ORIGINAL RESEARCH **PERIOPERATIVE MEDICINE**

Assessment of serum fractalkine level as potential role for complications in splenectomized and non-splenectomized patients with β-thalassemia major

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ABSTRACT

Introduction: Thalassemia is a serious genetic blood related condition characterized by a lack of or malfunctioning hemoglobin in the RBCs. One of the most widespread inherited disorders is beta thalassemia (β-TM). The high susceptibility to infection in these individuals' is related to high mortality and morbidity rates. Fractalkine is known as CX3C chemokine ligand 1 (CX3CL1), which contains a total of 371 amino acid, and participates in a number of functions, including repair of tissues after injury, immunological response during inflammation, and chemotaxis effet.

Methodology: A case control study, containing sixty patients recognized as suffering from β-TM, was conducted at Al Zahra Teaching Hospital, Najaf (Iraq). The patients were divided into two groups; splenectomized and non-splenectomized patients. The thalassemia disease was enrolled in the "Thalassemia Unit". Suitable statistical techniques were used to analyze the outcomes.

Results: In the current study, a significant increase in serum level of CX3CL1 was found in patients with β-TM, especially in splenectomized group (14.914 ± 2.636), (P = 0.01) and non-splenectomy (13.816 ± 2.686), (P = 0.05) group as compared with control group (12.26 ± 1.797).The linear regression analysis showed that a significant positive correlation in ferritin with serum CX3CL1 level in splenectomy patients with β-TM group. ROC curve for CX3CL1 that might be diagnosis of patients with β-TM with an AUC of 0.757 (95% CI: 0.623-0.890), sensitivity and specificity equally 75% and 83.3%.

Conclusion: Increase level of CX3CL1 due to its interaction with CX3CR1 receptor may lead to buildup of inflammatory cells in the β-TM patients, blocking CX3CL1 signaling may be helpful for patients with β-TM.

Abbreviations: AUC – Area Under Curve; β-TM - Beta Thalassemia; ROC - Receiver Operating Characteristic UIBC - Unsaturated Iron-Binding Capacity test; TIBC - Total Iron-Binding Capacity

Key words: Thalassemia, β thalassemia major, Fractalkine

1. INTRODUCTION

Thalassemia is a serious genetic condition of the blood, brought on by a lack of or malfunctioning hemoglobin. RBCs contain the crucial protein Hb.[1] One of the most

widespread inherited disorders in the entire globe is beta thalassemia (β-TM). Due to their high susceptibility to infection, these individuals' mortality and morbidity rates will rise if they contract an infectious condition. Repeated blood transfusions and iron chelation are the primary treatments for β-TM, while in certain people, a bone marrow transplant could be used to reverse the illness.[2]

Fractalkine, well-known as CX3C chemokine ligand 1 (CX3CL1), is produced as a 371 amino acid transmembrane protein that includes an 18 amino acid hydrophobic transmembrane region, a 37 amino acid intracellular C-terminal domain, a 241 amino acid glycosylated mucin-like stalk, as well as a 76 amino acid N-terminal chemokine domain. CX3CL1 is largely expressed in endothelium and smooth muscle cells, as well as neurons and epithelial cells in the lung, kidney, and gut tissue. CX3CL1 participates in a number of functions, including repair of tissues, immunological response, response to inflammation, and chemokine cell orientation.[3]

A chemokine CX3CL1 that functions as immune response and adhesion across its specific receptor on CX3CR1-expressing immune cells. A membrane-bound form and a soluble form of CX3CL1 are both feasible and can be modified into one another in vivo. The membrane-bound form of CX3CL1 undergoes conversion into a soluble form during inflammation. Immune cells cluster at the site of action as a result of CX3CL1 mobilization. When tissue injury occurs, CX3CL1 functions as adhesion molecules and chemical primers to manage the initial development of inflammatory disorders. In the pathological processes underlying atherosclerosis, osteoarthritis, and muscular injury, CX3CL1 plays crucial roles.[4,5]

