PROGNOSTIC BIOMARKERS IN THE MANAGEMENT OF SICKLE CELL DISORDER PATIENTS OF ODISHA.

Somali Das[1*], Bisnu Prasad Dash[2]

[1*]Doctoral Research scholar in F.M University, Balasore, Odisha Email:-Somali536@gmail.com
[2]Retd.Prof in F.M. University

***Corresponding Author: -** Somali Das
Doctoral Research scholar in F.M University, Balasore, Odisha Email:-Somali536@gmail.com

Abstract
The predictive and diagnostic information about the patient's health condition is provided by routinely monitoring the haematological and biochemical parameters. The current study focused at how hematobiochemical parameters affected the pathophysiology of sickle cell disease (SCD) patients. Finally, the hematobiochemical profiles of 167 SCD (HbSS) patients are investigated. SCD patients had reduced haemoglobin levels. They have elevated retics, HbF, and LDH. Billirubin, Hb, and LDH were found to be indicators of disease severity in this research. As a result, bilirubin, haemoglobin, and LDH can be used as biomarkers in sickle cell disease patients. Early diagnosis of LDH and monitering of these factors can result in improved patient outcomes in sickle cell disease patients.

Keywords - Hemoglobin, Bilirubin, Sickle cell, anemia, mutation, sickling

Introduction

Haemoglobin (Hb) is essential for transporting oxygen in the blood. Sickle Cell Disease (SCD), also known as Sickle Cell Anaemia (SCA), is a genetic haemoglobin condition caused by a gene mutation. The amino acid at position 6 of a normal beta globin chain is translocated inside the 11th chromosome of the q arm of the -chain of beta globin gene (adenine to thymine (GAG to GTG)).As a result, the haemoglobin tetramer's properties alter, resulting in polymerization and vaso-occlusive crisis. In sickle cell patients, this vaso occlusive crisis triggers panic pain crises. This pain episode lasts two to three days. That's why; regular monitoring of the hemato-biochemical parameters gives prognostic and diagnostic information regarding the health status of sickle cell disorder patients. Here, the haematological parameters are Haemoglobin (Hb), Haemoglobin Factor (HbF) and Retics. The biochemical parameters are Serum Glutamic Oxaloacetic Transaminase (SGOT), Serum Glutamic Pyruvic Transaminase (SGPT), Lactate Dehydrogenase (LDH), Bilirubin Total (BIT), and Bilirubin Direct (BID).

The monitoring of the hemato-biochemical parameters helps to predict the severity of the disease that helps to manage them easily. The anaemia levels are determined by the concentration of Hb in blood. The anaemia level predicts the severity of the SCD. Lower level of Hb indicates severe anaemia while moderate and a higher value indicates moderate and milder anaemia. Retics are the immature RBCs. The retics count correlates with future sickle cell disease severity. It acts as an useful marker to estimate the degree of erythropoiesis and the health of bone marrow in response to anaemia. HbF is the main oxygen career in foetus. But HbF inhibits Sickle Haemoglobin (HbS) polymerization that plays a vital role in vaso-occlusive crisis. SGOT and SGPT are transaminases. These are liver enzymes. Due to vaso- occlusion in liver, these enzymes were significantly increased in sickle patients. Severity of the disease can be managed the early prediction of the parameters.LDH is one of the enzymes of the glycolytic pathway that catalyses the conversion of pyruvate to lactate with concurrent conversion of NADH to NAD^+. It is a ubiquitous enzyme found in all tissues.LDH is usually elevated in sickle patients due to hyperhemolysis. LDH has been used as a marker of haemolysis. Billirubin is the metabolic by product of Hb. Bilirubin levels are high due to hyperhemolysis.Since, blood biochemistry has a critical role for early diagnosis of pathophysiology in the human body. Regular monitoring of the haematological and biochemical parameters, gives prognostic and diagnostic information regarding the health status of the patients in order to correlate the severity of the disease, organ damage and better crisis management.

The objective of the present study is to determine the prognostic biomarker for sickle cell disease patients found in all throughout Odisha. Sickle cell disease is widespread in central India and in the western parts of Odisha. Indian SCA patients have reportedly higher HbF concentration with higher retics and high LDH. Many researchers had worked on Odisha sickle patients and have drawn inferences like Low Hb , High HbF ,High Retics , High SGPT and High SGOT, High LDH , High Billirubin. Above findings are true but in order to manage them properly, we need to know the early detection of the organ health. This can only be known through some parameters, that acts as future indicators of any health issues of sickle patients. Since,the pathophysiology of the sickle patients is very complicated. Patients differ from individual to individual.

Prevalence of Sickle gene in Odisha

Dunlop and Mazumder found sickle haemoglobin in the Oriya people in 1952. Batabyal and Wilson (1953) proposed cases of sickle cell disease in Assam, each born to Orissa immigrant parents. Vella and Hart also cited a case from Malaya with Orissa ancestors in 1959. Das et al. (1967) and Roy & Roy Chaudhuri (1967) reported the existence of this gene in a few Koraput district tribes. This gene was found in abundance in Agharias of Orissa, as reported by Nanda et al. (1965), Samal et al. (1978), and Samal and Naik (1979) (1983). Praharaj et al. noted the prevalence of sickle cell beta-thalassaemia. The HbS gene is widely distributed (Kar et al. 1987).

