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Research Article

Correlation between serum ferritin level and liver function tests in thalassemic patients receiving multiple blood transfusions

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ABSTRACT

Background: Multiple blood transfusions are the mainstay of thalassemic patients in order to combat the severe anemia. These frequent blood transfusions result in the excessive iron deposition, leading to multiple injuries to a variety of organs in the body. In response to these injuries, the levels of various enzymes are disturbed. The whole phenomena usually involve the interrelation of one parameter with some other. The present study aimed to estimate the levels of serum ferritin and hepatic enzymes and to find out any possible correlation between them in thalassemic patients receiving multiple blood transfusions.
Methods: A total number of 90 thalassemic patients of both sexes ranging from 10-15 years, receiving multiple blood transfusions were included in the present study. Blood samples from all the patients were withdrawn and analyzed for the values of serum ferritin, hemoglobin and hepatic enzymes (serum alanine transaminase, serum aspartate transaminase, serum alkaline phosphatase). Pearson correlation coefficient was applied to observe correlation between serum ferritin level and hepatic enzymes. A P value of ≤ 0.05 was considered statistically significant.
Results: The overall values of serum ferritin, and hepatic enzymes (serum Alanine Transaminase, serum Aspartate Transaminase, serum Alkaline Phosphatase) were remarkably increased than their normal values. However, hemoglobin level was considerably decreased in thalassemic patients. A weak positive insignificant correlation was observed between serum ferritin with hepatic enzymes and hemoglobin in thalassemic patients.
Conclusion: Multiple blood transfusions cause iron overload in the body, which in turn, lead to increased serum ferritin levels in thalassemic patients. High levels of hepatic enzymes are somewhat correlated to serum ferritin concentration. However, the exact reason of elevated levels is still unclear. Further detailed studies should be conducted in order to identify the exact mechanism behind this and to search for the promising correlations of various parameters in thalassemic patients receiving multiple blood transfusions.

Keywords: Thalassemia, Blood transfusions, Serum ferritin, Hepatic enzymes, Correlation

INTRODUCTION

Thalassemias are the most prevalent single gene defects in human beings worldwide.[1] These refer to a group of hereditary blood disorders characterized by reduced or absent beta (β) globin chain synthesis, resulting in reduced hemoglobin (Hb) level in the Red Blood Cells (RBCs), decreased production of RBCs and severe anemia.[2] In general, thalassemias are categorized into alpha or beta thalassemia in relation to the engagement of particular polypeptide chain. The frequency of alpha thalassemia is very uncommon and found periodically in the different parts of the world. β-thalassemia, on the other hand, is the most common genetic disorder worldwide. The word thalassemia was derived from Greek word *thalas* which means sea and *emia* that stands for blood, indicating its prevalence in countries around Mediterranean region. According to thalassemia international federation, about 200000 patients of β-thalassemia are alive globally.[3] It is an alarming situation because it may become a very serious threat in next fifty years due to lack of genetic counseling and proper screening.[4]

In Pakistan, the gene frequency of β-thalassemia has been expected to be 5-8% with 8-10 million carriers.[5] A number of observations have determined the independent origin of β-thalassemia in various populations.[6] The disease is characterized by a number of signs and symptoms, but moderate to severe anemia is critical one,[7] caused by hemolysis and ineffective erythropoiesis, which are manifested by enhanced apoptosis of the maturing nucleated erythroid cells. Moreover, the anemia in this disease is microcytic and hypochromic in nature, with low Mean Corpuscular Volume (MCV) and mean corpuscular hemoglobin.[8] The other signs include skeletal and/or endocrine changes and spleenomegaly.[9] In addition, diarrhea, irritability, fever, feeding problems and gradual bulging of the abdomen due to spleen and liver enlargement are frequently observed in thalassemic patients.

