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# Effect of the Serum Level of Interleukin-6 and Interleukin-10 on Chemotherapy in Acute Myeloid Leukemia Iraqi Patients

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**ABSTRACT**

***Background:*** Acute myeloid leukemia (AML), a cancer of the myeloid line of blood cells, It is characterized by malignant clonal proliferation and differentiation of immature myeloid progenitor cells that differentiate into malignant myeloblasts which cannot function like normal blood cells. Normally, cytokines are secreted by different types of cells to regulate the immune response, but in AML patients, cytokines can be produced by both leukemic blasts and immune system cells and their role in the pathogenesis is not clear .Blood cells and their marrow based progenitors are exquisitely responsive to their environment, and cytokines are an essential part of it. The overexpression of cytokines in leukemia patients declines in complete remission suggesting that these events are dependent on AML activity. Aim of the study is to determine the serum levels of IL-6, IL-10 in newly diagnosed, under treatment and relapsed acute myeloid leukemia Iraqi patients and show their relation to response to chemotherapy and used as a prognostic markers in the evaluation of the therapy making them highly applicable to routine clinical laboratories.

***Methods:*** Serum levels of IL-6, IL-10 were estimated by using ELISA kit, in 120 patients with AML, That divided into 40 patients for each group (newly diagnosed, under treatment and relapsed), depending on the stages of the patients with chemotherapy, from February 2022 to April 2023 from Baghdad Teaching Hospital, Baghdad, Iraq. Forty healthy controls were also enrolled in this study.

***Results:*** IL-6 and IL-10 levels were significantly higher in newly diagnosed and relapsed patients with AML than in control group and their levels decreased when patients responded to chemotherapy.

***Conclusion:*** The current study showed that the serum concentrations of IL-6 and IL-10 in AML patients increased before chemotherapy and began to decreased after therapy. These results may be used as a prognostic markers for the success of chemotherapy and could offer an interesting approach for treatment of AML.

**KEYWORDS:** AML – Chemotherapy – ELISA – IL-6 – IL-10- relapsed – Under treatment- newly diagnosed.

**ARTICLE DETAILS**

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## INTRODUCTION

Acute myeloid leukaemia (AML) is a heterogeneous, most common type of acute leukaemia that involves mutation in haematopoietic and progenitors stem cells (HPSCs) leading to uncontrolled division, self-renewal and differentiation[1]. Thus, accumulating numbers of immature haemopoietic progenitors replace the normal red blood cells, white blood cells and platelets[2]. The resistance of leukemia cells to chemotherapy drugs becomes the main obstacle in the treatment of AML. The research on the mechanisms of drug resistance in AML is very active and a considerable efforts have been spent in identifying prognostic markers that might predict clinical outcomes in AML patients. Prognosis in AML patients has been related to the frequently observed defective function of their immune system that hampers the development of effective response against leukemic blasts[3,4]. Amongst several reasons of resistance, certain secretory factors released by the tumor cells into the microenvironment have been found to confer resistance towards chemo- and radiotherapy, besides promoting growth. Normally, cytokines are secreted by different types of cells in response to a variety of stimuli to regulate the immune response, but in AML

patients, cytokines can be produced by both leukemic blasts and cells of the immune system and their role in the pathogenesis of acute leukemia has not been fully clarified[5]. So this cytokine signaling is a feature of leukemia that may play a role in proliferation, blast survival, resistance to chemotherapy and patients" prognosis[6]. Interactions between pro-inflammatory and anti-inflammatory cytokines regulate cytokine response[7]. And any changes in one of these cytokines lead to compensatory mechanisms that alter the cytokine network[8]. Interleukin-6 (IL-6), one of the major cytokines in the tumor microenvironment[9]. Which is found at high concentrations and known to be deregulated in cancer. It promotes tumorigenesis by regulating all hallmarks of cancer and multiple signaling pathways, including apoptosis, survival, proliferation, angiogenesis, invasiveness and metastasis and most importantly, the metabolism[10,11]. Interleukin-6 (IL-6) is a cytokine with pleiotropic inflammatory effects, has demonstrated different effects on the growth of leukemic blasts[12]. On the other hand, IL-10 is the most important anti-inflammatory cytokine found within the human immune response. It is an inhibitor of Th1 cytokines, including both IL-2 and IFN- g and a deactivator of monocyte/macrophage proinflammatory cytokine synthesis[13]. The function of IL-10 has been detected in the leukemic cells of most ALL and AML cases, and it suppresses the immune reactions, suggesting that IL-10 could be associated with escape of leukemia cells from immune surveillance[14].

