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Risk factors for iron metabolic disorders in newly diagnosed B-Chronic lymphocytic leukemia patients

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Abstract---Due to the importance of increasing the incidence conditions of B cell chronic lymphocytic leukemia (B-CLL) that requiring accurate knowledge of the biology behind the pathogenesis and risks of this disease of each individual patient, this study was designed to investigate if hormonal alterations such as Hcpidin, Erythropoietin, and oxidant-antioxidant status may be useful in the understanding of B-CLL development in addition the relationship of these parameters with anemia in advance clinical stage in newly diagnosed patients with B-CLL. This study was conducted on 60 newly diagnosed patients with B-CLL and 30 control group .The socio-demographic information including (age and sex) and clinical data were collected for the period from 20 February until 25 December 2021. The result of the present study showed that out of the 60 newly diagnosed B-CLL patients, 39 was male and 21 was females, with the mean age (64.04 ± 5.6) years and out of the 30 controls group ,12 was males and 18 was females with the mean age of (63.33 ± 4.70) years .therefore, it was a higher prevalence p value ≤0.05 of newly diagnosed B-CLL patients in males (65.0 %) compare to females (35.0 %). According to Binet stage , the clinical data of newly diagnosed B-CLL patients were : the presence of (6.7%) patients within stage A , the presence of (43.3 %) patients within stage B and the presence of (50.0%) patients within stage C and (51.7 %) , additionally, clinical examination of the patients revealed a higher proportion of

lymphadenopathy (65.0%) followed by hepatomegaly and/or splenomegaly (51.7%) . Regarding to hormonal alterations , the mean levels of Hepcidin and Erythropoietin were elevated significantly in newly diagnosed patients with B-CLL in compare to the control group .on the other hand, the result of oxidant-antioxidant status showed that the patients have been significantly increase of Malondialdehyde compare to control group while Total antioxidant capacity levels were significantly decrease in newly diagnosed patients with B-CLL in compare to the control group . In conclusion , the contribution of Hepcidin and Erythropoietin hormones to the regulating of iron metabolisms, and the role of oxidant-antioxidant status were closely related with the pathophysiological and development of B-CLL.

Keywords---Hormonal alterations ; Hypoxia ; Lymphadenopathy.

Introduction

B-Chronic lymphocytic leukemia is one type of hematological malignancy, resulting from uncontrolled proliferation and clonal accumulation of small and mature B lymphocytes in bone marrow , peripheral blood, and other secondary lymphoid organs [1].The average annual incidence of B-CLL is greater than 6 per 100 million people, although the global median age at diagnosis has now exceeded 70 years. based on the duration of observation without treatment, males are approximately twice as susceptible as females. [2]. Iron is is an important component for synthesizing hemoglobin , it is involved in various fundamental physiological functions , like cellular proliferation and development, but also damages cells through oxidative stress formation which leads to deferent types of leukemia and then provides a severe threat to human health [3]. In B-CLL, iron metabolism is altered, including alterations in iron uptake, storage, and efflux, as well as dysregulation of the ferroportin–hepcidin regulatory axis. [4]. Therefore, understanding the etiology of iron homeostasis and iron disorders mechanisms has been improved by the discovery of hepcidin[5]. Hepcidin maintains iron homeostasis and stored iron for many physiological functions, such as erythropoiesis and oxygen transport, limits the toxicity of excessive iron . Hepcidin dysregulation that resulting from genetic inactivation, ineffective erythropoiesis, or inflammation causes iron overload or iron deficiency disorders, such as anemia of inflammation, and iron-loading anemia[6]. Erythropoietin (EPO) is an essential hemopoietic hormone produced by the kidney and liver in both adults and fetus,. This hormone is necessary for controlling erythropoiesis and homeostasis of erythrocytes by enhance the survival, proliferation, and differentiation of erythroid progenitor cells and regulating the numbers of red blood cells in the circulation. Tissue oxygenation regulates the production and release of EPO in response to hypoxia, as well as the expression of its receptor[4]. Epo dysregulation unavoidably leads to erythrocytes production disorder in the bone marrow [7].Abnormal endogenous erythropoietin production has been reported in lymphoproliferative disorder patients [8] . One results of this disorder causes anemia; Anemia is a common clinical characteristic that adversely affects the prognosis of B-CLL patients. Different factors contribute to the prevalence of anemia in B-CLL for example Leukemic bone marrow infiltration, autoimmune

diseases, and a poor nutritional state resulting in folic acid, vitamin B12, and iron deficiency [8]

