipolar, peptide, produced by multiple cell types including macrophages, neurons, and endothelial cells. [7,8] released in response to endothelial cell activation, that constrict blood vessels, provide the vascular system and perform a number of specialized metabolic and transport functions, while in contact with the sub endothelial matrix on the basal side. ET-1 production is induced by the factors such as, proinflammatory cytokines, growth factors, angiotensin-II, mechanical stress, peripheral tissue injury, and hypoxia. [7, 9] Plasma ET-1level is elevated in the SCD patients and mice both during and after a vaso-occlusive episode. [9]
Elevation of plasma homocysteine levels has been shown to be a risk factor for the endothelial cell damage and thrombosis, which are implicated in sickle cell disease (SCD)-related vaso-occlusion. [10] Therefore ET- 1 is included as a biomarker for the present study.

So, the purpose of this prospective research work is to determine the actual concentration of the plasma endothelin-1 (ET-1) level in the sickle cell disease patients and compared with the healthy controls.

2. Materials and Methods

Patients with HbS were identified in the records of the Department of Hematology and Clinical Biochemistry, Peoples Medical College and Hospital, PUMS, Bhanpur, Bhopal- M.P. - India. Healthy volunteers were identified, based on the records of the clinical records of the clinical investigation centers at Peoples Medical College and Hospital, Bhopal.

Total 111 subjects have been enrolled after applying inclusion and exclusion criteria and written informed consent was taken.

All the participants were in the steady state, characterized by absence of blood transfusion in a period of four months prior to blood draw. In addition, patients included in this study did not show any infection, hospitalization or vaso-occlusive event, and were not under antibiotics, corticosteroids or hyroxyurea (HU) treatment, the patient should not be taken the non-steroidal anti- inflammatory drugs used during the proceedings two weeks, BMI should not be >30, patients should not be positive serology of HIV, hepatitis-B or C, pregnancy, hypertensions, the patients and the controls should not be on any type of medications. The control group consisted of 111 healthy individuals recruited at the People's College of Medical Sciences & Research Centre, Bhopal; this group was characterized by absence of haematological disorders or inflammatory conditions.

All procedures followed had been in accordance and approved by the Research Ethics Committee of the People's College of Medical Sciences & Research Centre, Bhopal, and also with the Helsinki Declaration of 1975 and its revisions. Informed consent forms had been obtained from all patients.

Inclusion criteria for normal healthy subjects:
Normal healthy person are comprised of 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.

Screening tests for the sickle cell disease subjects:
To confirm any diagnosis, a sample of blood was examined under a microscope to check for large number of sickle cells, patient's history and blood cell counts i.e. RBC, WBC, and HCT, MCH, MCV, MCHC, had also been carried out. But in the present study, we have concentrated on Haemoglobin - (Hb), WBC, RBC, PCV and MCV count.

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 4 ml blood had been poured in EDTA anticoagulated vacutainer. The Sample was centrifuged at 3000 rpm for 10 minutes; serum was separated and immediately stored in deep freezer at – 20^{o}C until further analysis.

Methodology
Hematological values were measured on fully Automatic blood cell counter hematology analyzer (Coulter LH 780), and HmX Hematology Analyzer with Autoloader. The following parameters were subjected into analysis: hemoglobin concentration (HGB), erythrocyte parameters - red blood cell count (RBC), hematocrit (HCT) or packed cell volume (PCV), MCH, MCHC. Information related to the size, shape, and relative maturity of blood cells; leukocyte parameters and platelet parameters were collected.

In this study, we have concentrated on the variables like, Haemoglobin - (Hb), WBC, RBC, PCV and MCV count. All the haematological values were compared to the reference values. The ET-1 was determined by using enzyme-immunoassay (ELISA kit) method, specifically designed for direct measurement of plasma ET-1. The sensitivity of the assay is 0.5pg/ml, and the cross-sensitivity of the antibodies used is reported to be < 1% with big ET-1.

Statistical Analysis
Statistical analysis (calculations) of the data will be performed by using window based latest version of SPSS software, and the $p < 0.05$ will be considered statistically significant.