## MATERIAL AND METHODS

### Subjects

One hundred and twenty of Iraqi AML patients were enrolled in this study, they were sub grouped into newly diagnosed (40/120), under treatment (40/120) and relapsed (40/120). The samples were collected from Baghdad teaching hospital, Baghdad, Iraq, their ages was ranged from 15 to 75 years. Another 40 healthy individuals also enrolled as control. Based on a laboratory tests and medical inspection for both patients and control. This study was done during the period from January 2022 to April 2023. The study protocol was approved by the Ethics Committee of the Iraqi Ministry of Health/Al-Nahrain University and written informed consent was obtained from all participants before entering the study.

### Measuring of IL_6 level

A quantitative sandwich ELISA kit was used to measure IL_6 rendering to the instructions providing by producer SunLong Biotech Co., LTD; China.

### Measuring of IL_10 level

A quantitative sandwich ELISA kit was used to measure IL_10 rendering to the instructions providing by producer SunLong Biotech Co., LTD; China.

### Statistical analysis

Statistical analysis of Data were done by using SPSS program version 23 and figures are drawn by GraphPad prism 9. Results were expressed using simple statistical parameters such as mean, standard deviation and standard error. Differences between means were assessed by independent samples T test. A probability that equals or less than 0.05 was considered significant. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability) in this study.

### Results

The age range of AML patients was between (15-75) years old. Thirty eight cases (31.666%), out of (120) was up to forty years old and only two patient (1.666 %) was up to seventy years old, and the other patient is range in between, as shown in table (1), and the overall mean age was 43.2 years, Results of this study showed that the distribution of patients according to gender revealed that the prevalence of AML was (61.66) % for male and (38.33) for female, as shown in table (2). Smoking also recorded in this study, 57 (47.5%) smoker patients, and 63 (52.5%) non- smoker patients with p= 0.527 as shown in table (3). Approximately the patients were grouped according to FAB classification system, out of 120 AML patients were , Acute Promyelocytic Leukemia (APL) subtype (M3) subtype with percentage of (47.5 %) followed by (AML-M2) (24.375%) whereas the lowest one was in M6 and M7 as shown in table (4).

**Table.1. Distribution of age groups in AML patients**

| Age groups (year) | No. of patients | Percentage (%) |
|---|---|---|
| 10-20 | 9 | 7.5 |
| 20-30 | 26 | 21.666 |
| 30-40 | 13 | 10.833 |
| 40-50 | 38 | 31.666 |
| 50-60 | 19 | 15.833 |
| 60-70 | 13 | 10.833 |
| Over 70 | 2 | 1.666 |
| Total | 120 | 100 |
| Chi-Square ($\chi^2$) (P-value) | --- | 12.382 ** (0.0001) |
| ** (P≤0.01). | | |

**Table 2: Distribution of sample study according to Gender**

| Gender | No of patients | % |
|---|---|---|
| Male | 74 | 61.666 |
| Female | 46 | 38.333 |
| Total | 120 | 100 |
| Chi-Square ($\chi^2$) (P-value) | --- | 8.100 ** (0.0044) |
| ** (P≤0.01). | | |

**Table 3: Distribution of AML patients according to smoking**

| Smoking | No of patients | % | Chi-Square ($\chi^2$) (P-value) |
|---|---|---|---|
| Smokers | 57 | 47.5 | 0.40 NS (0.527) |
| Non smokers | 63 | 52.5 | |
| Total | 120 | 100 | |
| ** (P≤0.01), NS: Non-Significant. | | | |

**Table 4. Distribution of AML patients according to FAB**

| Subtype | No of patients | % |
|---|---|---|
| **M0** | 4 | 3.333 |
| **M1** | 11 | 9.166 |
| **M2** | 29 | 24.166 |
| **M3** | 57 | 47.5 |
| **M4** | 1 | 0.833 |
| **M5** | 18 | 15 |
| **M6** | 0 | 0 |
| **M7** | 0 | 0 |
| **Total** | 120 | 100 |
| Chi-Square ($\chi^2$) (P-value) | --- | 21.367 ** (0.0001) |
| ** (P≤0.01) | | |

Results explained that a significant increase serum level of IL-10 in newly diagnosed patients (16.12 ±0.91 pg/ml) compared with healthy group at the P value (P≤ 0.01), there are no significant differences between the control and under treatment group, but the level of IL-10 decreased in relapsed group as shown in table (4) and figure (1).