Materials and Methods

Study Subjects

The present study was conducted on 60 newly diagnosed B-CLL patients who were admitted to the Hematology center, Baghdad Teaching Hospital-Medical City. Before the collection of the blood samples, verbal informed consent of all patients and approval of the study was also obtained. Then, the socio-personal information including (age and sex) and clinical data were collected from the period 20 February until 25 December 2021. All patients were diagnosed as having B-CLL based on physical examination by a consultant hematologist, morphological assessment of blood films and flow cytometric immunophenotypic profile, and sometimes by bone marrow smear by aspirate examination and based on the guidelines of the International Workshop on Chronic Lymphocytic Leukemia/National Cancer Institute [9]. Thirty healthy volunteers, age and gender matched samples were taken as a control group .

Methods

Five ml of blood was obtained from newly diagnosed B-CLL patients and the control group and then collected in gel tube. Serum was then obtained after centrifugation of blood in a gel tube, and transferred to a new clean tube and stored in refrigerator at -20°C then used to laboratory investigations for measurement the levels Hepcidin , Erythropoietin , Malondialdehyde and Total antioxidant capacity by using Human ELISA Kit kit prepared by MyBioSource , USA. Additionally, The socio-demographic information including (age and sex) and clinical data were also collected.

Statistical Analysis

The results of Statistical in the present study were expressed as mean \pm standard deviation (SD) and considered statistically significant when the p value was ≤ 0.05 and the all analysis was done using the statistical analyzing system Graph Pad Prism Software to make the statistical analysis and for comparisons between B-CLL patients and control group.

Results

Personal characteristics distribution of study population

This study was conducted on 60 newly diagnosed patients with B-CLL, and the results showed there was a higher prevalence p value ≤ 0.05 in males (65.0 %) over females (35.0 %). Out of the 60 patients, 39 were male and 21 females, with 30 controls (12 males and 18 females). The mean age of patients was (64.54 ± 4.743) years, while the mean age of control was the (63.33 ± 4.700) years. (Table 1-1) .

Table 1-1: The personal characteristics of study population

Personal characteristics	Newly diagnosis B-CLL patients (n=60)	Control (n=30)
Gender, n (%)		
Male	39 (65.0 %)	12 (40.0 %)
Female	21 (35.0 %)	18 (60.0 %)
Age (years), mean \pm SD		
	64.04 \pm 5.6	63.33 \pm 4.70

The distribution of the patients according to Binet stage and clinical characteristics

In the present study , the clinical characteristics of newly diagnosed B-CLL patients based on Binet staging were : (6.7%) in stage A , (43.3 %) in stage B and (50.0%) in stage C and (51.7 %) , additionally, clinical examination of the patients revealed a higher proportion of lymphadenopathy (65.0%) followed by hepatomegaly and/or splenomegaly (51.7%) . (Table 1-2) .

Table 1-2 :The clinical data for the newly diagnosed B-CLL patients

Characteristics	Newly diagnosed B-CLL patients (n=60)
Binet staging , n (%)	
A	4 (6.7%)
B	26 (43.3%)
C	30 (50.0%)
Hepatomegaly – and /or splenomegaly, n (%)	
Yes	31(51.7%)
No	29 (48.3%)
Lymphadenopathy, n (%)	
Yes	39(65.0%)
No	21(35.0%)

Serum levels of Hepcidin

The present study showed the mean levels of the Hepcidin was significantly increased in newly diagnosed B-CLL patients (88.82ng/ml \pm 8.717) compare to the control group (40.48 ng/ml \pm 2.546) (Figure 1-1).

