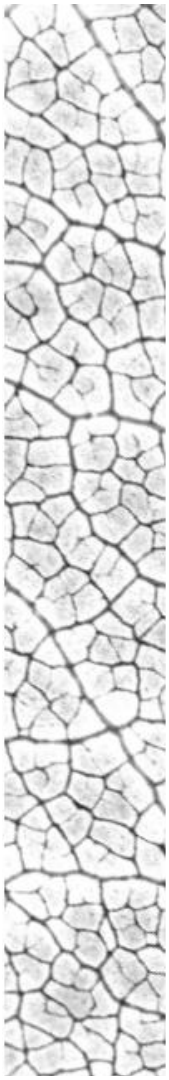

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The association of hepcidin with some inflammatory markers in β-thalassemia major patients of Basrah Province

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Abstract
Hepcidin is an antimicrobial peptide have an important role in iron metabolism especially in β-thalassemia major patients where receiving a big amount of iron during repeat blood transfusion , hepcidin expression regulated by iron state , hypoxia , inflammation . A case- control study was conducted at Basrah province (Hereditary blood disease center) during the period from 18 November 2019 to 15 April 2020. 60 patients with β thalassemia major and 28 healthy controls. Plasma levels of s. iron , ferritin ,hepcidin , high sensitive C-reactive protein , interleukin 1(IL-1α) , interleukin6 (IL-6), tumor necrosis factors (TNF-α) and transforming growth factor(TGF-β1) were determined using an enzyme linked immune-sorbent assay (ELISA) kits. The mean concentration of iron , ferritin , hepcidin , IL-1α ,IL-6, TNF-α and TGF-β1 P≤0.0005 , P≤.000 ,P≤ 0.028 , P≤0.01, P≤0.031 ,P≤ 0.006 ,P≤0.003 respectively .The statical analysis revealed a high significant correlation of hepcidin with iron , ferritin ,IL-1α, IL-6 ,TNF-α at level P≤0.015 ,P≤0.01, P≤0.027 ,P.≤,0.017, P≤0.015 respectively but there were no correlation with hs-crp and TGF-β1 at level P.≤0.265 ,0.232 respectively .

Keywords: thalassemia major, iron overload, ferritin, hepcidin, IL-1α, IL-6, TNF-α, TGF-β1

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INTRODUCTION

Thalassemia is inherited disease have multiple genetic forms including alpha and beta thalassemia (Taher and Saliba, 2017). Each types of thalassemia containing several types, thalassemia major is the most dangers and patient needs to blood transfusion but thalassemia minor is slight (Zarghamian et al. 2020), people with thalassemia major are dependent on regular blood transfusion which on the long term leads to iron overload (Tari et al. 2018). Hepcidin is innate antimicrobial agent which induced by invasive bacteria then limits bacterial proliferation by reducing iron in plasma and extracellular fluids (Barton and Acton, 2019). human hepcidin expression is high in liver, moderate in heart and brain, and lower in other organs (Krause et al. 2000). Hepcidin is expressed in blood, neutrophil, mononuclear (Wu et al. 2012) lymphocytes (Pinto et al. 2010) and in macrophages (Nemeth et al. 2004).

Hepcidin acts by regulating the cellular concentration of its receptor ferroportin, ferroportin is the cellular iron exporter (Donovan et al. 2005) and it exports iron into plasma from duodenal enterocytes which absorb dietary iron, macrophages of spleen and liver which recycle iron from old erythrocytes, and hepatocytes which release stored iron according to body needs (Abboud and Haile, 2000)Hepcidin expression by hepatocytes is modulated by three major pathway iron homeostasis, hypoxia and inflammation stimuli (Nairz et al. 2018).

Inflammatory response are essential to exclude pathogens but also lead to tissue damage .The inflammation-induce hepcidin possibly contributes to the alteration of iron metabolism under acute or chronic inflammatory disorders (Gozzelino and Arosio, 2016). Several cytokines including interleukin(IL)-6, activin B, interleukin-22 ,and interferon-α induced in response to inflammation are responsible for the up-regulation of hepcidin expression (Chung et al. 2010; Besson-Fournier et al. 2012; Smith et al. 2013) .It has been also reported that, interleukin-1 alpha (IL-1α) stimulates hepcidin transcription (Lai et al. 2008).

Interleukin 6(IL-6) infusion in humans induced hepcidin expression and decreased serum iron and transferrin saturation (Nemeth et al. 2004).IL-6 has been suggested to be a principal molecular to confer inflammation-related hepcidin transcription, which is

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Table 1. Level of serum iron and ferritin

Parameters	Groups	No	Mean	S.D±	P. value
S. Iron	Patients	60	85.426	17.307	.008
	Control	28	69.678	27.378	
S. Ferritin	Patients	60	4721.975	3664.592	.000
	Control	28	38.719	56.534	

mediated via signal transducer and activator of transcription (STAT)-binding site on the hepcidin promoter (Kanamori et al. 2019).

