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RELATIONSHIP BETWEEN HEPCIDIN LEVELS AND SOME BIOCHEMICAL MARKERS (MELATONIN, FERRITIN, TRANSFERRIN, IRON) IN PATIENTS WITH ANEMIA DISEASE

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INTRODUCTION:

Anaemia has been recognized to be one of the most common disorders mainly found in women. The biological cause of anaemia is because of the deficiency of iron in the body. Not only this, but the test results of anaemia shows that along with the deficiency of iron in the whole body, there occur inabilities in an adequate supply in the appropriate amount of iron in the body, which is mostly consumed by the bone marrow. The dysfunction occurs in the body when bone marrow finds a lack of iron in the body to consume and produce a sufficient amount of red blood cells for the maintenance of the oxygenation process of the tissues within the body. From the biological studies, it has been estimated that a liver peptide hormone called “hepcidin” is controlling the viability of iron. Anaemia has been a global health problem that is mainly associated with the young children and pregnant women.

Anaemia and hepcidin, the inter-relationship:

There is a strong biological inter-relationship between the functions of hepcidin and the cause of anaemia. Hepcidin has been considered to be the master regulator for the cause of systematic iron homeostasis. It highly affects the production of erythrocytes. With the increase in the levels of hepcidin causes the blockage of the absorption of intestinal iron and the recycling of the macrophage iron. It thereby causes iron restricted anaemia and erythropoiesis. With the decrease in the levels of hepcidin, it favours the iron supply to the bone marrow for an adequate production of the red blood cells and the synthesis of haemoglobin. The metabolism of hepcidin is profoundly modified in chronic kidney disease (CKD). We investigated its relation to iron disorders, inflammation and hemoglobin (Hb) level in 199 non-dialyzed, non-transplanted patients with CKD stages 1–5. All had their glomerular filtration rate measured by 51Cr-EDTA renal clearance (mGFR), as well as measurements of iron markers including hepcidin and of erythropoietin (EPO). Hepcidin varied from 0.2 to 193 ng/mL. The median increased from 23.3 ng/mL [8.8–28.7] to 36.1 ng/mL [14.1–92.3] when mGFR decreased from ≥60 to <15 mL/min/1.73 m2 (p=0.02). Patients with absolute iron deficiency (transferrin saturation (TSAT) <20% and ferritin <40 ng/mL) had the lowest hepcidin levels (5.0 ng/mL [0.7–11.7]), and those with a normal iron profile (TSAT ≥20% and ferritin ≥40), the highest (34.5 ng/mL [23.7–51.6]). In multivariate analysis, absolute iron deficiency was associated with lower hepcidin values, and inflammation combined with a normal or functional iron profile with higher values, independent of other determinants of hepcidin concentration, including EPO, mGFR, and albuminemia. The hepcidin level, although it rose overall when mGFR declined, collapsed in patients with absolute iron deficiency. There was a significant interaction with iron status in the association between Hb and hepcidin. Except in absolute iron deficiency, hepcidin’s negative association with Hb level indicates that it is not down-regulated in CKD anemia.

Expansion in the erythropoiesis due to any kind of haemorrhage can lead to the blockage of the hepcidin by a sensitive reduction in the saturation of transferring, release of erythroblast hormones and the hepcidin inhibitor "erythroferrone". A quantitative reduction in the erythropoiesis with the limitation in the consumption of iron leads to the increase in the stimulation of the transcription of hepcidin along with the saturation of transferrin (Guo et al. 2018). An abnormality in the synthesis of hepcidin is caused by the association of three conditions of anaemia. These conditions are Iron Refractory Iron Deficiency Anemia or IRIDA, chronic inflammatory disorder and acute anaemia and a rare case of hepcidin producing adenomas. Adenomas are mainly developed in the children's liver with the inborn errors of the metabolism of glucose. The rise in the levels of hepcidin causes an iron restricted and iron-deficient erythropoiesis in such conditions.

