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IRON OVERLOAD, OXIDATIVE STRESS AND ANTIOXIDANT CAPACITY IN BETA THALASSEMIA MAJOR PATIENTS ATTENDING A TERTIARY CARE HOSPITAL IN MIRAJ, MAHARASHTRA.

Biochemistry

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ABSTRACT

Background: Oxidative stress in patients with beta thalassemia major is mainly caused by tissue injury due to over production of free radical by secondary iron overload and alteration in serum antioxidant level. Present study is conducted to evaluate the iron overload in beta thalassemia major patients attending a tertiary care hospital in Miraj, Maharashtra.

Materials and Methods: A comparative cross sectional study is done to evaluate the iron overload in beta thalassemia major patients of the age less than or equal to 12 years attending the pediatric OPD who were currently being transfused and managed for the clinical symptoms and manifestations of the disease during January 2016 to December 2016. There were 47 beta thalassemia patients and 50 non beta thalassemia patients hence used as controls. Mean levels of the parameters were compared in both the groups. P value of less than 0.05 was taken as significant.

Results: Cases and controls were comparable in their demographic characteristics (for age P-0.888 ; for gender proportion P=0.902). Serum level of iron and ferritin was significantly higher in the patients as compared to controls (P<0.05) whereas total iron binding capacity was significantly lower in patients (P<0.05). Melandialdehyde (MDA) levels were also significantly higher in patients (P<0.05) whereas vitamin C levels were significantly lower in patients (P<0.05).

Conclusion: Iron overload is seen in beta thalassemia major patients who were currently being transfused. Iron overload leads to tissue injury by free radicals and hence oxidative stress in patients. This leads to increased lipid peroxidation (high MDA) and decreased antioxidant capacity (lower vitamin C levels).

Recommendation: Iron chelation therapy with monitoring of the parameters can help to reduce the iron overload and its complications in beta thalassemia patients.

KEYWORDS

beta thalassemia major, iron overload, oxidative stress, antioxidants.

INTRODUCTION:

Beta thalassemia is one of the most common autosomal recessive disorder worldwide.[1] Each year 8000 children with thalassemia born in India, contributing around 10% of the annual world incidence[2]. Beta thalassemia is characterized by the lack of beta globin chain synthesis, Hemoglobin A is absent, Hemoglobin F is 95-98% and Hemoglobin A_2 is 2-5 %.[3] β-thalassemia phenotypes are variable, ranging from the severe transfusion dependent thalassemia major to the mild form of thalassemia intermedia. Clinical Presentation of beta thalassemia major occurs between 6-24 months. Affected infants fail to thrive and become progressively pale.

β-thalassemia is a severe anemia, can be treated with blood transfusions which may lead into iron overload and organ failure[4]. Recent reports suggested that patients with beta thalassemia major suffered from oxidative stress and lipid abnormalities due to excess iron induced free radicals and which leads into antioxidants consumption which produces deficiency of antioxidants[5].

Present study is carried out to see the iron overload in terms of serum iron, serum ferritin and total iron binding capacity (TIBC) in beta thalassemia major patients who were recently transfused. Also to evaluate the lipid peroxidation in terms of levels of Malondialdehyde and antioxidant vitamin C levels.

MATERIALS AND METHODS:

This is a comparative cross sectional study carried out in patients who attended paediatric department.

Data collection period: The blood samples were collected from first encounters of all patients who had visited to tertiary care hospital during period of January 2016 to December 2016.

Inclusion Criteria:-

All patients having age less than or equal to 12 years attending Paediatric OPD of Tertiary Care Hospitals who were currently being transfused.

Exclusion Criteria: -

The exclusion criteria was patients with hepatitis B and C, Cytomegalovirus, Diabetes Mellitus, Spleeneoctomy, Hypothyroidism, Hyperthyroidism, Renal Failure, Haemoglobinopathy, Malaria and 2nd and more encounter to the Tertiary Care Hospital.

Sample Size:

There were total 97 subjects having age less than or equal to 12 years who were currently being transfused out of which 47 were Beta Thalassemia Major patients and 50 were non beta thalassemia patients hence used as controls.

Statistical Evaluation:

Data was statistically evaluated by employing z test.

Ethical Consideration:

An official approval was obtained from institutional ethical committee. All the Tenets of Helsinki was followed during the study.

Blood Collection:

Informed consent was obtained from all participants. The blood samples were collected from the Department of Paediatrics. To avoid contamination blood samples were withdrawn by using 20 gauge stainless steel disposable needles attached to 5 ml polythene disposable syringes, from anticubital vein with aseptic precautions. 5ml venous blood was taken from each subject at the time of recruitment and divided into three aliquots. 2ml of whole blood was transferred into tube containing heparin and then centrifuged at 3000 rpm for 5 minutes, plasma thus separated is used for the estimation of MDA and Vitamin C. Remaining 2ml was transferred into plain bulb for the estimation of Serum Iron, TIBC and Ferritin.

