C-REACTIVE (CRP) PROTEIN IN TRANSFUSION DEPENDENT THALASSAEMIC PATIENTS

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Background: In thalassaemic patients iron overload, secondary to blood transfusion, results toxic effects by producing reactive radicals. Iron overload can be studied using serum ferritin level which has a direct correlation with the body's iron status. While oxidative damage can be studied using biomarker of inflammation like hsC-reactive proteins. **Methods**: Blood samples of 55 thalassaemic patients (39 males, 16 females) were collected from Fatmid Foundation (Hyderabad). The samples were analysed for CBC, serum ferritin level and hsC-reactive proteins. **Results**: High mean serum ferritin levels was found in all the patients regardless of the frequency of blood transfusion (4774.2135±3143.3040 μg/L), indicating the iron overload. High mean hsC-reactive protein was found (2.5151±1.3712) with a positive correlation with ferritin (r= 0.8371198, *p*= 0.0000) and platelets (r= 0.43293443, *p*= 0.000962175). **Conclusion**: C-reactive proteins serve as biomarker of various inflammatory conditions, progression of cardiovascular diseases and as indicator of morbidity and mortality. High C-reactive proteins in these patients indicate ongoing iron overload toxicity related damage in these patients. The estimation of hsC-reactive proteins and other biomarkers of inflammation and oxidation may help in better management of these patients.
Keywords: Thalassaemia, transfusion, iron overload, ferritin, C-reactive proteins

INTRODUCTION

Thalassaemia are inherited recessive blood disorder which results due to an alternation in the rate of globin chain synthesis.[1–3] In thalassaemia, the genetic defect results in reduced rate of synthesis of one of the globin chains that make up haemoglobin (α, β, γ, δ) which results the imbalance, the excess chain thus produced tend to accumulate and produce unstable product, leading to hemolysis.[1] The release of heme portion of these precipitated unstable chains results oxidative damage to the cell membranes.[4]

The intramadullary premature destruction of RBCs results bone marrow hyperplasia and peripheral destruction results haemolytic anaemia, the processing of large number of abnormal RBCs also results splenomegaly.[5,6] These complication can be controlled with regular red blood cell transfusions, which allow normal development during childhood and extend survival.[6,7] Blood transfusion with correcting the problems of anaemia and reversal of ineffective erythropoiesis also result in iron overload.[8,9] Liver, heart, and endocrine glands are among the most affected organs in these forms of systemic iron overload.[10] Iron overloading with repeated transfusion, results in the saturation of transferrin and than results labile iron pool (LIP) (iron within cells, not bound to dedicated proteins) selectively in some organs.[11] This accumulation serves as marker for potential biological damage because the free metal ion can results the oxidative damage.[7,11,12] The majority of iron deposited in this way accumulate in the liver.[13] Normally there is no free detectable free iron in the plasma but in iron overload conditions as encountered in thalassaemia, when the circulating transferrin become saturated, free iron can bee detected in the plasma. This free low molecular iron is the actual problem, this results production of hydroxyl radicals and accumulate in various organs such as liver, spleen, heart, endocrine glands and results significant damage.[1,10,14]

Cell membrane peroxidation by the free ion in the labile pool is the main cause of organ damage in iron overload.[15] The iron in this free pool is readily available to catalytic reaction, generating free hydroxyl radicals and other ROS, which are responsible for the oxidative damage. Hydroxyl radicals induce lipid peroxidation of cellular organelles including mitochondria, lysosomes, and sarcoplasmic membranes.[16,17] Free ion laden peroxidation damage is evident in vivo in animals with iron overload and thalassaemic, sickle cell and haemochromatosis patients.[18] These reactive radicals can damage the cellular component like lipids, nucleic acids, proteins, and carbohydrates; causing impairment in cellular function and integrity.[17,19] Biomarkers of oxidative stress including plasma malondialdehyde (a marker of lipid peroxidation[20,21] and plasma protein carbonyls[22], a marker of oxidation to circulating proteins have been found increased in patients with β thalassaemia with iron overload.[20] Inflammatory biomarkers including C-reactive proteins and cytokines(IL-6) are found to be increased in various inflammatory conditions and have been found useful in studying thalassemia[20] and other disease states including heart disease and diabetes.[20,23]