Ferritin is the primary protein in the body that stores iron. Its synthesis is influenced via the presence of iron through interacting of cytoplasmic proteins bound to mRNA, which have been designated as iron regulatory proteins, and particular mRNA structural elements referred to as iron-responsive elements. Due to its ability to attach and to sequester intracellular iron, it plays an imperative role of preserving iron homeostasis. Regardless of whether iron excess is present or not, elevated serum ferritin levels are reported in a wide range of inherited and acquired diseases. In general, iron overload is indicated by rising ferritin levels along with rising transferrin saturation.[6]

Splenectomy was frequently performed to preserve greater hemoglobin levels. Clinical practice has progressively shifted to limit splenectomy to extremely specific triggers which include poor growth and development, hypersplenism with symptomatic leukopenia and or thrombocytopenia, or symptomatic huge splenomegaly. This is due to the greater risk of morbidity that comes with splenectomy. An increased risk of serious infections, including the potentially fatal post-splenectomy sepsis, is associated with splenectomy. Pneumococcal 23-valent polysaccharide, Hemophilus influenzae, and meningococcal polysaccharide vaccinations must be given to patients who will have splenectomies at least two weeks prior to the surgery. Splenectomy elevates the risk of hypercoagulability and a related increase in the occurrence of pulmonary hypertension, silent cerebral infarcts, venous thromboembolism and leg ulcers, for the reason that the spleen destroys procoagulant RBCs and platelets. Splenomegaly is brought on by extra-medullary hematopoiesis and raised RBC degradation, both of which raise demand for transfusions. In order to prevent this problem, splenectomy is frequently done, which lowers the necessity for blood transfusions. Splenomegaly and related adverse effects typically take longer to become noticeable with the highest level of care, pushing back the requirement for splenectomy into the second decade. It is well recognized that splenectomy may outcome in short-term as well as long-term issues like infections, hypercoagulability, and thrombosis. The existence of splenunculi (accessory spleens), which get bigger post splenectomy, may cause repetition of anemia, hence it is important to find and take out them following surgery.[7]

We assessed immunological inflammatory marker CX3CL1 and its interaction with thalassemia development, especially after splenectomy, which can provide novel methods for the therapy of the disease and its complications.

2. METHODOLOGY

A case control study design, involved sixty patients recognized as suffering from β-TM, and divided into groups containing either splenectomized or non-splenectomized patients. The treatment was based on blood transfusion in both groups. There were 15 males and 15 females in splenectomized group (Group S); and 12 males and 18 females in non-splenectomized group (Group NS). The thalassemia disease was diagnosed in the 'Thalassemia Unit' of Al Zahra Teaching Hospital, Najaf, from January 2022 to March 2022. The ages ranged from 7 to 20 y. Patients with no other disease were selected.

Another thirty healthy subjects, were carefully chosen as a control group (Group C), whose ages and genders were comparable. This group of patients included 11 males and 19 females. The inclusion criteria were: (1) age between 7 and 20 y; (2) Body Mass Index (BMI) between 25 and 30 kg/m^2; and (3) no ongoing drug therapy.

Table 1: Comparison between β-TM patients with control group				
Parameters	Group C (G1) (n = 30)	Group S (G2) (n = 30)	Group NS (G3) (n = 30)	P-value
Age (Y)	16.27 ± 4.118	16.80 ± 4.444	16.23 ± 5.066	P1 = 0.746 P2 = 0.728 P3 = 0.57
BMI (kg/m^2)	23.85 ± 4.059	17.87 ± 5.557	16.27 ± 4.062	P1 = 0.190 P2 = 0.001 P3 < 0.001
Ferritin (ng/mL)	122.26 ± 45.23	3987 ± 1519.4	2855.1 ± 1416.3	P1 = 0.001 P2 = 0.001 P3 < 0.001
Iron (µmol/L)	22.15 ± 5.16	53.89 ± 17.11	39.09 ± 7.88	P1 = 0.005 P2 = 0.005 P3 = 0.001
TIBC (µmol/L)	70.01 ± 9.35	60.98 ± 9.98	84.05 ± 11.13	P1 = 0.001 P2 = 0.001 P3 = 0.003
UIBC (µmol/L)	46 ± 12.87	32.08 ± 16.10	27.75 ± 15.43	P1 = 0.05 P2 = 0.001 P3 = 0.04
TS%	36.33 ± 12.06	64.74 ± 17.03	61.11 ± 12.45	P1 = 0.05 P2 = 0.001 P3 = 0.001
Transferrin (g/L)	0.18 ± 0.033	0.3 ± 0.01	0.16 ± 0.03	P1 = 0.01 P2 = 0.65 P3 = 0.01
CX3CL1 (ng/mL)	12. 26 ± 1.797	14.914 ± 2.636	13.816 ± 2.686	P1 = 0.062 P2 = 0.05 P3 = 0.01