Biochemical Parameters and Method of Estimation:

Sr.No.	Parameters	Method of Estimation
1	Serum Iron	Ferrozine Method[6]
2	Total Iron binding capacity	Ferrozine Method.[6]
3	Ferritin	Latex Enhanced Immunoturbidimetry[7,8]
4	Malondialdehyde	Keisatoh (1978)[9]
5	Vitamin C	Ayekyaw (1978)[10]

RESULTS:

Table No.1: Demographic profile of the study subjects.

Groups	Age(Years) (Mean±SD)	No. of males in a group(%)	Total
Patients	6.48±2.72	26 (55.31%)	47
Controls	6.56±2.81	26 (52.00%)	50
P value	0.898	0.902	Not significant

Table no. 1 shows that mean age of the patients was 6.48±2.72 years whereas that of controls was 6.56±2.81 but the difference is not significant. Also male proportion in patients group was 55.31% and that of control group was 52.00%. here also the difference is not significant. Hence the patients and the controls are comparable in their demographic characteristics.

Table No.2 : Biochemical Parameters in Patients and Control

Parameters	Patients n=(47) Mean±SD	Control n=(50) Mean±SD	P value
Serum Iron (µg/dl)	234.7±79.84	99.12 ±23.80	<0.05
TIBC (µg/dl)	214.62±35.88	299.18±21.16	<0.05
Ferritin (ng/ml)	2242.77±1049.5	45.5±21.65	<0.05
MDA (mg/dl)	2.05±0.67	0.92±0.28	<0.05
Vitamin C (mg/dl)	0.77±0.17	1.18±0.15	<0.05

Table no. 2 shows the biochemical parameters in patients and control. All the differences showed P value less than 0.05 hence all are significant differences.

Serum iron and ferritin was significantly higher in patients whereas TIBC is significantly lower suggesting iron overload in patients of beta thalassemia major as compared to controls. MDA is significantly high in patients whereas vitamin C levels are significantly low in them. This suggest oxidative stress, increased lipid peroxidation and decreased antioxidant levels.

DISCUSSION

β-thalassemia major is a severe transfusion dependent anemia caused due to complete absence of β-globin chain of hemoglobin which result in excess amount of α-globin chains which precipitates as inclusion bodies and cause premature breakdown of red blood cells.[11]

Clinical presentation of thalassemia major occurs between 6 to 24 months of age. Affected infants fail to thrive and become progressively pale. Feeding problems, diarrhea, irritability, recurrent bouts of fever, spleenomegaly may occur.

Regular blood transfusion is necessary to maintain hemoglobin concentration of 9.5 to 10.5 gm/dL and also to maintain normal growth and development. Multiple blood transfusions develop iron overload and related complications like growth retardation. Iron chelators are used to remove this excess iron. [12]

Excess iron in β-thalassemia major patients may develop oxidative stress due to iron induced free radical formation which results in antioxidant depletion. [13]

Thus present study was carried out to see iron overload in terms of serum iron, ferritin and total iron binding capacity. Lipid peroxidation suggested by MDA levels and antioxidant levels in terms of vitamin C. We found significant increase in (P<0.05) Serum Iron levels in β-thalassemia patients as compared to healthy controls.

Iron overload is due to increased intestinal iron absorption and multiple blood transfusions. In β-thalassemia major patients, GDF protein is produced as a result of mutations; this protein is inhibitor of peptide Hepicidin hormone and sends its reducing signal to the liver. After hepicidin reduction, iron absorption from the diet increased by Ferroportin. [14]

Beta thalassemia major is managed by blood transfusions. Frequent blood transfusions have increased life expectancy and improved quality of life for the thalassemia major patients. But chronic blood transfusions increases frequency of complications. Iron overload is an unavoidable complication suffered by thalassemic patients as a consequence of excessive number of blood transfusions. It is so common that it has been referred to a “second disease” during the treatment of first.

Iron is one way substance, there is no mechanism for iron excretion from body, and therefore iron starts builds up in β-thalassemia major patient's body. To excrete excess iron, chelating agents are used. [15]

Most of the government medical colleges and hospitals in India give packed cell transfusions free of cost to thalassemia major patients but there is no free supply of iron chelators and more than 80% of the patients cannot afford regular iron chelation therapy.

Inadequate chelation is one more cause of increased iron overload. [16]

Increased Serum Iron levels in β-thalassemia patients as compared to healthy controls are due to increased iron overload.

Similar results were obtained by Mohammad A.Al-Katan et al (2009)[17], Nishtha Saral et al (2015) [18], Sonali Bhagat et al (2012),[19] Aparna Sagare et al (2014[20]) and Rahul Ghone et al (2008)[20].