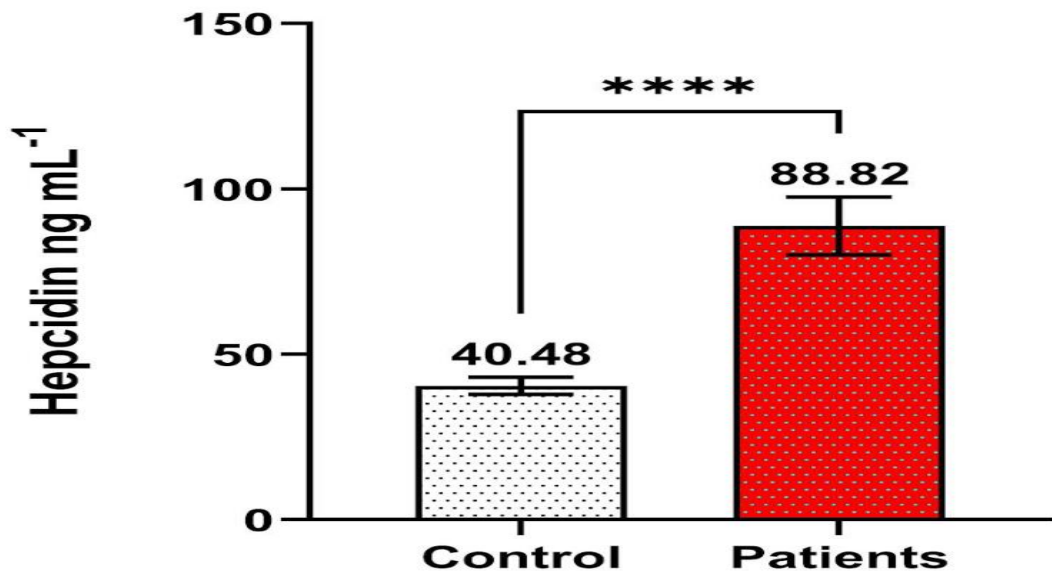


Figure 1-1 :Levels of Hepcidin between patients and control group

Serum levels of Erythropoietin

In the current study the mean levels of the Erythropoietin was significantly increased the newly diagnosed B-CLL patients (86.48 U/ml \pm 4.581) compared with control group (17.88 U/ml \pm 1.508) (Figure 1-2).

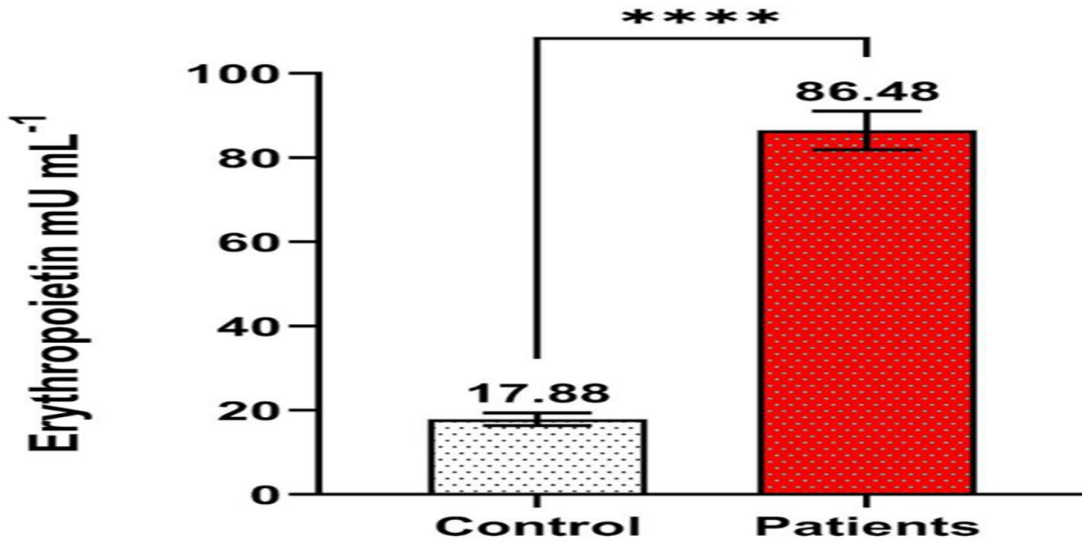


Figure 1-2 : Levels of Erythropoietin between patients and control group

Oxidant - Antioxidant status

The results of Malondialdehyde (MDA) levels was significantly increase in newly diagnosed B-CLL patients (4.331 pmol/ml \pm 0.309) compared to control group (2.275 pmol/ml \pm 0.271) while The results of Total antioxidant capacity (TAC) show a significantly decrease in newly diagnosed patients with B-CLL (11.74 U/ml \pm 0.911) compared to control group (17.93U/ml \pm 0.886) (Figure 1-3 and Figure 1-4).

