Research Article

C-Reactive Protein and Iron Status in Iraqi Patients with Acute Myeloid Leukemia Before and After Treatment

Khitam Abdul Wahab Ali[1]*, Alaa Fadil Alwan[2] and Hiba Ammar Mohammed[3]

[1]Clinical Biochemistry, College of Medicine, Al-Mustansiriyah University, Baghdad.

[2]Clinical Hematology, National Center of Hematology, Al-Mustansiriyah University, Baghdad.

[3]Clinical Biochemestry, Al-Muthana hospital, Ministry of Defense.

ABSTRACT:

Objectives: To assess serum C-reactive protein (CRP) and serum Iron level changes in patients with acute myeloid leukemia (AML) before and after one course of chemotherapy and compare their levels to controls and their contribution on the pathogenesis of AML. **Methods:** This present study includes Fifty-eight (58) patients (30 male and 28 female) with acute myeloid leukemia with age range (15-65 years). Forty three (43) patients continued the study (24 male and 19 female) divided into two groups: Group (1) Patients with AML before starting chemotherapy. Group (2) the patients after 4 weeks of chemotherapy. Patient's treatment was done according to international protocol which is called (3+7) when Daunorubicin was given in the first day to third day and Cyatrabine (Ara-C) was given from the first day to seventh day then evaluation is done on twenty eighth day to evaluate response of patients. This study conducted at the National Center of Hematology and Baghdad Teaching Hospital in the Medical City from February 2014 to June 2014. All patients were subjected to complete history and physical examination. Diagnosis as AML patients was established by complete blood count and blood film, bone morrow aspiration and biopsy, C-reactive protein, ferritin, iron, and total iron binding capacity. Forty-three (43) healthy subjects (24 male and 19 female) were included in the study mainly from medical staff and their families. They were age and sex matched to patients group and considered as controls as (Group 3). **Results**: Patient's serum samples were investigated before and after treatment and compared with its corresponding data of healthy control group and then statistically analyzed. Results revealed that: the prevalence of AML was higher in male than in female. Serum CRP levels increased in AML patients before and after treatment compared to newly diagnosed AML patients, while there were a significant increase in mean serum ferritin levels observed in (Group 2) compared to newly diagnosis patients (Group 1)($P<0.002$) and the levels were significantly higher in newly diagnosis group compared to healthy controls ($P<0.015$). Patients with (AML) during remission show significant decrease in iron levels compared to newly diagnosis group ($P<0.0001$), while levels in healthy controls recorded higher values than both (Group 2) and (Group 1) ($P<0.0001$). Serum total iron binding capacity **(**TIBC) levels showed a significant decrease in (Group 2) after treatment compared to (Group 1) before treatment ($P<0.0001$) but the levels were significantly higher in healthy controls compared to (Group 1) and (Group 2) ($P<0.0001$) **Conclusions:** C-Reactive Protein, Ferritin, Iron and Total Iron Capacity may be used as diagnostic criteria for acute myeloid leukemia and also can play an important role in pathogenesis of AML.

INTRODUCTION

Acute Myeloid Leukemia (AML) is a clonal hematopoietic disorder arising from the acquisition of genetic and epigenetic alterations, leading to a premature arrest of the normal differentiation of stem cells and to the accumulation of immature neoplastic cells in the blood and bone marrow[1]**.** Changes in white blood cells lead to impaired ability to fight infection and decrease the ability of the bone marrow to form red blood cells and platelets[2]**.** Rate of (AML) incidence raises in male than in female and with progressive of age[3]**.** The development associated with myelodysplastic syndromes (MDS), genetic disorders, acquired diseases, exposures to ionizing radiation and alkylating agents and exposure to anti-cancer chemotherapy[4]**.**

Patients with acute myeloid leukemia treated with anti-cancer drugs chemotherapy to damage and disrupting leukemia cells. Main induction therapy consists of cytarabine (Ara-C) and anthracycline based regimen "3 + 7" (daunorubicin 45 to 60 mg/m^2 per day intravenously for 3 days andcytarabine 100 mg/m^2 per day) for 7 days . It has been found that the complete remission (CR) rate is approximately 60% to 80% in newly diagnosed younger adult patients with AML treated with 3+7"[5]**.** The remission induction therapy in leukemia produces normal bone marrow function, thereby complete remission is defined when the patients have full recovery of normal peripheral blood counts with recovery of normal bone marrow cellularity, and less than 5% blast cells are present in the bone marrow"[6]**.**

Post remission therapy “consolidation therapy” is needed to damage remaining AML cells and prevent relapse[7]. Decreasing of rest leukemic cells accomplished by cytotoxic chemotherapy, leading to significant myelosuppression[8].

C-reactive protein (CRP), a plasma protein of the pentraxin family and an acute phase reactant, which displays high sensitivity as a general inflammation marker[9]. It is produced and secreted mainly by liver in response to cytokines such as interleukin-6[10], released from leukocyte within tumor microenvironment[11]. Serum level of C-reactive protein has a plasma half-life of 19 hours. It changes by at least 25% during inflammatory conditions, and even after trauma or surgery[12]. The association between CRP levels and acute myeloid leukemia risk influenced by multiple factors such as (location, age, gender). Blast cells growth could cause inflammatory response, thereby increasing CRP levels. Alternatively, chronic inflammation could lead to the development of cancer.CRP is a marker of inflammation, has a direct role in carcinogenesis[11].