On the other hand, The results explained that the serum levels of IL 6 before the chemotherapy in newly diagnosed group were significantly higher than those after chemotherapy and the control group (513.26±14.874), then in relapsed group (497.82±7.302) with the P value (0.0001). Increased IL-6 levels decreased when patients responded to induction chemotherapy in under treatment group (32.1±4.157) as shown in table (4) and figure (2).

**Table 4: Comparison between difference groups in IL-6 and IL-10.**

| Groups | | (Mean±SE) | |
|---|---|---|---|
| | | IL-6 (pg/ml) | IL-10 (pg/ml) |
| **Controls** | | 24.37±8.194A | 7.27±0.33 b |
| **AML Cases** | Newly Diagnosed | 513.26±14.874B | 16.12±0.91 a |
| | Treated | 32.1±4.157B | 7.61±0.40 b |
| | Relapse | 497.82±7.302B | 3.76±0.62 c |
| | Total | 347.73±8.77 | 10.65±3.012 |

**Different letter means significant differences (p<0.05), values are given by mean±SE. The first letter compared to controls, the 2nd letter multiple T-Test analysis within studied group variables. P. Value> 0.05: Non-Significant, P. Value<0.05; Significant differences**

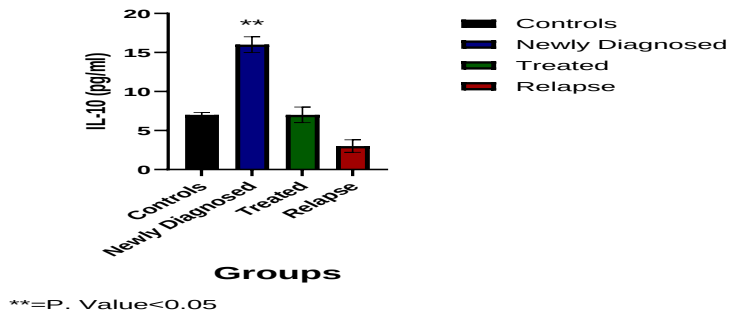


**Figure 1. Comparison between difference groups of AML in IL-10**

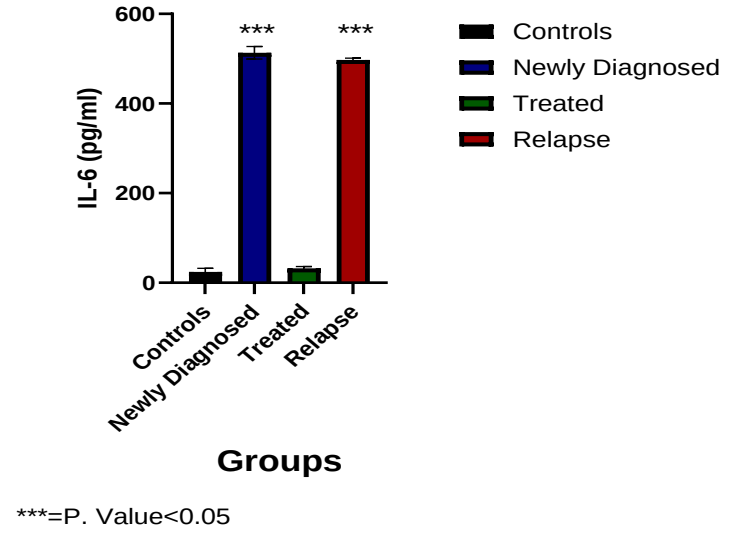


**Figure 2. Comparison between difference groups of AML in IL-6**

**ROC ANALYSIS**

ROC analysis for IL-10 recorded that in newly diagnosed ROC were Sensitivity %: 45, Specificity %: 95, confidence interval: 0.1 to 0.78, Area: 0.44±0.17, cutoff value: 265.06, p. value: 0.720. In treated group the Sensitivity %: 25, Specificity %: 98, confidence interval: 0 to 0, Area: 0±0, cutoff value: 255.45, p. value: 0.000. While relapsed group recorded Sensitivity %: 20, Specificity %: 99, confidence interval: 0 to 0, Area: 0±0, cutoff value: 255.45, p. value: 0.000. (Figure 3), (Table 6)