Methodology

167 sickle blood samples were collected through interaction with the patients and after getting the approval of the ethical committee. Blood samples were collected from different district hospitals. Samples were collected from both male and female subjects for study. This study was carried out from 2017 to 2020. Screening was done for all the patients and they were detected as sickle cell homozygous. Out of 167 patients, 103 were male and 64 Female patients. 95 Adults (60 male & 35 Female) and 72 pediatrics (43 male & 29 Female) patients. Patients were grouped as 01-10 YRS (61 patients) 11 - 20 YRS (58 patients) 21 - 30 YRS (32 patients) 31 - 40 YRS (7 patients) & 40 yrs (9 patients). All the patients were suffering from common symptoms like anemia, joint pains, fever, swelling of hand and pain, delayed growth, delayed puberty, Frequent infections, pain crisis, liver enlargement and splenomegaly. These patients come to the hospitals for regular transfusions. Further research work was carried out at P. G. Dept of Biosciences and Biotechnology, Fakir Mohan University, Balasore.

Confirmative sickling tests were done by following the method of Daland and castle (1948). One drop of whole blood was mixed with one drop of freshly prepared 2 % metabisulphite solution on a microslide. After mixing, a cover slip was placed properly and then sealed with molten wax to make it air tight. The slides were observed under the microscope using high power lens after one hr and after 24 hrs of sealing. Hematological parameters were calculated by using Automated Hematology Analyzer by Sysmex.

The Automated Hematology Analyzer from Sysmex was used to calculate the haematology parameters.

Hematology analyzers are frequently used to count blood cells for disease detection and monitoring in patient and research settings. Basic analyzers provide a three-part differential white blood cell (WBC) count along with a complete blood count (CBC). Hematology analyzers primarily employ three physical technologies: electrical impedance, flow cytometry, and fluorescent flow cytometry.

To increase the measurable parameters, these are combined with chemical reagents that lyse or modify blood cells. For instance, electrical impedance can separate red blood cells (RBCs), white blood cells (WBCs), and platelets according to volume. It is feasible to distinguish lymphocytes by nucleating an agent that shrinks lymphocytes more than other WBCs.

The traditional method of counting cells is electrical impedance, also known as Coulter's principle. Used in almost all hematology analyzers. Whole blood passes between the two electrodes through an opening so n arrow that he can only pass one cell at a time. Impedance changes as cells pass through. The change in im pedance is proportional to cell volume, resulting in cell number and volume measurements. Impedance an alysis returns CBC and WBC tripartite differences (granulocytes, lymphocytes, and monocytes), but disting uishes similarly size granular leukocytes (eosinophils, basophils, and neutrophils). A count rate of up to 10, 000 cells per second can be achieved and a typical impedance analysis can be performed in less than 1 mi nute.

Spectrophotometric analysis was done on the biochemical samples.

In contrast to a reference or blank sample, the amount of discrete wavelengths of UV or visible light that are absorbed by or transmitted through a sample can be determined using the analytical method of UV-Vis spectroscopy. The sample composition has an impact on this feature, potentially revealing what is in the sample and at what concentration. The energy of light has a fixed value that is inversely proportional to its wavelength. As a result, shorter light wavelengths carry more energy while longer ones carry less. Absorption is a physical phenomenon that occurs when a substance's electrons are promoted to a state with a higher energy level. In a substance, electrons in various bonding conditions require different amounts of energy to move them to higher energy states. This explains why different substances absorb light at different wavelengths. The light then travels through a sample in the spectrophotometer, using whichever wavelength selector is employed. It is crucial to measure a reference sample, sometimes known as the "blank sample," for all analyses. This reference sample might be a cuvette filled with the same solvent that was used to create the sample. Due to quartz's transparency to the majority of UV rays, quartz sample holders are necessary. Because molecular oxygen in the air absorbs light with wavelengths less than 200 nm, one may also consider air to be a filter. For observations with wavelengths less than 200 nm, a unique and more expensive setup is needed, typically including an optical system filled with pure argon gas. Humans can see a range of visible light, from around 380 nanometers, which we perceive as violet, to 780 nanometers, which we perceive as red.

The wavelengths of UV light are about 100 nm shorter than those of visible light. Since light can be defined by its wavelength, UV-Vis spectroscopy, which seeks out the precise wavelengths that correlate to maximal absorbance, can be used to evaluate or identify various substances.
A detector is then utilised to transform the light into a detectable electronic signal after it has passed thr ough the sample. Detectors are often built using semiconductors or photoelectric coatings.

Value of LDH activity has been calculated by using LDH Kit method established by Weishaar H. D. et. al. (1975).LDH catalyses the reduction of pyruvate with NADH to form NAD. The rate of oxidation of NADH to NAD is measured as a decrease in absorbance, which is propotional to the LDH activity in the sample. Absorbance was taken by using UV spectrophotometer at 340 nm. (Weishaar H. D. et. al. (1975) Med. Welt 26:387)
SGPT - ALT catalyzed the conversion of alpha ketoglutarate to pyruvate and L - glutamate. LDH catalyzed the oxidation of reduced cofactor. The rate of Decrease in absorbance of the reaction mixture at 340 NM, due to the oxidation of the reduced cofactor, is directly propotional to the ALT activity. (J. Clin. Chem. Clin. Biochem., 1986, Vol 24:481)

SGOT - The aminotransferases are widely distributed in animal tissues. Both AST and ALT are normally distributed in human plasma.
AST help in the conversion of L- Aspartate and 2 - oxaloglutarate to form Oxaloacetate and L - glutamate.The rate of Dec in absorbance of the reaction mixture at 340 NM, due to the oxidation of the reduced cofactor, is directly propotional to the AST activity. (Burtis, C.A.,Ashwood,E.R., editors. Tietz textbooks of clinical chemistry. 2nd Edition Philadelphia, W.B. Saunders company, 1994,p. 790-795).