During immune activation, ferritin is known as an acute phase reactant because of its intracellular iron storage abilities[13] . By hepatocytes, and also by other cell types, including macrophages and cancer cells it is produced and secreted[14]. Therefore, serum ferritin levels elevated in infection, systemic inflammation, and malignancies[15]. Evidences were suggested that , there was association between high body iron stores and the risk of developing cancer[16]. So, increased in serum ferritin might indicate the exists of malignant disease spatially in acute and chronic leukemia[17]. Acute myeloid leukemia commonly associated with iron overload[18]. Many factors are participating to the hyperferritinameia associated with AML , inflammation chemotherapy, blood transfusion and ferritin hepatic clearance disorders[19]. In other study on malignant cells predicted that malignant cells need a high requirement of iron due to the rapid division of the cells. Tumor cells were changed routes of the uptake of iron. These routes may be important in achieving raised iron levels under this condition[20].

Recent study predicted that iron is a risk factor for different types of cancers mainly due to its prooxidant activity . Non–protein-bound iron ("free" or catalytic iron) is a prooxidant, as its participation in the redox cycling which is associated with the generation of reactive oxygen species (ROS) such as the hydroxyl radicals. ROS are highly reactive molecules capable of oxidative damage to DNA[21]. Increased cellular iron may cause tumorigenesis. Neoplastic cells were higher iron requirements than normal cells, therefore decreasing iron level was developed as efficient strategies in chemotherapy and from malignant cells themselves[18].

Total iron binding capacity (TIBC) determines the maximum amount of iron that serum proteins can bind. TIBC assay measure the total number of transferrin binding sites per unit volume of plasma or serum. Normally, almost all the binding capacity is due to transferrin. One third of plasma TIBC is saturated with iron[22]. Plasma TIBC rises in iron deficiency, but often tends to be low in patients with iron overload and in protein losing states such as infections, neoplasms, anemia of chronic disease and after trauma[23]. Elevated TIBC, were associated with increased risk for developing various types of cancer such as acute myeloid leukemia[21].

Subjects and methods

The prospective cohort study conducted at the National Center of Hematology in Al-Mustansiriyah University and Baghdad Teaching Hospital in the Medical City from February 2014 to June 2014. This study was approved by scientific committee of Mustansiriyah Medical College . Questioner history and consent was obtained from all patients prior to study, fifty-eight (58) patients (30 male, and 28 patients female) aged between (15-65 years).

Inclusion criteria included patients with newly diagnosis of AML, age between (15-65 years), and no history of illness, while the exclusion criteria included patients of AML with subtype M_3 ,age of patients was under 15 years and above 65 years , patients with relapse and refectory of AML and Frail patients not suitable for chemotherapy.

Fifteen patients (15) out of fifty-eight (58) were excluded from the study because preventing to take chemotherapy, went to another center outside Baghdad, loss of follow up or early death during period of study. After exclusion of fifteen patient, forty three patients (43) contained the study, (24 male and 19 female). Patients divided into two groups: Group (1): Patients with AML before starting chemotherapy. Group (2): Patients after 4 weeks of chemotherapy. Group (3): Forty-three (43) healthy subjects (24 male and 19 female) were included in the study mainly from medical staff and their families. They were age and sex matched to patients group and considered as controls.

All Patients were subjected to complete history and physical examination. The diagnosis was established by complete blood count and blood film, bone morrow aspirated and biopsy, liver function tests, and renal function tests. Other parameters were done in this study such as ferritin, CRP, S. iron, and total iron binding capacity.

Patient's treatment was done according to international protocol which is called (3+7) when Daunorubicin was given in the first day to third day and Cyatrabine (Ara-C) was given from the first day to seventh day then evaluation is done on twenty eighth day to evaluate response of patients. Five milliliters (5 ml) of venous blood were taken from each patients and controls. Blood samples were put in plain polyethylene tube and allowed to clot at room temperature for thirty minutes, then samples were centrifuged at (3000 rpm) for (10 min). The obtained serum were frozen at -20^{0}C to be analyzed later, hemolyzed samples were discarded.

Latex Agglutination Slide Test was used for the Qualitative and Semi-quantitative Determination of serum C-Reactive Protein (CRP) in Non-diluted(manufactured by Human-Germany). Thismeasurement depend on the immunological reaction between C-reactive protein (CRP) of a patient specimen serum and the corresponding anti-human CRP antibodies bound to latex particles. In the test cell of the slide, the positive reaction is reflected by a visible agglutination of the latex fractions[24].

Estimation of serum ferritin levels was done by immunoenzymometric assay required essential reagents such as antibodies with affinity and specificity (enzyme and immobilization) using kit (manufactured by Monobind- UAS)[25].

Serum iron concentration was determined by iron Colorimetric test with Lipid Clearing Factor (LCF) using kit(manufactured by Human-Germany)[26]

The principle of this assay based on the reaction of Fe^{+3}, chromazurol B (CAB),and cetytrimethylammonium bromide (CTMA) to produce colored ternary complex at the absorption 623 nm. There is direct proportional between the concentration of iron in the sample and the intensity of the color production.