In normal concentration the iron is found to be completely bound to the transport proteins and is

non toxic and there is no free iron in the circulation, in conditions of iron overload, when transferrin become fully saturated, non transferring bound iron (NTBI) become detectable in the plasma, and results the production of reactive radicals and results deposition of iron in the organs.[7,24–27] As iron accumulates and exceeds body needs, production of apoferritin is accelerated to provide means for storing iron in non-toxic forms as ferritin or haemosiderin. When ferritin storage accelerated, it is disintegrated to haemosiderin, but again its an protective measure, but it also results release of hydrolytic enzymes from lysosomes and damage to the cells.[28] The measurement of plasma or serum ferritin is the most commonly used indirect estimate of body iron stores. Normally, ferritin concentrations decrease with depletion of storage iron and increase with storage iron accumulation.[29]

MATERIAL AND METHODS

The study has been designed to evaluate the status of hsC-reactive proteins in thalassaemic patients, in relation to the iron overload using serum ferritin levels as indicator of iron overload which results the oxidative damage and degenerative changes.

Blood samples of 55 thalassaemic patients (39 males, 16 females) were collected from Fatmid Foundation (Hyderabad). All the patients are at regular transfusion, having at least one transfusion every month, while some of them are on chelation therapy (n=35). One of the patients having splenectomy has been excluded from study. Samples were collected in both EDTA. K_2 and plain tubes and serum has been separated immediately and stored at -40 ºC for further analysis.

Blood CBC has been performed using coulter (NIHON KOHDEN). Serum hsCRP was estimated using immunoenzymatic assay (TYPE3) kit from Monobind Inc. Lake Forest, C 92630, USA. Serum Ferritin was estimated using Immunoenzymometric sequential assay (TYPE4) kit from Monobind Inc. Lake Forest, C 92630, USA.

RESULTS

The results are summarized in Tables 1–4.

Table-1: Mean values for CBC, hsCRP, Transferrin and Ferritin in transfusion dependent thalassaemia patients (n=55)

Parameter	Mean±SD
hsC-Reactive Proteins (µg/ml)	2.5151±1.3712
WBC (10^3/µl)	8.1182±5.3031
Lymphocytes (%)	47.8236±13.1163
Neutrophils (%)	43.8455±11.9879
Platelets (10^3/µl)	237.6000±130.3306
Transfusion Frequency (days)	18.6364±6.5585
Ferritin (µg/L)	4774.2135±3143.304

Table-2: Mean values for CBC, hsCRP, Transferrin and Ferritin in transfusion dependent thalassaemic patients for three groups on basis of blood transfusion frequency (n=55)

Transfusion Interval	15 days	20 days	30 days
hsC-Reactive Proteins (µg/ml)	2.1336±1.2047	2.4407±1.2490	2.6983±1.4942
WBC (10^3/µl)	9.7091±5.1764	7.6867±5.5347	7.7379±5.3016
Lymphocytes (%)	49.4727±14.5847	43.2733±13.8338	49.5517±12.0365
Neutrophils (%)	42.4182±12.5698	48.1400±14.3257	42.1655±10.2229
Platelets (10^3/µl)	183.0909±106.2915	223.2000±100.0209	265.7241±147.0517
Ferritin (µg/L)	4130.6645±3791.7466	4467.2073±2393.4881	5177.1145±3267.1852

Table-3: Correlation between hsCRP and other biochemical parameters (n=55)

	WBC	Lymphocytes (%)	Neutrophils (%)	Platelets	Transfusion Frequency	Ferritin
Pearson Correlation	0.13292264	0.043878317	0.02607116	0.43293443	0.000	0.8371198
Sig. (2-tailed)	0.333324026	0.750417233	0.850139883	0.000962175	0.262365	0.000

Correlation is significant at the 0.01 level (2-tailed).