Bilirubin-Bilirubin are broken down in the reticuloendothelial system through apoptosis. This gives Bilirubin as their metabolic by product upon removal of Iron.This billirubin is transferred to the liver and is known as indirect bilirubin.
In the liver, bilirubin is conjugated to the glucuronic acid by the enzyme Uridyl diphosphate glucuronyl transferase to form direct bilirubin or conjugated bilirubin.

Total Bil is the sum total of direct and Indirect bilirubin.
The concentration of the bilirubin is measured, based on the reaction of bilirubin with diazo reagent to form a colored compound called azobilirubin.
The diazo reaction is accelerated by the addition of surfactant as a solubilizing agent. The increase in absorbance at 546 nm due to azobilirubin is directly propotional to the total bilirubin concentration.
The intensity of the color formed is directly propotional to the amount of bilirubin present in the sample. (Jendrassik L. & Grof P. Biochem 2.297, 81 (1938))

Results

The sample of 167 patients is statistically analysed in table-1. The mean Hb is low in total sickle patients. The mean Hb for male is significantly more than females. The mean HbF is high in total cases and the mean HbF of females is higher than males. The mean retics for total cases is high and the mean retics for male is higher than female patients. The mean SGOT is high in total sickle cases. Males show higher SGOT levels while females show normal SGOT levels. The mean SGPT level is lower in total sickle cases and males have higher SGPT levels than females. The LDH is higher in sickle cases but males have higher LDH than females. The total Bilirubin is higher in sickle cell cases while males have higher bilirubin levels than females. The Direct bilirubin is high in total sickle cases while males show higher values than females.

Table 1 Statistical analysis of sample

	parameters	TOTAL CASES	TOTAL CASES	
			MALE	FEMALE
sample size		167	103	64
MEAN (SD)	Hb	9.12 (1.93)	9.18 (1.93)	9.02 (1.94)
MEAN (SD)	Hb F	16.42 (7.59)	16.21 (5.64)	16.76 (6.06)
MEAN (SD)	RETICS	9.14 (5.6)	9.73 (5.58)	8.2 (5.09)
MEAN (SD)	SGOT	43.22 (23.4)	45.58 (25.31)	39.29 (19.78)
MEAN (SD)	SGPT	27.88 (28.38)	29.36 (33.07)	24.62 (17.77)
MEAN (SD)	LDH	919.44 (541.15)	988.07 (609.77)	808.98 (386.68)
MEAN (SD)	BIT	2.55 (1.88)	2.84 (2.02)	2.03 (1.47)
MEAN (SD)	BID	0.56 (0.66)	0.65 (0.78)	0.45 (0.38)

Table 2 portraits the statistical Analysis of data for adults and paediatrics. This classification gives a vivid insight into the spread of samples. The mean Hb for Adults group is higher than paediatrics sickle patients. Adults male show high Hb counts than adults female. Paediatrics female shows high Hb value than paediatrics male group. Paediatrics group show high HbF value than adults group. Adults male shows high HbF than adults female. Paediatrics female shows high HbF than paediatrics male. Paediatrics group shows high retics count than adults group. Adults male and paediatrics male shows high retics than adults female and paediatrics female respectively. Adults group shows higher SGOT than paediatrics group. Adults male and paediatrics male shows higher SGOT than adults female and paediatrics female respectively. SGPT of paediatrics group is higher than adults group. Adults male shows higher SGPT than adults female. Paediatrics male shows higher SGPT value than paediatrics female. Adults group shows higher LDH than paediatrics group. Adults male and paediatrics male shows high LDH than adults female and paediatrics female respectively. Total billirubin is higher in adults group than paediatrics group. Adults male and paediatrics male shows higher total billirubin than adults female and paediatrics female respectively. Direct billirubin is high in adults group than paediatrics group. Adults male and paediatrics male shows higher direct billirubin than adults female and paediatrics female respectively.

Table 2 Statistical Analysis of data for adults and paediatrics

	parameters	TOTAL ADULTS	`TOTAL ADULTS		TOTAL PEDIATRICS	TOTAL PAEDIATRICS	
			MALE	FEMALE		MALE	FEMALE
sample size		95	60	35	72	43	29
MEAN (SD)	Hb	9.18(2.23)	9.5 (2.3)	8.62 (2.01)	9.05 (1.46)	8.74 (1.13)	9.51(1.77)
MEAN (SD)	Hb F	16.38(5.88)	17.27(5.72)	14.87(5.92)	16.47(5.72)	14.73(5.23)	19.05(5.5)
MEAN (SD)	RETICS	8.99 (5.42)	9.32 (5.68)	8.43 (4.97)	9.35 (5.86)	10.31(6.9)	7.92(5.3)
MEAN (SD)	SGOT	43.64 (24.24)	46.24(27.31)	39.17(17.23)	42.67(22.41)	44.86(22.14)	39.43(22.8)
MEAN (SD)	SGPT	26.81 (22.87)	29.04 (26.71)	22.98(13.59)	28.52 (34.2)	29.81(40.66)	26.6 (21.89)
MEAN (SD)	LDH	959.26 (547.23)	1014.68(609.44)	864.26(410.72)	866.896(532.24)	950.95(615.47)	742.25 (350.94)
MEAN (SD)	BIT	3.05 (2.02)	3.36 (2.1)	2.47(1.77)	1.88(1.42)	2.1(1.68)	1.52(0.75)
MEAN (SD)	BID	0.69 (0.82)	0.77(0.98)	0.54(0.43)	0.4 (0.29)	0.44(0.32)	0.34(0.25)