ROC data analysis for IL-6 showed that in newly diagnosed were Sensitivity %: 100, Specificity %: 87, confidence interval: 0.9 to 1.04, Area: 0.97±0.04, cutoff value: 6.51, p. value: 0.000. In treated group the Sensitivity %: 95, Specificity %: 100, confidence interval: 1 to 1, Area: 1±0, cutoff value: 28.88, p. value: 0.000. While relapsed group recorded Sensitivity %: 100, Specificity %: 87.5, confidence interval: 1 to 1, Area: 1±0, cutoff value: 10.52, p. value: 0.000. (Figure 3), (Table 5)

**Table 5: ROC curve analysis (Wilson/Brown method) Sensitivity% and 1-Specificity %, confidence interval, Area (Mean± SE) and Probability of IL-6, IL-10 in AML patients groups p<0.05.**

| Group | Sensitivity % | Specificity % | 95% confidence interval | Area ± SE | Cut off | P. Value |
|---|---|---|---|---|---|---|
| **IL-10** | | | | | | |
| Newly diagnosed group | 45 | 95 | 0.1 to 0.78 | 0.44±0.17 | 265.06 | 0.720 |
| Treated group | 25 | 98 | 0 to 0 | 0±0 | 255.45 | 0.000 |
| Relapsed group | 20 | 99 | 0 to 0 | 0±0 | 255.45 | 0.000 |
| **IL-6** | | | | | | |
| Newly diagnosed group | 100 | 87 | 0.9 to 1.04 | 0.97±0.04 | 6.51 | 0.000 |
| Treated group | 95 | 100 | 1 to 1 | 1±0 | 28.88 | 0.000 |
| Relapsed group | 100 | 87.5 | 1 to 1 | 1±0 | 10.52 | 0.000 |

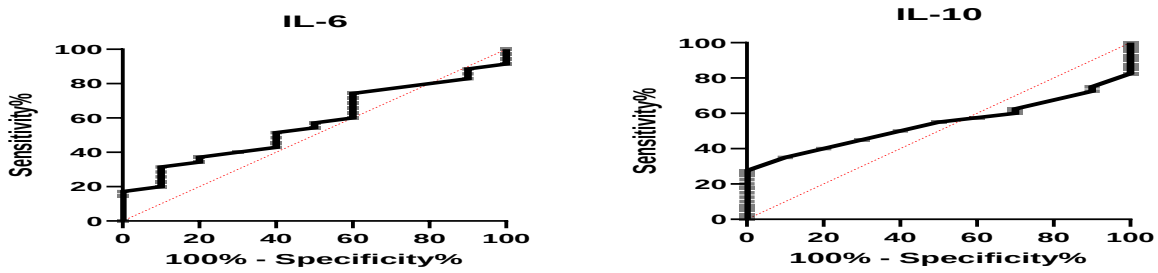


**Figure (3): AML ROC curve analysis (Wilson/Brown method) Sensitivity% and 1-Specificity %, of IL-6, IL-10 p<0.05**

## DISCUSSION

The cytokines are soluble molecules carrying specific information for the target cells. They provide target cells with specific information about organism conditions, and cause a specific response. The response may be stimulated and activated in case of inflammation or tissue damage, causing proliferation or apoptosis[15]. In healthy BM microenvironment, hematopoietic stem cells (HSCs) are maintained in a balance between quiescence, self-renewal, and differentiation to ensure life-long steady-state hematopoiesis and replenishment of the blood effector cell population under stress conditions such as infection, acute and chronic inflammation, aging or bleeding [16]. During infection and inflammation, an array of cytokines, including Interleukin together with hematopoietic growth factors [17, 18], control the switch from steady-state to emergency hematopoiesis [19,20]. In patients with preleukemic and leukemic conditions, including AML, the tight regulation of these cytokines is impaired, leading to aberrant cytokine secretion[21,22]. Thus, aberrant cytokine signaling is a feature of leukemia that may contribute to proliferation, blast survival, resistance to chemotherapy and patients' prognosis[23,24]. Recently some researches showed that deviating interleukin signaling pathways could be responsible for a significant contribution to the growth, resistance, and relapse of AML[25]. Results explained that a significant increase serum level of IL-10 in newly diagnosed patients (16.12 ±0.91 pg/ml) compared with healthy group at the P value (P≤ 0.01), there are no significant differences between the control and under treatment group, but the level of IL-10 decreased in relapsed group as shown in table (4) and figure (1). [18]agreed with this result, who demonstrated that AML new diagnostic (ND) patients also had increased plasma IL-10 levels compared with AML Complete Remission( CR) patients and the control group. Our results were in accordance with [26] as they found that the peripheral serum level of IL-10 in AML ND patients was much higher than that in complete remission patients or controls.

