Study of Hepcidin Activity in Acute Leukemic Patients

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Abstract:
Acute leukemia is the term used to describe the development of immature bone marrow-derived cells (blasts), which may have an impact on the peripheral circulation or solid organs. This study aimed to evaluate the hepcidin activity in erythropoiesis in patients with acute leukemia (ALL, AML). This study was conducted between November 2021 to May 2022 at the Imam Hussein Center for Oncology and Hematology (Al-Husseini Hospital) in Karbala and Baghdad Teaching Hospital in the Medical City in Baghdad. (65) samples were collected from patients with acute lymphocytic leukemia, both lymphatic and myeloid types, and (36) of the samples as a control group. Our results show that the statistical analysis of hepcidin is high significant difference (P≤ 0.05) in acute leukemia patients compared to healthy people. As well as a significant difference (P≤ 0.05) in iron and ferritin levels in acute leukemia patients compared to the control group.
Keywords: Hepcidin, Acute Leukemic, blast

1. Introduction

Acute leukemia is the term used to describe the development of immature bone marrow-derived cells (blasts), which may have an impact on the peripheral circulation or solid organs. Acute leukemia needs at least 30% of bone marrow blast cells, according to long-standing conventional belief. Nevertheless, in more recent recommended categorization systems, blast cell counts for a number of leukemia categories have been reduced to 20%, and where particular morphologic and cytogenetic criteria are present, no minimum blast cell percentage is required (Albano et al .,2006). The kind of blood cell implicated in this sort of cancer is another classification. During the process of hemopoiesis, hematopoietic stem cells divide and develop into cells of the myeloid and lymphoid lineages (Kondo, 2010). While lymphocytic leukemia is brought on by T and B lymphocytes, neutrophils, basophils, and eosinophils are myeloid cells, and granulocytes and monocytes (macrophages) are involved in malignancy (Haouas et al., 2010).
Acute lymphoblastic leukemia is a kind of leukemia that affects lymphoid progenitor cells in the bone marrow, blood, and extramedullary areas (ALL). Even while children are involved in 80% of ALL cases, adults are also at considerable health risk (Howlader et al .,2016). while Acute myeloid leukemia (AML) is a kind of malignancy that is distinguished by the infiltration of proliferative, clonal, abnormally differentiated, and occasionally poorly differentiated hematological system cells into the bone marrow, blood, and other organs (Döhner et al.,2010).
The liver produces hepcidin, a 25-amino-acid peptide hormone that controls iron levels. Hepatocytes are the main producers of hepcidin, although it may also play a role in the local autocrine and paracrine regulation of iron fluxes in other tissues and cells, including macrophages, adipocytes, and the brain, due to its low levels of expression in these tissues and cells (Valore, Ganz.2008). It controls the amount of iron that is taken up by absorbing intestinal cells, erythrocyte-recycling macrophages, and iron-storing hepatocytes and supplied to blood plasma. Ferroportin, the lone cellular iron exporter that carries iron from all iron-transporting cells to the plasma, is attached to by hepcidin and rendered inactive. Iron storage and plasma iron act as a traditional endocrine feedback mechanism to induce hepcidin production.
The fact that inflammation also stimulates hepcidin suggests that it could be involved in innate immunity. The suppression of hepcidin by increased erythropoietic activity results in greater iron absorption and release from storage, balancing the supply of iron with the rise in demand. Hepcidin is suppressed by the hormone erythroferrone, which is produced by erythroblasts driven by erythropoietin. (Ganz, 2016) The accumulation of radiolabeled hepcidin in ferroportin-rich tissues shows that hepcidin is eliminated from those tissues by receptor-mediated endocytosis. (2005) Rivera et al. Hepcidin controls ferroportin-mediated cellular iron export to plasma and extracellular fluid. The hepcidin receptor and ferroportin are the only two known cellular iron exporters in vertebrates.
The only known iron exporter in vertebrate cells is ferroportin (Fpn), which controls cytosolic iron levels and exports iron to plasma to preserve iron homeostasis. Iron and other transition metals may influence ferroportin1 expression (FPN1). Fpn posttranslationally may be changed by internalization and degradation mediated by hepcidin. .(Troadec et al.,2010).
Hepcidin plays a crucial role in iron metabolism because it may regulate the export of iron from cells. The primary iron export protein ferroportin (FPN), which is responsible for hepcidin's activity, does this. By breaking down FPN, hepcidin prevents iron export from cells like macrophages and enterocytes.

