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Estimation of IL-6 and IL-10 Levels in Patients with Iron Deficiency Anemia

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ABSTRACT

The study included a process of diagnosing and differentiating (60) cases of iron deficiency anemia patients (30) moderate iron deficiency anemia and (30) severe iron deficiency anemia, who are residents of the city of Kirkuk and its suburbs, and their ages ranged from 18-30 years of both sexes (30 males). And 30 females (with a control group of healthy (30) of whom (15 males and 15 females) and their ages ranged from 18-30 years. Anemia was diagnosed by following several basic measures such as the total blood picture count, which included the compacted cell volume (PCV), hemoglobin level, the RBCs red blood cell count, as well as the corpuscular hemoglobin rate (MCH) and the corpuscular volume rate (MCV) in addition to Other discriminatory measures such as serum iron and ferritin Serum Hepcidin, a marker of iron deficiency anemia, was significantly lower in the case of IDA patients. It has been found that IL-6 plays an important role in hepcidin regulation particularly in IDA. In the case of IDA, the level of IL-6 was significantly decreased. And we observed elevated IL-10 in IDA patients. We found that IL-10 has a role in hepcidin inhibition through IL-6. Inhibition.

Keywords- iron deficiency anemia, hepcidin, IL-10, IL-6

I. INTRODUCTION

The human body can conserve iron in a number of ways including recycling iron after red blood cells are broken down and retaining iron in the absence of an excretion mechanism. However, iron absorption is limited to 1 to 2 mg per day and most of the iron required per day is about 25 mg supplied through recycling by macrophages that phagocytose erythrocytes Controlled by hepcidin, a hormone that maintains iron in the entire body Within normal ranges, avoiding both iron deficiency and excess(1).

Iron acquisition mechanisms are tightly regulated by hepcidin, a peptide hormone that is primarily synthesized by the liver. It has a role as an acute-phase reactant that modulates fluctuations in plasma iron levels caused by absorbing enterocytes and macrophages in the spleen by binding to and degrading ferroportin, which releases iron from cells(2).

Iron is required by the host to mount an effective immune response. In humans with a healthy immune system, nonspecific and humoral immunity is affected by iron deficiency in several ways. In nonspecific immunity, macrophage activity is impaired against bacteria in iron deficiency; there is also a decrease in both the number of T lymphocytes and T lymphocyte mitogenesis in iron deficiency. In response to a number of different microbes, this change is largely correctable with iron repletion. Humoral immunity appears to be less affected by iron deficiency than in iron-deficient humans(3). It is known that protein-energy malnutrition is associated with significant impairment of cellular immunity, phagocytic function, cytokine production and an altered immune response. Deficiencies of individual nutrients such as zinc, selenium, iron, copper, important vitamins A, C, E, B6 and folic acid affect immune responses Altered immune responses have a great deal of practical and general health(4) Evidence in the past decades shows that iron is an essential component of the normal development of the immune system, and its deficiency affects the ability to have an adequate immune response. The role of iron in immunity is essential for the proliferation of immune cells and malnutrition, especially lymphocytes, associated with the generation of a specific response to infection(5).

Interleukin-6-6 is a pro-inflammatory cytokine that is produced by a variety of macrophage, monocyte, T-cell, B-cell, and endothelial cell types(6). That interleukin-6 can stimulate hepcidin transcription in hepatocytes but not in macrophages has an important role in the haematopoiesis immune response and response to inflammatory processes(7). IL-6 is an endogenous biochemical that is active during B-cell maturation and the inflammatory process. IL-6 is produced by macrophages and monocytes in response to other inflammatory cytokines containing tumor necrosis factor (TNF) beta and interleukin 11(8) In host defense, acute IL-6 expression plays a major role through the activation of different cell populations. IL-6 is initiated by a wide range of acute phase proteins fibrinogen, hatoglobin, C-reactive protein, hepcidin and antichymotrypsin when acting on hepatocytes(9) Induction of CD8+ T cells by IL-6 is beneficial in Useful in the production of T cells and activation of hematopoietic stem cells and the maturation of megakaryocytes in platelets is stimulated by IL-6 in hematopoiesis NF-kappa receptor B activation is

activated by IL-6 production in bone marrow stromal cells which is important for activation and differentiation between bone resorption and osteoporosis(10).