Multiple blood transfusions along with chelation therapy are mainstay in thalassemic patients. Over the past few decades, regular blood transfusions and iron chelation therapies have substantially improved the quality of life, and extended the life with an average of 20 years.[10] However, these recurrent transfusions may result in iron overload and a new range of complications like cholelithiasis and hepatotoxicity could emerge in adolescents and young adults.[11]

Iron is primarily stored in the liver that possess about 70% of the total body contents.[12] Ferritin is the principal iron storage protein, present normally in the liver, spleen, bone marrow and with minute quantity in the blood, where it store iron in a non-toxic and safe form, and transport it to the target areas.[13] Owing to the multiple blood transfusions in thalassemic patients, iron load may exceed the storage and detoxifying capacity of ferritin, ultimately lead to the deposition of free iron in blood and tissues.[14] This iron burden is usually reflected by increased serum ferritin levels, the long term control of which correlates with long-term survival. Estimation of serum ferritin concentration is the index of measuring total iron in the body.[15] In previous studies, a good correlation between serum ferritin and hepatic iron concentration has been reported in multiple blood transfused thalassemic patients.[12] In addition, the correlations between serum ferritin levels and thyroid gland damage have been found to be inconclusive in the studies conducted so far. However, there is paucity of data available regarding the correlation of iron overload and liver damage in thalassemic patients. Therefore the present study has been planned to determine the level of serum ferritin and liver enzymes and to develop the possible correlation between the serum ferritin concentrations and the level of liver enzymes in thalassemic patients receiving multiple blood transfusions.

METHODS

The study was conducted on ninety thalassemic major patients of both sexes males and females ranging from 10-15 years old, selected from Allied hospital, Ali Zeb foundation, and thalassemia centre of Red Crescent Hospital, Faisalabad. The entire selected patients were receiving multiple blood transfusions with chelation therapy.

After the approval of Institutional Ethical Committee (IEC), informed consent forms were obtained from each subject. Blood samples were withdrawn from all the thalassemic patients, sera were separated in small aliquots and stored at -4°C till analysis. The samples were analyzed in the laboratories of the department of biochemistry, Punjab Medical College, Faisalabad.

The following parameters were examined in thalassemic patients.

Liver function tests

Measurement of serum alanine transaminase (ALT)

The concentrations of alanine transaminase were determined by commercially available liquiUV Test kits (Human Gesellschaft fur Biochemica und Diagnostica mbH, EC 2.6.1,2, Germany). The absorbance against water blank was recorded at A505 (Biosystem, BTS-330). The ALT concentrations of samples were calculated by kinetic method[16] in semi-automated chemistry analyzer (Microlab 300, Merck) and results were expressed as IU/L.

Measurement of serum aspartate transaminase (AST)

The AST levels were determined by commercially available liquidUV kits (Human Gesellschaft fur

Biochemica und Diagnostica mbH, EC 2.6.1.1, Germany). The absorbance against water blank was recorded at A505. The AST concentrations of the samples were calculated by using standard factor.[16]

Measurement of serum alkaline phosphatase (ALP)

The concentrations of ALP were calculated with the help of alkaline phosphatase liquicolor kits (Human Gesellschaft fur Biochemica und Diagnostica, mbH, EC 3.1.3.1, Germany), by using following formula.

U/L = 2760×ΔA 405 nm/min SEMI-MACRO

Absorbance of the sample against blank was determined at 405 nm wavelength. The reference values of ALP were taken as described by Schlebusch et al.[17]

Measurement of serum ferritin

Serum ferritin levels were determined by commercially available Accu-Bind Elisa Microwell kits (Monobind Inc. Lake Forest, CA, USA). Absorbance in each well was measured at 450nm in a microplate reader.

All the data were analyzed using SPSS® (Statistical Package for Social Sciences, version 17.0) and presented as Mean ± SEM for all quantitative variables like (ALT, AST, ALP and serum ferritin). Pearson correlation coefficient was applied to observed correlation between serum ferritin level and liver function tests. A P value ≤0.05 was considered statistically significant.

RESULTS

The average Body Mass Index (BMI) of all subjects enlisted in this study was 15.44 ± 0.28 (kg/m^2). Individual average values for male and females patients were 15.78 ± 0.42 (kg/m^2) and 15.01 ± 0.35 (kg/m^2) respectively. In general, 42 (46.7%) patients were lying in normal weight category, whereas 48 (53.3%) patients were underweight (Figure 1).

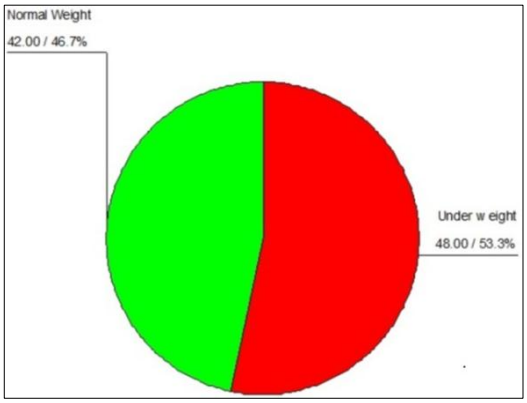

Figure 1: Body mass index of the patients.