Estimation of Serum Total Iron Binding Capacity (TIBC) was measured using kit manufactured by (Human-Germany)[27]. TIBC in serum is saturated with a further concentration of Fe^{+3} ions. Unbound iron (increase) is absorbed by aluminium oxide and precipitated.

Data were analyzed using the available statistical package of SPSS-22 (Statistical Packages for Social Sciences- version 22),and presented in simple measures of frequency, percentage, mean, standard deviation, and range (minimum-maximum values).

The significance of difference of different means (quantitative data) were tested using Students-t-test for difference between two independent means or Paired-t-test for difference of paired observations (or two dependent means), or ANOVA test for difference among more than two independent means. The significance of difference of different percentages (qualitative data) were tested using Pearson Chi-square test (χ^2-test) with application of Yate's correction or Fisher Exact test whenever applicable. Pearson correlation was calculated for the correlation between two quantitative variables with its t-test for testing the significance of correlation. The correlation coefficient value (r) either positive (direct correlation) or negative (inverse correlation) with value <0.3 represent no correlation, 0.3-<0.5 represent weak correlation, 0.5-<0.7 moderate strength, >0.7 strong correlation. In addition to correlation the r^2 was calculated (The coefficient of determination), i.e. when value of r=0.58, then r^2=0.34, this means that 34% of the variation in the values of y may be accounted for by knowing values of x or vice versa. Statistical significance was considered whenever the P value was equal or less than 0.05.

RESULTS AND DISCUSSION

Regarding assessment of the importance of age and gender for all subjects were shown in **Table (1):** the prevalence of age in AML group was(40-59) years and (≥ 65 years) (30.2%) were higher than other age groups. These results predicting that AML is more common in elderly. So, AML is generally a disease of old age[28]. These findings coincides with previous report by[29] .This table showed regarding to AML patient's gender in male (24) (55.8%) higher than in female (19) (44.2 %). Thisresults predicted that higher prevalence of AML observed in men. This result is agreed with[30] study.

Table 1: Age gender distribution of studied groups

Characterization		AML		Control	
		No.	%	No.	%
Age(years)	<20	7	16.3	8	18.6
	20-39	10	23.3	10	23.3
	40-59	13	30.2	12	27.9
	≥65	13	30.2	13	30.2
	Mean ±SD(Range)	43.0 ±18.6	(15-65)	43.0 ±18.6	(15-65)
Gender	Male	24	55.8	24	55.8
	Female	19	44.2	19	44.2
*Significant difference between proportions using Pearson Chi-square test at 0.05 level					

Results of this study demonstrated that the screening test for C-reactive protein(CRP) levels in the most patients showed positive test while all controls showed negative test therefore the comparison is done between two groups of AML patients, as shown in **Table (2) , Figure(1)**. CRP levels showed significant differences in two groups of AML : Newly diagnosis and after treatment in all titers of CRP in(mg/L) (≤6 x >6, ≤12 x >12, ≤24 x >24) P-Value (0.014,0.045,0.013) respectively.

Table 2: Mean C-Reactive Protein Levels for Studied Groups

		AML Before		AML After	
		N0	%	No	%
CRP (mg/L)	0	8	18.6	3	7.0
	6	6	14.0	1	2.3
	12	-	-	2	4.7
	24	7	16.3	4	9.3
	48	22	51.2	33	76.7
P value comparing (CRP=<6 x >6)	0.014*				
P value comparing (CRP=<12 x >12)	0.045*				
P value comparing (CRP=<24 x >24)	0.013*				
*Significant difference between proportions using Pearson Chi-square test at 0.05 level					