Data represented as Mean ± SD; SD= Standard deviation; CX3CL1= Fractalkine, P1 = P-value(G2×G3), P2 = P-value (G1×G3), P3 = P-value (G1×G2).

Blood samples were taken and placed in a serum separator tube. The samples were then allowed to clot for 15 min at room temperature before being centrifuged for 15 min at 3000 X. Serum samples were then kept at 20 °C. Using a kit of common enzymatic techniques, the concentration of serum iron was determined. An enzyme-linked immunosorbent assay (kit) was used to measure the levels of serum ferritin and CX3CL1. A specific equation was used to calculate the BMI.

Statistical analysis

Means and standard deviations are used to express all data. The statistical data was analyzed using SPSS 26. The differences between groups were compared using one-way ANOVA and Fisher's least significant difference (LSD) test.   P ≤ 0.05 was considered significant. The relationship between CX3CL1 and biochemical parameters was determined using Pearson's correlation coefficient. We used receiver operating characteristic (ROC) - area under curve (AUC) for diagnosis of β-TM via CX3CL1 marker.

3. RESULTS

The study consisted of 60 enrolled patients and 30 healthy subjects. Their mean age, BMI, iron status, and CX3CL1 are summarized in Table 1, with non-significant differences in age in all the study groups. BMI had a significant difference between patient groups (Groups S and NS) and Group C.

We found a significant increase in the ferritin, iron, TS% and CX3CL1 levels, and significantly lower unsaturated iron-binding capacity (UIBC) test in patients with β-TM, in the Groups S and NS when compared to the Group C.

transforming growth factor beta-1 (TGF-β1) as well as reactive oxygen species.[14]

The sensitivity and specificity of CX3CL1 equals 75% and 83.3%, respectively, with an AUC of 0.757 (95% CI: 0.623-0.890; P = 0.002), and cut-off value 12.099 (ng/mL) of serum CX3CL1 in patients with β-TM.

The study found a significant increase in the total iron-binding capacity (TIBC) level (P = 0.001), while ferritin, iron, UIBC, TS% and transferrin revealed a significant decrease in Group S compared with Group NS. The linear regression analysis showed that a significant positive correlation in serum level ferritin, while BMI have negative correlation with serum CX3CL1 level in Group S, as revealed in Table 2, also in Table 3 we found a positive correlation in Group NS of serum ferritin, iron, TIBC, and TS% with CX3CL1; while, age, BMI, UIBC and transferrin found a negative correlation with CX3CL1 level.

However, as shown in Table 4 and Figure 2, the ROC curve for CX3CL1 can help in appropriate diagnosis of CX3CL1,[12] which may drive the migration of CX3C chemokine receptor type 1 (CX3CR1)⁺ non-classical monocytes (NCM) to the lungs, which sustains the local fibrotic process;[12] CX3CR1 must be present to effectively and repeatedly patrol the luminal side of the vasculature and eliminate cell injury and debris.[13] Functional tests showed that

4. DISCUSSION

In this study we found the increase level of serum CX3CL1 in children with β-TM. As far as the best of our knowledge it was the first study about the co-localized actions of CX3CL1 with epithelial cells, leading to a chemo-attractant gradient and accelerated non-classical monocyte endothelial transmigration, additionally serving as an autocrine and paracrine factor that contributes to the oxidative stress as well as inflammation.[8]

