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Hepatitis-B and C in Sickle Cell Hemoglobinopathies of Western Odisha, India

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ABSTRACT: ***Background-*** *Sickle Cell Disease (SCD) is a serious health problem in Western Odisha, India. One of the most presenting symptoms is anemia and blood transfusion (BT) before painful crisis. Infections in Sickle Cell Disease are many and Hepatitis is one of them which again related to transfusion dependent transmission.*

Material & Method- *Sickling reduction test, alkaline agarose gel electrophoresis (pH- 8.6), CBC, biochemical parameters like BIL-T & BIL-D, SGOT, SGPT, ALP, serum creatinine, serum urea were performed Cation exchange high performance liquid chromatography (HPLC) by VARIANT-II was used to detect and quantify various Hb fractions based on individual retention time (RT). Screening test for hepatitis-B and hepatitis-C virus was done by kit of hepacard on sandwich principle of enzyme-linked immunosorbent assay (ELISA).*

Result- *A total 500 sickle cell haemoglobinopathy cases, of which 382 were sickle cell anemia (HbSS) and 118 cases were sickle cell trait (HbAS). Out of 382 cases of HbSS, 11 (2.9%) cases were positive for hepatitis-B and 2(0.5%) cases were found to be positive for hepatitis-C. Out of 118 cases of sickle cell trait, 2 (1.7%) cases were found to be positive for hepatitis-B.*

Conclusion*-Rise in Liver Function Test could be due to Hepatitis B and C infection in the Sickle Cell Diseases and trait cases. Hematological parameter is not influenced by this infection.*

KEYWORDS- *Hepatitis B, Hepatitis C, Sickle Cell Disease, Sickle Cell Trait, Liver Function Test*

I. INTRODUCTION

Hepatitis virus infection is a global public health problem. All human hepatitis viruses are RNA viruses, except for hepatitis B, which is a DNA virus. It is estimated that there are 240 million Hepatitis B virus (HBV) carriers in the world, of whom roughly 600,000 die annually from HBV-related liver disease (1)(2). WHO estimates that about 3% of the world's population have been infected with Hepatitis C virus (HCV) and that there are more than 170 million chronic carrier who are at risk of developing liver cirrhosis and/or liver cancer (3) (4) (5).The average estimated carrier rate of hepatitis B virus (HBV) in India is 4%, with a total pool of approximately 36 million carriers. (6) and prevalence of Hepatitis C virus in India is about <2.5%.

Sickle Cell Disease is an inherited hemoglobin disorder cause due to substitution mutation (GAG>GTG) on 6^{th} codon of Beta globin gene leading to change of amino acid from Glutamic Acid to Valine. In India prevalence of Sickle Cell Disease of 1-40 % and The State of Odisha falls in the high prevalence zone (21-40%)(7).

Viral hepatitis in sickle cell haemoglobinopathy has been found recently to be important observation. These patients are at increased risk of parenterally transmitted hepatitis i.e. Hepatitis-B and hepatitis-C infections due to repeated blood transfusions and injections. The signs and symptoms of viral hepatitis and sickle cell disorders are also similar. This study is being undertaken to know the possible disease course and prognosis in sickle cell patients which may help for better management.

II. MATERIALS AND METHODS

Place and period of study

The study was conducted in department of Medicine and Sickle Cell Clinic, Veer Surendra Sai Institute of Medical Science & Research (VIMSAR), BURLA during the period of September 2012 to September 2014. After obtaining necessary written consent from the subject, blood samples were collected in K_2 EDTA Vaccutainers (BD Peripherals, Franklin Lales, NJ USA). Basic laboratory investigations like Sickling reduction test (sodium metabisuplhite reduction test), Complete Blood Count (CBC) using Sysmex KX 21 (Sysmex Corporation, Kobe, Japan), alkaline agarose gel electrophoresis (pH- 8.6), biochemical parameters *viz.*, serum bilirubin (BIL-T & BIL-D), serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), Alkaline Phosphatase (ALP), serum creatinine (CRT-J), serum urea (UREA) using Cobas Integra 400 Plus (Roche Diagnostics Ltd., Rotkreuz, Switzerland) were performed as per the manufacturer's instruction and standard protocols. Cation exchange high performance liquid chromatography (HPLC) by VARIANT-II hemoglobin testing system using the CDM 5.1^{TM} software (Bio-Rad Laboratories, Hercules, CA, USA) was used to detect and quantify various Hb fractions based on individual retention time (RT). Their detailed history including past history of jaundice, blood transfusion, parenteral injection etc, was taken and clinical examination done.