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(Ganz, Nemeth.2012). An 84-amino-acid precursor called prepropeptide is created by the hepcidin gene. A mature peptide of 25 amino acids is produced from the precursor by processing it via two cleavages. (Poli et al.,2014) . A FPN extracellular loop important in ferroportin ubiquitination, disulfide bridging, and proteasomal degradation is where hepcidin-25 interacts. FPN1 is downregulated as a consequence of the dissolution of the hepcidin/FPN1 complex, which induces intracellular iron sequestration. (Qiao et al., 2012).

2. Patients and Methods

65 samples were collected from leukemia patients in the Imam Hussein Center for Oncology and Hematology (Al-Husseini Hospital) in Karbala and Baghdad Teaching Hospital in the Medical City in Baghdad. Samples were collected from November 2021 to May 2022. (5ml) venous blood samples were collected from acute leukemia patients.

Control group The control group consisted of 36 individuals, including children and adults, who appeared to be in good health and did not complain of any blood problems. These samples were collected if the subjects did not receive any medication and did not have any chronic or acute blood diseases.

Blood samples were collected from patients by drawing 5 ml of venous blood in the arm using a compressor around the arm, and withdrawing it using a sterile syringe after sterilizing the place of withdrawal with alcohol using a cotton swab. They were divided into two groups: the first blood was placed in a 3 ml gel tube after coagulation for 15-20 minutes at room temperature, centrifuged at 3000 rpm for 10 minutes and the second section was placed 2 ml of blood in an EDTA tube. The blood is gently mixed and then used in blood tests. Patients' samples were distributed by gender, age group and type of disease. 20 male (55.56%) control group and 34 (52.31%) patients. Females 16 (44.44%) control group, 31 (47.69%) patients. As for the age groups, they were divided into three categories: (9 months – 20);(21-40);(41-62). As for the type of disease 40(61.54%) patients were diagnosed with acute lymphoid leukemia and 25 (38.46%) with acute myeloid leukemia.

3. Results and Discussion

The results of our study appeared an increase in the level of hepcidin in the serum of leukemia patients in general. The results showed an increase in hepcidin ($p<0.05$) in the serum of patients with lymphocytic leukemia when compared to myeloid leukemia and the control group (745.24±9.8 ; 634.80±2.6 ; 261.79±21.3) respectively. The results also showed that there was a significant increase in the level of hepcidin among patients with lymphocytic and myeloid leukemia. As shown in the table (1-1) below.

The results of the current study showed an increase in the level of ferroportin in the serum of leukemia patients. The results showed an increase in ferroportin ($p<0.05$) in the serum of patients with lymphocytic leukemia compared with myeloid leukemia and the control group (11.13±1.8; 7.95±0.6; 5.58±0.4) respectively.

The current study showed an increase in iron level in the serum of patients when compared to the control group. and involved that there is a noticeable increase in iron in the serum of leukemic patients when compared to the control group (117.25±2.8 ;107.67±1.8 ; 91.50±6.9) mg/dL respectively. There is also a clear significant difference between lymphocytic and myeloid leukemic patients, as shown in the table.

The results of our study appeared that there is a significant increase ($p\leq0.05$) in the level of ferritin in the blood for patients. The results showed that acute myeloid leukemic patients had a high ferritin level when compared to lymphocytic leukemia patients and the control group (712.43±8.8 ; 833.10±3.0; 48.86±2.2) (ng/ml) .The results also showed that there was an increase in ferritin level among patients with myeloid and lymphocytic leukemia (833.10±3.0 ; 712.43±8.8), As shown in the table.

Table (1-1) Hepcidin, Ferroportin, Ferritin and Iron concentration acute in leukemic patients groups (Acute Myeloid Leukemic Patients, Acute lymphoid Leukemic Patients) and control group.