A patient suffering from Iron Refractory Iron Deficiency Anemia or IRIDA does not respond to the oral supplementation of iron or sometimes responds partially to the intravenous iron. Hepcidin suppression anaemia mainly includes congenital sideroblastic anaemia, congenital dyserythropoietic anaemia and thalassemia syndrome. The paradigm of thalassemia occurs with the release of the erythroferrone from the huge amount of immature erythroid cells. This results in the suppression of hepcidin with the overload of secondary iron. This further leads to the worsening of an ineffective anaemia and erythropoiesis (Chen et al. 2021). The increase or decrease in the levels of hepcidin allows a better analysis of the control of erythropoiesis. This is because the erythropoiesis iron can be considered as the major factor for the maturation of the erythroid cells. There are many conditions, as discussed in the following study, which leads to the cause of anaemia.

Iron-erythropoiesis connections:

It has been experimentally estimated that the process of the production of the red blood cells approximately consumes almost 80% of the circulating iron for the synthesis of haemoglobin of the matured erythroblasts. 20 to 25 mg per day iron is being recycled by the macrophage. A limited amount of 1 to 2 mg per day iron is being derived from the intestinal absorption. The function of the kidney hormone erythropoietin is to control the proliferation of the erythroid progenitor as well as the early phase of the terminal erythropoiesis. For the regulation of hepcidin, it is requires an interconnection among the liver endothelial sinusoidal cells. These cells produce bone morphogenic protein for the activation of the hepatocytes. Hepatocytes produce hepcidin. It has been analyzed that BMP2 and BMP6 are the two vital BMPs. It up-regulates the hepcidin. BMP6 is mostly iron-dependent, and BMP2 mainly appears as a low iron responsive pathway (Aksan et al. 2019). The NephroTest study is a prospective hospital-based cohort that has enrolled patients with any diagnosis of CKD stages 1 through 5, recruited from three nephrology departments. Patients younger than 18 years, dialysis patient or with a kidney transplant, and pregnant women were excluded. Between January 2000 and January 2012, NephroTest included 1095 patients after they had provided written informed consent. The NephroTest study design was approved by the relevant ethics committee (Direction Générale pour la Recherche et l'Information, Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé MG/CP09.503) and adheres to the Declaration of Helsinki. Patients not receiving EPO and intravenous iron were included in this analysis if a plasma sample was available for them. More precisely, 114 men were drawn from the NEPHROTEST cohort

samples from 2000 to 2004 and all women of the same period were included (n=48).

Additionally, 37 women with plasma samples available from the 2006 to 2008 period were added to obtain a study population that was balanced with respect to sex. Hepcidin was measured for these 199 patients. They were similar to the overall study population with respect to age and mGFR distribution (Appendix 1). Biopsy-proven nephropathy was identified in 21% of all patients, but fewer than 10% of those with diabetes. Clinical criteria based on a history of urinary albumin >300 mg/g creatinine and of other microangiopathy damage (retinopathy or neuropathy) were used to classify diabetes patients who had not had renal biopsies with diabetic glomerular nephropathy. Other diabetes patients were classified with another nephropathy type, most probably vascular.

Anaemia with abnormal levels of hepcidin:

The cause of anaemia can be categorized on the basis of the levels of hepcidin, as it has been observed that anaemia is caused by the lowering and rise in the hepcidin levels. It has been intuitive that a persistent increase in the levels of hepcidin is caused by the blockage of the absorption of iron. It causes deficiency of iron, resulting in anaemia. On the other hand, ineffectiveness in the erythropoiesis has been characterized by the anaemia due to iron overload and low levels of hepcidin. These two categorized resultant anaemia is the outcome of the opposite pathophysiology mechanism. The first type of anaemia is caused due to the inhibitory effect that is being exerted by the hepcidin on the iron absorption. Along with this, the recycling process leads to the systematic deficiency of iron. In the 2nd type of anaemia, anaemia is caused due to the suppression of hepcidin by the expansion of the abnormal erythropoiesis (Ndevahoma et al. 2021).