The interleukin-10 (IL-10) anti-inflammatory cytokine, IL-10 gene is located on chromosome 1 at q31-32 (11). It is produced by immune cells such as natural killer cell(12). During infection, IL-10 production increases in primary macrophages, and iron availability to erythroid developing cells ultimately depends on macrophages, thus a higher concentration of IL10 plays a key regulatory role with a direct anti-inflammatory effect as well as an indirect effect on iron restriction through up-regulation. Hepcidin(1) and there is a study that demonstrated the effect of IL-10 treatment leads to a decrease in the expression of hepcidin MRNA in serum(13). IL-10 affects other interleukin production, such as inhibiting IL-6 and IL-1 synthesis and in the macrophage(14).

II. MATERIALS AND WORKING METHODS

This study was carried out in Al-Ahlia laboratories in Kirkuk between December 2020 until March 2021. The study included 90 samples of iron deficiency anemia patients, their ages ranged between 18-30 years, and the fields were divided According to the severity of the injury to:

1. ***The control group (C):*** This group includes healthy people who do not suffer from anemia, and their number is 30 people.
2. ***The second group (A1):*** This group includes people suffering from anemia (iron deficiency of moderate severity, and their number is 30 patients).
3. ***The third group (A2):*** This group includes people with anemia (iron deficiency) of high severity, and their number is 30 patients. 10 ml of venous blood was withdrawn for anemia disease (iron deficiency) and for healthy people in Kirkuk private laboratories for the period from September 20, 2020 until March 15, 2021, 2.5 ml of blood was placed in tubes containing anticoagulant (EDTA tubes) for the purpose of conducting a blood picture examination The complete CBC, as for the other part, was placed in tubes containing gel tubes and left at room temperature for 20 minutes, then placed in a centrifuge (3000 cycles per minute) for 10 minutes to obtain the serum. After that, the serum was transferred to dry tubes and added Plain tubes are stored at -20°C until laboratory tests are performed (ferritin, hepcidin, interleukin-6 and interleukin-10).

III. STATISTICAL ANALYSIS

Statistical analysis of the current study was carried out using spss program (16 versions) through the T test for variance at the 0.05 probability level.

IV. RESULTS AND DISCUSSION

Table No. 1: Shows the levels of IL-6 and IL-10 in the sera of patients with iron deficiency anemia and control.

Statistical significance (p value)	Severe iron deficiency anemia group (30)	Medium number of iron deficiency anemia patients group (30)	control group number)30)	immune variant
	Mean ± SD	Mean ± SD	Mean ± SD	
≤ 0.01 **	1.4227 ± 0.686	3.418 ± 1.946	6.623 ± 1.293	IL-6
≤ 0.01 **	22.532 ± 2.274	17.986 ± 3.595	6.506 ± 0.906	IL-10

The relationship of iron deficiency anemia to interleukin-6 levels

The relationship of iron deficiency anemia with interleukin-6 levels, the results of our study indicated a significant decrease in interleukin-6 concentrations in poor patients Moderate iron deficiency blood, in addition to severe anemic patients compared to the group control, and the study agreed with(15). with a significant decrease in Interleukin-6 levels in the anemic group were 20.18 pg/ml compared to the non-anaemic group Infected patients were 192.56±421.99 pg/mL.s.

This study agreed with a study conducted in Egypt (16). As serum IL-6 levels were significantly lower in patients with. Iron deficiency anemia compared to the control group, and it was found that there is a significant positive relationship between. Serum iron and IL-6 and hepcidin, although there was no significant relationship between serum iron and other immunological parameters. The study agreed with the researcher (17) where IL-6 levels were low in 27 cases.

The results differed with (18) and his study concluded that the levels of Low iron in the blood is significantly associated with higher levels of IL-6 in the blood among adults. And it differed with (19) in his study of the relationship of interleukin-6 and erythropoietin and the decrease of iron in the blood in laboratory mice, where it was shown that there is an inverse relationship of interleukin-6 and hemoglobin with an increase in erythropoietin, where the anemia group recorded levels of interleukin-6 (86.4±21.3 pg/ml) compared with the control (6.8±6.5 pg/ml) attributing the researcher to the reason that there was no unmodified

