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RESEARCH ARTICLE

QUALITATIVE EVALUATION OF HUMORAL IMMUNITY OF β-THALASSAEMIA PATIENTS BY IMMUNOFIXATION TEST

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ABSTRACT

Background: adaptive (humoral) immune system is one of the constituents of the immune system of β-thalassaemia patients to prevent infections; many studies described a variation of immunoglobulin levels. This study was designed for qualitative detection of abnormal band patterns, hence immunoglobulin states, in the serum of β-thalassaemia patients.
Patients and method: The study was done as a cross- sectional study; it was performed from June 2014 to September of 2014 in Diyala province/ Iraq. Simple random sampling of patients of different age groups from Thalassaemia Center was used to include patients in the study.
Immunofixation test was done at the lab of the researches at College of Medicine / Diyala University using Interlab S. r. l. Immunofixation test. The effect of many factors, including hepatitis, splenectomy, iron overload, and others, were evaluated.
Results: The total enrolled patients were 40, male gender comprises about 2 third of the enrolled patients and most of the included patients were from age group below 15 years. Immunofixation tests reveal no abnormal immunoglobulin bands were detected in all patients.
Conclusion: Patients with β-thalassaemia appear to have an intact humoral immune system, whatever the criteria of the patients.

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INTRODUCTION

The thalassaemias are the commonest inherited disorders in humans. They are a group of disorders of haemoglobin and most of them result from mutations that involve either α or β globin genes.(1) The disease in the affected patients can progress to a number of complications such as infection, cardiac failure, and liver diseases, which consequently are associated with a higher rate of mortality.(2)

Infectious complications constitute the second most common cause of mortality and a main cause of morbidity in β-thalassaemia. There are various causes of infection, including iron overload, repeated blood transfusion, splenectomy iron chelation therapy, low level of zinc, and aberration of function in the immune system. An aberration in the immunity system could increase the risk of leukemia and lymphoma.(2)

Many studies have been carried out to evaluate the possible changes of the immune system in thalassaemic patients, considering the humoral and cellular immune system ; but no consistent defect in white cells or immune function had been documented.(3) The abnormalities observed to date are both quantitative and functional, and concern several components of the immune response. More specifically, the changes in T-lymphocyte subsets include a greater number and activity of suppressor T-cells (CD8), reduced proliferative capacity, and a number and level of activity of helper T-cells (CD4) leading to decreased CD4/CD8 ratios, as well as defective activity of Natural Killer (NK) cells.(4-9) B lympocytes are characterized by increased numbers, high activation, and impaired differentiation.(4,7,9,10) Impairment of immunoglobulin secretion accompanied by increased levels of IgG, IgM, and IgA, has also been noticed.(4,11,12)

The Immunofixation kits are intended for the qualitative immunological identification of proteins (monoclonal proteins, M proteins) in human serum and urine using the Micro-gel instrument. Immunofixation Electrophoresis (IFE) is a procedure that separates the serum proteins by electrophoresis,

followed by treatment of the proteins with specific antiserum against IgG, IgA, IgM, IgD, IgE, kappa, and lambda. If A M-protein is present, a precipitation band will form. The Gel is washed with saline to extract all un-precipitated proteins, then stained, destained, and dried.[13] Immunofixation electrophoresis technique has been introduced in the laboratory in 1976 for the study and identification of polyclonal and monoclonal gammopathies.[14]

Considering the prevalence of beta thalassaemia in our country, we investigated the humoral immune system for abnormal bands as a probable cause of increased risk of infection in these patients.

PATIENTS AND METHODS

This single-center study of patients with β-thalassaemia was a cross- sectional study, it was carried out within 4 months (June 2014 to September 2014) in Diyala province/ Iraq. The total registered thalassaemia patients at the center of thalassaemia at Al- Batool teaching Hospital were 375 patients, Forty (11%) of them were enrolled in the study by simple random sampling, the acceptable margin of error of the sample size was taken at 15% of the expected 50% proportion with 95% of Confidence Interval.

The enrolled patients had different age groups, including adults. Before carrying out the present study, an official permission was undertaken from the hospital manager and an oral informed consent was taken from the participating patients/ their near relatives.