Screening test for hepatitis-B virus (detection of HBsAg) in Sickle Cell disease cases was done by kit of hepacard on sandwich principle of enzyme-linked immunosorbent assay (ELISA). HBV profile testing by A.HBeAg (Hepatitis- B Envelope antigen), B.IgM AntiHbC(Hepatitis-B core antibody), C. AHBe (Anti Hepatitis B Envelope antigen), D. AHBs(Anti Hepatitis B Surface antigen). Briefly A. Determination of antibody to hepatitis-B envelope antigen (Anti-HBe) Interpratation (as per the manufacturer's instruction and standard protocols) done by Reference value (Units(s/co)); i. Positive: ≤1.01, ii. Equivocal: 1.01-1.10, iii. Negative: ≥1.11, B. Quantification of IgM Anti Hbc(Hepatitis-B core antibody); Interpretation has been performed (as per the manufacturer's instruction and standard protocols), The reference range (Units (OD ratio)) i. Specimens with values≥1.11 are considered positive, ii. Specimens with value 0.90-1.11 are considered equivocal, iii. Specimens with value≤0.90 are considered negative, C. Determination of antibody to hepatitis-B envelope antigen (Anti-HBe); Interpratation (as per the manufacturer's instruction and standard protocols) done by Reference value (Units(s/co)) i. Positive: ≤1.01, ii. Equivocal: 1.01-1.10, iii. Negative: ≥1.11. D. Anti hepatitis-B surface antigen determination (AHBs); Interpretation (as per the manufacturer's instruction and standard protocols) done by Reference range (Units(miu/ml)) i. Negative:<10.0, ii. Positive≥10.0 . Same screening test used for the qualitative detection of hepatitis-C antibodies in human serum by enzyme-linked immunosorbent assay (ELISA) using the major immunoreactive antigens (recombinant antigens) of the HCV genome namely Core, NS3, NS4, and NS5. HBV DNA and HCV RNA quantification was not done due to affordability factor. For avascular necrosis of femur and splenomegaly in Sickle Cell Disease patients X-ray hip joint and USG Abdomen and pelvis were done respectively.

Inclusion criteria

1. The patients having symptoms suggestive of sickle cell haemoglobinopathy such as joint pain, fever, anemia, jaundice were evaluated for the same and confirmatory tests were done.
2. Patients with sickle cell disease as well as sickle cell trait with or without history of blood transfusion with or without clinical features of liver disease were screened for serum markers of Hepatitis-B & Hepatitis -C virus.

Exclusion criteria

Sickle cell hemoglobinopathy with malaria, dengue, leptospirosis, pregnancy, HIV & age less than 15 years.

Statistical Analysis Methods

For comparison between groups, ANOVA was used as appropriate by using GraphPad InStat Version 3.00 for Windows. Association of clinical signs with HBsAg positive and HCV positive was done by regression analysis. All regression analysis was performed by using SPSS version-16.0 for windows. *p* value of <0.05 was considered to be statistically significant.

III. RESULT

A total 500 sickle cell haemoglobinopathy cases where studied during the study period from September 2012 to September-2014, of which 382 where sickle cell anemia (HbSS) and 118 cases with sickle cell trait (HbAS). Out of 382 cases of HbSS, 11 (2.9%) cases were positive for hepatitis-B and 2(0.5%) cases were found to be positive for hepatitis-C. Out of 118 cases of sickle cell trait, 2 (1.7%) cases were found to be positive for hepatitis-B.

The mean age of patients with sickle cell anemia was 25.8 with a standard deviation of 7.4. The mean age of cases with sickle cell trait was 26.6 with a standard deviation of 5.5. There was no statistical significance between the mean age and the genotype as ($p>0.05$). Of the 382 sickle cell anemia cases studied 212 case were males (55.5%) and 170 case females (44.5%). Of the 118 cases with sickle cell trait 50 were males (42.4%) and 68 case were females (57.6%). There was no statistical significant in the sex distribution of both sickle cell anemia and sickle cell trait cases ($p >0.05$). The most common presentation at the time of registration was vaso-occlusive crisis (83.76 %), anemia (81.41 %), blood transfusion (62.82 %) was followed by splenomegaly (62.6%), Jaundice (60.2), hepatomegaly (41.1%), and fever was in (39.5%) of cases.