association between serum iron and IL6 The apparent discrepancy between the human and mouse data may be explained by the confounding effect of iron treatment as serum iron is significantly associated with IL-6 in children with CKD One of the mechanisms by which IL-6 contributes to the development of anemia is that the association between iron status and erythrocyte volume (MCV) has been described in children with CKD and may explain the improvement in MCV that we observed in both IL-6-deficient mice. IL-6 rapidly lowers serum iron levels by mobilizing transferrin-bound iron to hepatocytes via transferrin receptors. In higher non-human mammals, IL-6 deficiency causes reversible anemia with low proportion of nucleated red blood cells in the bone marrow and low serum iron levels. Furthermore, IL-6 has been shown to increase ferritin synthesis and decrease transferrin synthesis. In patients with ACD, the proliferation and differentiation of erythrocyte precursors is impaired, tumor necrosis factor-α (TNF-α), interleukin-1 (IL-1), and interleukin-6 disrupt erythrocyte growth by inhibiting these precursors in human bone marrow(20). Activation of inflammatory cytokines, in combination with their defensive effects on erythropoiesis, may lead to the lower pathological erythropoietin levels observed in patients with inflammatory disease(21). In one analysis, an increased number of elevated inflammatory markers was associated with decreased erythropoietin in anemic patients, regardless of hemoglobin levels. Iron metabolism and anemia of chronic disease. The average adult human contains 2 to 4 g of iron. Iron regulates 3 major aspects of erythroblast function They are iron absorption and storage and heme biosynthesis. This control is exerted by iron-regulating proteins sensitive to intracellular iron levels and are able to control translation of the messenger mRNA by interacting with specific iron-responsive elements in the mRNA of erythroid 5-aminolaevulinic acid synthase (rate-limiting heme biosynthesis), ferritin, and the receptor transferrin(22).

The relationship of iron deficiency anemia with interleukin 10 levels. The results of the study indicated a significant increase in the levels of interleukin 10 in patients. Those with moderate and severe iron deficiency anemia, and the results of our study agreed with the study of(23), who demonstrated that increased iron availability was a prominent feature of patients with Suffering from anemia and it has been proven that the excessive iron that can be obtained can promote blood formation. Erythropoiesis, elevated hepcidin induced by decreased IL-10 and IL-22 was an important reason to increase iron supply, these results from human and mouse studies suggest that an imbalance in Inflammatory and immune cytokines contribute to the promotion of excessive erythrocyte production in some patients with increased red blood cell count and elevated hemoglobin levels had an increased rate of soluble transferrin receptor stfr in serum induced by globulin precursors Significantly due to an increase in the speed of iron utilization, at the same time, total iron in the blood increased In the study samples compared to the control, anemia may result from stimulation of ferritin translation by IL-10 in cells of the reticuloendothelial system and successive incorporation of metabolically available iron into protein, This reduces the amount of iron metabolically available to erythrocyte progenitor cells, resulting in an imbalance Heme biosynthesis and the subsequent development of anemia, however, cannot be observed significant changes in Serum iron levels or transferrin saturation, despite increased levels of ferritin and stfr is gradually increased during IL-10 treatment, this could be because: stfr is more parameterized Sensitivity to reflect the need for iron in tissues and/or bone marrow from serum iron levels Transferrin saturation or increased stfr levels were observed in the treated patients At the highest dose of IL-10 it may also be affected by the regulatory effect of IL-10 on tfr mrna expression. A study (24) showed that IL-10 was significantly elevated in IDA patients compared to the control group and that the concentration of IL-10 shows a clear association between IL10 and anemia. It was found (25). that the anti-inflammatory cytokine IL-10 stimulates tfr-mediated iron uptake in active monocytes, affecting the levels of tfr-mediated iron uptake. Tfr mrna, surface expression, and iron uptake, thus stimulating iron acquisition mediated by tfr.

This result was also supported by(26) who showed a statistically significant $p<0.05$ that showed that the in vitro cytokine IL10 was higher in production in patients with IDA compared to the control where the level of IL-10 was in both groups (2.5± F 0.5 and 3.4 ± 0.5 ng/mL), respectively. Iron retention increased IL-10 production in patients, 4.1 F 0.6 and 3.7 F 0.6; Compared with a control group, IL-10 production was increased in the presence of an iron concentration of 50% (2.9 ± 0.6 ng/ml); At higher iron doses, there was no difference for IL10 production immunized without iron (2.7 F 0.6 and 2.6 F 0. ng/ml, respectively(27) showed significant increases in the in vitro pro-inflammatory cytokine of IL-10 from patients with IDA compared to the control group.

The results of the current study differed with(28) that iron deficiency reduces serum levels of IL-10, IL-12 and IFN-g in (uninfected) C56BL/6 mice, but that the decrease was partly due to nutritional deficiency.