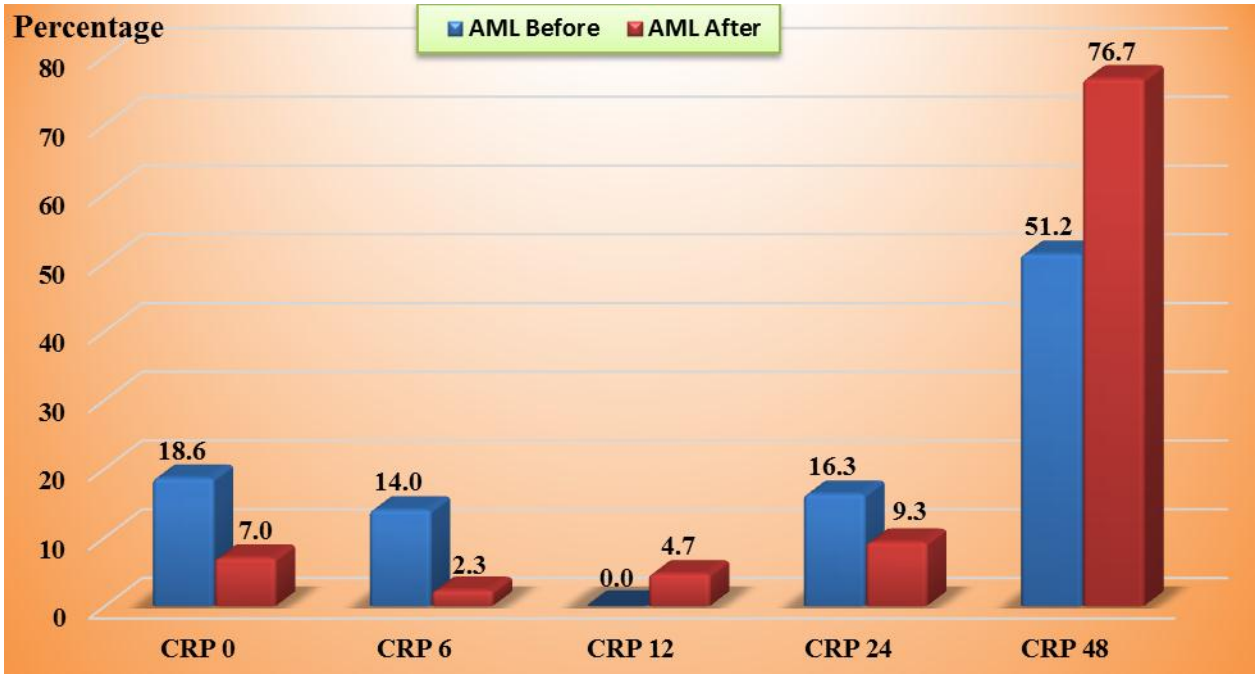

Fig. 1: Mean C-Reactive Protein (mg/L) in Acute Myeloid Leukemia Patients Before and After Treatment

The results of this study predicted that there were a significant increase in serum C- reactive proteins (CRP) levels in patients with acute myeloid leukemia before treatment because of the response to tumor necrosis, local tissue damage, or associated inflammation. These results were agreed with same results mentioned in[31] and[32] studies**.**

After treatment, infections in the immunocompromised host as a result of chemotherapy, were associated with elevation occurrence of neutropenic complication, which influences to response to chemotherapeutic, and cause morbidity and mortality. Additionally, malignant process itselfcauses increasing in CRP levels in spite of the presence of systemic bacterial infections[33]**.** These findings were in agreement with[34] study.

Regarding serum ferritin levels changes in AML patients show a significant increase in patients during remission compared to newly diagnosis , values were statistically significant (849.1±777.6; 624.0±197.68ng/ml , respectively [P<0.002] and the mean values were significantly higher in newly

diagnosis patients compared to controls (624.0±197.68; 132.4±138.7 ng/ml ,respectively [P<0.015]) and significantly increase in the patients during remission compared to controls (849.1±777.6; 132.4±138.7 ng/ml , respectively [P<0.0001]) as shown in the**Table (3), Figure (2).**

Table 3: Mean Serum Ferritin Levels for Studied Groups

Serum Ferritin (ng/ml)	AML Before	AML After	Controls
Number	43	43	43
Mean±SD	624.0±197.68	849.1±777.6	132.4±138.7
Standard Error of Mean	30.146	118.588	21.157
Range	300-1200	410-5704	40.9-982
P value compared to Control	0.015*	0.0001*	-
P value compared to AML After	0.002*	-	-
*Significant difference between two independent means using Student-t-test at 0.05 level			

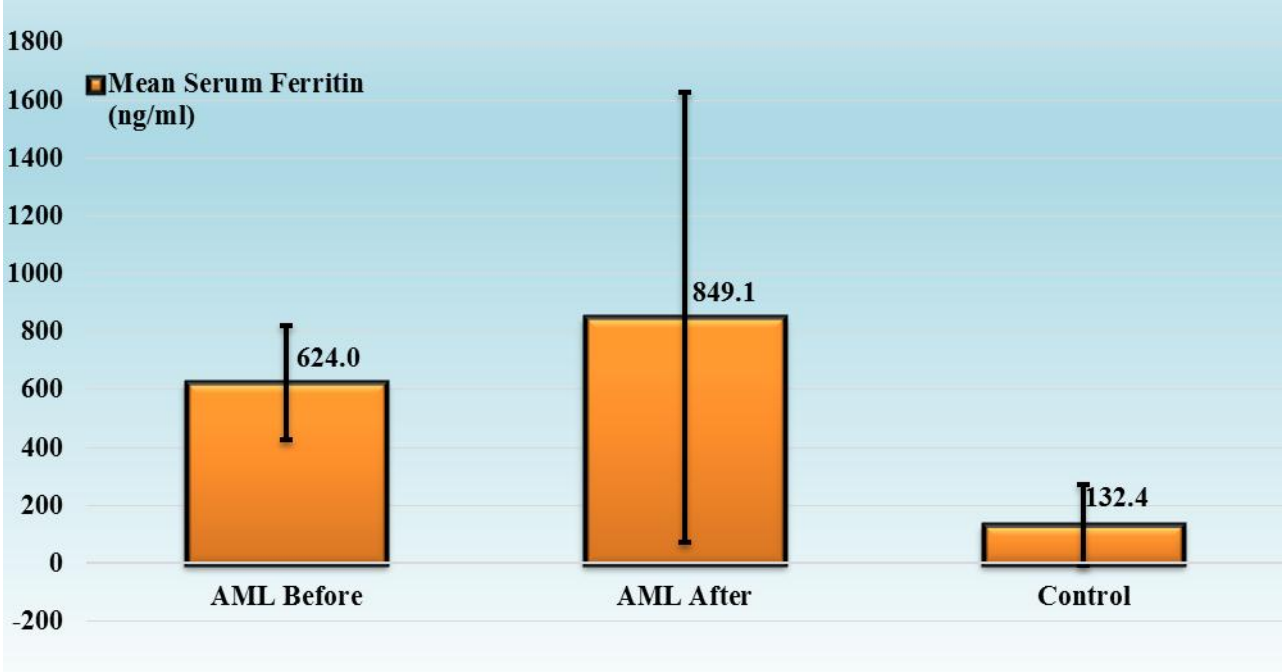

Fig. 2: Comparison of the Mean of Serum Ferritin Levels (ng/ml) in Acute Myeloid Leukemia Patients Before Treatment, After Treatment, and Controls