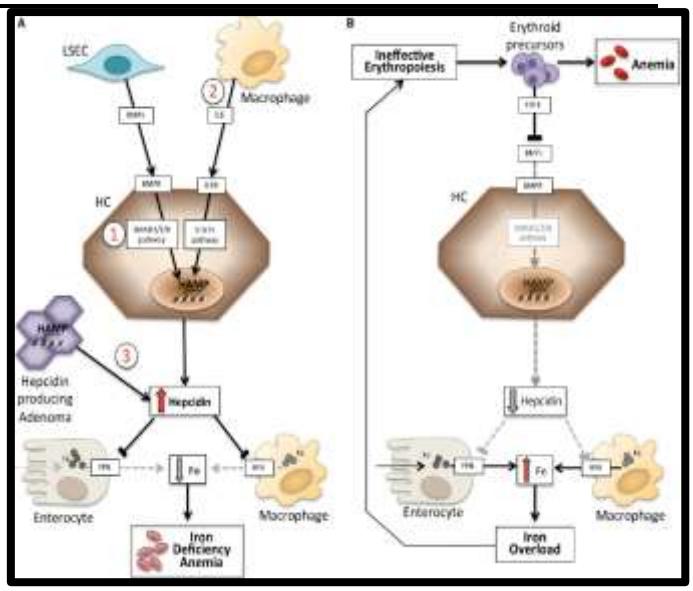

Figure 1: Schematic representation of low and high hepcidin level
(Source: Pagani et al. 2021)

Anaemia with high levels of hepcidin:

The occurrence of anaemia due to high levels of hepcidin can be included in the two inherited rare disorders. This inherited rare disorder can be considered in the hepcidin producing adenomas and iron refractory iron deficiency anaemia.

We studied crude and mGFR-adjusted relations of hepcidin with age, gender, ethnicity, diabetes and diabetic nephropathy, body mass index (BMI), mGFR, the urinary protein to creatinine ratio (PCR), albuminemia, C-reactive protein (CRP), oral iron treatment, erythropoietin, ferritin, TSAT, TIBC, and the combined iron marker; we used ANOVA to compare categorial variables and Pearson's correlations for quantitative variables. The hepcidin determinants were then analysed by multivariate regression analysis that included age, gender, center, albumin, BMI, CRP, mGFR, EPO, oral iron therapy, and the combined iron marker. Finally, we tested the association of Hb levels with the hepcidin concentration treated continuously after adjustment for other Hb determinants. These included the combined iron marker (or ferritin), gender, diabetes, BMI,

mGFR, oral iron treatment, albuminemia, CRP, and angiotensin converting enzyme inhibitors/angiotensin receptor blockers. Potential interactions between hepcidin and the combined iron marker (or ferritin) in the relation with Hb were also tested. In all analyses, hepcidin was transformed by its square root to meet the criterion of a normal parameter.

For covariates with less than 3% missing observations (albumin and CRP), the median value was imputed in the multivariate analysis. A missing data category was created for the combined iron marker and EPO. Statistical analyses were performed with SAS 9.2 (SAS Institute Inc., Cary, NC, USA) and R 2.15 (R Foundation for Statistical Computing, Vienna, Austria, 2012).