In this study from 382 SCD cases 240 (62.82 %) are on BT and among 240 only 9 (3.75 %) are of HBV positive; other 142(37.18 %) were of no BT and only 2 (1.4 %) infected with HBV. From 382 SCD cases only 2 (0.53 %) cases were infected with HCV.

Out of the 500 cases with sickle cell haemoglobinopathies, the LFT parameters were raised in 68 cases. Out of 11 cases HBV positive cases in HbSS 5 cases (45.4%) were in the recovery stages and 6 (54.5%) were in the late acute stage with low infectivity.

Comparison of the hematological parameters (by ANOVA) between HBsAg positive, HBsAg negative cases and HCV positive cases in HbSS showed there was no statistical significance found between the three groups of cases ($p>0.05$) [Table- I]. Comparison of LFT showed that it was raised in HCV positive cases and HBsAg positive case whereas in cases negative for both showed lowered LFT parameters. These differences were found to be statistically significant [Table-II]. Comparison of clinical parameters showed that the VOC/year and requirement for BT were not statistically significant but there was a trend of increasing in these two parameters. Rate of hospitalization was more in cases with HCV(+) compared to patients with HBsAg (+) and negative for both [Table-3]. Multiple regression analysis revealed that in SCA genotype patient with HBsAg(+)has positive correlation with the incidence of jaundice($p<0.003$) whereas the incidence of fever, anemia, splenomegaly, hepatomegaly and voc was independent of HBsAg positivity ($p>0.05$) [Table-III].

In 2 HBsAg positive cases with HbAS one case (50%)was in the recovery stage and the other (50%) was in late acute stage with low infectivity. Comparison of the hematological parameters between HBsAg positive and HBsAg negative cases revealed no significant difference ($p>0.05$) [Table-IV]. LFT showed that S.Bilirubin, SGOT and SGPT were significantly raised in HBsAg positive cases than without it ($p<0.05$) [Table-V].

IV. DISCUSSION

There are episodes of jaundice in sickle cell disease (SCD) patients which may be misleading in many cases. This is because it may either be part of the chronic haemolysis they experience or due to transfusion related hepatotropic viral infections (HBV/HCV), thus the need for Hepatitis B viral testing among SCD patients who receives transfusion therapy (12).

In this study, 500 cases of sickle cell haemoglobinopathies were studied 13 cases were HBsAg + ve and 2 cases were found to be HCV +ve. Out of which 382 cases were sickle cell anemia of which 11 were found to be positive for HBsAg (2.9%) and 2 case were positive for HCV(0.5%) in the screening test for anti HCV antibody. Out of the 118 cases of sickle cell trait only 2 cases were found to be positive for HBsAg (1.7%)

Vichinsky et al **(8)** defined active hepatitis-B as liver function tests above normal range, together with a positive antigen (HBsAg, HBeAg) test or positive IgM core antibody, in conjuction with negative HBSAb test. All the cases in this study were either in recovery stage (positive anti HBs) or in late acute stage with low infectivity(negative Anti IgM antibody and negative Anti HBe). Thus this did not significantly affect the disease course.

In this study done on the hematological parameters of HBsAg positive and negative cases in HbSS showed that there was no stastistical significance $p>0.05$ and this study was consistent with the study done by Nsiah K et.al. (9). Thus it was shown in this study that haematological parameters was not changed considerably in patients with HBsAg positive in HbSS. Raised bilirubin levels, predominantly unconjugated , are universal in sickle cell patients due to chronic hemolysis. Total bilirubin concentrations are usually less than 6mg%, with no other clinical or laboratory evidence of liver disease (Johnson et al,1985). (10)

In this study done on the liver function tests of HBsAg positive and negative cases in HbSS showed that that S.Bilirubin, SGOT, SGPT increased in cases with HBsAg positive than without it($p<0.05$).Similar studies conducted by Nnebe-Agumadu et al (11) showed that eventhough no statistically significant difference was attributable to the presence of HBV was observed between the mean values of the serum enzymes amongst groups,isolated elevation in enzymes levels were noted and randomly distributed in various group with and without HBV markers. Similar studies by Nsiah k et. al (8) showed that the mean levels of S.Bilirubin, SGOT, SGPT were significantly higher in HBV positive cases, but were not statistically significant.