The sample of 167 patients is plotted with respect to age..Figure 1(a) plots the graph between Haemoglobin (Hb) and age. It clearly shows that SCD is lethal with lower age. The Hb is high in >40 age group followed by 11- 20 age group, 21-30 age group, 1 -10 year age group and 31 – 40 age group. In Hb 13.17% of sickle cases found severely anaemic, 37.12 % of cases found mild anaemic, 34.73% of sickle cases found within the normal range and 14.97 % of sickle cases found above normal range. This is predicted with very less number of cases in age above 40 years. HbF or Fetal Haemoglobin versus age is plotted in Figure 1(b). It indicates that abnormally higher values of HbF are seen in sickle cell patients. The HbF level is high in >40 age group followed by 31 -40 age group , 1 – 10 age group, 21-30 group and 11-20 age group. Retics (%) versus age plot is plotted in Figure 1(c). It indicates that the abnormally higher retics (%) is seen amongst sickle cell patients. In Retics count, no sickle cases come below the normal case, 4.79% of cases come within the normal case and 95.2 % of cases found above the normal range.Retics (%) is seen not to change with age. Retics is high in 21-30 year age group followed by >40 year group, then, 31- 40 group, 1-10 year age group and 11-20 year age group. Figure 1(d) indicates the relationship between Serum Glutamic-Oxaloacetic Transaminase (SGOT) and age. SGOT is high in 31-40 age group followed by 11-20 year group, 1-10 year group, 21-30 group and lastly >40 group.In SGOT, 0.59% of sickle cases found below normal range, 52.69% of cases found within the normal range and 46.7 % of cases found above normal range. Sickle cell patients shows inverse relationship with age, while Serum Glutamic Pyruvic Transaminase (SGPT) is seen not vary or deviate much from normal. SGPT was seen to be abnormally lower. SGPT versus age is plotted in Figure 1(e). In SGPT, no sickle cases found below the normal range, 80.83% of cases found within the normal range and 19.16 % of cases found above normal range.SGPT is high in 11-20 year age group, followed by 1-10 year age group, 31-40 group, 21-30 age group, and lastly >40 age group. Figure 1(f) represents the plot between lactate dehydrogenase (LDH) and age. LDH is high in >40 age group, followed by 11-20 year group, 31-40 year group, 1-10 year group and lastly 21-30 age group.In LDH, no sickle cases found below the normal range, 12.57% of cases found within the normal range and 87.42% of sickle cases found above the normal range. Figure 1(g) represents the plot between Bilirubin Total (BIT) and age. In BIT, no sickle cases found in less than normal range, 16.56% found in between the normal range and 83.43 % of cases found above normal range.BIT is high in 11-20 year group followed by 21-30 year group, 31-40 group, >40 group then lastly 1-10 group. Figure 1(h) represents the plot between Bilirubin Direct (BID) and age. BID is high in 11-20 year followed by 21-30 year group, >40 year group, 31-40 year group and lastly 1-10 year group.In BID, 1.81% of cases found below normal range, 18.18% of cases found within the normal range and 80% of cases found above normal range.

	parameters	01-10 YRS	11 - 20 YRS	21 - 30 YRS	31 - 40 YRS	>40 yrs

sample size		61	58	32	7	9
MEAN (SD)	Hb	8.96 (1.37)	9.29 (2.1)	9.15 (2.32)	8.67 (2.58)	9.37 (2.26)
MEAN (SD)	Hb F	16.54 (5.94)	15.72 (5.87)	16.35 (5.1)	17.31 (6.3)	19.68 (6.24)
MEAN (SD)	RETICS	8.91 (5.63)	8.88 (6.13)	10.12(5.01)	8.95 (6.75)	9.11 (3.06)
MEAN (SD)	SGOT	43.11 (20.53)	46.59 (26.46)	40.07 (25.91)	46.67 (12.54)	30.76 (13.67)
MEAN (SD)	SGPT	28.39 (35.19)	28.78 (25.83)	25.14 (24.05)	26.14 (6.26)	23.49 (12.92)
MEAN (SD)	LDH	895.28 (564.48)	928.15 (412.87)	880.89 (590.7)	918.51 (438.01)	1164.81 (947.33)
MEAN (SD)	BIT	1.73 (1.11)	3.26 (2.23)	2.97 (1.93)	2.43 (2.04)	2.18 (1.4)
MEAN (SD)	BID	0.4 (0.29)	0.74 (0.96)	0.59 (0.55)	0.54 (0.62)	0.51 (0.32)
		01-10 YRS	11 - 20 YRS	21 - 30 YRS	31 - 40 YRS	>40 yrs