Serum ferritin concentrations were estimated in this study for AML groups and found that there was an increase in values of serum ferritin in newly diagnosed patients and in remission patients but still under chemotherapy when compared to healthy controls. These results were agreed with[35] results**,** who found that serum ferritin levels among patients newly diagnosed or on remission stage were significantly increased which may indicate that leukemia cell could affect iron metabolism leading to iron overload .

Other agreement by[36] who suggested that the highest levels of ferritin were found in AML patients under chemotherapy course treatment.

There was a growing in evidences which predicted that iron overload is common in patients with hematologic malignancies[37]**,** and the excessive iron body stores are known to interfere with natural body defenses and the increase in body stores of iron lead to increase growth rate of cancer cells[38]**.**

Previous study by[39] was predicted factors that contribute the increase in serum ferritin levels in acute myeloid leukemia including the followings:

- All patients of acute myeloid leukemia are anemic and have an elevation in the amounts of iron storage which are presented by further serum ferritin levels.
- In large mass of leukemic cells elevated the production of ferritin, this leads to raise in serum ferritin levels.

- Treatment by chemotherapy leads to damage most of the cells in the body, which lead to release of abnormal amounts of ferritin. There was no correlation between the elevation in the circulating of ferritin during chemotherapy with the amount of blood transfused or the degree of liver damage.
- The elevation in ferritin levels is a marker of acute phase response, and this acute phase usually founds in the acute myeloid leukemia due to increase in the concentrations of ferritin in the body.

In addition, results of iron study showed that the mean serum iron levels in acute myeloid leukemia patients during remission were significantly lower compared to newly diagnosis patients (12.57±1.98; 15.11±2.32 mmol/L), respectively [P<0.0001] .In the same time these mean iron values were significant lower in both newly diagnosis and during remission patients compared to controls (15.11±2.32;12.57±1.98; and 23.19±2.66 mmol/L, respectively [P<0.0001]) as shown in the **Table (4), Figure(3).**

Table 4: Mean Serum Iron Levels for Studied Groups

Serum Iron (mmol/L)	AML Before	AML After	Controls
Number	43	43	43
Mean±SD	15.11±2.32	12.57±1.98	23.19±2.66
Standard Error of Mean	0.354	0.301	0.406
Range	10.60-20.10	8.81-16.30	19.02-28.00
P value compared to Control	0.0001*	0.0001*	-
P value compared to AML After	0.0001*	-	-
*Significant difference between two independent means using Student-t-test at 0.05 level			