Groups Parameters	ALL	AML	Control
	Mean±S.E		
Hepcidin	745.24±9.8 c	634.80±2.6b	261.79±21.3 a
Ferroportin	11.13±1.8 c	7.95±0.6 b	5.58±0.4 a
Ferritin (ng/ml)	712.43±8.8 b	833.10±3.0 c	48.86±2.2 a
Iron (mg/dL)	117.25±2.8 c	107.67±1.8 b	91.50±6.9 a

*Similar letters is not significant difference at P≤ 0.05 level. Different letters is significant difference at P≤ 0.05 level. (ALL) acute lymphoid leukemia ,(AML) acute myeloid leukemia

In the table (1-2) showed Hepcidin, Ferroportin, Ferritin and Iron concentration in leukemic patients groups (AML, ALL) and control group according to gender. The results for males were that there was a significant increase in the hepcidin concentration among the lymphoid and myeloid patients in male. There was also a significant increase between the patients and the control. In the case of females, there was also a significant difference between the leukemic patients and between the patients and the control. It is clear from the above table that the level of hepcidin in females has higher significant differences than males among patients with acute myeloid leukemia.

As for the relationship of ferroportin with gender, there is a significant increase among patients with lymphoblastic and myeloid leukemia, and there is also a significant increase among patients groups and control for males and females. The table showed

that the level of ferroportin in females has higher statistically significant differences than in males among patients with acute myeloid leukemia

For the relationship of ferritin with gender, there is a significant increase between lymphoid and myeloid leukemia patients, and there is a significant significant increase between patients and control for males. As for females, there is no significant difference between patients with myeloid leukemia, but there is a significant difference between leukemia and control patients. The table involve the level of ferritin in males has a higher statistically significant difference than in females between patients with acute meyloid leukemia.

The table (1-2) appeared the relationship of iron with gender, and there was a significant increase among patients with lymphoblastic and myeloid leukemia, and there was a significant increase between patients and control for males and females. The above table showed that the iron level in males has a statistically significant difference than in females between patients with acute lymphocytic leukemia.

Table (1-2) Hepcidin, Ferroportin, Ferritin and Iron concentration in leukemic patients groups (AML, ALL) and control group according to gender.

Groups Parameters	Gender	ALL	AML	Control
		Mean±S.E		
Hepcidin	Male	664.48±12.5	±6.3271.29	251.68±3.9
	Female	849.76±9.7	816.56±5.8	276.40±2.5
$LSD_{(0.05)}$		**28.508**		
Ferropoten	Male	8.04±0.6	±0.45.04	4.75±0.6
	Female	15.12±0.3	±0.39.40	6.78±0.7
$LSD_{(0.05)}$		**2.832**		
Ferrintin (ng/ml)	Male	822.90±7.4	1500.00±11.2	54.79±5.6
	Female	569.47±8.2	499.64±4.6	42.89±7.2
$LSD_{(0.05)}$		**306.519**		
Iron (mg/dL)	Male	135.00±7.4	107.67±8.1	99.20±1.6
	Female	64.00±9.2	110.23±6.4	83.80±2.7
$LSD_{(0.05)}$		**24.706**		
(ALL) acute lymphoid leukemia ,(AML) acute myeloid leukemia				

Table (1-3) showed the relationship of Hepcidin parameter with age in leukemia patient groups and the control group, where the results showed for the age groups from 9 months-20 years, the group from 21-40 and the age group from 41-62 years that there was a high significant difference between cancer patients and between patients and control.

The relationship of the Ferropoten parameter with age in the groups of leukemia patients and the control group, where the results showed for the age groups from 9 months - 20 years, there was a significant difference between cancer patients and between patients and control and the group from 21-40 and there was a significant difference between leukemia patients, but There is no significant difference between patients and control. As for the age group of 41-62 years, there is a high significant difference between cancer patients and between patients and control.

As for the relationship of Ferrintin parameter with age in leukemia patient groups and the control group, where the results showed for the age groups from 9 months-20 years, the group from 21-40 and the age group from 41-62 years that there was a high significant difference between cancer patients and between patients and control

As for the relationship of iron parameter with age in the group of leukemia patients and the control group, where the results for the age groups from 9 months to 20 years showed a high significant difference between leukemia patients and between patients and control, and the group from 21-40, there is no significant difference between leukemia patients and between patients and control. The age group is 41-62 years, there is no significant difference between leukemia patients, but between patients and control, there is a significant difference.

Table (1-3) Hepcidin, Ferroportin, Ferritin and Iron concentration in acute leukemic patients groups (AML, ALL) and control group according to age groups.

