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Evaluation of Interleukins 12 and 13 Levels in Beta Thalassemia Major Patients and their Relations to Viral Hepatitis C

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Abstract

Background Several immunological defects can be found in patients with β-thalassaemia, among them is disturbance in the production of some cytokines.

Objectives To evaluate the levels of interleukin-12 and 13 and its relation with Hb, packed cell volume, serum ferritin and between them in β-thalassemic major patients with or without viral hepatitis.

Methods A case control study was conducted on 48 patients with β-thalassemia major divided into two groups; Group I comprised 24 infected with viral hepatitis C and group II comprised 24 patients with no infection; in addition twenty healthy age- and sex-matched subjects were studied as control group. Five ml of venous blood sample were collected; two ml put in EDTA tube for complete blood count and 3 ml in plain tube for biochemical lab investigations; 300 µm from the left-over serum was taken and divided into two tubes; one for estimation of interleukin-12 level and the other for estimation of interleukin-13 using ELISA Reader device.

Results All thalassemic patients with or without viral hepatitis had low level of interleukin-12 and had high level of interleukin-13. Interleukin-12 was much lower in those infected with hepatitis C virus than those with no infection and the reverse with interleukin-13.

Conclusion β-thalassemia major patients had decreased level of interleukin-12 and increased level of interleukin-13 which was more prominent in hepatitis positive thalassemic patients. Inverse correlation was noticed between interleukin-12 and 13 levels in thalassemic patients.

Key words IL-12, IL-13, β-thalassemia major, viral hepatitis.

List of abbreviation: IL-12 = Interleukin 12, IL-13 = Interleukin 13, Hb = Hemoglobin, PCV = Packed cell volume, EDTA = Ethylene diamine tetra acetic acid, CBP = Complete blood picture, HIV = Human immunodeficiency virus, HBV = Hepatitis B virus, HCV = Hepatitis C virus, ELISA = Enzyme-linked immunosorbent assay.

Introduction

β-Thalassemia is a heterogeneous hemoglobin (Hb) disorder characterized by the absence or reduced synthesis of the β-globin chain that causes globin chain imbalance [(1)]. Several immunological defects can be found in patients with β-thalassaemia, among them is disturbance in the production of some cytokines. Decreased interleukin (IL)-12 level and increased IL-13 level was documented in patients with β-thalassaemia major [(2)].

The mechanism of these abnormalities is not clarified, also the role of immunologic alternations on the clinical course of β-thalassemia is not established, although they have been considered relevant to infectious episodes that these patients suffer [(3)].

A major cause of morbidity and mortality in β-thalassemic patients is infections; these could be the results of functional alteration in the immune system due to multiple blood transfusions [(4)]. Repeated blood transfusions will

expose them to dangerous infections such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV) [5]. HCV is responsible for 80-90% of post transfusion hepatitis in β-Thalassemic patients [6].

The objectives of this study was to evaluate the levels of interleukins-12 and 13 in β-thalassemic major patients with or without viral hepatitis

Methods

A case control study was conducted on 48 patients with homozygous β-thalassemia major who were attending Al-Karama Teaching Hospital for receiving blood transfusion and treatment for the period from December 2013 to April 2014.

β-thalassemia patients were divided into two groups; group I comprised 24 infected with HCV and group II comprised 24 with no infection. In addition, twenty healthy age- and sex-matched subjects were included in the study as control group. Peripheral venous blood sample was collected from anticubital fossa from each patient and control subjects. Patients sample were collected just before transfusion. Two ml put in EDTA tube for complete blood count and three ml was collected in the plain tube, which then immediately centrifuged for 10 minutes at 3000 rpm to separate the serum. From the leftover sample, 1 ml of serum was taken and divided into two tubes; one for estimation of IL-12 level and the other for estimation of IL-13, and they were stored at -20^{o}C in central lab. of Al-Nahrain Medical College until time of assay of IL-12 and IL-13. One hundred µl of serum was taken for IL-12 and other 100 µl for IL-13 assay by using ELISA kit applies a technique called a quantitative sandwich immunoassay. The microtiter plate provided in this kit has been pre-coated with an antibody specific to IL-12 and the other specific for IL-13; and polyclonal antibody preparation were used manufactured by CUSABIO BIOTECH co.

The procedure was carried out in accordance with the manufacturer's instructions. Standards or samples are then added to the appropriate microtiter plate wells with a biotin-conjugated polyclonal antibody preparation specific for IL-12/P40 and Avidin conjugated to Horseradish Peroxidase (HRP) is added to each microplate well and incubated. Then a TMB (3, 3', 5, 5'tetramethyl-benzidine) substrate solution is added to each well. Only those wells that contain IL-12/P40, biotin-conjugated antibody and enzyme-conjugated Avidin will exhibit a change in color.