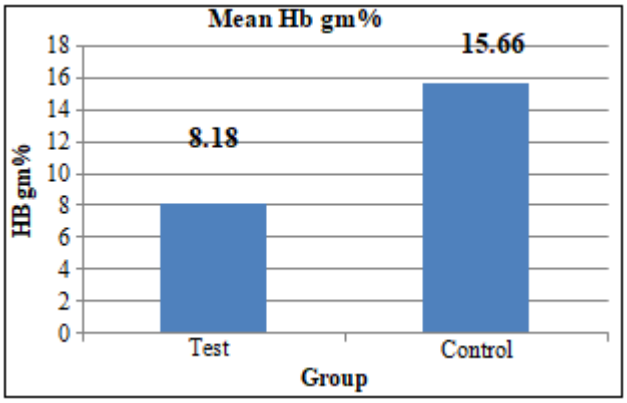
3. Results

In this case controlled study, we enrolled 111 test patients and 111 are the healthy controls, here we found that, the variations in the variables in the SCD patients as compared to the controls. The variables Hb in the SCD patients is very

much (highly) significantly decreased as compared to the controls (8.18 ±1.58: Control 15.66 ± 0.85) and P < 0.0000 showing highly significant, showed in Table no-1 and Graph -1.

Table 1: Hemoglobin in Sickle cell disease

Group	Mean	SD	t-statistics	P-value	Significance
Test	8.18	1.58	43.80	0.0000	Highly significance
Control	15.66	0.85			



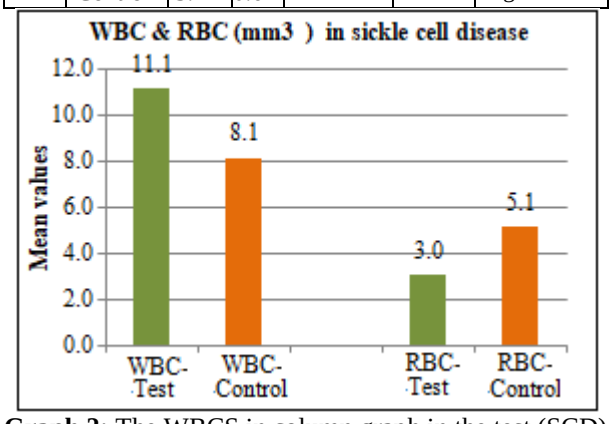
Graph 1: The column graph in the test (SCD) group was found declined (8.18gm %) as compared to healthy controls (15.66gm %).

Complete Blood Count

The complete blood count is determined; besides all blood components we have considered here only WBC, RBC, PCV and MCV. In the prescribed (Table no-2 and Graph -2) study, the WBCs in the tests groups were found to be elevated (11.13 ± 4.85; 8.14 ± 1.68) very significantly high as compared to the controls, and the calculated P value is P < 0.0000 showing very highly significant. But, the RBCs are declined significantly low in the test (SCD) group as compared to its control healthy group, (3.05 ± 0.77; 5.12 ± 0.61), the calculated P value is also P < 0.0000 showing very highly significant.

Table 2: WBC and RBC count in Sickle cell disease

	Group	Mean	SD	t-statistics	p-value	Significance
WBC	Test	11.13	4.85	6.15	0.0000	Highly significant
	Control	8.14	1.68			
RBC	Test	3.05	0.77	22.21	0.0000	Highly significant
	Control	5.12	0.61			

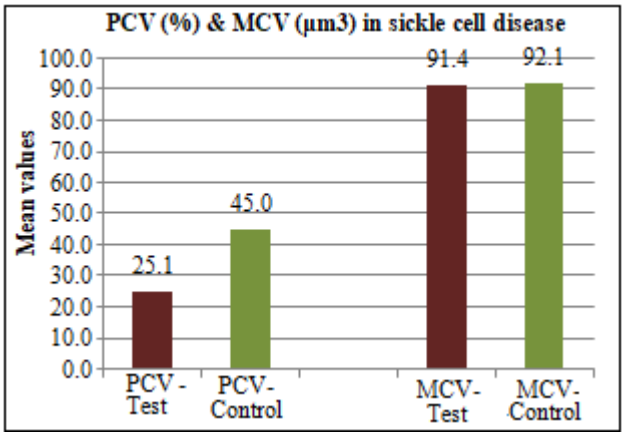


Graph 2: The WBCS in column graph in the test (SCD) group was found to be elevated (11.1mm^3) as compared to healthy controls (8.1). But the RBCs are declined significantly (3.0 mm^3) in the test (SCD) group as compared to its control healthy group (5.1).