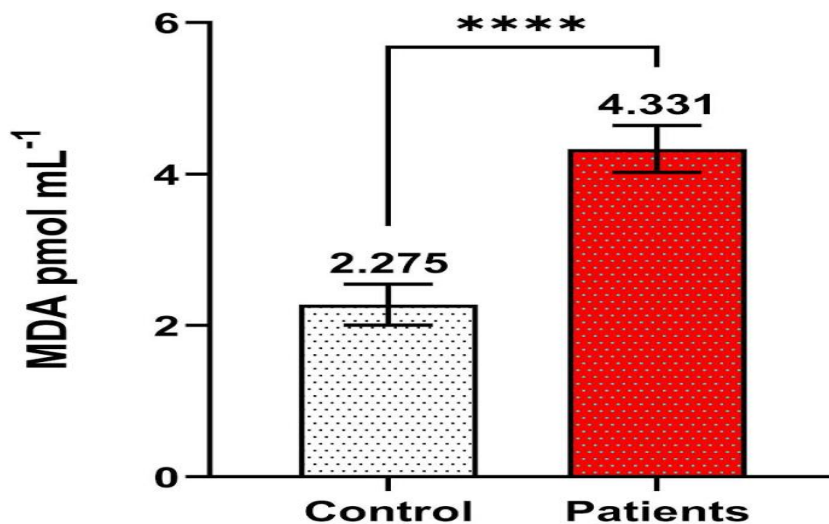


Figure1-3 :Assessment of Malondialdehyde levels between patients and control group

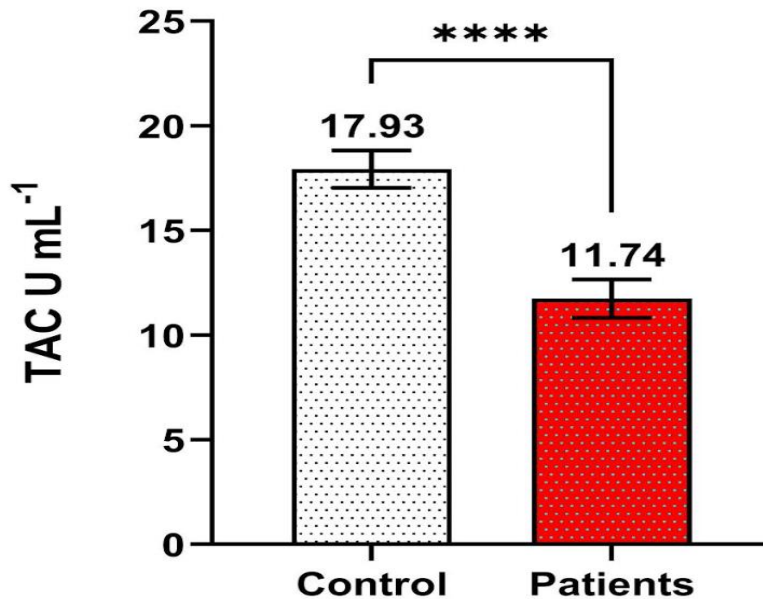


Figure1-4:Assessment of Total antioxidant capacity levels between patients and control group

Discussion

The distribution of newly diagnosed B-CLL patients according to sex in present study (Table 1-1) showed there were significant difference between the incidence of males and females patients. When male have been higher prevalence p value ≤ 0.05 in compared to female ones, in addition, the mean age of the patients lies in the elderly age. The results of present study are in agree with Jakšić *et al.*, they found that the incidence of B-CLL is increase in male than in female, with the ratio being about 2:1[10]. other study demonstrated that the incidence of B-CLL increase with an aging in elderly phase and ratio of B-CLL is predominant in males more than female addition to the onset of disease at adult individual [11,12]. Clinical staging for most malignancies is the important process to determine how far the disease has progressed to guide appropriate treatment. therefore, most of the malignancies were classified based on the staging, size of cancer, and the degree to which it has spread [13]. Anemia and thrombocytopenia may be observed in 15-30% of patients while Lymphadenopathy can be detected in approximately 80% of cases, with bilateral and symmetrical enlargement of the cervical and axillary lymph nodes. In around 50 percentage of cases, mild to moderate splenomegaly is present, but hepatomegaly is less common and It is evident that there is a wide range of variability in each result of the clinical staging of newly diagnosed B-CLL patients distribution. This may be attributed to the fact that some of the patients were with stage B and other patients with higher percentage in stage C [14,15]. Therefore, the overall picture reflected an interplay between values attributed to each stage.