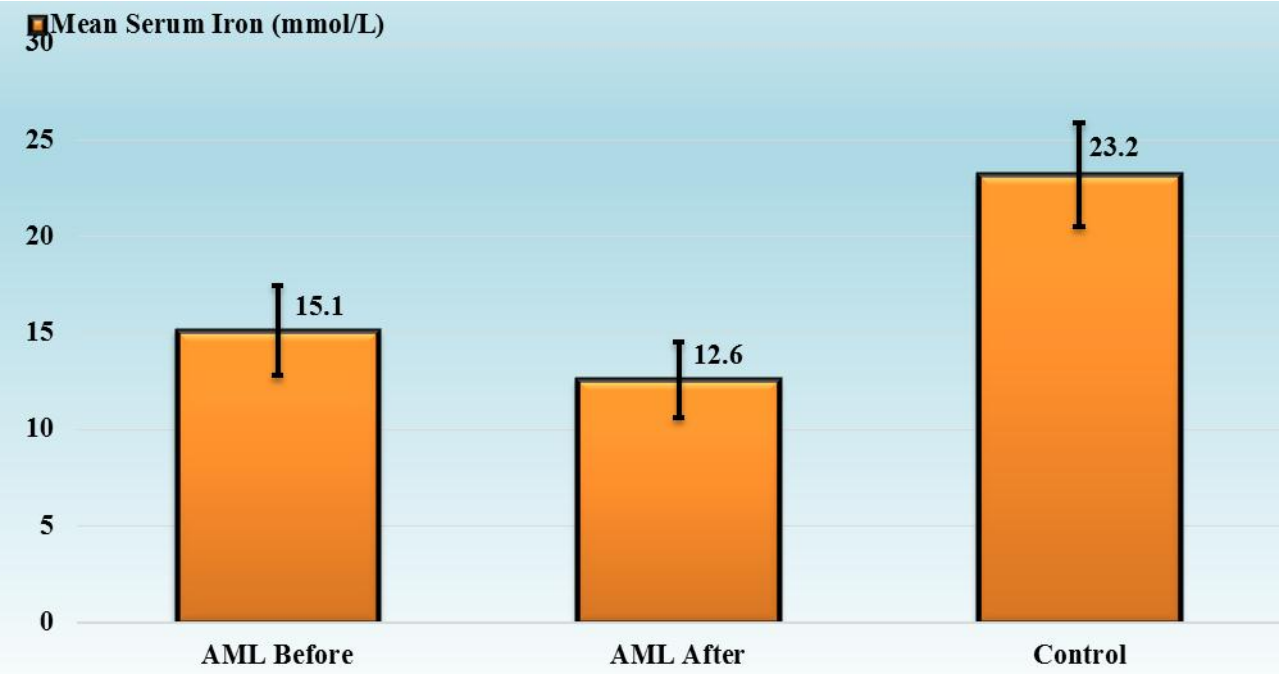

Fig. 3: Comparison of Mean Serum Iron (mmol/L) in Acute Myeloid Leukemia Patients Before Treatment, After Treatment, and Controls

The evaluation of deregulations of iron metabolism is very important in serum iron studies, especially iron deficiency and iron excess. Physiological function of iron is importance to produce red blood cells and to use as antimicrobial defense[40].

Regarding to serum iron levels investigation in this study, the results showed a significant decrease in serum iron levels in pre and post chemotherapy treatment comparing to healthy subjects, as shown in Table (5) and Figure (5)**.** Several previous studies also indicated that reducing in serum iron levels in AML patients may due to iron deficiency anemia and acute and chronic infections. These results come in agreement with previous results reported by[41] who predicted that serum iron levels decreased in acute leukemia cases.

Other agreement with these results was predicated by[42]**,** who observed that iron was thought to be a risk factor for cancer development in epidemiological studies in humans. The reducing in serum iron

level leads to interfere with the vital functions and increased mortality risk**.** Before and after chemotherapy treatment, serum iron is effected by several factors including iron absorption from diets; infection, inflammation, and diurnal variation. Patients with AML have inflammation which caused reducing in the iron availability to cells[43.]

Other related study showed that two biological effectors change the plasma iron concentration : infection and inflammation .Serum iron concentrations were affected by inflammatory factors that released from cells of immune system during the inflammatory process. Inflammation stimulate the movement of iron from the plasma pool into storage sites in macrophages, this explain the reduction in iron concentrations with the releasing of the inflammatory factors and lead to reduce in the hormone erythropoietin production, reduce response to erythropoietin, and interference with iron metabolism. Finally, anemia of inflammation caused reducing in serum iron levels[44]**.**

Estimation of total iron binding capacity **(**TIBC) observed that there were a significant decrease in mean serum TIBC levels in acute myeloid leukemia patients during remission compared to newly diagnosis patients , values were statistically significant (37.63±7.63; 51.32±4.78 mmol/L, respectively [P<0.0001]) , and the mean significantly decrease in the newly diagnosis compared to controls (51.32±4.78; 58.24±5.27M mol/L, respectively [P<0.0001]) and significantly decrease in the patients during remission compared to control (37.63±7.63; 58.24±5.27mmol/L, respectively [P<0.0001]) as shown in the **Table (5), Figure (4).**

Table 5: Mean Serum TIBC Levels for Studied Groups

TIBC (mmol/L)	AML Before	AML After	Controls
Number	43	43	43
Mean±SD	51.32±4.78	37.63±7.63	58.24±5.27
Standard Error of Mean	0.729	1.163	0.804
Range	42.00-69.00	15.00-60.20	49.00-69.00
P value compared to Control	0.0001*	0.0001*	-
P value compared to AML After	0.0001*	-	-
*Significant difference between two independent means using Student-t-test at 0.05 level			