Table 3: Packed cell volume and Mean cell volume in Sickle cell disease:

Variable	Group	Mean	SD	t-statistics	p-value	Significance
PCV	Test	25.14	5.52	31.72	0.0000	Highly significant
	Control	45.04	3.62			
MCV	Test	91.41	9.79	0.65	0.5188	Not significant
	Control	92.10	5.53			

In the above table-3, in the tests group; the packed cell volume levels were significantly very low i.e. 25.14 ± 5.52 as compared to the controls 45.04 ± 3.62; so the lower PCV level was found to be highly significant, and the Mean cell volume were slightly decreases but is was reported not significant in the test (91.41± 9.79; 92.10 ± 5.53) as compared to the controls.

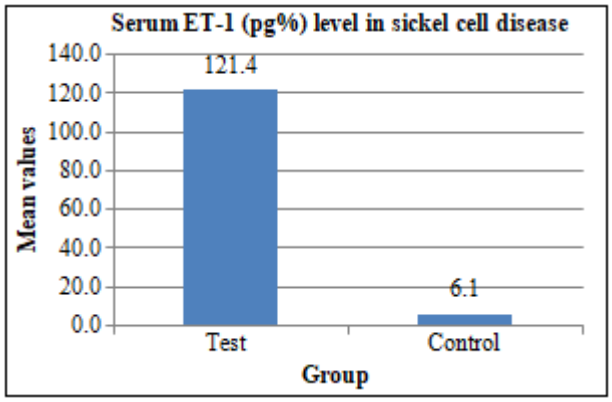


Graph 3: The packed cell volume levels were significantly declined in the test (25.1) as compared to the inclined controls (45.04). But the mean cell volume graph is not showing the measure difference in the test (91.41) and controls (92.10).

Table 4: Serum Endotheline -1 in Sickle cell disease:

Group	Mean	SD	t-statistics	p-value	Significance
Test	121.44	11.28	48.57	0.0000	Highly significant
Control	22.32	6.11			

In the above prescribed table no-4 the serum ET-1 levels were found to be increased in the test group (121.44 ± 11.28) as compared to the controls (22.32 ± 6.11); and the statistically calculated- P< 0.0000 which was showing highly significant.



Graph 4: In the graph, the test group was found to be elevated (121.4pg %) as compared to the control (22.32 pg %)

4. Discussion

In the present study, we highlighted on the blood hemoglobin level, CBC components like WBC, RBC, PCV, MCV and Serum Endotheline -1 in Sickle cell disease. Here the hemoglobin levels were found to be decreased in the sickle cell disease as compared to that of the controls, along with this, the packed cell volume and red cell indices, were also decreased in the SCD patients. These variables were also decreases in homozygous sickle cell disease and also decrease in males than the females in both cases than the controls in sickle cell disease, but the white blood cell count was found to be increased significantly high in cases as compared to the controls in sickle cell disease. [11-16]

Decreased level of hemoglobin and RBCs were found due to the sickling (polymerization) and unsickling causes the red blood cell membrane of RBCs in blood cells. [12]

The serum endothelin-1 is the another variable in sickle cell disease, which is significantly very high in the cases as compared to the controls. [17-22]

5. Conclusion

In this study, we reached to conclude that, the variables like blood hemoglobin, RBC, WBC, PCV and Sr. ET-1 were showing extremely significant difference between mean of cases and healthy controls. So our hypothesis gets strengthened and strongly reached to conclusive position that the serum ET-1, which could be used as a reliable biomarker for the SCD.

6. Conflict of interest

None declared.