[19]observed a significantly higher increase in the frequency of IL-10 + secreting cells in AML ND patients than the healthy controls. [27]found the same results of our study that the expression of cytokines including IL-5, IL-6, IL-8, IL-10, TNF-α, TNF-β, IL-17F, and IL-22 (P < 0.05) was higher at the time of initial AML diagnosis in patients in the remission groups compared with the levels in these patients after two chemotherapy sessions. [22]agreed with our study but they used gene expressing IL-10 mRNA from the BM samples of the AML patients. They revealed that the IL-10 expression in the newly diagnosed AML patients was higher than the controls, this indicated the leukemic cells in bone marrow could secret IL-10 in an autocrine way as the local secretion of IL-10 in the tumor growth process, may cause the loss of the sensitivity to cytotoxic T lymphocytes and make the growth of tumor cells easier. Level of IL-10 in under treatment group was significantly decreased when compared with the newly diagnosed AML group, but still higher. They concluded that this may be due to the chemotherapy which inhibits the growth and activity of the leukemic cells and affects the function of the patients' immune system [20,22]. [28]matched the results when observed that the concentrations of TNF-α, IL-6 and IL-10 were significantly higher in AML patients than in the healthy volunteers in both groups of age. Moreover, plasma levels of IL-6 and IL-10 were associated with patient survival. Park *et al.* (2006) [29]recorded that the cytokine levels of bone marrow T cells decreased significantly at the time of complete remission (CR) after chemotherapy, compared to the cytokine levels before the start of chemotherapy, this results are similar to our results that showed that the serum level of IL-10 are decreased in relapsed group after chemotherapy. Another local study are disagreed with our results, [30]which explain significant increase serum level of IL-10 in pre- and post-treatment patients compared with healthy group, but there are no significant differences between the two group (pre- and post-treatment). [31]did not find any significant differences in the IL-10, IL-4, or IFN-γ levels of circulating lymphocytes derived from AML patients. Abd el-Hafez *et al.*, (2018)[32] said that IL-6 and IL-10 levels were significantly higher in newly diagnosed AML patients than in control group and their levels decreased when patients responded to induction chemotherapy, that similar to our results. The role of IL-10 in carcinogenesis has recently been found to be complex and pleiotropic[33], but there is still a strong body of evidence that IL-10 plays an important immunosuppressive role[34], allowing malignant cells to evade immune surveillance, and promote cancer growth and dissemination[35]. IL-10 has been detected in the leukemic cells of most ALL and AML cases and it suppresses the immune reactions, suggesting that IL-10 could be associated with escape of leukemia cells from immune surveillance[14]. [36]mention that the TH17 cells expressed IL-10 receptor α and may affect AML cells indirectly through their immunoregulatory effects, [37]conclude that the effect of

intensive AML chemotherapy differ between circulating T cell subsets, relative frequencies of TH17 cells are not affected by chemotherapy and this subset may affect AML cells indirectly through their immunoregulatory effects but probably not through direct effects of IL17-A.

