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Original Article

"Hematological and Biochemical analysis of β-thalassemic Major patients"

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ABSTRACT

β-thalassemia is a single gene disorder requiring regular multi-blood transfusions. In patients with β-thalassemia major impaired biosynthesis of the beta globin leads to accumulation of unpaired alpha-globin chain. Repeated blood transfusions, ineffective erythropoiesis and increased gastrointestinal iron absorption lead to iron overload in the body. Thus shortened red cell lifespan and iron overload cause functional abnormalities in various organ systems. An attempt was made to study hematological and biochemical parameters in β-thalassemia major patients in order to assess the present status of their organ functions as well as test for transfusion transmitted infection (HBV, HCV & HIV) were performed, for this blood samples were collected just before scheduled blood transfusion from 58 β-thalassemia major children who were on regular blood transfusion and chelation therapy and 50 blood samples from healthy children belonging to same age group. Highly significant difference was noticed for CBC count including deteriorated liver and kidney functions test in patients as compared to controls. Not a single positive case of HBV, HCV & HIV were observed.

Conclusion: β-thalassemia major patients were associated with multiple renal abnormalities and deranged liver enzymes due to continuous blood transfusion and iron overload. Appropriate chelation therapy and regular monitoring of the status of iron overload is very much necessary.

Keywords:
β-thalassemia major, iron overload, LFT, KFT.

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Introduction

Thalassemia is one of the major hemoglobinopathies among the population all around the world. It is single gene hereditary disorder in humans. In thalassemia there is impaired production of alpha or beta chains of hemoglobin are. If the production of alpha chains is impaired the condition is called alpha thalassemia and if the production of beta chain is impaired the condition is called beta thalassemia. (Asha Shah, 2004).

β-thalassemia represents a group of recessively inherited hemoglobin disorders characterized by reduced synthesis of β-globin chain. Three classes of β-thalassemia have long been recognized clinically, β-thalassemia major, intermedia and minor (Thein, 2004). β- Homozygous state presents with variable degree of anemia from early childhood and are generally transfusion dependent, a condition clinically known as thalassemia major. β-heterozygous cases (thalassemic minor) are almost asymptomatic with normal or slightly reduced levels of hemoglobin. However an intermediate condition which may have either heterozygous or homozygous pattern of inheritance, requires minimal or no blood transfusion and has milder clinical course than thalassemic major but is severe enough as compared to thalassemic minor. It manifests generally after two years of age and does not require regular

transfusion therapy. (Rund, 1997, Tyagi, 2003).

Reportedly, there are about 240 million carriers of β-thalassemia worldwide and in India alone the number is approximately 30 million with a mean prevalence of 3.3%. (Yashis, 2007, Verma, 1992, Yagnik, 1997).But among certain communities and religions like Punjabi's, Sindhi's, Bengali's, Jams and Muslim's the incidence of β-thalassemia trait ranges between 8-15 %. (Marwah and Lal, 1994)

Thalassemic major child is born if both parents carry a hemoglobinopathy trait, since there is a 25% chance with each pregnancy for an affected child. Once a child is diagnosed to have thalassemia homozygous disorders, he/she has to take lifelong treatment. Management includes regular filtered packed red cell transfusion, chelation therapy for iron overload, management of complications of iron overload and transfusion, including osteoporosis, cardiac dysfunction, endocrine problem, hepatitis B and C, HIV infection etc.