Thalassemia is the most prevalent inherited genetic illness, which can be fatal or extremely disabling; for which there is no effective treatment yet.[9] Hemolysis and insufficient erythropoiesis are both features of the genetically inherited hemoglobinopathy commonly referred to as β-thalassemia. The β-thalassemia major (TM) and β-thalassemia intermedia (TI), respectively, are created via a total or partial reduction in the synthesis of β-globin chains.[10] Lifelong red blood cell transfusions, iron chelation, splenectomy, or allogeneic hematopoietic stem cell transplantation (HSCT) are all necessary for the treatment of β-thalassemia. Vascular endothelial cells express the protein CX3CL1, and pro-inflammatory cytokines like tumor necrosis factor (TNF), interleukin-1 (IL-1), and interferon (IFN) strongly promote its expression. Perforin and granzyme

**Table 2: Correlation Between CX3CL1 with parameters in patients of Group S**

Variables	r	P-value
Age (y)	0.070	0.630
BMI (kg/m²)	-0.322*	0.022
Ferritin (ng/mL)	0.442**	0.001
Iron (μmol/L)	0.221	0.124
TIBC (μmol/L)	-0.073	0.616
UIBC (μmol/L)	-0.042	0.775
TS %	0.146	0.312
Transferrin (g/L)	0.073	0.616

UIBC - Unsaturated Iron-Binding Capacity test; TIBC - Total Iron-Binding Capacity; TS - T statistic

Table 3: Correlation between CX3CL1 with parameters in patients of Group NS

| Variables	r	P-value
Age (y)	-0.009	0.944
BMI (kg/m²)	-0.028	0.818
Ferritin (ng/mL)	0.111	0.362
Iron (μmol/L)	0.032	0.795
TIBC (μmol/L)	0.072	0.552
UIBC (μmol/L)	-0.064	0.601
TS %	0.066	0.587
Transferrin (g/L)	-0.072	0.552

UIBC - Unsaturated Iron-Binding Capacity test; TIBC - Total Iron-Binding Capacity; TS – T statistic
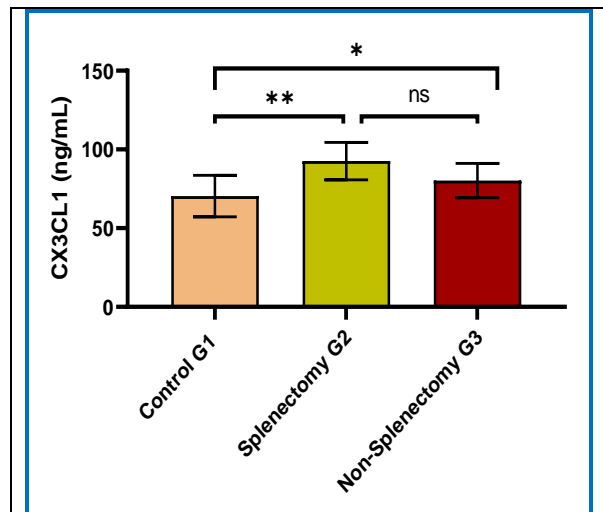
Figure 1: Comparison of serum CX3CL1 levels between control group and patients.

Table 4: Receiver Operating Characteristic-Area Under Curve analysis of the CX3CL1 in β-TM patients

Variable	Cut-off concentration	Sensitivity %	Specificity %	AUC	95% CI of AUC	P-value
CX3CL1 (ng/mL)	12.099	75.0	83.3	0.757	0.623-0.890	0.002
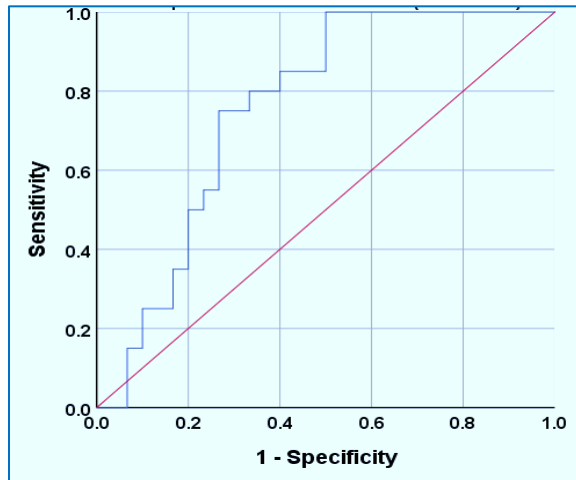
Fig 2: ROC curve of CX3CL1 in β-TM patients

are released by cytotoxic lymphocytes. Natural killer cells and terminally differentiated cytotoxic T cells all express unique receptor CX3CR1 to the CX3CL1.[11]