The mean values of liver enzymes (ALT, AST, ALP) and serum ferritin are depicted in Table 1. Data from the table revealed that the mean ± S.E. values of serum alanine transaminase (ALT) were 112.10 ± 6.82 for all 90 thalassemic patients, among males and females these values were 116.03 ± 9.56 and 107.20 ± 9.71 respectively with insignificant difference (P = 0.523) between them.

Table 1: Mean ± S.E. values of different liver enzymes and serum ferritin in thalassemic patients.

Parameters	Unit	Male	Female	Total	P value
Serum alanine transaminase (ALT)	IU/L	116.03 ± 9.56 (10.30-378)*	107.20 ± 9.71 (26-253)*	112.10 ± 6.82 (10.30-378)*	0.523
Serum aspartate transaminase (AST)	IU/L	113.16 ± 8.27 (45-315)*	101.63 ± 8.02 (28-236)*	108.03 ± 5.81 (28-315)*	0.327
Serum alkaline phosphatase (ALP)	IU/L	441.88 ± 23.30 (34-807)*	462.38 ± 21.53 (216-783)*	450.99 ± 16.05 (34-807)*	0.529
Serum ferritin	µg/L	4601.65 ± 187.58 (716.33-7616)*	4996.28 ± 184.60 (2360-7070)*	4777.04 ± 133.54 (716.33-7616)*	0.143
Hemoglobin (Hb)	g/dL	7.65 ± 0.18 (4.23-10.43)*	7.88 ± 0.18 (4.53-9.97)*	7.75 ± 0.12 (4.23-10.43)*	0.354

*Denotes the minimum and maximum values

The total mean values of serum aspartate transaminase (AST) were 108.03 ± 5.81, in males (113.16 ± 8.27) and in females (101.63 ± 8.02) with no statistical difference (P value = 0.327) between them. The overall mean concentration of serum alkaline phosphatase (ALP) was 450.99 ± 16.05, with mean values 441.88 ± 23.30 for males, and 462.38 ± 21.53 for females. An insignificant (P = 0.529) difference for ALP were observed among males and females.

The average values for serum ferritin were 4777.04 ± 13 for all thalassemic patients. The concentration of ferritin

were (4601.65 ± 187.5) in males, and (4996.28 ± 184.6) in females with insignificant difference (0.143) among them.

The severity of ferritin level in all thalassemic patients is presented in Figure 2. According to the data, 85 (94.4%) patients had severe (<2500 µg/L) grade ferritin level whereas moderate (1000-2500 µg/L) and mild (<1000 µg/L) ferritin level were observed only in 4 (4.4%) and 1 (1.1%) patients respectively.

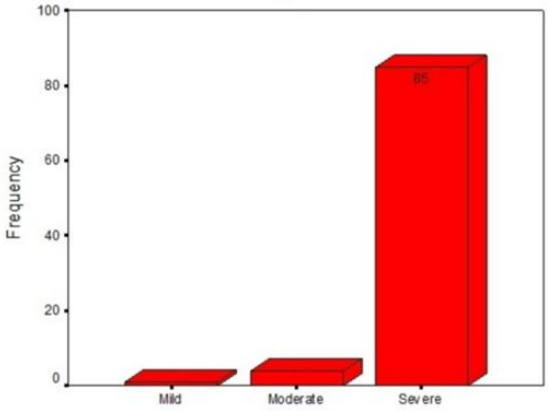

Figure 2: Severity of ferritin level in 90 thalassemic patients.

Many attempts were made to find out any possible correlation between liver enzymes (ALT, AST, ALP) and Hb with serum ferritin by performing Pearson correlation and Chi-square tests. A very weak positive correlation (Pearson correlation, 0.067) was observed between serum ferritin and Hb level, which was statistically insignificant (P = 0.266**)** (Figure 3). Similarly, weak insignificant positive correlations were observed between serum ALT (Pearson Correlation 0.097; P = 0.181), serum AST (Pearson Correlation 0.045; P = 0.335) and serum ALP (Pearson Correlation 0.036; P = 0.364) with serum ferritin levels which are shown in the figures (Figure 4, 5 and 6) respectively.