In this study comparison of the clinical parameters (VOC, Hospitilization, blood transfusion showed that there was a increasing trend with blood transfusion but was not statistically significant. In case of rate of hospitalization ,it was found to be statistically significant($p<0.05$).In a study by Nnebe-Agumadu et al (11) to have stated that there was no statistical significance between increased hospitalization, VOC and requirement for blood transfusion among HBsAg positive cases when compared to HBsAg negative cases in HbSS. In a study by Samuel S et al (12) found out that sickle cell patients are not a major risk of hepatitis- B viral infection due to repeated transfusion therapy because of the use of properly screened donor blood.However,there remains significant risk by donations from infected donors who have not yet developed detectable HBsAg levels.

In this study comparison of hematological parameters between HCV (+) and HCV(-) cases found no statistical significance as $p>0.05$. The liver function test between HCV(+) and HCV(-) cases found that the LFT parameters were significantly raised in HCV(+) cases when compared to without it, this correlated with studies done by Jose P et al (13).

Clinical parameters (VOC, Hospitalization, blood transfusion)between HCV(+) and HCV(-)showed that there was a increasing trend with blood transfusion but was not statistically significant($p>0.05$). It was found significant that the relationship between increased hospitalization and HCV infection($p<0.05$)was similar to the studies by Jose P et al (13)

Hematological parameters of hepatitis-B positive and Hepatitis- B negative cases of HbAS was found that there was no statistical significance($p>0.05$). Liver function test among Hepatitis-B positive and Hepatitis-B negative cases among HbAS, showed S.Bilirubin, SGOT, SGPT was increased significantly in HBsAg positive cases than without it($p<0.05$). Surprisingly similar studies have not been conducted on HbAS in this part of the country.

V. CONCLUSION

The lower prevalence of the HBsAg in SCD patients seen in this study could be due to better HBsAg screening techniques in blood transfusion centers. HBV infection during transfusion should not be completely excluded. There remain significant risks from infected donors who have not yet developed detectable HBsAg levels. HBV screening and vaccination should therefore be a part of the management of SCD patients. Elevated LFT level in sickle cell cases positive for HBsAg and HCV. Sickle Cell cases negative for both HBsAg and HCV also have elevated LFT which necessitate intermittent follow up. Thus further studies are required to know the long term prognosis in such patients.

VI. CONFLICT OF INTEREST:

The authors declare there is no any conflicting interest

VII. ACKNOWLEDGEMENT-

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Table I
Haematological parameters Of HBsAg positive, HBsAg negative cases and HCV positive cases in HbSS

BLOOD PARAMETERS	MEAN±SD			*p* value
	HCV(-) & HbsAg(-)	HbsAg(+)	HCV(+)	
WBC(m/mm^3)	11.3±3.9	11.5±3.4	14.05±6.29	0.8807
RBC(M/mm^3)	3.3±0.9	3.69±0.66	3.5±0.18	0.1312
HGB(g/dl)	8.67±2.6	8.83±2.6	9.8±0.28	0.8511
HCT (%)	26.33±6.5	29.43±4.99	33.8±4.24	0.1172
MCV(fl)	81.8±10.9	78.5±4.3	84.5±0.70	0.3178
MCH(pg)	28.08±4.8	25.2±1.85	26±0.28	0.069
MCHC(g/dl)	34.2±4.7	32.05±1.32	31.05±0.35	0.1229
RDW	13.3±2.2	15.13±0.8	15.15±2.33	0.0578
LYM(%)	31.1±8.1	31.6±7.5	31.6±11.3	0.6422
MON(%)	2.08±2.6	3.22±2.7	5.15±1.20	0.132
NEU(%)	66.8±9.02	63.5.3±15.3	62.45±13.3	0.5604
PLT(m/mm^3)	242.5±83.8	252.9±70.8	201±57.98	0.6841

