Thalassemia is described as a heterogeneous group of autosomal recessive inherited anemia resulting from either reduced level or absence of the globin chains of hemoglobin (Hb) that form the heterotetrameric structure. Thalassemia is classified into the following 2 main subclasses on the basis of Hb structure: (a) $\beta$-thalassemia, which result from functional mutations in genes that code for $\beta$-globin genes and (b) $\beta$-thalassemia, which result from mutations in genes that code for $\beta$-globin genes.1,2

Several parameters and statuses such as carrier identification, genetic counseling programs, and prenatal diagnosis are required for thalassemia prevention.3 The incidence of symptomatic individuals is estimated at 1 in 100 000 and 1 in 10 000 worldwide and in the European Union countries, respectively.4

Thalassemia is prevalent in different geographical regions and ethnic groups. The disorder is mostly prevalent in the Eastern Mediterranean, South Asia, and South East Asian countries.5

According to the latest reports from the Prenatal Diagnosis Team for Prevention of Thalassemia program of Iran, approximately 4% to 5% of Iranians are the carriers of $\beta$-thalassemia. This rate is more in some provinces, for example, in Mazandaran—a northern province of Iran, where about 10% of the population are carriers.6

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Overproduction of CXC chemokines CXCL1, CXCL9, CXCL10 and CXCL12 in $\beta$-thalassemia major or patients

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BACKGROUND AND OBJECTIVES: $\beta$-thalassemia major is one of the most frequent hematological genetic disorders, worldwide. Chemokines are the main components of the immune system and play fundamental roles in pathogenesis of inflammatory disorders. Therefore, the present study aimed to examine whether serum CXC chemokines are altered in $\beta$-thalassemia major patients.

DESIGN AND SETTINGS: We enrolled 63 $\beta$-thalassemia patients and 80 controls in this cross-sectional study, which was performed during 2012-2013 in Kerman, Iran.

METHODS: We enrolled 63 $\beta$-thalassemia patients and 80 controls in the present study. Patients were selected from referrals to Samenolhojaj clinic for thalassemia, Kerman, Iran. The circulating levels of CXCL1, CXCL9, CXCL10, and CXCL12 were detected by enzyme-linked immunosorbent assay in thalassemia patients and healthy controls immediately after blood collection. Data were analyzed by $\chi^2$, t-test, and analysis of variance statistical methods and using SPSS, version 13 (SPSS Inc., Chicago, IL).

RESULTS: The results of the study demonstrated a significant elevation of CXCL1, CXCL9, CXCL10, and CXCL12 in thalassemia patients than in control. These results also demonstrated that serum chemokine levels are related to transfusion duration and post-transfusion viral infections.

CONCLUSION: According to the results obtained, it can probably be concluded that chemokines are also involved in the pathogenesis of $\beta$-thalassemia major and its clinical complications in addition to several other parameters.
Infection and immune system abnormalities are most often considered as the main causes of morbidity and mortality in β-thalassemia. A wide range of immune abnormalities are associated with β-thalassemia in patients with a history of multiple transfusions.7-12 Multiple transfusions are responsible for immune abnormalities. In addition to causing iron overload, repetitive transfusions lead to continuous alloantigenic stimulation. Additionally, transfusion increases the risk of transmission of viruses with immuneosuppressive properties, such as cytomegalovirus, human immunodeficiency virus, Ebstein–Barr virus, hepatitis B virus (HBV), and hepatitis C viruses (HCV).13 Chemokines play key roles in pathogenesis of inflammatory disorders of the immune system.14,15 They are functionally active in several important biological activities, which vary from recruitment of immune and hematological cells to the injured and infected tissues, stem cell homing, and mobilization to making balance between angiogenesis and angiostasis. Chemokines are classified into the following four subclasses according to the position of cystein motifs in their structure: C, CC, CX3C, and CXC.16-20 Although, elevated serum levels of TNF-α and IFN-α (critical molecules involved in inflammatory responses) are well evidenced in thalassemia,21,22 sufficient information regarding the role of chemokines is not available in pathogenesis of thalassemia. Therefore, we hypothesized that a relationship between serum chemokines and inflammatory responses in thalassemia patients may be established because of the presence of active inflammation in these patients. Thus, the present study aimed to determine the serum levels of chemokines CXCL1, CXCL9, CXCL10, and CXCL12 in β-thalassemia major patients.