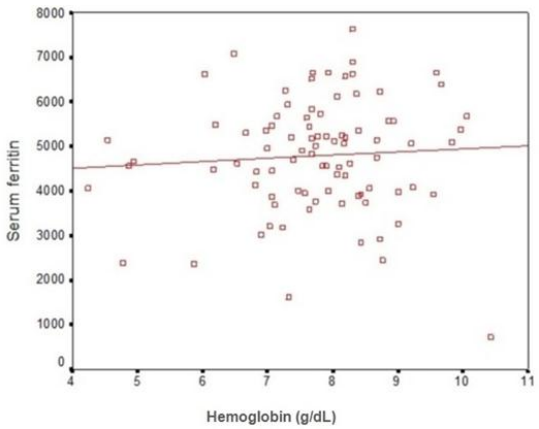

Figure 3: Scatter plot demonstrating weak positive insignificant correlation between serum ferritin and hemoglobin (Hb) levels in thalassemic patients.

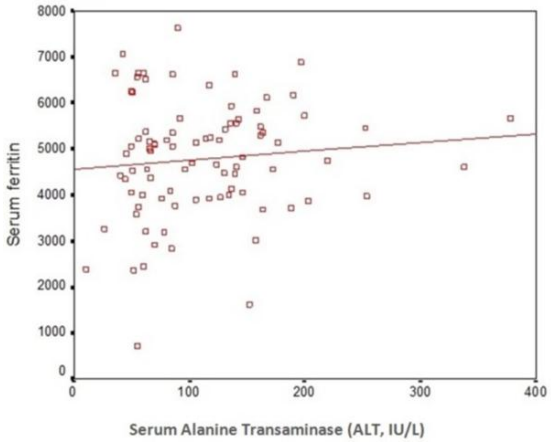

Figure 4: Scatter plot demonstrating weak positive insignificant correlation between serum ferritin and serum alanine transaminase (ALT) levels in thalassemic patients.

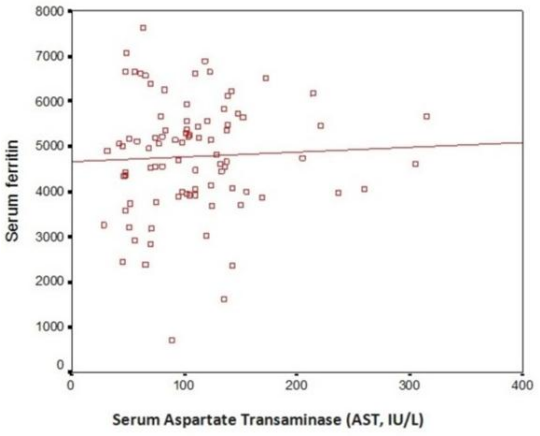

Figure 5: Scatter plot demonstrating weak positive insignificant correlation between serum ferritin and serum aspartate transaminase (AST) levels in thalassemic patients.

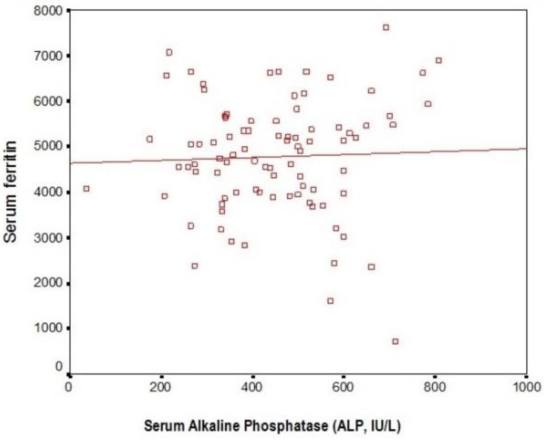

Figure 6: Scatter plot demonstrating weak positive insignificant correlation between serum ferritin and serum alkaline phosphatase (ALP) levels in thalassemic patients.