Iron overload is the life limiting complication commonly found in thalassemics.(Wangruangsattit S.et.al. 1999).The progressive iron overload in β-thalassemia major patients is consequence of ineffective erythropoiesis, increased gastrointestinal absorption of iron, lack of physiologic mechanism for excreting excess iron, and above all multiple transfusions. A unit of red blood cells transfused contains approximately 250mg of iron, while the body cannot excrete more than 1mg of iron per day. The iron which exceeds the iron binding capacity of transferrin appears in the plasma as non transferrin bound iron, which is highly toxic to the tissues. (Giardina PJ and Grady RW, 2001). Iron overload- produces reactive oxygen species that damage the heart (cardiomyopathy), liver (fibrosis and cirrhosis),nervous system, can lead to diabetes mellitus, hypothyroidism, hyperparathyroidism as well as adrenal and pituitary insufficiencies.(Rund D, Rachmilewitz E.,2005). Deferroxamine, Deferiprone and Deferasirox are few commonly used iron chelator in India. Due to high cost of such chelators most of the patient cannot afford this. Regular blood transfusion is available in most of the countries, is lifesaving and improves short time quality of life. But iron chelation therapy is essential for long term survival (S Mallik, C Chaterjee et.al.2009)

Beside this, Transfusion transmitted infection (TTI) is a major challenge to the transfusion services all over the World. The problem of transfusion transmitted infection (TTI) is directly proportional to the prevalence of the infection in the blood donor's community. In India HIV, HBV, HCV, Syphilis, Malaria, Human T-lymphocyte virus (HILv-1 and HILv-2) and bacterial infection are important causes of concern (Bhasin R et.al. 2003).Blood transfusion transmitted infection (TTI) occurs in patients who are dependent on blood transfusion. Multiple blood transfusions are required mainly in patients of thalassemia, Sickle cell anemia, hemophilia, aplastic anemia, patients of chronic hemodialysis etc.

Thus the most important cause of mortality and morbidity in these patients is organ failure related with shortened red-cell life span ,rapid iron turnover and tissue deposition of excess iron. These are major factors responsible for functional and physiological abnormalities in β-thalassemic major patients as well as number of transfusion transmitted infections. An attempt was made to study hematological and biochemical parameters in β-thalassemic major patients attending the private and Muncipal hospital of Akola in order to assess the present status of their organ function as well as prevalence of blood transfusion transmitted infections among them.

Materials and Method:

58 samples were analyzed for the study which was conducted in research lab of dept. of Biochemistry at Shri Shivaji College, Akola. Sample collection was done from Hedgewar Blood Bank, Thalassemic unit of Indrani hospital and Muncipal hospital of Akola.

Study of Biochemical parameters including Serum urea, Serum creatinine, Serum bilirubin, SGPT, Serum Alkaline phosphatase, Serum iron, TIBC were done on Robonik Biochemical analyzer while hematological study was done on ERMA made Fully automatic Cell counter and Sodium, Potassium, Calcium analysis was done on Sod-Pot analyzer(Roche 9180 electrolyte analyzer). Seroprevelence of Hepatitis B, Hepatitis C and HIV were done by using Acon One step immunoassay Rapid test, Flavicheck HCV, Rapid immunochromatographic test for HCV antibodies and Aspen One step immunoassay

Rapid test respectively.

Result & Discussion:

Total 58 blood samples of β-thalassemic patients were collected from Hedgewar Blood Bank, Thalasemic Unit of Indrani hospital just before scheduled blood transfusion. The age range of patients lies between 5 to16 years. All the patients were on regular blood transfusion as well taking the oral iron chelator i.e. Kelfer. Similarly a camp was organized at Shri Shivaji High School, Akola and 50 blood samples as a control from students were collected belonging to same age range. Complete blood count and biochemical analysis were performed. The hematological data was tabulated in table no.1 while biochemical data was shown in table no.2 which was statistically analyzed using Z-score test.