Groups Parameters	Age (years)	ALL	AML	Control
		Mean±S.E		
Hepcidin	9 months-20	913.26±3.3	331.29±3.4	245.38±2.6
	21-40	213.32±2.9	448.15±4.1	225.22±3.7
	41-62	536.92±5.2	1066.55±3.7	301.65±6.1
$LSD_{(0.05)}$		**59.973**		
Ferroportin	9 months-20	13.22±0.9	5.95±0.6	2.93±0.3
	21-40	4.93±1.1	6.23±0.2	4.96±0.1
	41-62	9.05±0.7	11.53±1.7	6.73±0.02
$LSD_{(0.05)}$		**1.261**		
Ferritin (ng/ml)	9 months-20	655.27±25.6	166.23±12.3	50.00±15.6
	21-40	639.99±15.1	1008.47±9.7	47.22±11.2
	41-62	767.17±14.0	903.47±10.7	49.37±10.2
$LSD_{(0.05)}$			**66.74**	
Iron (mg/dL)	9 months-20	139.00±6.4	109.50±8.2	97.50±5.2
	21-40	89.50±5.8	104.00±7.7	82.50±1.3
	41-62	114.00±11.6	99.14±6.9	79.11±2.6
LSD(0.05)			28.175	
(ALL) acute lymphoid leukemia ,(AML) acute myeloid leukemia				

Our results showed high level for hepcidin hormone in acute leukemic patients (AML,ALL) $p<0.05$ in

contrast with control group which consistant with (Hamad et al., 2019) . Hepcidin and serum ferritin typically react to inflammation and changes in iron stores in a similar manner,(Kemna et al.,2005) . It is known that hepcidin binds to ferroportin, causing external iron linked to transferrin or serum ferritin to decrease and intracellular iron to be retained in macrophages (Muckenthaler et al., 2008).It thus brings the intriguing issue of whether higher hepcidin levels in the presence of transfusional iron loading may prevent excessive parenchymal iron overload and consequent organ damage. Iron issues are often brought on by variations in hepcidin concentrations, as opposed to ferritin, a marker of iron storage. The dysregulation of hepcidin-ferroportin axis showed high hepcidin and uncontrolled ferroportin (FPN) hyperactivity at childhood ALL which may point to the impaired down-regulation of FPN by hepcidin (Hsi-Che et al., 2020).Nemours studies that discovered significantly higher levels of hepcidin in patients compared to the healthy group provided an explanation for the same outcomes, reporting that the increase in hepcidin levels may protect the body from excessive iron parenchymal and organ damage in the presence of iron loading (Liu et al ., 2016; Vinchi et al .,2020;Ali et al .,2022).

All living things need iron for their essential metabolic processes. A multitude of comorbidities with poorer prognoses and more expensive treatment choices are linked to iron imbalance, which still affects over 25% of the global population. This is despite several public health programs and in-depth research over the years. Hepcidin is largely responsible for controlling the homeostasis of systemic iron. By binding to ferroportin, the only known iron exporter, the highly conserved hepcidin lowers plasma iron concentrations and induces iron retention in macrophages and enterocytes (Nemeth et al., 2004).

Iron and erythropoietic activity govern the homeostatic levels of hepcidin. Hepcidin synthesis is increased by iron overload and inflammation, but hepcidin expression is decreased by anemia and hypoxia (Chauhan et al ., 2014).Other investigations demonstrated that chronic disorders like leukemia, which can lead to iron malfunction with hypoferremia and anemia, greatly elevated serum hepcidin levels during inflammation and infection (Langer, Ginzburg .2017);(Sonkar et al.,2018).

The current study showed an increase in iron in the blood serum of patients, and these results were similar to the studies he conducted (Wande et al .,2020). Iron is an essential element for biological processes. Iron homeostasis is regulated through several mechanisms, from absorption by enterocytes to recycling by macrophages and storage in hepatocytes. Iron has dual properties, which may facilitate tumor growth or cell death (Coates,2019). Cancer cells exhibit an increased dependence on iron compared with normal cells. Macrophages potentially deliver iron to cancer cells, resulting in tumor promotion. Mitochondria utilize cellular iron to synthesize cofactors, including heme and iron sulfur clusters. However, highly increased iron concentrations result in cell death through membrane lipid peroxidation, termed ferroptosis (Olcay et al., 2017). Therefore, the risk of an imbalance in the entire iron homeostasis should be taken into account. The toxicity of iron results from the Fe2 + forms of iron, which are highly reactive and cause rapid oxidant damage of proteins and DNA, permanently changing the structure of proteins and genetic material (Koskenkorva-Frank et al., 2013)the intensity of chemotherapy results in an increase toxic forms of iron, occurring simultaneously with increased serum iron, ferritin, hepcidin, and ferroportin concentrations (Coates et al., 2014)