We found significant decrease in (P<0.05) TIBC levels in β-thalassemia patients as compared to healthy controls.

Transferrin is protein used for transport form of iron and shows short term of fluctuation. Transferrin can be measured indirectly as the ability of the plasma to bind iron so called total iron binding capacity (TIBC). The iron deposits in thalassemics who have received multiple blood transfusions fully saturate transferrin, thus TIBC of plasma decreases. TIBC is reliable test as iron overload marker.[78] Decreased TIBC levels in β-thalassemia patients as compared to healthy controls are due to fully saturation of transferrin by excess iron.

Similar results were obtained by Mohammad A.Al-Katan et al (2009) [21], Nishtha Saral et al (2015)[18], Sonali Bhagat et al (2013)[19], Kuldeep KG et al (2011[22]) and Rahul Ghone et al (2008)[20].

We found significant increase in (P<0.05) Ferritin levels in β-thalassemia patients as compared to healthy controls. The liver is the major site of iron overload containing 70% or more of body iron content. Liver iron correlates closely with total body iron in transfusional iron overload and total body iron.

Ferritin is the main iron storage protein in the body. Iron overload is usually manifested by a high serum ferritin levels. In any events, when serum ferritin is greatly increased, whatever the reason, there is cause for concur an increasingly aggressive iron chelation treatment should be given.[23] Increased Ferritin levels in β-thalassemia patients as compared to healthy controls are due to increased iron overload.

Similar results were obtained by Amit Kumar et al (2013) [23], Dr.Devaki RN et al (2015) [15], Nadeem Ikram et al (2004) [24], Sonali Bhagat et al (2013)[19] and Amna Faruqui et al (2014)[25].

We found significant increase in (P<0.05) MDA levels in β-thalassemia patients as compared to healthy controls. Absence of β-globin chains leads to accumulation of unpaired α-globin chains is a primary reason for the cellular oxidative damage and also iron overload. As a result of both high plasma iron and high intracellular non-hemoglobin iron, transferrin gets fully saturated and consequently “free” iron begins to accumulate in tissues and blood.

Normally the H_2O_2 produced in the body is converted into innocuous compounds by the action of enzyme catalase and peroxidase. But when free ferrous iron is available it reacts with H_2O_2 and produce hydroxyl radical ($OH^{\bullet}$) which is an extremely reactive species.[20]

In thalassemia there is excess production of reactive oxygen intermediates which leads to oxidative stress. If the production of ROS exceeds the capacity of enzymatic and non-enzymatic antioxidant system to scavenge these species, then oxidative stress occurs. The oxidative stress may contribute to shortened life span of erythrocytes, primary and secondary amenorrhoea, hypogonadism, osteoporosis, heart failure, endocrine abnormalities like diabetes, hypothyroidism, liver failure and ultimately early death.

MDA is the end product of the primer reactions that lead to the significant oxidation of such polyunsaturated fatty acids in cellular membranes and thus serves as a reliable marker of oxidative stress. Increased MDA levels in β-thalassemia patients as compared to healthy controls are due to increased iron induced oxidative stress and

lipid peroxidation.[26]

Similar results were obtained by Mohsen Rageb Tolba et al (2015)[27], Vinita Belsare et al (2015)[28], Seruni KU et al (2011)[29], K. Goswami et al (2005)[30] and Elham Abed Mahdi et al (2014)[31].

We found significant decrease in ($P<0.05$) Vitamin C levels in β-thalassemia patients as compared to healthy controls. Vitamin C is a water soluble, dietary essential vitamin. It acts as a reducing agent, cofactor, and also a powerful antioxidant. It is a chain breaking antioxidant protects Low Density Lipoprotein (LDL) from being oxidized.[32]

In thalassemia major Vitamin C levels decreases due to its protective effect against free radicals and oxidative stress.[21] Prakash A. et al (2013)[33] found 3 cases of Scurvy in β-thalassemia major patients. Thasinas Dissayabutra et al (2005)[34] found that Vitamin C deficiency is common in β-thalassemia major patients and supplementation of Vitamin C along with Vitamin E is more beneficial than Vitamin E alone to β-thalassemia major patients.

Decreased Vitamin C levels in β-thalassemia patients as compared to healthy controls are due to increased iron induced oxidative stress and antioxidant defense against it.

Similar results were obtained by Bassm N. Aziz et al (2009)[(35)], MA. Livera et al (1996)[36], Claster S. et al (2009)[17].

CONCLUSION:

In conclusion our study revealed that beta thalassemic patients have severe iron overload and hyperferritinemia which induce oxidative stress and depletion of endogenous antioxidants. There is need for better chelation of excess iron and supplementation of antioxidants.

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