METHODS

Study subjects

In the current study we enrolled 63 β-thalassemia major patients and 80 controls. The patients were selected from referrals to Samenolhojaj especial clinic for thalassemia, Kerman, Iran. All patients suffered from the homozygous form of β-thalassemia major, and regularly received transfusion to maintain their Hb at a balanced level. Peripheral blood specimens were collected approximately 24 hours before transfusion with washed cells to be sure that the circulating blood belongs to the patient and is not the transfused blood. Patients and controls with any acute illness or pathological injuries were not enrolled in the study. This project was approved by the regional ethical committee of Rafsanjan University of Medical Sciences. Written consent forms were also filled out by the parents of all patients and controls prior to sample collection.

Chemokine assay

The circulating levels of CXCL1, CXCL9, CXCL10, and CXCL12 were detected by enzyme-linked immunosorbent assay (R&D systems, UK) in thalassemia patients and healthy controls immediately after blood collection. Ninety-six–well EIA/RIA (enzyme immunoassay/radio Immunoassay) plates were coated with a capturing monoclonal antibody against CXCL1, CXCL9, CXCL10, and CXCL12 chemokines and were then blocked with a mixture of 1% bovine serum albumin (BSA). The supernatants were diluted in reagent diluents (1% of BSA) and incubated for 24 hours at room temperature. The detection antibody was then diluted in reagent diluents and incubated for 2 hours at room temperature. Antibody binding was detected with streptavidin-conjugated horseradish peroxides and developed with a substrate solution. A standard curve was generated for each set of samples assayed and was made from 7 points of a twofold dilution series. Each standard or sample was assayed in duplicate. The sensitivity of kits was 2 pg/mL, and inter- and intra-assay assessments of reliability of the kits were conducted.

Statistical analysis

The statistical analysis of the differences between groups was determined by χ², t test, and analysis of variance using SPSS, version 13 (SPSS Inc., Chicago, IL) to achieve a power of 90%. A P value of less than .05 was considered significant. The statistical analysis of the demographic characteristics demonstrated no marked difference between the mean age and gender of the participants. The average age of the patients and controls was 16.1 (7.1) and 16.1 (6.9) years, respectively (P=.85). Of the patients, 37 were female and 26 were male. In the control group, 36 cases were female and 44 were male (P=.9), (Table 1). No significant difference was observed in age and gender between patients and controls. The results of the present study demonstrated that all of the studied CXC chemokines (CXCL1, CXCL9, CXCL10, and CXCL12) were significantly elevated in thalassemia patients than in control. Data analysis revealed statistically significant differences between β-thalassemia patients and controls in all of the studied chemokines. The mean serum level of CXCL10 was 273.4 (76.3) pg/mL and 116.79 (33.53) pg/mL in β-thalassemia patients and controls, respectively. A significant difference was observed between patients and controls (P<.05; Figure 1). The results of our study also indicated that the...
Table 1. Demonstrates some of clinical characteristics of study participants, either patient or control.

| Variable             | Thalassemia patients | Control |
|----------------------|----------------------|---------|
| Age (y)              | 16.1 (7.1)           | 16.1 (6.9) |
| Gender               | Male 37              | 44      |
|                      | Female 26            | 36      |
| Familial history     | 17.3 (2.0)           | -       |
| Alcohol abuse        | 5                    | -       |
| Hb (g/dL)            | 9.5 (0.9)            | -       |
| Desferrioxamine      | All                  | -       |
| treatment            |                      |         |
| Transfusion interval (d) | 29 (6)            |         |
| Transfusion volume (mL) | 450 (60)           | -       |
| Iron                 | 130.5 (37.2)         | -       |
| TiBc                 | 237.6 (102)          | -       |
| Ferritin             | 3129.6 (1973.5)      | -       |

The mean serum level of CXCL1 was 248 (113.1) pg/mL and 156.3 (34.5) pg/mL in β-thalassemia patients and controls, respectively (P<.03, Figure 2).

The mean serum level of CXCL12 was 292 (11.1) pg/mL, 276.3 (15.2) pg/mL, 253 (18.1) pg/mL, and 162 (112.1) pg/mL, respectively, in thalassemia patients who were transfused for more than 100 months. The statistical analysis of data showed a significant difference between chemokine serum levels in patients transfused for less and more than 100 months’ duration (Table 2). No difference was observed when the patients were compared for the post-transfusion HCV and HBV infections (Table 2).