Table No.1: Hematological Parameters

Sr. No.	Hematological Parameters	Control group (n=51)	β-thalassemic major patients (n=58)	Z-score	P value (level of significance)
1.	WBC X 10^3/ul	8.07±0.26	11.19±0.09	17.24	HS
2.	RBC X 10^6/ul	4.38±0.05	3.19±0.02	29.96	HS
3.	Hgb g/dl	12.09±0.13	7.58±0.11	36.69	HS
4.	HCT %	35.52±0.43	23.75±0.21	35.87	HS
5.	MCV fl	81.04±0.51	74.35±0.40	14.62	HS
6.	MCH Pg	27.77±0.37	23.84±0.42	9.85	HS
7.	MCHC g/dl	34.15±0.41	32.12±0.61	3.93	HS
8.	RDW %	14.64±0.10	18.55±0.30	19.33	HS
9.	PLT X 10^3/ul	277.80±9.19	361.74±5.41	11.49	HS

Key: HS= Highly significant (p< 0.001), S= Significant (p<0.01)

Table No. 2: Biochemical Parameters

Sr. no.	Biochemical parameters	Control group (n=51)	β-thalassemic major patients (n=58)	Z-score	P value (level of significance)
1.	Urea mg/dl	27.52±1.17	30.83±1.03	3.01	S
2.	Creatinine mg/dl	0.78±0.02	0.92±0.03	4.94	HS
3.	Sodium meq/L	135.57±0.46	150.46±0.48	31.42	HS
4.	Potassium meq/L	3.7±0.04	4.94±0.12	14.50	HS
5.	Calcium mg/dl	9.61±0.05	9.05±0.03	12.38	HS
6.	SGPT Units/ml	23.58±1.04	37.13±0.57	16.81	HS
7.	Alkaline phosphatase KA units	5.56±0.17	9.36±0.20	19.82	HS
8.	Total Bilirubin mg/dl	0.63±0.04	1.29±0.02	19.02	HS
9.	Direct Bilirubin mg/dl	0.16±0.01	0.38±0.006	20.99	HS
10.	Serum iron ug/dl	88.15±1.79	182.09±1.63	54.67	HS
11.	TIBC ug/dl	276.92±31.52	237.65±29.34	9.50	HS
12.	Transferrin saturation %	32.20±5.84	77.85±11.68	38.81	HS

Key: HS= Highly significant (p< 0.001), S= Significant (p<0.01)

β-thalassemia major is an inherited, autosomal recessive hemoglobinopathy that results in a large number of hematological, biochemical and systemic abnormalities. The β-thalassemic children included in the present work showed a significantly altered hemogram, especially in red blood cell mass and related indices (Hb, RBC,HCT and MCV,MCH).Our hematological findings in β-thalassemic patient s were found to be in similar in view as that of Rigano et.al. (2001) that showed in their study significantly altered hemograms with severe anemia, thrombocytosis and leucocytosis of β-thalassemic Sicilian patients. Yassin et.al. (2013) had studied over β-thalassemic Palestinian patients and reported severe anemia with decreased hemoglobin, HCT along with significant thrombocytosis and leucocytosis among the patients as compared to controls.

A rise in iron indices observed in β-thalassemic patients may be due to erythrocytes

hyperhemolysis and due to chronic blood transfusion.Similar results were found in the study of Asma K .*et.al.*(2003). The acute iron overload found in beta-thalassemia can lead to an iron intestinal hyperabsorption and to an abnormal molecular iron form (non-transferrin-bound: NTBI) accumulation. NTBI has hepato and cardio-cytotoxic properties. Furthermore, NTBI contributes to the formation of free radicals and increases hemolytic process (Borgna–Pignatti *et al.*, 2004). We observed highly significant iron increase leading to iron overload, in patients as compared with mean serum iron levels of 182.09 vs 88.15 ug/dl respectively. Mean value of TIBC in patients and control group was found to be 237.65 and 276.92 ug/dl whereas mean value of transferrin saturation was observed to be 77.85 and 32.20 % respectively.

Our result revealed a significant increase in serum urea level in experimental group as compared to control group.(30.83 and 27.52 mg/dl respectively).The concentration of creatinine in serum is the most widely used and commonly accepted measure of renal function in clinical medicine (Persone *et. al.* 1992).Our study showed highly significant difference in creatinine concentration in the experimental group as compared to control group.(0.92 and 0.78 mg/dl respectively).The increasing level of urea and creatinine in β-thalassemic patients possibly due to higher iron deposition in their kidneys, shortened red cell lifespan and excess iron which causes functional and physiological abnormalities in various organ systems in thalassaemia patients.