Table 1: Anemia categorized in accordance to the level of hepcidin

High hepcidin anaemia		
Hereditary	**Online Mendelian Inheritance in Myelodysplastic syndrome**	**Prevalence**
Anaemia of the chronic inflammation (Anemia of the chronic disease)		Common
Anaemia of the acute inflammation		Common
Hepcidin producing adenomas (Acquired)	#232200	Rare
Iron Refractory Iron Deficiency Anemia or IRIDA	#206200	Rare
Low hepcidin anaemia		
Hereditary, Iron loading anaemia	**Online Mendelian Inheritance in Myelodysplastic syndrome**	**Prevalence**
Low risk of MDS with the ringed sideroblasts		Rare
Sideroblastic anaemia (Acquired)	#300751	Rare
Congenital dyserythropoietic anaemia	#224100	Rare
β thalassemia	#613985	Common

(Source: Pagani et al. 2021)

Iron refractory iron deficiency anaemia or IRIDA:

IRIDA is known as a rare and recessive disorder. It is being categorized by the hypo-chromic microcytic anaemia, an unacceptably high or normal level of hepcidin and low saturation of transferrin. This deficiency is caused by the mutation of TMPRSS6. TMPRSS6 has known a gene that mainly encodes type 2 serine protease called matriptase 2. The mutation caused due to TMPRSS6 is being spread with the gene. It mainly affects various domains and specifically the catalytic domain. The trans-membrane protease is mainly expressed in the liver that inhibits the transcription of hepcidin with the cleavage of the cell surfaces; the BMP co-receptors are called hemojuvelin. It thus attenuates the signalling of BMP with the synthesis of hepcidin (Yacoub et al. 2020).

The function of TMPRSS 6 is most essential in the case of the occurrence of the deficiency of iron. It mainly allows compensatory mechanisms of the increase in the absorption of iron. IRIDA is found in an individual from the birth, which is mainly diagnosed at a young age. The cause of the disease is mainly associated with the basic cause that is iron deficiency, typical results of the iron parameters within the body and rise in the suspicion of disease. The percentage of the saturation of the transferring gets highly reduced, which is mostly found to be 10%. Eventually, the cause of the deficiency of iron leads to an increase in the levels of the serum ferritin. This effect can be reflected in the increase of the accumulation of ferritin in the macrophage because of the high level of hepcidin. It thus induces the storage of iron in the sequestration. From the genetic 6test, it can be identified that the TMPRSS 6 mutation in some cases can be found to be splicing, frame shift and nonsense mutation. From the diagnosis, it can be observed that the levels of the serum hepcidin are high from the normal and consistent with an increase in ferritin (Huang et al. 2016).

In some patients, it has been observed that a single TMPRSS 6 mutated allele is reported with the phenotypes of the refractory

iron deficiencies. In the tests, a spectrum is observed that says a condition from the classic and severe IRIDA. This is due to the fact that TMPRSS 6 mutations occur to increase the susceptibility of the deficiency of iron and is conferred with the single polymorphic or mutational change. A classic IRIDA can be considered to be a normalized hepcidin based on the iron parameters that mainly include the ratio of the saturation of transferrin. The optimal treatment of the IRIDA is not specific or under-defined. Oral iron content is always found to be ineffective, as the iron is not absorbed by the bone marrow. If a sufficient amount of Vitamin C is added, it allows the sporadic responses. The intravenous iron can be induced with a partial response at a comparatively slower rate with respect to the acquired iron deficiency (Huang et al. 2016).

Table 2: The experimental therapies that target the ferroportin-hepcidin axis

Mechanism		Compounds
Compounds that help in the decrease in the level of hepcidin or increase in the function of ferroportin		
Class I	Reduction in the signalling pathways with the stimulation in the level of hepcidin	• Non-anti-coagulant heparin • Anti HJV Mono-clonal antibodies • BMP receptor inhibitor • Anti-BMP 6 Mono-clonal antibodies • Anti IL 6-R, anti-IL-6
Class II	Hepcidin binder	• Anti hepcidin gene or HAMP Mono-clonal antibodies • Oligo nucleotides aptamer
Class III	Interference with the hepcidin-FPN interactions	• Anti ferroportin or FPN Mono-clonal antibodies • Guanosine 5' di-phosphate
Compounds that help in the decrease in the level of hepcidin or decrease in the function of ferroportin		
Class I	Hepcidin mimics	• Hepcidin analogues • Mini-hepcidin
Class II	• Blockage of the hepcidin receptors • Blockage of the hepcidin inhibitors • Activation of the hepcidin	• Ferroportin inhibitors • Anti TMPRSS 6 (siRNA) • BMPs (pre-clinical study)
Class III	Others	• Inactivation of the bone marrow transferring receptor 2 • Protoporphyrin IX (inhibition of the heme oxygenase) • Human transferring infusion