The blood samples were aspirated from thalassaemia patients just before giving new blood transfusion. The serum was isolated immediately by centrifuge and frozen at -70 c. Immunofixation test was done at the lab of researches at College of medicine / Diyala University by using Interlab S. r. l. Immunofixation test.

Immunofixation Electrophoresis

The rule of immunofixation electrophoresis is depending upon protein separation by electrophoresis, then detection of specific proteins via antigen-antibody precipitation creation. Dilutions of a specimen were placed on separate tracks (fingers) on a cellulose acetate slide six fingers shaped, and then separation of the main groups of protein by electrophoresis was done. One of the fingers of the slide is managed with a chemical fixative liquid to fixate all the proteins and form a reference pattern of electrophoresis for the sample.

The remaining fingers of the slide are immunofixed by different specificities antisera: IgG, IgA, IgM- heavy chains; anti-lambda (bound and free) and anti-kappa light chains. The resulting antigen-antibody complex will become insoluble if the proportion of antibodies to antigens is precipitated and appropriate.

The rate of precipitation is influenced by the temperature, pH, and ionic force of the solution and proportions of the reactants. After immunofixation, the slide was processed to remove excess soluble proteins through a washing step. Precipitated proteins then stained. The excess of the stain is removed by a de-staining step. The test protocol done according to the manufacturer's instructions.

The image below shows the normal pattern.[13]

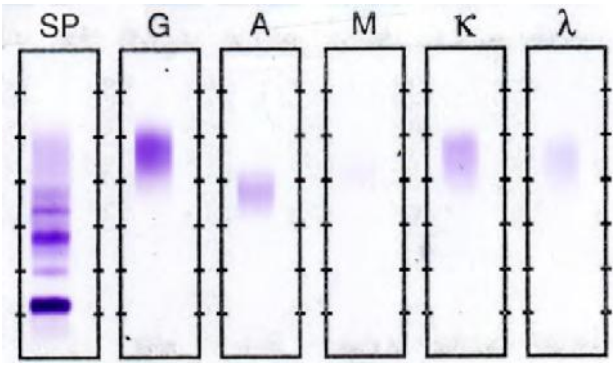

Figure 1

Studied variables

For evaluation of the effect of iron overload on Immunofixation test results, the patients were classified into two categories: 1st one include those who have a serum ferritin level < 2500 µg/L and the2nd category with serum ferritin 2nd category with serum ferritin levels ≥ 2500 µg/L.

Viral hepatitis was diagnosed serologically by enzyme linked immunosorbant assay (ELISA) by BioTeck (USA made), hence the sample was divided into hepatitis C, hepatitis B, both B and C, and non- hepatitis group, then we evaluate their effects on the results of the study. The relationship of immunofixation test results with many other criteria was also considered, including type of thalassaemia, consanguinity marriage, repeated serious infections, blood transfusion reactions, splenectomy, White Blood Cell (WBC) count, and used chelating agents.

A self- administered questionnaire was used to gather demographic information from recruiting patients.

Statistical analysis

Data were analyzed through the application of descriptive statistical analysis that include frequency and percentage by using electronic calculator.

RESULTS

The total enrolled patients were 40, male gender cover about 2 third of the whole sample, and most of them from age groups below 15 years (table 1).

Table 1 Characters of the sample of the study

Age/ sex	Male number (%)	Female number (%)	Total number (%)
< 5yr	7 (17.5)	5 (12.5)	12 (30)
5yr- < 10yr	7 (17.5)	2 (5)	9 (22.5)
10yr- <15yr	8 (20)	3 (7.5)	11 (27.5)
≥ 15yr	5 (12.5)	3 (7.5)	8 (20)
Total	27 (67.5)	13 (32.5)	40 (100)

Immunofixation tests for abnormal immunoglobulin bands were negative in all patients, so that immunoglobulin abnormalities were not detected in the current study whatever the criteria of the patients. Normal humoral immune status was obvious from these results in all types of β-thalassaemia and it was not affected by consanguinity marriage, hepatitis, and

splenectomy, unrelated to blood transfusion reactions and infections, not associated with WBC count and serum ferritin level derangement, and not affected by chelating agents (table 2).