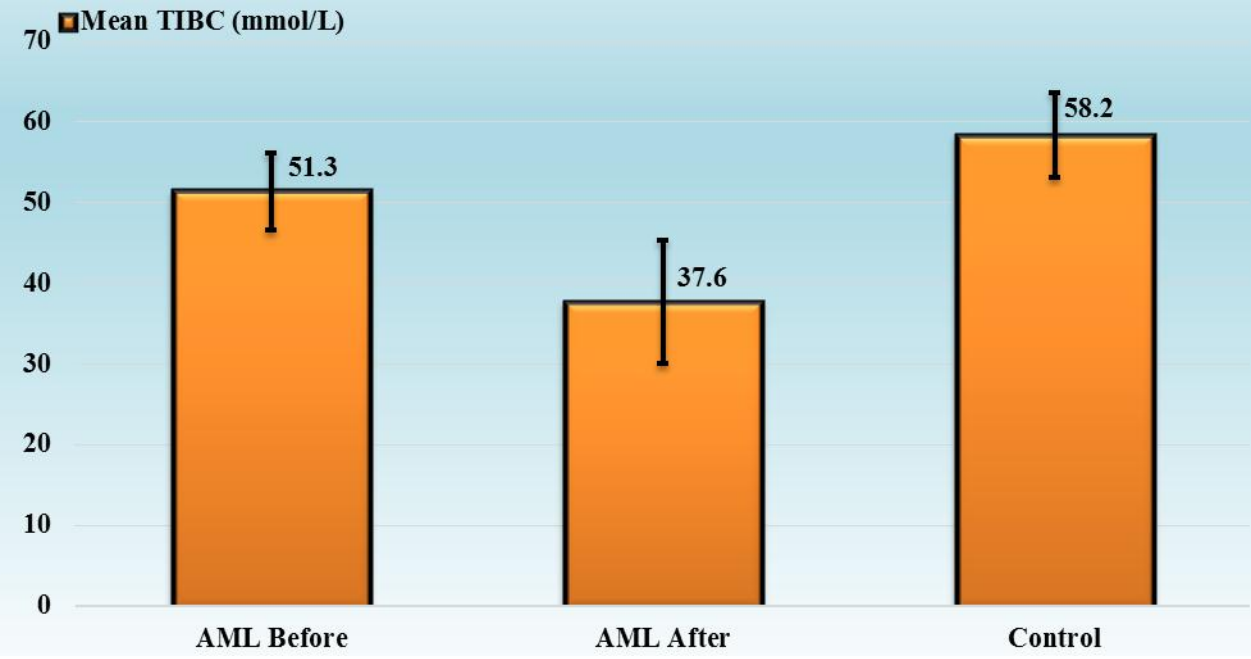

Fig. 4: Comparison of the Mean Serum TIBC Levels (mmol/L) in Acute Myeloid Leukemia Patients Before and After Treatment and Controls

In Acute myeloid luekemia patients before and during chemotherapy, total iron binding capacity was lower than in the controls. These results demonstrated that TIBC levels were significantly decreased in AML patients after four weeks with chemotherapy treatment ,these findings coincides with previous report which suggested that the production of iron binding proteins is became weak pre, during, and

post chemotherapy and decrease the ability of the liver to absorb from the circulation nontransferrin bound iron[45].

Total iron-binding capacity (TIBC) presents the availability of iron-binding sites, which is influenced by factors: iron status, malnutrition, inflammation, chronic infection, and cancer. Patients with hematologic disorder including AML cannot mobilized and utilize iron, which is stored in excess in reticulo-endothelials system leading to decrease in serum (TIBC)[44].

REFERENCES

1. Patriarca A., Salutari P. and Di S., 2015. The Impact of Molecular Genetic in Acute Myeloid Leukemias. Journal of Blood Disorders and Transfusion, pp. 1-12.
2. Betty Ciesla, 2012. Hematology in practice 2. ed., Philadelphia : F.A. Davis, cop.
3. Forman, D. et al., 2003. Cancer prevalence in the UK: Results from the Europreval study. Annals of Oncology, 14, pp.648–654.
4. Pierrakos, C. & Vincent, J.-L., 2010. Sepsis biomarkers: a review. Critical care (London, England), 14, pp.R15.
5. Pallis, M. et al., 2011. Analysis of the interaction of induction regimens with p-glycoprotein expression in patients with acute myeloid leukaemia: results from the MRC AML15 trial. Blood Cancer Journal, 1(6), p.e23.
6. Ramalingam, R. & Lancet, J.E., 2003. Management of Acute Myelogenous Leukemia in the Elderly. Journal of the Moffitt Cancer Center, 10(6), pp.1–5.
7. Linker, C., 2003. Autologous Stem Cell Transplantation for Acute Myeloid Leukemia. Bone Marrow Transplantation., 31(1), pp.731–738.
8. Panovska-stavridis, I., 2010. Immunophenotyping of the Blast Cells in Correlations with the Molecular Genetics Analyses for Diagnostic and Clinical Stratification of Patients with Acute Myeloid Leukemia : Single CenterExperience.
9. Salazar, J. et al., 2014. C-reactive protein: Clinical and epidemiological perspectives. Cardiology Research and Practice, 2014(Mi).
10. Ingle, P. V. & Patel, D.M., 2011. C- reactive protein in various disease condition - an overview. Asian Journal of Pharmaceutical and Clinical Research, 4(1), pp.9–13.
11. Guo, Y.-Z. et al., 2013. Association between C-reactive protein and risk of cancer: a meta-analysis of prospective cohort studies. Asian Pacific journal of cancer prevention : APJCP, 14, pp.243–8.
12. Fonseca, L. a M. et al., 2013. Review article C-reactive protein : clinical applications and proposals for a rational use ☆. Rev Assoc Med Bras, 59(1), pp.85–92. Available at: http://dx.doi.org/10.1016/S2255-4823(13)70434-X.
13. Cray, C., Zaias, J. & Altman, N.H., 2009. Acute phase response in animals: A review. Comparative Medicine, 59(6), pp.517–526.
14. Weiss, G. & Goodnough, L.T., 2005. Anemia of chronic disease. The New England journal of medicine, 352, pp.1011–1023.
15. Arneson, W. & Brickell, J., 2007. Clinical chemistry : a laboratory perspectiveNo Title 1st ed., Philadelphia: Philadelphia : F.A. Davis Co.
16. Nakagawa, H. et al., 2014. Inverse correlation between serum interleukin-6 and iron levels among Japanese adults: a cross-sectional study. BMC hematology, 14(1),pp.6.
17. Abd, N. et al., 2010. Prognostic Impact of Elevated Serum Hyulronic Acid , Ferritin and Interleukin-6 in Patients with Acute Myeloid Leukemia. , 6(10), pp.423–432.
18. Zhang, C. & Zhang, F., 2014. Iron homeostasis and tumorigenesis : molecular mechanisms and therapeutic opportunities. Protein & Cell.
19. Kroot, A. & Viljoen, M., 2011. Acute Phase Proteins: Ferritin and Ferritin Isoforms, Acute Phase Proteins - Regulation and Functions of Acute Phase Proteins. In P. F. Veas, ed. Acute phase proteins - regulation and functions of acute phase proteins. South Africa: InTech, pp. 154–186.
20. Heath, J.L. et al., 2013. Iron deprivation in cancer-potential therapeutic implications. Nutrients, 5, pp.2836–2859.
21. Fonseca-Nunes, A., Jakszyn, P. & Agudo, A., 2014. Iron and cancer risk-a systematic review and meta-analysis of the epidemiological evidence. Cancer Epidemiology Biomarkers and Prevention, 23(January), pp.12–31.
22. Van Vranken, M., 2010. Evaluation of microcytosis. American Family Physician,82,pp.1117–1122. 23.Holley, A.K. et al., 2011.
23. Manganese superoxide dismutase: Guardian of the powerhouse. International Journal of Molecular Sciences, 12, pp.7114–7162.