Reversing the negative effect of underfeeding on IL-10 levels, mean IL-10 concentrations of C mice showed lower than those reported in the studies (174: 3G73: 5 pg/ml), however, the values were higher than those reported by the other investigators (15.6). pg/mL in 39/40 samples and 43.6 pg/mL in one sample

The differences in IL-10 serum levels between our study and those reported by other researchers could be due to either differences in mouse strains or environmental conditions. The effect of iron deficiency on serum levels of IL-10 depends on two factors:

splenomegaly and thymic atrophy. Both of these disorders are very common in laboratory animals and possibly also in humans(29).

Thymic atrophy is a highly sensitive marker of generalized protein-energy malnutrition and individual micronutrient deficiencies in humans. Normal thymus, at approximately normal IL-10 levels.

However, mice with both splenomegaly and thymic atrophy had mean IL-10 levels that were not different from those of mice with thymic atrophy alone(28).

While it was found(28) that iron deficiency has a general effect. Detrimental effect on cells that secrete the IL10 cytokine.

REFERENCE

[1] Mohamed, M. S. A. (2019). Evaluation of Plasma Interleukin-10 Level in Sudanese Iron Deficiency Anemia Patients among Khartoum State (Doctoral dissertation, Sudan University of Science and Technology).

[2] Nemeth, E.; Tuttle, M.S.; Powelson, J.; Vaughn, M.B.; Donovan, A.; Ward, D.M.; Ganz, T.; and Kaplan, J. (2004). Hepcidin regulates cellular iron efflux by binding to ferroportin and inducing its internalization. Science, 306, 2090–2093.

[3] Beard, J.L. (2001). Iron Biology in Immune Function, Muscle Metabolism and Neuronal Functioning. The Journal of nutrion, 4(3):568-579.

[4] Chandra RK. Nutrition and the immune system from birth to old age. Eur J Clin Nutr 2002; 56: 73–76.

[5] Beard, J.L. (2001). Iron Biology in Immune Function, Muscle Metabolism and Neuronal Functioning. The Journal of nutrion, 4(3):568-579.

[6] Salman AH, Iqbal MN, Bakir WA. The In Situ Expression of IL-6and IL-1β in breast cancer patients. Iraqi J Pharm Sci. 2008 Nov; 17 (2):55 – 62.

[7] Tripsianis G, Papadopoulou E, Romanidis K, Katotomichelakis M, Anagnostopoulos K, Kontomanolis E, et al. Overall Survival and Clinicopathological Characteristics of Patients with Breast Cancer in Relation to the Expression Pattern of HER-2, IL 6, TNF-α and TGF-β1. Asian Pac J Cancer Prev. 2013; 14(11): 6813 –6820. Available from http:// dx.doi.org DOI: 10.7314/APJCP.2013.14.11.6813 infectious disease. J Nutr 2001; 131: 6168–6355.

[8] Fuster, J.J. and Walsh, K., 2014. The Good, the Bad, and the Ugly of interleukin-6 signaling. The EMBO journal, p.e201488856.

[9] Wang, Y., van Boxel-Dezaire, A.H., Cheon, H., Yang, J. and Stark, G.R., 2013. STAT3 activation in response to IL-6 is prolonged by the binding of IL-6 receptor to EGF receptor. Proceedings of the National Academy of Sciences, 110:16975-16980.

[10] Grossman, R.M., Krueger, J., Yourish, D., Granelli-Piperno, A., Murphy, D.P., May, L.T., Kupper, T.S., Sehgal, P.B. and Gottlieb, A.B., 1989. Interleukin 6 is expressed in high levels in psoriatic skin and stimulates proliferation of cultured human keratinocytes. Proceedings of the National Academy of Sciences, 86:6367-6371

[11] Sheikhpour E, Noorbakhsh P, Foroughi E, Farahnak S, Nasiri R, Neamatzadeh H. A Survey on the Role of Interleukin-10 in Breast Cancer: A Narrative. Rep Biochem Mol Biol. 2018 Jul; 7 (1): 30 – 37

[12] Sung W – W, Lee H. The role of interleukin-10 in the progression of human papilloma virus - associated lung carcinoma. Onco Immunology .2013 Jul [2013]; 2 (9): e25854-1 - e25854-3. Available from http:// dx.doi.org DOI: 10.4161/once.25854