Table 2 Relationship of results of immunofixation test with the criteria of β- thalassaemiapatients

Patient criteria		Results of immunofixation test Normal Number (%)	Abnormal [a] Number (%)	Total Number (%)
Type of thalassaemia	Major	24 (60)	0	24 (60)
	Intermedia	12 (30)	0	12 (30)
	Minor	4 (10)	0	4 (10)
	Total			
Consanguinity marriage	Positive	28 (70)	0	28 (70)
	Negative	12 (30)	0	12 (30)
	Total			
Hepatitis	Hepatitis B	4(10)	0	4 (10)
	Hepatitis C	4 (10)	0	4 (10)
	Both B and C	1(2.5)	0	1 (2.5)
	Not infected	31(77.5)	0	31 (77.5)
	Total			
Repeated serious infections [b]	Positive	0	0	0
	Negative	40 (100)	0	40 (100)
	Total			
Blood transfusion reactions	Positive	3 (7.5)	0	3 (7.5)
	Negative	37 (92.5)	0	37 (92.5)
	Total			
Splenectomy	Positive	12(30)	0	12 (30)
	Negative	28 (70)	0	28 (70)
	Total			
Leukocyte count [c]	Elevated	24 (60)	0	24 (60)
	Decreased	1 (2.5)	0	1 (2.5)
	Normal	15 (37.5)	0	15 (37.5)
	Total			
Level of serum ferritin	< 2500 µg/L	20 (50)	0	20 (50)
	≥ 12500 µg/L	17 (42.5)	0	17 (42.5)
	Missed data	3 (7.5)	0	3 (7.5)
	Total			
Used chelating agent	Deferoxamine	2 (5)	0	2 (5)
	Deferasirox	27 (67.5)	0	27 (67.5)
	Both	3 (7.5)	0	3 (7.5)
	None	8 (20)	0	8 (20)
	Total			

[A] Abnormal results indicate abnormal immunoglobulin bands by immunofixation.
[b] Any infection need hospitalization.
[c] This count was done at the same time of sample collection, taken normal value was 4000-11,000/m3

DISCUSSION

In view of the fact that adaptive (humoral) immune system is the major constituent of the immune system of β-thalassaemia patients to prevent infections; estimation of serum level of immunoglobulin of thalassaemia patients became of a great importance, on the other hand, many studies described the variation of immunoglobulin levels. This study showed that patients with β- thalassaemia appear to have an intact humoral immune system in all groups of patients with no factor affecting it.

Qualitatively, the serum levels of IgA, IgM, and IgG were normal in βthalassaemia patients in all age groups, this results are concordant to a previous study in Iran showed normal levels of IgM and IgG, but with variation of IgA according to age groups,[15] and disagreed to a prior study in Shiraz which showed an elevated levels of IgA, IgM, and IgG.[16]

It is important to mention that no any patient had a history of repeated serious infections needed hospitalalization before and during the current study.

The present study showed that removal of spleen didn't affect the state of immunoglobulin. In Turkey, a study indicated that there was no substantial alteration of the levels of immunoglobulin between splenectomized and not splenectomized groups, which is in agreement with our study. A studies on β-thalassaemia patients in India and Iran also found IgA and IgG levels in splenectomized and not splenectomized patients did not demonstrate a significant change compared with the group of control.[15,17] It was theorized that splenectomy may push the other lymphoid tissues to compensate for major immunoglobulin synthesis,[16] but it was clear that it is not practiced in the current and the above mentioned studies.

A previous study suggested that iron overload plays a role in altering the immune system of β-thalassaemia patients [18] by enhancing migration of T- helper cells to the lymph nodes and gut, leading to an increased IgG value, [19] the current study does not seem to support the idea of changing immunoglobulin qualitative analysis among groups varying in their iron burden, this was supported by Vergin *et al*. 1997 which showed that iron overload does not affect the humoral immune system of thalassemic patients.[17]

As mentioned above, there was no an alteration in serum immunoglobulin in thalassaemia patients in this study and other studies may support or contrast these findings, this probably seems to be as a result of dissimilarities of various studies in characteristics regarding the age groups, state of nutrition, and socioeconomic state of the concerned population

CONCLUSION AND RECOMMENDATION

The study showed no qualitative derangement of the humoral immune system, whatever patient's criteria; being in contrast to many previous observations. Surveillance for infections in patients with β-thalassaemia is crucial in view of impaired immunity and the tendency to infection suggested by other studies while additional studies are required for evaluation and reviewing of the constituents of the immune system, including adaptive immune system.

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