24. Al-Qurashi M. Abdulrahman and Tarek E. Hodhod, 2013. The association of CageA-positive. Journal of American Science,9(4), pp.355-361.
25. Burtis, C.A., Ashwood, E.R. & Tietz, N.W., 1999. Tietz textbook of clinical chemistry 3rd ed., Philadelphia: Philadelphia : W.B. Saunders, ©1999.
26. Ahyayauch H., Wafae S., Adela R., et al.,2013. Effects of chronic and acute lead treatments on the biophysical properties of erythrocytes membranes, and a comparison with model membranes. FEPS Open Bio, 3, pp.212-217.
27. Williams, H.L. et al., 1980. Improved radiochemical method for measuring ferrochelatase activity. Clinical Chemistry, 26, pp.153–156.
28. Krug, U. et al., 2011. The treatment of elderly patients with acute myeloid leukemia. Deutsches Ärzteblatt international, 108(4), pp.863–70.
29. Bacârea, A., 2012. Diagnosis of Acute Myeloid Leukaemia. In S. Koschmieder, ed. Myeloid Leukemia-Clinical Diagnosis and Treatment. Romania:InTech,p.296.
30. Van der Helm, L.H. et al., 2013. Azacitidine might be beneficial in a subgroup of older AML patients compared to intensive chemotherapy: a single centre retrospective study of 227 consecutive patients. Journal of hematology & oncology, 6, p.29.
31. Wang, C.-S. & Sun, C.-F., 2009. C-reactive protein and malignancy: clinico-pathological association and therapeutic implication. Chang Gung medical journal, 32, pp.471–482.
32. Delavigne, K. et al., 2014. Hemophagocytic syndrome in patients with acute myeloid leukemia undergoing intensive chemotherapy. Haematologica, 99, pp.474–480.
33. Biswal, S. & Godnaik, C., 2013. Incidence and management of infections in patients with acute leukemia following chemotherapy in ergenal wards. Ecancermedicalscience, 7, pp.1–16.
34. Endo, S. et al., 2012. Usefulness of Presepsin (Soluble CD14 Subtype) as a Diagnostic Marker for Sepsis. Nihon Kyukyu Igakukai Zasshi, 23(October), pp.27–38.
35. Zhang, X. et al., 2014. Elevated Serum Ferritin Levels in Patients with Hematologic Malignancies. , 15, pp.6099–6101.
36. Kennedy, G. a. et al., 2013. A prospective phase II randomized study of deferasirox to prevent iatrogenic iron overload in patients undertaking induction/consolidation chemotherapy for acute myeloid leukaemia. British Journal of Haematology, 161, pp.794–801.
37. Armand, P. et al., 2012. Does iron overload really matter in stem cell transplantation? American journal of hematology, 87(February), pp.569–72.
38. Kang, J.O., 2001. Chronic iron overload and toxicity: clinical chemistry perspectives. Clinical Laboratory Science, 14(3), pp.209–219.
39. Abd, N. et al., 2010. Prognostic Impact of Elevated Serum Hyulronic Acid , Ferritin and Interleukin-6 in Patients with Acute Myeloid Leukemia, 6(10), pp.423–432.
40. Camaschella, C., 2013. Iron and hepcidin: a story of recycling and balance. Hematology / the Education Program of the American Society of Hematology. American Society of Hematology. Education Program, 2013(Table 1), pp.1–8.
41. Demir, C. et al., 2011. Altered serum levels of elements in acute leukemia cases in Turkey. Asian Pacific Journal of Cancer Prevention, 12, pp.3471–3474.
42. Sheiekh, N., Abid, R. & Bashir, T., 2009. Changes in The Serum Iron, Aminotransferases and Blood profile of The Pre-Treatment Leukemia Patients. Pakistan Journal of Science, 61(2), pp.85–90.
43. Beard, J.,2004 Indicators of the iron status of populations: free erythrocyte protoporphyrin and zinc protoporphyrin; serum and plasma iron, total iron binding capacity and transferrin saturation; and serum transferrin receptor. Assessing the Iron Status of Populations, pp.75–94.
44. Van Vranken, M., 2010. Evaluation of microcytosis. American Family Physician, 82, pp.1117–1122.
45. Therapy, I. et al., 2014. Evaluation of Iron Overload in Acute Leukemia , 36(4), pp.281–292.