Elevated cellular iron levels are a result of increased iron absorption and reduced iron efflux in leukemia cells. Leukemia patients also have an elevated systemic iron pool, which is made worse by repeated red blood cell transfusions. Numerous experimental and epidemiological investigations have shown a connection between iron metabolism dysregulation and the development and progression of leukemia(Kennedy et al., 2014; Benadiba, et al.,2017; Hagag, et al .,2018). Due to their high need for iron to support their fast multiplication, leukemia cells are far more vulnerable to iron deprivation than normal cells (Callens et al .,2010).

The significance of iron in the development and spread of cancer has been apparent in recent years (Torti et al.,2013; Shen et al., 2021). In particular, iron chelating drugs constitute a viable anticancer therapy since tumor cells use more iron than healthy cells do (Forciniti et al.,2020). In both hematological and solid malignancies, iron chelators have been shown by several authors to decrease proliferation and trigger apoptosis (Calabrese et al.,2020; Amano et al.,2020). Deferiprone (DFP) and deferasirox (DFX), two iron chelators that have been licensed for use in leukemic patients and are capable of preventing the growth of cancer cells (Messa et al., 2010);(Zhang et al .,2019) are two examples.To promote tumor growth, cancer cells change the usual processes of managing iron. In vivo tests (Greenberg .1976), epidemiological research (Fonseca-Nunes et al .,2014), and cancer susceptibility in hemochromatosis patients (Kowdley .2004) provided evidence for iron overload's role in causing cancer some time ago, but the underlying molecular pathways have been more well recognized for ten years (Torti et al 2013). Iron overload is a typical observation in leukemic patients, in part because cancer patients frequently require high doses of red blood cell transfusions due to natural erythropoiesis impairment and chemotherapy-induced anemia. The "withholding response," which is the organism's reaction to restricting iron bioavailability, has been the main focus of therapeutic advancements based on iron metabolism up to this point.

Iron intake, storage, and export indicators frequently

coexist with iron excess. TFR1 expression, also known as CD71, is often higher in leukemic cells than in normal counterparts. The amount of CD71 expression in AML, whether it is minimally differentiated or not, may be directly connected with the degree of differentiation (Liu et al., 2014).

In this study, serum ferritin was increased in patients when compared with the control group. These results are similar with those reported (Genena et al.,2015; Hamodat et al ., 2020). A significant difference in ferritin levels between healers and non-healers reflecting the level of serum acute phase ferritin, which is typically linked to iron storage, can aid in disease stage prediction (Luznik et al. 2008; Armand et al., 2007; Hamad et al., 2019). The increase in ferritin concentrations for all patients is caused by broken cancer cells releasing ferritin into the bloodstream. (Kemna et al., 2005).

Serum ferritin, the iron storage analogue, is commonly elevated in leukemic patients and is detrimental to overall and relapse-free survival in chemotherapy-treated patients (Baker et al., 2014; Tachibana et al., 2018; Bertoli et al.,2019; Ihlow et al.,2019) as well as in recipients of allogeneic stem cell transplants (Artz et al., 2016). Ferritin is made up of 24 polypeptide heavy chain (FTH) and light chain subunits (FTL). FTH appears to be an NF-B downstream effector that, in an inflammatory setting, inhibits Jun N-terminal kinase (JNK) to decrease TNF-driven apoptosis (Kamata et al ., 2005) NF-B and pro-oxidant pathways are included in a gene expression profile linked to FTH overexpression in AML patients, which causes resistance to chemotherapy (Bertoli et al.,2019). AML frequently exhibits dysregulation of the ferroportin-hepcidin axis, which results in decreased iron outflow. Notably, core binding factor (CBF) AML subgroups are regularly reported to have low ferroportin expression, which appears to be associated with better outcomes and increased chemotherapeutic sensitivity

4. Conclusion

We noticed through our study that acute lymphocytic leukemia was more prevalent in children, and myeloid leukemia was more in the elderly, and it was noted that the hormone hepcidin was higher than in acute lymphocytic and myeloid leukemia patients, and this is considered a risk factor as it is an oxidizing factor and thus leads to the activation of free radicals. Also, high iron, ferritin and ferroportin were observed in patients when compared with the control group, and these are considered risk factors.

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