Table-4: Number of patients and their percentage in three groups on the basis of hsCRP concentration (n=55)

	Number of patient	Percentage
hs-CRP (≤1)	11	20.00%
hs-CRP (1> & ≤2)	10	18.18%
hs-CRP (>2)	34	61.82%

DISCUSSION

Blood transfusion and iron chelation therapy has improved treatment of thalassaemic patients in the recent years, but iron overload and its toxicities are also common among them.[20] Iron overload resulted cardiovascular complications are one of the common cause of death in thalassaemic patients.[8,30,31] Secondary to repeated blood transfusion, iron in excess of transferrin saturation, results free iron as

NTBI (non transferring binding iron).[12,32] NTBI accelerate the production of appoferritin to provide means of storing the iron in non-toxic form as ferritin or haemosiderin Plasma or serum ferritin measurement is the most commonly used indirect estimate of body iron stores, this reflect iron stores over the wide range of normal values as well as extreme and has direct positive correlation with iron overload.[33–38] In the present study ferritin level has been used as indicator of iron overload. The mean serum ferritin levels for the total sample has been found 4774.2135±3143.3040 (µg/L) and increasing mean quantities have also been observed with increasing frequency of blood transfusion. In three groups on the basis of blood transfusion frequency of once in 15 days, 20 days and 30 days, mean ferritin levels were 4130.6645±3791.7466 (µg/L), 4467.2073 ±2393.4881 (µg/L) and 5177.1145±3267.1852 (µg/L) respectively. The mean serum ferritin level in all three groups has been more than 1000 µg/L, which point to iron overload.[37] A good cardiac diseases free survival has been observed in the patients having ferritin levels less than 2500 ηg per millilitre.[39]

Iron in NTBI start to become deposited in the organs and is unstable, serve as precursor for various chemical reactions to produce free reactive radicals as hydrogen peroxide which results the peroxidation damage to cellular components, mainly in cell membrane.[4,7,40,41] Peroxidation products have been found increased in animals and thalassaemic patients with iron overload.[18] This iron laden insult to the tissues has been monitored using the hsC-reactive proteins as biomarker of inflammation. Serum C-reactive protein concentrations closely follow the course of the acute-phase response to inflammation or tissue necrosis and theoretically provide barometer for many disease processes.[42] C-reactive proteins and cytokines have been used by a number of workers as biomarker of inflammation in thalassaemia[20] as well for other diseases as pyogenic infections including pneumonia, infective pulmonary exacerbation in cystic fibrosis,[43,44] diabetes, hepatitis and as marker for the development of cardiovascular diseases, a major morbid complication of thalassaemia.[45,46] With a high mean hsC-reactive proteins (2.5151±1.3712) in all three groups, a direct positive correlation has been found between hsC-reactive proteins and ferritin r=0.8371198, p=0.0000[47–49] and platelets r=0.43293443, p=0.000962175. It has been found in the present study that a greater proportion of the patients (61.82%) are lying in the group having C-reactive proteins concentration more than 2 µg/ml and more than 50% of them (n=18) having C-reactive proteins more than 3 µg/ml, lying in high risk group.

No relative leukocytosis has been observed with a mean white cell count of 8.1182±5.3031. Lymphocyte concentration is found slightly increased 47.8236%±13.1163 with lower neutrophils concentration 43.8455%±11.9879. This observed lymphocytosis may be a attributed to repeated transfusion as has been reported previously.[33]

CONCLUSION & RECOMMENDATIONS

It is proposed that the other molecular markers of oxidation as malondialdehyde and protein carbonyls have to be estimated, and negative stress on antioxidant may also be evaluated. This may help in understanding the ongoing inflammatory processes and a better management of these patients.

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