The Hb is high in >40 age group followed by 11- 20 age group, 21-30 age group, 1 -10 yrs age group and 31 – 40 age group, The HbF level is high in >40 age group followed by 31 -40 age group , 1 – 10 age group, 21-30 group and 11-20 age group.Retics is high in 21-30 yrs age group followed by >40 yrs group, then, 31- 40 group, 1-10 yrs age group and 11-20 yrs age group.SGOT is high in 31-40 age group followed by 11-20 yrs group, 1-10 yrs group, 21-30 group and lastly >40 group. SGPT is high in 11-20 yrs age group, followed by 1-10 yrs age group, 31-40 group, 21-30 age group, and lastly >40 age group.LDH is high in >40 age group, followed by 11-20 yrs group, 31-40 yrs group, 1-10 yrs group and lastly 21-30 age group. Total billirubin is high in 11-20 yrs group followed by 21-30 yrs group, 31-40 group, >40 group then lastly 1-10 group. Direct billirubin is high in 11-20 yrs followed by 21-30 yrs group, >40 yrs group, 31-40 yrs group and lastly 1-10 yrs group.

Independent-Samples Mann-Whitney U Test Summary

Total N	167
Mann-Whitney U	3354.000
Wilcoxon W	8710.000
Test Statistic	3354.000
Standard Error	303.702
Standardized Test Statistic	.191
Asymptotic Sig.(2-sided test)	.849

Table 6

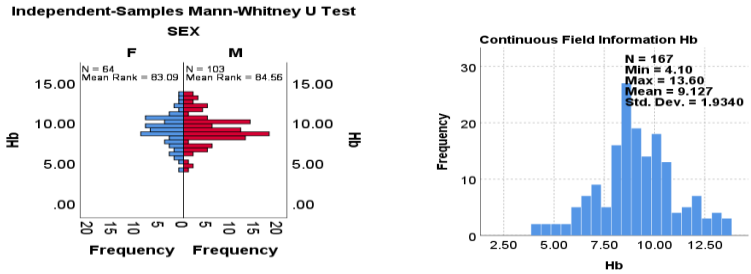


Fig- 3(a) & 3 (b) The plot between frequency and Hb for the MWUT is placed in Figure 3 (a). Figure 3 (a) indicates that the mean rank for males is more than females. Figure 3 (b) indicates the continuous field information for Hb.

Independent-Samples Mann-Whitney U Test Summary

Total N	167
Mann-Whitney U	3104.000
Wilcoxon W	8460.000
Test Statistic	3104.000
Standard Error	303.739
Standardized Test Statistic	-.632
Asymptotic Sig.(2-sided test)	.527

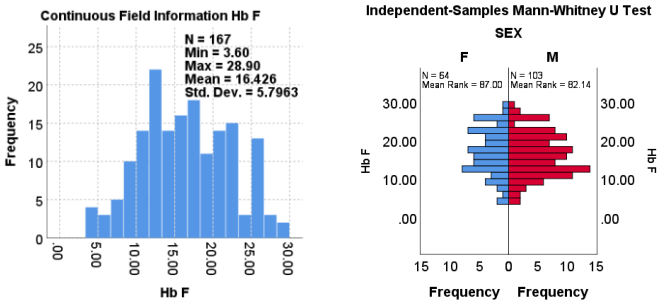
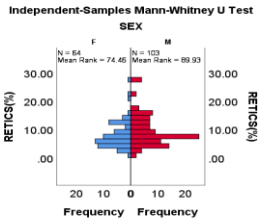


Table- 7

Fig- 4 (a) & 4 (b). The plot between frequency and Hb F for the MWUT is placed in Figure 4 (a). Figure 4 (a) indicates that the mean rank for males is less than females. Figure 4 (b) indicates the continuous field information for Hb F.

Independent-Samples Mann-Whitney U Test Summary

Total N	167
Mann-Whitney U	3906.500
Wilcoxon W	9262.500
Test Statistic	3906.500
Standard Error	303.684
Standardized Test Statistic	2.010
Asymptotic Sig.(2-sided test)	.044



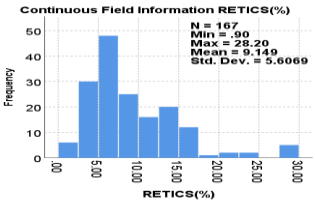
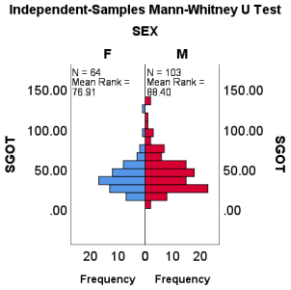


Table -8

The plot between frequency and retics (%) for the MWUT is placed in Figure 5 (a). Figure 5 (a) indicates that the mean rank for males is more than females.Figure 5 (b) indicates the continuous field information for retics (%).