leukemic cells are highly active in terms of their metabolic requirement. Their survival and proliferation depend on the supply of important micronutrients and

macronutrients. One of these important micronutrients is iron [16]. The results in figure (1-1) are in agreement with study by Arvedson *et al.*, they showed that Hepcidin levels were elevated in newly diagnosed B-CLL patients compared with a control group in addition it elevated in many cancer populations raising the possibility that functional iron deficiency may play a role in the etiology of anemia in B-CLL [17]. Hepcidin controls iron uptake and release from iron-recycling or iron-storing cells, hence regulating plasma iron levels. Hepcidin exerts its effects via its receptor, ferroportin, a cellular iron exporter. Hepcidin is regulated by plasma iron concentrations, body iron stores, infection and inflammation, and erythropoiesis, resulting in systemic iron homeostasis [18,19]. In contrast, blocking the action of Hepcidin in certain malignancies can suppress the progression of the tumour [20]. On the other hand, iron-sequestering drugs was inhibited tumour growth by starving cancer cells of iron. The increased iron requirement in cancer cells leads to changing the expression of proteins involved in the iron supply and iron export in order to adapt to their metabolic requirements [21].

The results of Erythropoietin levels are in agreement with Capalbo *et al.*, they noted that Erythropoietin levels in serum were significantly increased in B-CLL patients in compare to control group [22]. additionally ,it is in agreement with the study by Beguin *et al.* , who showed the elevation of Erythropoietin was appeared adequate for the degree of anemia [23]. In addition, a defective endogenous Erythropoietin production has also been described in patients with lymphoproliferative diseases [8,24]. Katz *et al.*, reported the role of Erythropoietin in the biology and prognosis of some malignancies as an anti-neoplastic effect such as in certain lymphoproliferative diseases [25] finally, anemia is a frequent complication of advanced B-CLL and several cytokines known to inhibit erythropoietin formation are produced by B-CLL [23]. Although Iron is biologically essential for nearly all organisms but also potentially toxic. Iron's toxicity is caused by its Fe²⁺ forms, which are highly reactive and cause rapid oxidative damage to proteins and DNA, hence permanently altering the structure of proteins and genetic material [26]. The biology of cancer cells requires metabolic reprogramming for survival in a harsh environment, including nutrient deprivation, reactive oxygen species (ROS) generation, and oxygen deprivation. Several other metabolic fluxes, most notably the pentose phosphate pathway and mitochondrial metabolism, are emerging as important for cancer in maintaining redox balance, in addition to the extensively studied glycolytic metabolism.[27].The results in figure (1-3) are in agree with several recently studies , Zhevak *et al.* , indicated that patients with B-CLL experience increased oxidative stress (OS) by concomitantly increasing reactive oxygen metabolites production and the relative deficiency of the antioxidant defence system [28]. and also agree with Bakan *et al.*, they demonstrated increasing MDA levels in the plasma of CLL patients, which may not only be the result of increased production but also a failure of the antioxidant defence [29]. Furthermore , MDA levels were shown to be higher in B-CLL lymphocytes than in healthy lymphocytes, mainly because of a decrease in the antioxidant enzyme activities of intracellular protection enzymes such as SOD and CAT, indicating that the intracellular level of MDA is a biomarker of disease progression [30].in addition the result in figure (1-4) are in accordance with a previous study, Salimi *et al.* , reported that total antioxidant capacity levels were significantly decrease in patients with B-CLL

compare to control group [30]. The mechanisms by which OS may promote cancer growth are not yet completely understood, and in B-CLL, as well as other neoplasms, it is unknown whether OS is a primary cause of the disease or only a secondary effect. OS in B-CLL is concurrently produced by increased ROS production, related mostly to the mitochondrial activity of B-CLL cells, and insufficient antioxidant defences.[32,33]. The decreased antioxidant capacity found in the sera of our B-CLL patients may be related to a consumption of antioxidants and/or to a reduced efficacy of antioxidant defences due to the systemic excess of ROS that is common in cancer[34]. Accumulating evidence by two important studies suggested that increased OS and the relative deficiency of the antioxidant defence system are involved in crucial steps of B-CLL development[28,35]. Resultantly, it may be involved in initiation and enhancement of multiple stage carcinogenesis, cell apoptosis, proliferation, differentiation, and immune function suppression, and it contributes to genomic DNA, RNA instability that resulted in gene mutations through cell division, and a final result is development of B-CLL [36-38].

Conclusion

Identification of the clinical features, hormonal disorders such as Hepcidin and Erythropoietin as well as evaluation of oxidative stress biomarkers and antioxidant defenses may be important for the early diagnosis of B-CLL patients and evaluation of tumor development.

Conflicts of Interest

The authors declare no conflict of interest .

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