(Source: Pagani et al. 2021)

Anaemia of hepcidin and producing adenomas:

The condition of anaemia of hepcidin and producing adenomas can be considered as an extremely rare condition in medical science that occurs mainly in the adult patients. The disease is caused by glycogen storage disease 1a, which is a recessive disorder. The reason behind this is the deficiency of the glucose-6 phosphatase. This catalyzes the reaction that occurs between gluconeogenesis and glycogenolysis. The most commonly known symptom that is caused by the reaction is hypoglycemia. Treatment of the disease causes some serious symptoms, such as liver adenomas and anaemia. Anaemia caused is hypochromic and microcytic, refractory and iron deficient with respect to the oral iron treatment. Adenoma tissues are found to be positive for the hepcidin mRNA. The normal and surrounded tissues mainly show the suppression of hepcidin due to the ectopic and uncontrolled production of hepcidin (Goyal et al. 2017).

Anaemia with low levels of hepcidin:

Low levels of hepcidin or ineffective erythropoiesis are the main features of an iron loaded anaemia. The prototype of the case is β thalassemia. β thalassemia is a genetic and excessive disease that is caused due to the mutation of the β globin genes. It further causes the production of the α globin chain and anaemia. The production of the α globin chain leads to the precipitation of the Hemi chromes in bone marrow, which damages the maturing of the erythroid precursor. It leads to ineffectiveness in erythropoiesis. This symptom is probably seen at the time of thalassemia intermedia or non-transfusion dependent thalassemia (Stojkovic Lalosevic et al. 2020).

The erythropoiesis of thalassemia intermedia can be categorized on the basis of the prevalence of the immature cells that manly release the erythroferrone for the inhibition of

the liver hepcidin. The suppression of hepcidin is mainly mediated by an increase in the cytokine erythroferrone, which is synthesized by the erythroblasts upon the stimulation of the EPO. ERFE genes are released upon the circulation as well as the sequesters BMPs, mostly the BMP 6. The hepcidin level is found to get decreased by some special mechanisms at the time of a low risked myelodysplasia with the ringed sideroblasts. Myelodysplasia is a clonal disorder that is caused due to the mutation of the spliceosome gene called SF_3B_1 (Goyal et al. 2017).

Prevalence of Anemia in Iraq:

Iron deficiency is the leading cause of anaemia. It has been observed in Iraq, women suffered mainly from the fall of haemoglobin levels in the blood, resulting in anaemia (Research Gate, 2021).

Hepcidin produced helps in the reduction of the iron entry to the plasma from the absorptive duodenal cell as well as the iron recycling macrophage through the blockage of the export of iron. It also leads to the degradation of the iron exporter "ferroportin". Hepcidin leads to the regulation of the systematic iron homeostasis along with the regulation of the plasma iron. Due to this, the hepcidin strongly affects the erythropoiesis, which possibly leads to the development of anaemia. The study says the prevalence of anaemia in Iraq is 14%, which is a low rate. An exceptional case is there for the non-pregnant women with an anaemia rate of 20.7%.