Independent-Samples Mann-Whitney U Test Summary

Total N	167
Mann-Whitney U	3749.500
Wilcoxon W	9105.500
Test Statistic	3749.500
Standard Error	303.767
Standardized Test Statistic	1.493
Asymptotic Sig.(2-sided test)	.135



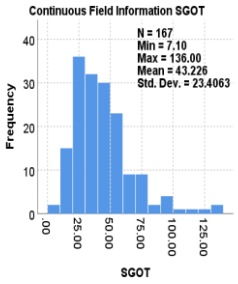
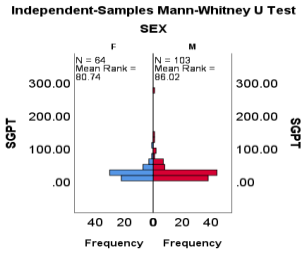


Table -9

The plot between frequency and SGOT for the MWUT is placed in Figure 6 (a). Figure 6 (a) indicates that the mean rank for males is more than females. Figure 6 (b) indicates the continuous field information for SGOT.

Independent-Samples Mann-Whitney U Test Summary

Total N	167
Mann-Whitney U	3504.500
Wilcoxon W	8860.500
Test Statistic	3504.500
Standard Error	303.750
Standardized Test Statistic	.686
Asymptotic Sig.(2-sided test)	.492



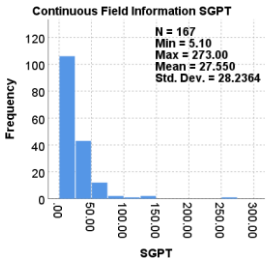
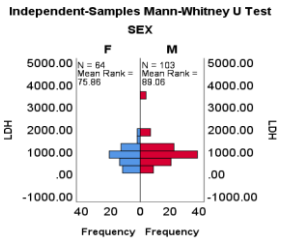


Table- 10

The plot between frequency and SGPT for the MWUT is placed in Figure 7 (a). Figure 7 (a) indicates that the mean rank for males is more than females. Figure 7 (b) indicates the continuous field information for SGPT.

Independent-Samples Mann-Whitney U Test Summary

Total N	167
Mann-Whitney U	3817.000
Wilcoxon W	9173.000
Test Statistic	3817.000
Standard Error	303.631
Standardized Test Statistic	1.716
Asymptotic Sig.(2-sided test)	.086



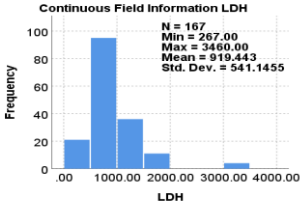
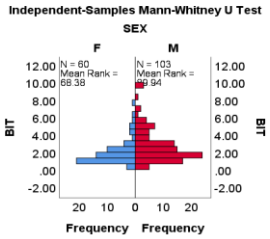


Table- 11

The plot between frequency and LDH for the MWUT is placed in Figure 8 (a). Figure 8 (a) indicates that the mean rank for males is more than females. Figure 8 (b) indicates the continuous field information for LDH

Independent-Samples Mann-Whitney U Test Summary

Total N	163
Mann-Whitney U	3907.500
Wilcoxon W	9263.500
Test Statistic	3907.500
Standard Error	290.599
Standardized Test Statistic	2.813
Asymptotic Sig.(2-sided test)	.005



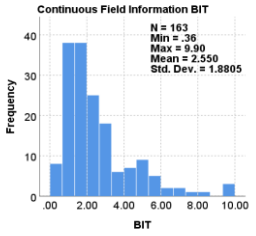
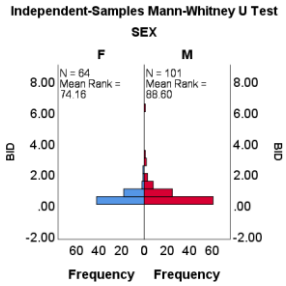


Table -12

The plot between frequency and BIT for the MWUT is placed in Figure 9 (a). Figure 9 (a) indicates that the mean rank for males is more than females.Figure 9 (b) indicates the continuous field information for BIT.

Independent-Samples Mann-Whitney U Test Summary

Total N	165
Mann-Whitney U	3798.000
Wilcoxon W	8949.000
Test Statistic	3798.000
Standard Error	298.861
Standardized Test Statistic	1.894
Asymptotic Sig.(2-sided test)	.058



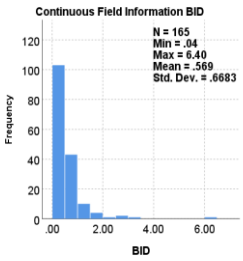


Table – 13

The plot between frequency and BID for the MWUT is placed in Figure 10 (a). Figure 10 (a) indicates that the mean rank for males is more than females. Figure 10 (b) indicates the continuous field information for BID.

The use of Mann-Whitney U Test (MWUT) is to test the weather the variables affect male and female equally or not. The null hypothesis for Hb is the distribution of Hb is the same across categories of sex. The parameters for this hypothesis test are placed in table 6. The asymptotic sig. (2-sided test) gives a value of 0.849. Thus, indicating the null hypothesis is to be accepted.

The use of Mann-Whitney U Test (MWUT) is to test the weather the variables affect male and female equally or not. The null hypothesis for Hb F is the distribution of Hb F is the same across categories of sex. The parameters for this hypothesis test are placed in table 7. The asymptotic sig. (2-sided test) gives a value of 0.527. Thus, indicating the null hypothesis is to be accepted.

The use of Mann-Whitney U Test (MWUT) is to test the weather the variables affect male and female equally or not. The null hypothesis for retics (%) is the distribution of retics(%) is the same across categories of sex. The parameters for this hypothesis test are placed in table 8. The asymptotic sig. (2-sided test) gives a value of 0.044. Thus, indicating the null hypothesis is to be rejected.