Previous research indicated that patients with interstitial lung disease (ILD) had considerably higher plasma levels of CX3CL1,[12] which may drive the migration of CX3C chemokine receptor type 1 (CX3CR1)⁺ non-classical monocytes (NCM) to the lungs, which sustains the local fibrotic process;[12] CX3CR1 must be present to effectively and repeatedly patrol the luminal side of the vasculature and eliminated cell injury and debris.[13] Functional tests showed that NCM migrated more readily in ILD samples when CX3CL1 was present. When there is kidney fibrosis, CX3CR1+NCM promote a release of fibrotic mediators, such as collagen-I and transforming growth factor beta-1 (TGF-β1) as well as reactive oxygen species.[14]

The results of the current study agree with a number of previous studies, which have demonstrated that patients with numerous inflammatory disorders, such as atherosclerosis, asthma, diabetes mellitus, and rheumatoid arthritis, have immunohistochemistry expression or circulating levels of CX3CL1, that are much greater than in the general population.[15] CX3CL1 levels were observed to be more elevated in type 2 diabetic mellitus patients than in controls, returning as a chemoattractant and an adhesion factor, CX3CL1 regulates the inflammatory cell staffing in addition to colonization at areas of inflammation, including rising white adipose tissue in obesity as well as diabetic mellitus. It may also be involved in monocyte adhesion to adipocytes. Expression of CX3CL1 by human adipocytes and stromal

vascular cells has been correlated with obesity, insulin resistance, and diabetic mellitus.[16]

Previously it was found that rheumatoid arthritis patients' synovial fluid had higher levels of CX3CL1 than healthy individuals. In rheumatoid arthritis, CX3CL1 drives T cell-dependent proliferation of synovial fibroblasts.[17] Ruze et al. found that atherosclerosis patients' artery walls with atherosclerotic plaque had considerably greater levels of CX3CL1 expression,[15] low shear stress-induced atherosclerotic lesions have increased CX3CL1 expression.[18] CX3CL1 was significantly higher in patients with small lung cancer, due to their chemotactic action on cancer cells, CX3CL1 plays significant roles in the occurrence and progression of malignancies.[19] The authors also reached the conclusion that CX3CL1 plays a role in the pathophysiology and development of certain inflammatory diseases and cancers. CX3CL1 orchestrates the recruitment of inflammatory cells and their colonization at locations of inflammation by acting as a chemo-attractant and an adhesion factor.[20]

Only a limited number of studies, have demonstrated the importance of CX3CL1/CX3CR1 axis signaling in the etiology of epilepsy and the related cell death,[21] in surgically eradicated brain samples taken from people with mesial temporal lobe epilepsy (MTLE), these investigations have revealed higher expression of CX3CL1. By inhibiting the axis with the anti-CX3CR1 antibody, electrical epileptic seizures' effect on microglial activation, neurodegeneration, and neuroblast formation are diminished.[22]

5. CONCLUSION

The results of our study show that elevated levels of CX3CL1 in β-TM patients, whether splenectomized or non-splenectomized, may be associated with increased apoptotic and inflammatory markers including IL-1 and TNF-α. Additionally, the iron status was both negatively and positively correlated with CX3CL1 in β-TM patients. CX3CL1 deficiency might display anti-inflammatory action in β-TM by inhibiting macrophage proliferation and polarization. According to the analysis above, CX3CL1/CX3CR1 function has a complicated regulation mechanism and merits further investigation in the future.

6. Ethical issues

Medical Ethics Committee of the Faculty of Science – University of Kufa (2022/1/16-n/181) and the Health Directorate of the administration of Thalassemia Unit in AL-Zahra Teaching Hospital in AL- Najaf, Iraq.

7. Data availability

The numerical data generated during this research is available with the authors.

8. Acknowledgement

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9. Conflict of interest

The authors declare no conflict of interest.

10. Authors' contribution

All authors took equal part in concept, conduct the study, data collection, data analysis. manuscript writing, editing and correction, final approval

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