DISCUSSION

The present study was undertaken to evaluate the circulatory levels of some CXC chemokines in β-thalassemia patients and age- and gender-matched controls. The study showed elevated serum concentrations of CXCL1, CXCL9, CXCL10, and CXCL12 in thalassemia patients than in age- and gender-matched controls. This supports previous reports showing elevated serum levels of TNF-α, IL-1α, and IFN-α in thalassemia patients. A correlation was also reported between the severity of clinical symptoms and the cytokines level in β-thalassemia patients. In Iran, thalassemia patients were previously transfused with packed red blood cells, which sometimes is contaminated with other biological and cellular fractions including, leukocytes, plasma protein deposits, fragmented cells, and several other unknown materials. These are all antigenic and are able to stimulate the cellular immune system, which may lead to an elevated chemokine expression by tissue and cellular sources. Some of the previous studies
showed that reactive T-cells are an enriched source of CXC chemokines, and some studies revealed an increased number of these cells in β-thalassemia patients. Elevated absolute counts and percentages of cells with CD3+/DR+, CD3+/CD25+, and CD3+/CD71+ phenotypes were observed in these patients, supporting our finding that the levels of chemokines. This study established a relationship between the transfusion intervals and the level of chemokines, revealing that chemokines increased if the patients had received more transfusions. This probably could be because of sensitization and further chemokine production by immune cells, bone marrow, and reticuloendothelial system (macrophages, dendritic cells) because these cells are known as the most important sources of cytokines such as TNF-α, IFN-α, and chemokines. This study confirms our results that showed elevated levels of pro-inflammatory CXC chemokines (CXCL1 and CXCL10) in response to TNF-α in vitro cultures. The increased concentrations of these chemokines in thalassemia patients may also be attributed to the regulatory role played by TNF-α in up-regulation of chemokine. Moreover, Kori et al. claimed a significant increase in both absolute numbers and percentages of activated T and NKT cells in thalassemia patients (especially elderly patients). Thus, the simultaneous elevation of these cell phenotypes and levels of CXC chemokine might possibly be related to each other. Therefore, massive transfusion, especially in elderly patients, probably increases the risk of multiple exposures to different antigens—in fact, an elevated chemokine level. Again, Salasa and co-workers showed that peripheral blood mononuclear cells from thalassemia patients secrete more IFN-α than the control group following stimulation. Having chronic HCV infection or treatment with IFN-α could be another explanation for increased IFN-α production by stimulated lymphocytes. IFN-α, in turn, probably up-regulates the IFN-α–inducible chemokines in these patients. Although, IFN-α is known as the most potent inducer of CXCL9 and CXCL10 synthesis, the production of these chemokines is co-stimulated by TNF-α and lipopolysaccharides.

It is widely evidenced that in anemia, hematopoietic stem/progenitor cells express a subset of surface receptors for inflammatory cytokines, and several studies reported that the direct action of cytokines on hematopoietic cell lines in vitro can impair erythroid lineage development and further decrease erythroid progenitor cells. This hypothesis could probably be extended to proinflammatory chemokines, which need to be analyzed in further investigations. Hence, it...
Table 2. Demonstrates the chemokines serum levels in accordance to the transfusion intervals and post-transfusion infections. HBV: Hepatitis B virus, HCV: Hepatitis C virus.

| Variable                        | CXCL1 (pg/mL) | CXCL9 (pg/mL) | CXCL10 (pg/mL) | CXCL12 (pg/mL) |
|---------------------------------|---------------|---------------|---------------|---------------|
| Duration of transfusion (mo)    |               |               |               |               |
| Less than 100                   | 212 (13.1)    | 147.3 (17.9)  | 146.8 (33.5)  | 106.2 (49.1)  |
| More than 100                   | 292 (11.1)    | 276.3 (15.2)  | 253 (18.1)    | 162 (112.1)   |
| Type of infection               |               |               |               |               |
| HCV                             | 293 (18.2)    | 348 (16.2)    | 278.2 (18.5)  | 276 (48)      |
| HBV                             | 303 (28.3)    | 329.4 (31.2)  | 261.3 (63.2)  | 251.3 (51.6)  |

*Significant difference with patients received less than 100 mo transfusion.

is necessary to analyze more precisely the expression of specific receptors for these chemokines, e.g., CXCR1, CXCLR3, and CXCR4, on progenitor cells in thalassemia patients.43

Conflict of interest
None of authors of this study claimed conflict of interest.

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