The enzyme-substrate reaction is terminated by the addition of a sulphuric acid solution and the color change is measured at a wavelength of 450 nm±2nm spectrophotometrically.

The concentration of IL-12/P40 in the samples is then determined by comparing the O.D. of the samples to the standard curve. The same procedure was done for IL-13 except that the microtiter plate provided in this kit has been pre-coated with an antibody specific to IL-13 and then the same steps will be done.

Statistical analysis of data was performed with SPSS version 21 (2013) and Excel professional edition 2010 programs.

Results

The mean value of IL-12 in thalassaemic patients was lower in group I and group II than controls and it was much lower in patients belongs to group I than in those in group II ($P < 0.05$); accordingly 15 out of 24 patients (62 %) of group I versus 2 out of 24 patients (8%) of group II had markedly reduced IL-12 level (Table 1 and fig. 1). While 7 out of 24 patients (29%) of group I versus 10 out of 24 patients (41%) of group II had moderately reduced IL-12 level and only 2 out of 24 patients (8%) of group I versus 12 out of 24 patients (50%) of group II had minimal reduction of IL-12 level (Table 2 and fig. 3).

Table 1. Interleukins 12 and 13 in β-thalassaemia patients and control groups

Interleukin (pg/ml)	Group I mean±Sd N=24	Group II mean±Sd N=24	Controls mean±Sd N=20	P1	P2	P3
12	3.52 ± 2.2	6.32 ± 1.3	18.45 ± 6.41	0.033	<0.001	<0.001
13	1089.1 ± 618.4	864.5 ± 493.1	14.08 ± 66.3	0.11	<0.001	<0.001

P1: Group I vs. Group II, P2: Group I vs. controls, P3: Group II vs. controls

Table 2. Distribution of reduction in IL-12 categories according to the IL-12 quartiles

Reduction in IL-12	Group I		Group II		P Value
	No.	%	No.	%	
Markedly reduced	15	62.5	2	8.3	< 0.001
Moderately reduced	7	29.3	10	41.7	
Minimally reduced	2	8.3	12	50.0	
Total	24	100	24	100	

The mean level of IL-13 was significantly lower in controls than in group I and in group II ($P < 0.05$) and it was much higher in group I than in group II (Table 1 and fig. 2); accordingly 11 out of 24 patients (45 %) of group I versus 6 out of 24 patients (25%) of group II had markedly high IL-13 level.

Eight out 24 patients (33%) of group I versus 7 out of 24 patients (29%) of group II had moderately high IL-13 level and 5 out of 24 patients (20%) of group I versus 11 out of 24 patients (45%) of group II had minimal increase in IL-13 level (Table 3 and fig. 4).

Table 3. Distribution of increment in IL-13 categories according to the IL-13 quartiles

Increment in IL-13	Group I		Group II		P Value
	No.	%	No.	%	
Markedly High	11	45.8	6	25.0	0.15
Fairly high	8	33.3	7	29.2	
Minimally high	5	20.8	11	45.8	
Total	24	100	24	100	

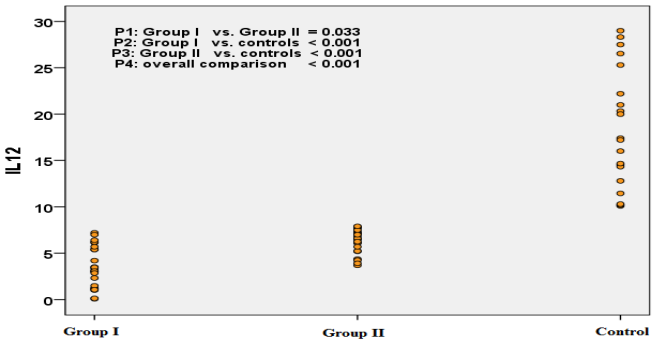

Fig. 1. Distribution of IL12 among the studied groups

There was an inverse insignificant correlation between serum ferritin and IL-12 in both studied thalassemic groups, in hepatitis C positive and in hepatitis C negative and positive insignificant correlation between serum ferritin and IL13 in both studied thalassemic groups, in hepatitis C positive and in hepatitis C.

Discussion

The current study showed that mean serum level of IL-12 in groups I and II β-thalassemic patients.

This may be attributed to many factors such as splenectomy, iron overload, repeated exposure to foreign antigens at the time of blood transfusion and the use of chelating agents may induce profound deleterious effects on the immune cells that secret this cytokines in β-thalassemia patients [2,3,7].