Note. WBC: white blood cells; RBC: red blood cell; HGB: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular Hb; MCHC: mean corpuscular Hb distribution width PLT: platelet; HCT :haemocrit; LYM:lymphocyte; MON;monocyte; NEU;neutrophil

Table II
Liver function test in HBsAg(+), HBsAg(-)cases and HCV(+)cases in HbSS

LIVER FUNCTION TEST	MEAN±SD			p value
	HBsAg(-) & HCV(-)	HBsAg(+)	HCV(+)	
S.BILIRUBIN (T) (mg/dl)	1.50±1.25	3.35±1.21	4.25±0.35	<0.0001
S.BILIRUBIN (D) (mg/dl)	0.44±0.4	0.9±0.5	0.3 ±0	<0.0001
SGOT (AST) (U/L)	35.7±18.9	75.2±39.4	160±56.56	<0.0001
SGPT (ALT) (U/L)	32.73±16.15	84.22±67.5	145±21.21	<0.0001
ALP (U/L)	125.1±19.7	135.0±45.2	116.5±27.5	0.091

Note-BIL-T: bilirubin total; BIL-D: bilirubin direct; SGOT;Serum glutamic oxaloacetic acid; SGPT;Serum glutamic pyuruvic trasaminase; ALP;Alkaline phosphatase

Table III
Clinical parameters in HbsAg(+) , HbSAg(-) and HCV(+) in HbSS

Clinical parameters	MEAN±SD			p value
	HBsAg(-) &HCV(-)	HbsAg(+)	HCV(+)	
VOC/YEAR	2.1±1.1	2.4±1.7	3.5±0.7	>0.05
BT/YEAR	1.6±1.2	2.3±1.9	2.5±0.7	>0.05
HOSPITILIZATION/YEAR	1.4±0.5	1.9±1.7	3.0	<0.05

Note- VOC- Vaso-Occlusive Crisis; BT-Blood transfusion

Table IV
Hematological parameters in HbSAg positive and HbSAg negative cases in HbAS

BLOOD PARAMETER	MEAN±SD		p value
	HBsAg(+)	HBsAg(-)	
Hb(g/dl)	13.3±0.6	12.3±2.2	0.5218
TLC((m/mm^3)	7.3±2.5	7.8±2.6	0.7953
RBC(M/mm^3)	4.6±0.53	4.6±0.7	0.9760
HCT(%)	41.0±2..4	38.6±5.6	0.5473
MCV(fl)	90.0±5.3	84.3±5.4	0.1518
MCH(pg)	29.2±2.2	26.9±2.6	0.2181
MCHCg/dl)	32.5±0.6	31.2±3.03	0.5664
RDW	10.3±0.14	11.2±1.4	0.3694
LYM(%)	20.5.±0.70	29.2±7.1	0.0895
MON(%)	0.5±0.70	0.3±0.6	0.6864
NEU(%)	79±1.4	70.5±7.08	0.0943
PLT(m/mm^3)	257.5±88.4	238.9±65.5	0.6934

Note. WBC: white blood cells; RBC: red blood cell; HGB: hemoglobin; MCV: mean corpuscular volume; MCH: mean corpuscular Hb; MCHC: mean corpuscular Hb distribution width PLT: platelet; HCT :haemocrit; LYM:lymphocyte; MON;monocyte; NEU;neutrophil

Table V
Liver function tests in HBsAg positive and HBsAg negative cases in HbAS

LIVER FUNCTION TEST	MEAN±SD		p value
	HBsAg(+)	HBsAg(-)	
S.Bilirubin (T) mg/dl	4.35±1.06	0.92±0.3	<0.0001
S.Bilirubin (D) mg/dl	0.6±0.42	0.31±0.17	<0.05
SGOT (AST) U/L	72.5±53.03	28.12±10.0	<0.0001
SGPT (ALT) U/L	78.5±55.9	28.2±7.9	<0.0001
ALP (U/L)	125±9.9	121.5±10.4	>0.05

Note-BIL-T: bilirubin total; BIL-D: bilirubin direct; SGOT;Serum glutamic oxaloacetic acid; SGPT;Serum glutamic pyuruvic trasaminase; ALP;Alkaline phosphatase