DISCUSSION

Thalassemias result due to a large number of molecular defects that may alter the expression of one or more globin genes. So far, nearly about 200 point mutations and rarely some deletions on chromosome 11 have been observed in β-globin gene.[18] The total body iron stores are maintained normally within a range of 200-1500 mg in humans with an average iron concentration of 13 mg/kg in men and 5 mg/kg in women.[19] In general, each unit of blood contains approximately 200 mg of iron, but this level is steadily increased to many times by regular blood transfusions in thalassemic patients.[20]

Previous studies revealed that the rate of iron absorption from gastrointestinal tract (G.I.T) is approximately 3-4 times greater in β-thalassemic patients than normal.[21] In non-transfused severe thalassemic patients, the abnormal iron absorption from G.I.T. leads to iron accumulation about 2-5 gm per year, which in turn depends upon the erythroid expansion. In this way, iron is stored in thalassemic patients by the transfusion-related iron overload as well as increased iron absorption from G.I.T.[21]

Iron is primarily stored in the form of ferritin in hepatocytes. Ferritin is a protein of 450 kDa consisting of 24 subunits (L and H types), which are present in almost all types of cells. Inside the ferritin shell, iron ions combine with phosphate and hydroxide ions to form crystallites complex which can store about 4500 iron (Fe^{+3}) ions.[22]

Serum ferritin is secreted in an iron-free form to a level of about 3000 µg/L from macrophages, but above this value, increasing proportions of iron-laden ferritin leaks from hepatocytes.[23] Moreover, the concentration of iron-laden ferritin reaches to many folds in the presence of any kind of infection or cancer.[24]

For estimating the iron toxicity level, the cut-off point of serum ferritin varies from 1000-3000 µg/L in different studies.[12] Furthermore, the standard values of serum ferritin level also vary to a wide range in males (10-220 µg/L) and in females (10-85 µg/L) in normal circumstances.

Serum ferritin and iron levels have very good correlation,[12] for this reason, serum ferritin concentration is generally used to estimate the iron overload in β-thalassemic patients.[25] In the current study, the average serum ferritin level (4777.04 ± 133.54) was considerably higher than its peak value (1000 µg/L) suggesting a very high iron overload due to multiple blood transfusions in thalassemic patients. Moreover, the average hemoglobin level (7.75 ± 0.12 gm/dL), on the other hand, was remarkably decreased than the average normal values (12-14 gm/dL), indicating the severe anemia in these patients. The correlation between serum ferritin and Hb level was positive though very weak and insignificant in thalassemic patients.

Alanine aminotransferase (ALT) is an enzyme belonging to the family of transaminases, produced mainly in the liver where it catalyses the transfer of amino groups between L-alanine and glutamate. The level of ALT is increased in any hepatic injury or it may be high before the appearance of hepatic diseases like jaundice.[26] Moreover, some researchers[27-30] described the high level of ALT in thalassemic patients receiving multiple blood transfusions. Similarly, we have also found the elevated levels of ALT in the current study, Hence, the results of the present study, are in line with other studies.[27-30]

Aspartate aminotransaminase (AST) is also a member of transaminase family, found in almost all tissues of the body. Typically, it is present in liver and sometimes in heart, coupled with microsomal and cell membranes[31] AST is a hydrolase enzyme responsible for removing phosphate groups from a variety of molecules including nucleotides, proteins, and alkaloids. Moreover, it is also liable for the transfer of amino and keto groups among alpha-amino acids and alpha-keto acids. AST displays its powerful activity in an alkaline environment and is particularly concentrated in liver, bones, and in lesser amounts in intestine, placenta, kidney and intestine. Higher levels are seen in extrahepatic bile obstruction, intrahepatic cholestasis, infiltrative liver disease and hepatitis.[32]

In addition, higher levels of AST are observed in thalassemic patients.[29,30] In the current study, we have found the elevated levels of AST as well. The results of the present study are thus concurrent with the results of other studies.[29,30]

The high level of serum hepatic enzymes (ALT, AST, ALP) can be correlated to some extent with the elevated serum ferritin level, which in turn, depends upon the iron overload due to multiple blood transfusions in thalassemic patients. The results of our study support this weak correlation. Hence, it can be concluded that the high levels of hepatic enzymes are possibly due to the hepatic injury, caused by iron overload in thalassemic patients receiving multiple blood transfusion. A number of researchers[2,33] have the same point of view regarding the high level of hepatic enzymes in thalassemic patients. Some investigators[2] have described the proposed mechanism of action but the exact mechanism is still unclear. Hence, further detailed studies should be conducted in order to explore the exact reason behind this in future and to find out the promising correlations in thalassemic patients receiving multiple blood transfusions.

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Conflict of interest: None declared
Ethical approval: The study was approved by the institutional ethics committee

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