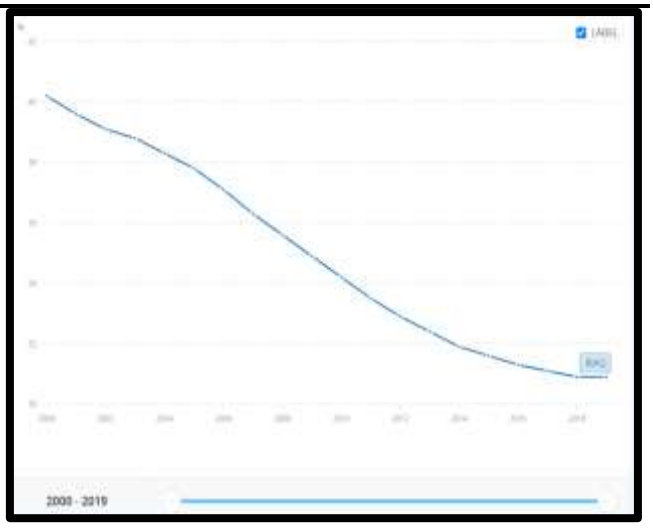

Figure 2: Prevalence of anaemia among the pregnant women in Iraq
(Source: Data.worldbank.org, 2021)

From the above graph, it has been estimated that there has been a sharp and monotonic decrease in the slope that says in Iraq, there is a huge decrease in the rate of anaemia among women.

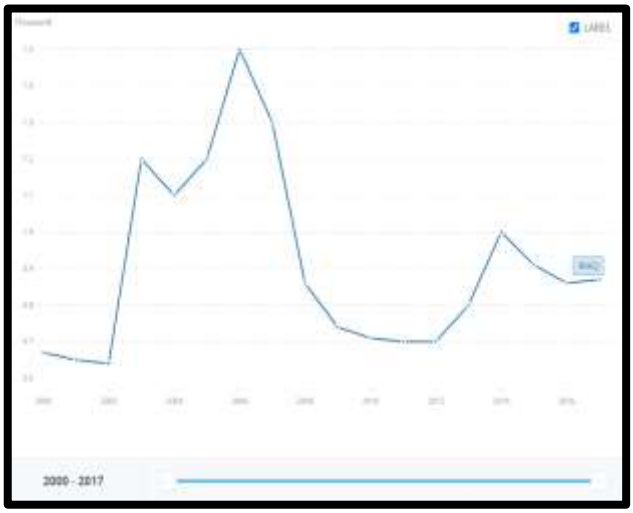

Figure 3: Number of the maternal deaths in Iraq
(Source: Data.worldbank.org, 2021)

From the above graph, it has been estimated that there has been a significant rise in the mortality rate of people due to anaemia in 2006 that significantly lowered from 2010 to 2012 and raised in 2014. From the mid of 2015, the mortality rate had a constant curve.

Table 3: Iraq estimation of the prevalence of anaemia in the individuals to get affected

Population group	The proportion of the population with the cause of anaemia is %	Public health problems
Pre-school aged children	22.9	Moderate
School belonging children	20.1	Moderate
Pregnant women	37.9	Moderate
Lactating women	25.8	Moderate
Non-pregnant women belonging to the age group 15 to 49 years	19.9 Mild	

(Source: Humanitarianresponse.info, 2021**)**

CONCLUSION:

From the overall discussion conducted in the comprehensive report, it can be concluded that spectacular advances that are made in the experiment or thesis help in a more depth understanding of the regulations of the iron metabolism in the body. The study says there is a major inter-relationship between the level of hepcidin and the cause of anaemia. This is mainly associated with the lowering and increases in the levels of hepcidin. In these scenarios, it is expected that dysfunctioning and dysregulation in the levels of hepcidin may cause anaemia. Apart from this, in the study, the mechanical ways by which the inter-relationship among the levels of hepcidin causing anaemia is showed depicts major functions of the bio-chemical makers such as iron, transferrin and ferritin that results in the anaemia disease. A sufficient amount of iron within the body helps in the sufficient amount of hepcidin production in the liver. It acts on its receptor known as "ferroportin", which is a trans-membrane Iron exporter protein. It is highly expressed on the hepatocytes, macrophage and enterocytes.

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