The use of Mann-Whitney U Test (MWUT) is to test the weather the variables affect male and female equally or not. The null hypothesis for SGOT is the distribution of SGOT is the same across categories of sex. The parameters for this hypothesis test are placed in table 9. The asymptotic sig. (2-sided test) gives a value of 0.135. Thus, indicating the null hypothesis is to be accepted.

The use of Mann-Whitney U Test (MWUT) is to test the weather the variables affect male and female equally or not. The null hypothesis for SGPT is the distribution of SGPT is the same across categories of sex. The parameters for this hypothesis test are placed in table 10. The asymptotic sig. (2-sided test) gives a value of 0.492. Thus, indicating the null hypothesis is to be accepted.

The use of Mann-Whitney U Test (MWUT) is to test the weather the variables affect male and female equally or not. The null hypothesis for LDH is the distribution of LDH is the same across categories of sex. The parameters for this hypothesis test are placed in table 11. The asymptotic sig. (2-sided test) gives a value of 0.086. Thus, indicating the null hypothesis is to be rejected. .

The use of Mann-Whitney U Test (MWUT) is to test the weather the variables affect male and female equally or not. The null hypothesis for BIT is the distribution of BIT is the same across categories of sex. The parameters for this hypothesis test are placed in table 12. The asymptotic sig. (2-sided test) gives a value of 0.005. Thus, indicating the null hypothesis is to be rejected.

The use of Mann-Whitney U Test (MWUT) is to test the weather the variables affect male and female equally or not. The null hypothesis for BID is the distribution of BID is the same across categories of sex. The parameters for this hypothesis test are placed in table 13. The asymptotic sig. (2-sided test) gives a value of 0.058. Thus, indicating the null hypothesis is to be rejected.

Discussion

Hb

Many researchers had worked on Odisha sickle patients and have drawn inferences like low Hb in all sickle patients. The mean Hb for male is significantly more than females. Adults male show high Hb counts than adults female. Paediatrics female shows high Hb value than paediatrics male group. Low hemoglobin in sickle patients were also studied previously by ,Vidhyanand et al,2017 ,Tripathi et al, 2018,Sandor et al,2017, ,Samal et al,2019,SATYA, 2019, Verma etal.,2020 , Mohanty et al,2020. The mean Hb is low in total sickle patients could be due to increased hemolysis and frequent recurrent infections in the sickle cell disease patients.

HbF

The mean HbF is high in total cases and the mean HbF of females is higher than males. Adults male shows high HbF than adults female. Paediatrics female shows high HbF than paediatrics male. HbF is generally high in sickle patients. All the sickle cases are above the normal range. A higher level of fetal hemoglobin was found in both sexes in present study . Our study is found similar with the data of Satya, 2019 , Mohanty et al,2020 , Verma et al., - 2020. A baby growing in the womb has high levels of HbF. The level of HbF usually drops to tiny amounts about 6 months after birth. Especially, In India, sickle cell disorder patients show High levels of hemoglobin F after birth & amp;amp; its high level is maintained in later age also. Alexandra,2019, described Fetal hemoglobin (HbF) modulates the phenotype of sickle cell anemia by inhibiting deoxy sickle hemoglobin (HbS) polymerization. Combination therapy with hydroxyurea and recombinant erythropoietin — rather than treatment with hydroxyurea alone — has been shown to further elevate hemoglobin F levels and to promote the development of HbF- containing F-cells. Fetal hemoglobin (HbF) is a physiologic protein tetramer that is crucial for a developing fetus to survive in uterus. Maternal hemoglobin has a relatively lower affinity for oxygen, and thus allows for an efficient transfer of oxygen from maternal to fetal blood. In addition to fulfilling a critical physiologic role, HbF is also known to alleviate symptoms of sickle-cell disease (SCD).

Retics

The mean retics for total cases is high and the mean retics for male is higher than female patients. Paediatrics group shows high retics count than adults group. Adults male and paediatrics male shows high retics than adults female and paediatrics female respectively. Hyperhemolytic crisis may cause high production of reticulocytes, furthermore, this type of anemia destroys red blood cells before they would normally die. Our data is similar with the data of Brahme et al.,2016, Nagose et al, 2018. A reticulocyte is a young red blood cell which is manufactured in the bone marrow that stays there, until they develop into red blood cells and enter the blood. For most of the people, the number is very low because most reticulocytes stay in the bone marrow, moreover, they have higher reticulocyte count, because their body has to make more red blood cells due to anemia, since, bone marrow has to work overtime to replace them.

SGOT

The mean SGOT is high in total sickle cases. Males show higher SGOT levels while females shows normal SGOT levels. Adults group shows higher SGOT than paediatrics group. Adults male and paediatrics male shows higher SGOT than adults female and paediatrics female respectively. High SGOT levels are seen may be due to hemolysis. Our study supports the previous studies of Tripathi P et al. 2016, study shows a significant higher values of AST in the SCA patients. Garg D et al. 2018, in 74% subjects was found above normal level and 26% subjects were found within reference range. Possible reason for elevated transaminases (AST) levels may be acute intra hepatic cholestasis or due to massive accumulation of sickle cells in hepatic sinusoids and stasis causing serious damage to hepatocytes and Kupffer cells. Serum enzymes (AST) and significantly increased as compared with control which indicates likelihood of continuous ongoing hemolysis in sickle cell population. Augustina, 2016, showed aspartate amino transferase significantly higher in multi and rarely blood transfused subjects when compared with controls. This mild elevation of the liver function tests might be due to transient red cell aplasia and increased haemolysis which are common in SCA patients. It has been observed that liver enzymes activities are elevated in SCA patient on blood transfusion. Mohanty AP et al, 2020, showed elevated AST levels. Meher et al, 2019., the SCA cases had significantly higher AST, than that of the controls . Liver enzymes of AST, was elevated during severe VOC as compared to mild VOC. This may be due to hyperhemolysis; there is an increase in liver enzyme activity to neutralizing heme toxicity.