7. Funding Sources

Nil

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References

[1] Vikas K. Gupta, Nilima Singh, S.W. Masram, S. K. B. Patil; Status of some Haematological and Antioxidant Parameters in SCA patients of Chhattisgarh Region, Journal of Medical Science and Clinical Research, 7, July 2015. Vol. 03.
[2] Brianna Lutz, B.S., Steffen E. Meiler, Yuan-Xiang Tao et. al, Transl Perioper Pain Med. 2015 July 26; 2(2): 8–17.
[3] 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, 2004.
[4] Kanter J, Kruse-Jarres R. Management of sickle cell disease from childhood through adulthood. Blood Reviews. 2013; 27:279–87.
[5] Steinberg, M.H., 2009. Genetic etiologies for phenotypic diversity in sickle cell anemia. Scientific World Journal (Special Issue: Hemoglobinopathies), 46-47.
[6] M. Nouraie, J.S. Lee, Y. Zang, T. Kanias, et, al. The relationship between the severity of hemolysis, clinical manifestations and of death in 415 patiens with sickle cell anemia in the US and Europe, Haematologica (2012).
[7] Prasad VS, Palaniswamy C, Frishman WH. Endothelin as a Clinical Target in the Treatment of Systemic Hypertension. Cardiology in Review. 2009:17.
[8] Ans J, Boyd R, Wang Y, Endothelin-1 expression in vascular adventitial fibroblasts. American Journal of Physiology - Heart and Circulatory Physiology. 2006; (290):700-708.
[9] Earley SN. Hypoxia-induced pulmonary endothelin-1 expression is unaltered by nitric oxide. Journal of Applied Physiology. 2002; 92:1152–8.
[10] Balasa VV, Kalinyak KA, Bean JA, Stroop D, Gruppo RA. Hyperhomocysteinemia is associated with low plasma pyridoxine levels in children with sickle cell disease. J Pediatr Hematol Oncol, 2002; 24:374–379.
[11] Akinsegun Akinbami, Adedoyin Dosunmu, Adewumi Adediran, Olajumoke Oshinaike, Phillip Adebola, Olanrewaju Arogundade; "Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria" BMC Research Notes; 2012, 5:396
[12] Omoti CE: Haematological values in sickle cell anaemia in steady state and during vaso-occlusive crises in Benin city, Nigeria. Ann Afr Med 2005, 4(2):62–67.
[13] Kaul DK, Fabry M, Windisch P, Baez S, Nagel R: Erythrocytes in sickle cell anaemia are heterogenous in their rheological and haemodynamic characteristics. J Clin Invest 1983, 72:22–31.
[14] Kraff-Jacobs B, Williams J, Soldin SJ: Plasma erythropoietin reference ranges in children. The journal of Paediatrics 1995, 126(4):601–603.
[15] Akinbami. "Haematological values in homozygous sickle cell disease in steady state and haemoglobin phenotypes AA controls in Lagos, Nigeria." Ann Afr Med., 2012; 1: 396.
[16] Lei H, Karniadakis GE. "Quantifying the rheological and hemodynamic characteristics of sickle cell anemia." Biophys J 2012; 102: 185-94.
[17] Rybicki AC., Benjamin L j., increased level of endothelin-1in plasma of sickle cell anemia patients. Blood. 1998; 92(7): 2594 – 2596.
[18] Sitki Ergul, Chris Y. Brunson, Jim Hutchinson, Amany Tawfik, Abdullah Kutlar, R. Clinton Webb, et al. " Vasoactive factors in sickle cell disease: In vitro evidence for endothelin-1 mediated vasoconstriction" American Journal of Hematology ; 2004, 76: 245- 251.
[19] Klug P., Kaye N., Jensen W. "Endothelial cell and vascular damage in sickle cell disorders" Blood cells; 1982, 8: 175- 184.

[20] Kasschau MR. Barbino GA, Bridges KR, Golan DE " Adheshion state of neutrophils and erythrocytes to fibronectine." Blood; 1996; (87); 771.
[21] Tharaux PL., Hagege I., Placier S., Vayssairat M., Kanfer A., Girot R., et al. "Urinary endothelin-1 as a Marker of renal damage in sickle cell disease." Nephrol Dial Transplant 2005; 20:2408-2413 PIMD: 16144850.
[22] Werdehoff SG, Moore RB, Hoff CJ, Fillingim E, Hackman AM. "Elevated plasma endothelin-1 levels in sickle cell anemia: relationships to oxygen saturation and left ventricular hypertrophy."Am J. Hemaol. Jul; 1998, 58(3):195-9.