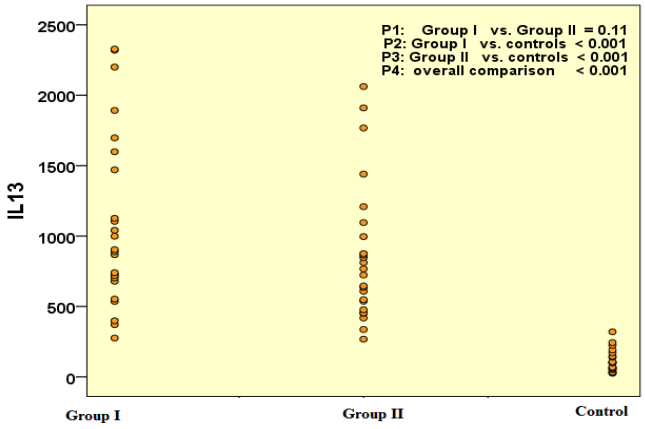

Fig. 2. Distribution of IL-13 among the studied groups

The decrement in the level of IL-12 in β-thalassemic patients with HCV infection as compared to those who were free from infection may be attributed to that HCV infection may suppress IL-12 production [2,8-10]. The inverse, yet, insignificant correlation between serum ferritin and IL-12 in both β-thalassemic groups may be ascribed to that iron overload per se had a negative effect on IL-12 p40 gene expression in neutrophils [11].

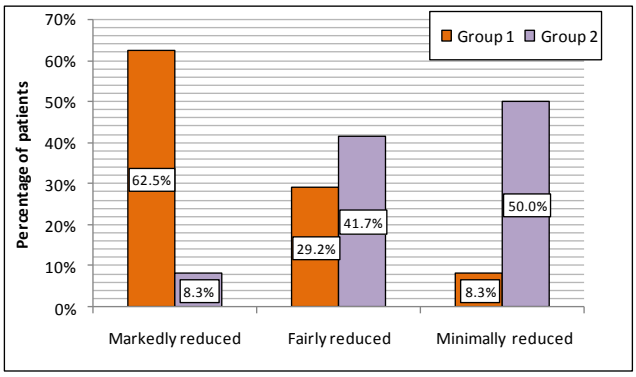

Fig. 3. Distribution of patients according to the reduction in IL-12 level

The current study showed that mean serum level of IL-13 was significantly higher in both groups of β-thalassaemia than that in controls, this could be ascribed to that in thalassemia the chronic transfusion program will result in continuous antigenic stimulation and iron overload with consequent abnormality in cell mediated immunity such as reduce CD4/CD8 ratio, T-cell subset anomalies and alteration in T-cell number and function [2,3,7]. This iron overload will induce oxidant stress and inflammation and will lead to organs injury [12,13]. Since IL-13 is an anti-inflammatory cytokine so its secretion will increase as aconsequence to inflammatory processes that occurs in thalassemia. In the current study serum iron along with IL-13 were higher in group I compared to group II; this may be attributed to the oxidative effect of iron overload which increase the secretion of anti inflammatory cytokines (IL-13) [12,13].

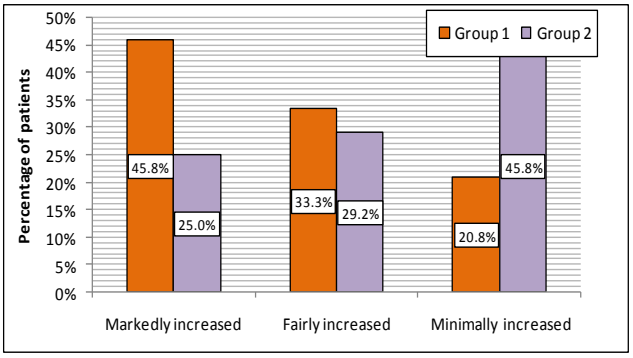

Fig. 4. Distribution of patients according to the increment in IL-13 level

There was positive insignificant correlation between serum ferritin and IL-13 in both studied thalassemic groups. The oxidative effect of iron overload in thalassemia can initiate tissue injury and/or inflammation, and this may result increase the secretion of anti inflammatory cytokines (IL-13) [12,13].

In conclusion, β-thalassemia major patients had decreased level of IL-12 and increased level of IL-13 which was more prominent in hepatitis positive thalassemic patients. There was a significant inverse correlation between IL-12 and IL-13 level in β-thalassemic patients.

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Author contributions

Conception and design, data collection, analysis and interpretation, writing and revision of the manuscript by Hashim; collection of cases and lab investigations, checking medical and surgical history by Dr. Alrawi and Hashim; and evaluating the levels of interleukins 12 and 13 by Jamal and Hashim.

Conflict of interest

None.

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