SGPT

The mean SGPT level is lower in total sickle cases and males have higher SGPT levels than females. SGPT of paediatrics group is higher than adults group. Adults male shows higher SGPT than adults female. Paediatrics male shows higher SGPT value than paediatrics female. Liver abnormality release alanine aminotransferase (ALT), which makes useful test for detecting liver damage. Hemolysis also raises ALT levels in SCD or might be due to the leakage of these enzymes from the cytoplasm and mitochondria of the liver tissue following hepatic injury which is common in sickle cell anaemia(Nsiah et. al., 2011) (Brahme ,2016) (Tripathi P et al. in 2016). Garg D et al. in 2018, described possible reasons for elevated transaminases (ALT) levels, that may be acute intra hepatic cholestasis or due to massive accumulation of sickle cells in hepatic sinusoids and stasis causing serious damage to hepatocytes and Kupffer cells. Serum enzymes (ALT) were significantly increased as compared with control which indicates likelihood of continuous ongoing hemolysis in order to neutralize heme toxicity in sickle cell population(Asaolu, 2010) (Meher et al, 2019). Augustina in 2016 described the alanine amino were significantly higher in multi and rarely blood transfused subjects when compared with controls. This mild elevation of the liver function tests might be due to transient red cell aplasia and increased haemolysis which are common in SCA patients.

LDH

The LDH is higher in sickle cases but males have higher LDH than females. Adults group shows higher LDH than paediatrics group. Adults male and paediatrics male shows high LDH than adults female and paediatrics female respectively. In LDH, no sickle cases found below the normal range, Alzahri, 2015. They found that LDH is high. Serum LDH is currently being used as a marker for the risk of vaso-occlusive crisis (VOC) and pain crises in sickle cell disease patients. It is known that LDH is a useful biomarker for intravascular hemolysis. Therefore, the gold standard is serum LDH. (Meher et al, 2019) (Ehtesham, 2020). LDH could be a biomarker not only for hemolysis but also for mortality in sickle cell disease. Kato et al,2013, serum LDH and plasma hemoglobin released during hemolysis & studied whether LDH works as a mortality biomarker in sickle cell disease. LDH level could be a useful tool in diagnosing sickle cell painful crises. LDH were statistically higher. Thus, LDH appear to act as good prognostic markers to assess and follow-up in cases of sickle cell crises(Verghese, 2019). The other markers of intra- or extra-vascular hemolysis that are used routinely include reticulocyte count, unconjugated bilirubin concentration, aspartate aminotransferase, serum haptoglobin, and plasma hemoglobin. The physiopathology of vaso-occlusion in SCD includes endothelial activation and secretion of adhesive molecules. It has been shown that increased levels of active von Willebrand factor, a hyperadhesive molecule better known for mediating platelet adhesion, is correlated with higher LDH activity Chen et al.,2011.

Billirubin

The total Billirubin is higher in sickle cell cases while males have higher billirubin levels than females. The Direct billirubin is high in total sickle cases while males shows higher values than females. Adults male and paediatrics male shows higher total billirubin than adults female and paediatrics female respectively. Adults male and paediatrics male shows higher direct billirubin than adults female and paediatrics female respectively. This study shows significant higher values of total bilirubin in the SCA patients might be due to increased breakdown of red blood cells (Tripathi et. al. 2016) (Meher et al, 2019)(Mohanty AP et al, 2020)(Asaolu, 2010) or might be due to transient red cell aplasia and increased haemolysis which are common in SCA patients (Augustina , 2016).

Conclusion

The present study evaluated the effect of hematobiochemical parameters on the pathophysiology of the sickle cell disease (SCD) cases and maximum hospitalizations were seen in winter seasons due to severe painful crisis and acute anemia. Splenomegaly and hepatomegaly were most common symptoms found in Odisha patients. The clinical symptoms of Odisha sickle cell disease patients reveal microcytic anaemia due to iron deficiency and the pathophysiology varies from individual to individual. The changes in LDH and bilirubin go along with the changes in hematological parameters. SCD is characterized by a wide variety of abnormal levels of biomarkers that have been identified and associated with different pathological conditions. Except few biomarkers, none of them provides prognostic information regarding the management of the sickle disease.

In this study, Billirubin, Hb and LDH may be considered as an indicator of the severity of the disease. So, Bilirubin, Hb & LDH can be considered as a biomarker for the sickle cell disorder patients. Early detection of LDH and correction of these variables can ensure better patient outcomes in the health of sickle cell disease patients.

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Conflict of Interest – There is no conflict of Interest.
Ethical Clearance – This study was approved by institutional Ethical Committee.

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