Electrolyte levels are tightly controlled by several hormones and by the kidneys, which are primarily responsible for retaining and removing electrolytes when necessary and keeping them in a constant state of balance. An electrolyte imbalance can lead to serious health issues, including eventual death if not corrected. The most common imbalances occur with sodium and potassium (Kamal *et.al.* 2013). Our findings revealed that there was highly significant increase in serum Sodium and Potassium in the patient group as compared to control group (150.46 and 135.57 meqL, 4.94 and 3.70 m eq/L respt).There was a significant decrease observed in the mean value of serum calcium in experimental group as compared to control.(9.05 and 9.61 mg/dl restp).Increase in serum Sodium and potassium and decrease in serum calcium was observed on 70 β-thalassemic patients by Hina Akram and Tabassum Mahboob (2004).

Frequent blood transfusion can also lead to iron overload in liver. Liver has a large capacity to produce proteins, which bind the iron and store it in the form of ferritin, therefore it can produce severe iron overload .Thus our study was also aimed to assess the liver status of β-thalassemic patients.Highly Significant change was observed in the SGPT level of patients as compared to control group (37.13 and 23.58 u/L respectively),similarly highly Significant difference was also observed in total and direct billirubin of experimental group as compared to control group (1.29 & 0.63 mg% and 0.38 & 0.16 mg% respectively) and highly significant increase in alkaline phosphatase was also noticed in experimental group as compared to control(9.36 & 5.56 KA units respectively)

Abnormal liver function represented by elevated levels of SGOT, SGPT and serum alkaline phosphatase, which was observed more frequently in patients with iron overload than in patients with a lower level of iron (Wanachiwanamin et.al.2003).

Regular blood transfusion improves the overall survival of patients with β-thalassemia, it carries a definite risk of infection with blood borne viruses (Amarapurkar DN *et.al.*, 1992, Mirmomen S *et.al.* 2006) and complication like secondary hemosiderosis, liver failure and renal failure. Infections are major complication and constitute the second most common cause of mortality and morbidity. In our study fortunately no single patient was found to be positive for Hbs antigen, anti HCV antibodies and anti HIV antibodies.

In case of hepatitis B, since an effective vaccine is available, immunization against this virus before transfusion management would effectively protect against transfusion transmitted hepatitis B virus. However since no such vaccine is so far available against Hepatitis C virus, the only effective protective measure is provision of HCV negative blood for transfusion. Therefore, screening of transfused blood for HCV as well as for HIV should be done mandatory, by using most sensitive screening methods with least possible false negative results. (Mohd. Y *et.al*, 2004). The decrease in seropositivity amongst multitransfused patients is because of implementation of measures such as, donor education, strict standards for donor selection criteria, improved serological screening protocols, improved blood collection and transfusion technique. (Vidja PJ, *et.al.* 2011)

Incidence of HIV positivity has decreased due to strict mandatory screening of all blood bags as well as by decreasing the window period by using improved technology. Prevalence of HCV is still there in frequent blood recipients like thalassemia major patients. This may be due

to late starting of screening for HCV antibody as well as no vaccine is available for protection against HCV. (Bhavsar H *et al.*, 2011)

Conclusion:

β-thalassemic patients present multiple renal abnormalities which may be due to iron overload. Thus appropriate chelation therapy and regular monitoring of the status of iron overload is very much necessary. Well balanced nutrition, patient education, diet counseling and supplementation therapy of calcium and vitamin-D for high risk group of β-thalassemics is strongly recommended. Ideally all patients of thalassemia major should complete vaccination for hepatitis B before starting transfusion or as soon as possible. Since increased titer of antibodies is protective against Hepatitis B viral infection. Though we didn't get any hepatitis positive case, references proved that prevalence of HCV infection is much higher as compared to HBV and HIV infections in β-thalassemic patients.

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