The TGF-β, members include TGF-β proteins , activins, nodals ,bone morphogenetic proteins (BMPs) and growth differentiation factors (GDFs) , TGF-β, activins and BMPs ,are able to induce hepcidin expression under iron overload condition(Shanaki et al. 2016).TGF-β1 is activated by iron overload and induces hepcidin mRNA expression in primary hepatocyte and mouse liver in vitro (Chen et al. 2016).

TNF-α reduction the serum hepcidin-25, but the effect of TNF-α was indirect where use hepcidin inhibitor therapy was accompanied by a decrease in serum IL-6, in vitro experiments stimulation with the cytokine combination of IL-6+TNF-α induced weaker hepcidin expression than did with IL-6 alone (Song et al. 2013).

This work done because there was no work determined the role of the inflammatory cytokines or markers in regulation of hepcidin related with iron state in thalassemia patients .

MATERIALS AND METHODS

Data collection

A specially designed questionnaire was used in present study which includes the date of birth, sex, splenectomy , and symptoms related to infection .

Patients group

The study included 60 patients divided into 33 female (55٪) and 27 male (45٪) . All patients had a history of admission to Hereditary Blood Diseases (HBD), these patients were assessed initially (clinically and by selected laboratory data).

Exclusion Criteria: the present study excluded the patients with splenectomy, Hepatitis C or B virus infections, Fever and the patients that have other diseases.

Control group

This group included 28 ages and gender matched healthy individuals with normal Hb pattern, with no fever or infection and no history of hemoglobinopathies and recent evidence of infection like upper respiratory infection.

Methods

Samples collection: 5ml of blood were collected from both patients with β-thalassemia major and control , sera were separated by centrifugation of blood for 6 minutes at 3000xg , the following investigations were done for each patient with βTM and for control group serum iron concentration measured automatically (by Abbot ARCHITECT plus ,Germany) , ferritin by (Cobas c4000 , Germany) , hs-CRP , TNF-α level , TGF-β1 level ,IL-1α , IL-6 by sandwich ELISA kits.

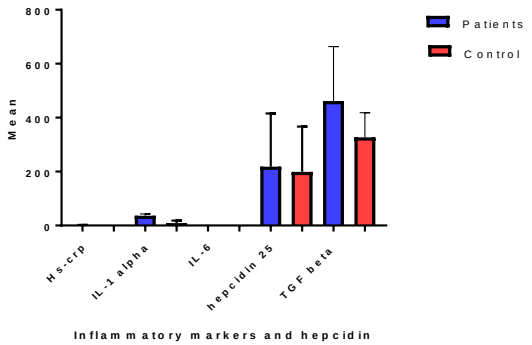

Fig. 1. Level of hepcidin and inflammatory markers in patients and control

Statistical analysis

The data were entered using the statistical IBM SPSS version 26. The data were summarized using: mean, standard deviation and P. value, for quantitative variables. Statistical differences between the groups were tested using student- t test to compare levels of s. iron, ferritin ,hs-crp , IL-6 ,TNFα and TGF-β1 . Correlations were done by using person test (two-tailed) between of hepcidin with iron and ferritin and inflammatory markers.

RESULTS

Level of serum iron and ferritin

Highly significant increase (P≤ 0.008, 0.000) was recorded in serum iron and ferritin respectively in patients with TM in comparison with control.

Level of hepcidin and inflammatory markers

Data documented that there were a significant differences (P≤0.003, 0.031, 0.006, 0.028) in hepcidin, IL-6, TNF-α and TGF-β1 respectively but no significant differences (P≤ 0.228) in hs-crp in patients compared with control, **Table 2** and **Fig. 1**.

Correlation of hepcidin with serum iron and ferritin

Documented data revealed a negative correlation of hepcidin 25 with iron and ferritin, **Table 3**.

Correlation between hepcidin and inflammatory markers

The comparison study revealed that there was a significant correlation of hepcidin with IL-6, IL-1α and TNF-α at levels (P≤0.027, P≤0.017, P≤0.015)

Table 2. Level of hepcidin and inflammatory markers in patients and control

Parameters	Groups	Mean	S.D±	P. value
Hepcidin 25	Patients	357.977	78.117	.028
	Control	317.751	79.903	
Hs- crp	Patients	1.063	1.862	.228
	Control	.6189	.755	
IL-1α	Patients	19.257	6.624	.010
	Control	15.857	2.512	
IL-6	Patients	1.883	1.145	.031
	Control	1.293	1.246	
TNF-α	Patients	21.290	5.257	.006
	Control	18.271	2.939	
TGF-β1	Patients	604.430	318.679	.003
	Control	391.550	262.834	

Table 3. Correlation of hepcidin 25 with s. iron and ferritin

		Iron	Ferritin
Hepcidin 25	R value	-.119	-.066
	P value	.269	.539

Table 4. Correlation of Hepcidin 25 with Hs-crp, IL-1α ,IL-6 ,TNF-α and TGF-β1

		Hs-crp	IL-1α	IL-6	TNF-α	TGF-β1
Hepcidin 25	R value	.120	.236*	.254*	.259*	.131
	P value	.265	.027	.017	.015	.223

* Correlation is significant at the 0.05 level

respectively, meanwhile no significant correlation between hepcidin with hs-crp and TGF-β1 at levels(P≤ 0.265 and P≤ 0 .223)respectively, **Table 4**.