[13] Huang, P., Wang, J., Lin, X., Yang, F.F., Tan, J.H. (2017). Effects of IL-10 on iron metabolism in LPS-induced inflammatory mice via modulating hepcidin expression. European Review of Medicine and Pharmacological Sciences, 21(15):3469-34

[14] Tian K, Zhang R, Wang X. Association of interleukin-10 polymorphisms and haplotypes with the risk of breast cancer in northern China. Int J Clin Exp Pathol. 2017 Apr; 10(6):6989 – 699

[15] Emiralioglu, N., Yenicesu, I., Sari, S., Egritas, O., Poyraz, A., Pasaoglu, O. T., . . . Dalgic, B. (2015). An insight into the relationships between prohepcidin, iron deficiency anemia, and interleukin-6 values in pediatric Helicobacter pylori gastritis. European journal of pediatrics, 174(7), 903-910.

[16] Hassan, T. H., Badr, M. A., Karam, N. A., Zkaria, M., El Saadany, H. F., Rahman, D. M. A., ... & Selim, A. M. (2016). Impact of iron deficiency anemia on the function of the immune system in children. Medicine, 95(47)

[17] Ekiz, C., Agaoglu, L., Karakas, Z., Gurel, N., & Yalcin, I. (2005). The effect of iron deficiency anemia on the function of the immune system. The Hematology Journal, 5(7), 579-583.

[18] Nakagawa, H., Tamura, T., Mitsuda, Y., Goto, Y., Kamiya, Y., Kondo, T., . . . Hamajima, N. (2014). Inverse correlation between serum interleukin-6 and iron levels among Japanese adults: a cross-sectional study. BMC hematology, 14(1), 6-6. doi: 10.1186/2052-1839-1

[19] Akchurin, O., Patino, E., Dalal, V., Meza, K., Bhatia, D., Brovender, S., . . . Kumar, J. (2019). Interleukin-6 contributes to the development of anemia in juvenile CKD. Kidney international reports, 4(3), 470-483.

[20] shu, T., Jing, C., Lv, Z., Xie, Y., Xu, J., & Wu, J. (2015). Hepcidin in tumor-related iron deficiency anemia and tumor-related anemia of chronic disease: pathogenic mechanisms and diagnosis. European journal of haematology, 94(1), 67-73.

[21] Raj, D. S. (2009). Role of interleukin-6 in the anemia of chronic disease. Paper presented at the Seminars in arthritis and rheumatism

[22] Fujiwara, T., Okamoto, K., Niikuni, R., Takahashi, K., Okitsu, Y., Fukuhara, N., . . . Nakamura, Y. (2014). Effect of 5-aminolevulinic acid on erythropoiesis: a

preclinical in vitro characterization for the treatment of congenital sideroblastic anemia. Biochemical and biophysical research communications, 454(1), 102-108.

[23] Tilg, H., Ulmer, H., Kaser, A., & Weiss, G. (2002). Role of IL-10 for induction of anemia during inflammation. The Journal of Immunology, 169(4), 2204-2209

[24] Nussenblatt, V., Mukasa, G., Grace, A.M., Garrett, N.E. and Semba, R.D. (2001). Anemia and Interleukin-10, Tumor Necrosis Factor Alpha, and Erythropoietin Levels among Children with Acute, Uncomplicated Plasmodium falciparum Malaria. Clinical and Diagnostic Laboratory Immunolology, 8(6): 1164–1170

[25] Ludwiczek, S., Aigner, E., Theurl, I. and Weiss, G. (2003). Cytokine-mediated regulation of iron transport in human monocytic cells. Blood, 101(10):4148-4154

[26] Bergman, M., Bessler, H., Salman, H., Siomin, D., Straussberg, R., & Djaldetti, M. (2014). In vitro cytokine production in patients with iron deficiency anemia. Clinical Immunology, 113(3), 340-344.

[27] Zaghloul, A., Al-Bukhari, T., Bajuaifer, N., Shalaby, M., Al-Pakistani, H., Halawani, S. H., . . . Wassif, G. A. (2016). Introduction of new formulas and evaluation of the previous red blood cell indices and formulas in the differentiation between beta thalassemia trait and iron deficiency anemia in the Makkah region. Hematology, 21(6), 351-358.

[28] Kuvibidila, S., & Warrier, R. P. (2004). Differential effects of iron deficiency and underfeeding on serum levels of interleukin-10, interleukin-12p40, and interferon-gamma in mice. Cytokine, 26(2), 73-81.