DISCUSSION

Iron overload develop in thalassemia major from increased intestinal iron absorption signaled by ineffective erythropoiesis, while it can also be secondary to regular transfusions where the human body lack a physiological mechanisms for removal of the excess iron load resulting from blood transfusion (Taher and Saliba, 2017). Present result revealed that iron and ferritin increased highly in β-TM and this is due to both repeated RBC transfusions and hyper absorption of iron (Shen et al. 2015). or these results reflect inadequate chelation (AL-Saidi and Zubair, 2016) . This result was in accordance with the previous reports like as (Al-Hakeim et al. 2020) in Najaf city (Obeid et al. 2018) in Baghdad.

Hepcidin is an important regulator of iron homeostasis, which is involved in various metabolic pathways of iron metabolism (Meier-Ewert et al. 2001)data showed that there was a high elevation in hepcidin level in β-TM patients in comparison with control and this result may be due to inflammatory conditions (AL-Saidi and Zubair, 2016), this condition return to alloantigen stimulation of repeat blood transfusion in those patients , this result inducted by (Kaddah et al. 2017) were showed that serum hepcidin is elevated in children with β-thalassemia ,but this elevation is more evident in TM patients with severe iron overload. Other study inducted by Origa et al. (2007) showed that the hepcidin levels were elevated in thalassemia major more than intermediate as compared with control. This study disagreement with (AL-Saidi and Zubairwere, 2016) done in Baghdad on patients with iron overload , all of the patients had low hepcidin level and this due to all patients are irregularly transfused and this lead to stress erythropoiesis. .

Present study illustrated that hs-crp levels increased in patients but were not significantly in patients compared with control, the present results may be in response to elevated ferritin (Said et al.2014). Previous studies indicated this results, like in Saudi Arabia (Shanaki et al. 2016) and in Iran (Ratha and Altaei, 2013) but other studies disagreement with present result like (Morabito et al. 2007) in sulymania.

IL-1α levels was increased significantly in βTM and this may be due to inflammatory condition resulting from repeat blood transfusion ,this study is in agreement with(Yaylim et al. 2001) whom showed significant increasing in IL-1α in β-TM patients in comparable with control.

Data documented that there was a significant increasing in IL-6 levels in βTM patients compared with healthy control, this result may be due to inflammation occurring with frequent blood transfusions. increased IL-6 might be ascribed to the transfusion-related continuous antigenic stimulation and iron overload with consequent macrophage activation(Yaylim et al. 2001) This study is in agreement with (El-Rasheidy et al. 2016).

Present work demonstrated that were a high significant increase in TNF-α level in βTM patients compared with control, this result may be due to iron overload and antigenic stimulation induced by chronic transfusion therapy(Maras et al. 2018). This result agreed with (Al-Hakeim et al. 2020).

TGF-β1 in β-TM patients have a significant increase compared with healthy control and higher TGF-β1 levels in thalassemia patients might be an extraordinary cause of disturbing iron metabolism, expressed by elevated serum ferritin levels (Al-Hindy et al. 2020), this result agreed with (Politou et al. 2020) but disagreement with (Al-Dedah et al. 2018).

The current work reported that there was no correlation between hepcidin with ferritin and iron, this result is matching with (Kattamis et al. 2006) where showed that hepcidin not correlate with indices of iron stores, such as, ferritin, and transferrin saturation. In addition result of (Haghpanah et al. 2015)compatible with present result were showed that no statistically significant correlation between the level of serum hepcidin and ferritin ,this result due to the dominant role of erythropoiesis compared to iron overload in regulation of hepcidin in patients with thalassemia (Haghpanah et al. 2015).

Documented data revealed that hepcidin have negative correlation with hs-crp level and TGF-β1, this result inducted by (Shen et al. 2015) were reported that

no significant correlations were observed between Hepcidin and hs-crp.

The same study showed a positive correlation between serum hepcidin and IL-1α levels which in agreement with (Means et al. 2004), IL-6 and TNF-α.

CONCLUSION

hepcidin can be a good marker of severe iron overload in patients with thalassemia major. In addition inflammatory markers can be a potential markers of sever iron overload ,and infection or inflammation in those patients . The level of hepcidin strongly correlated with IL-1α, IL-6 , TNF-α but not with TGF-β1 or hs-CRP.

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