The results explained that the serum levels of IL 6 before the chemotherapy in newly diagnosed group were significantly higher than those after chemotherapy and the control group (513.26±14.874), then in relapsed group (497.82±7.302) with the P value (0.0001). Increased IL-6 levels decreased when patients responded to induction chemotherapy in under treatment group (32.1±4.157) as shown in table (4) and figure (2). Suggesting that the measurement of IL-6 concentrations may be valuable in the evaluation of therapeutic effect. [27]found the same results of our study that the expression of cytokines including IL-5, IL-6, IL-8, IL-10, TNF-α, TNF-β, IL-17F, and IL-22 ($P < 0.05$) was higher at the time of initial AML diagnosis in patients in the remission groups compared with the levels in these patients after two chemotherapy sessions. [32]Abd el-Hafez *et al.,* (2018) said that IL-6 and IL-10 levels were significantly higher in newly diagnosed AML patients than in control group and their levels decreased when patients responded to induction chemotherapy, that similar to our results. Another study by [38] also agreed with our results, which demonstrated that the serum concentrations of IL-6 in AML Newly Diagnosed (ND) and relapsed patients were significantly higher compared to control group and the concentration fall in remission. [28]matched the results when observed that the concentrations of TNF-α, IL-6 and IL-10 were significantly higher in AML patients than in the healthy volunteers in both groups of age. Moreover, plasma levels of IL-6 and IL-10 were associated with patient survival. These results were combatable with [39] as he found that there was a significant increase in the serum level of IL-6 concentration in the ND and relapsed patient group, while in remission group, serum IL-6 fall within the normal range when compared with control group. Also study by [40,41,42,26] agreed with our results however they used plasma from bone marrow samples as they found markedly elevated concentration of IL-6 in bone marrow samples of AML- ND patients. [43]showed that IL-6, TNF and IL-2 levels were higher in myeloid malignancy groups than controls. Serum cytokines shown to be positively associated with each other and could signify to poor outcome of the myeloid malignancy patients. [44]showed that IL-6, IL-10, and IL-8 levels were significantly different in the AML cohort, and among the various cytokines tested, only elevated IL-6 levels predicted lower survival in the patients. Importantly, based on extensive clinical records, the elevated IL-6 levels may be valuable as a marker of the disease and as a target for new therapies for pediatric patients with AML. On the other hand, [45]didn't confirm these results as they found that the serum level of the IL-6 of the AML study group was significantly lower than the control may be due to the high level of IL-37 which has a negative correlation with IL-6 and the absence of the sepsis in AML study group. [46]disagree with our results as they found that IL-6 serum level in the AML patient group was lower than control group. The source of the elevated IL-6 within the BM niche is AML blasts auto secreting IL-6. IL-6 auto secretion has been demonstrated previously in solid tumors and in adults with AML[47,48,49,50]. An alternative source of IL-6 production is normal stromal cells or monocytes within the BM. It is possible that through paracrine signals, AML blasts are driving IL-6 production by these normal cells[51]. Our observation of decreased IL-6 levels in plasma collected during remission would be compatible with either explanation but the lack of correlation between IL-6 levels, and BM blast percentage at diagnosis supports a paracrine process. Chemoresistance contributes to poor survival and high relapse risk in acute myeloid leukemia (AML). As a pro-inflammatory cytokine, interleukin-6 (IL-6) plays a vital role in the chemoresistance of malignancies[52]. However, the underlying mechanisms of chemoresistance in AML have not been widely studied, we will disscuse some of them, the potential biological mechanisms by which elevated IL-6 levels could reduce survival in pediatric patients with AML. Since STAT3 signaling is known to regulate anti apoptosis gene expression, one possible mechanism could be by promoting chemoresistance[53]. Another possible mechanism by which IL-6 promotes relapse could be by supporting the LSCs, that a higher proportion of cells in the LSCe subpopulation had increased pY-STAT3 levels following treatment with exogenous IL-6 compared with non–stem AML cells. , so realized that IL-6–induced pY-STAT3 activity could potentially help cancer cells, particularly the critical LSC subpopulation, survive initial rounds of chemotherapy and allow them to subsequently expand, leading to relapsed disease[44].This hypothesis is supported by previous study showing increased levels of IL-6–induced pY-STAT3 at relapse compared with those at diagnosis[54]. [55]showed another mechanism, that bone marrow stromal cells (BMSCs) promoted chemoresistance in AML cells via the activation of the IL-6/STAT3/OXPHOS (The mitochondrial oxidative phosphorylation (OXPHOS) pathway. These findings exhibit a novel mechanism of chemoresistance in AML cells in the bone marrow microenvironment from a metabolic perspective. In this study, the co-culturing with BMSCs heightened OXPHOS levels in AML cells, thus promoting chemoresistance in these cells. The relationship among serine phosphorylated STAT3 (pS-STAT3), OXPHOS, and chemosensitivity of AML cells induced by BMSCs was demonstrated by the STAT3 activator and inhibitor, which upregulated and downregulated the levels of mitochondrial pS-STAT3 and OXPHOS, respectively. AML cells remodeled to secrete more IL-6, which augmented mitochondrial OXPHOS in AML cells and stimulated their chemoresistance. IL-6 knockout in HS-5 cells impaired the ability of these cells to activate STAT3, to increase

OXPHOS, or to promote chemoresistance in AML cells. Because lipid metabolism, which contributes to chemoresistance in AML, is enhanced by IL-6 in skeletal muscle cells, another mechanism by [52]hypothesized that IL-6 promotes the chemoresistance of AML by promoting lipid metabolism when realized that lipid transport-associated genes were upregulated in the high IL-6 receptor expression group and inhibition of fatty acid (FA) uptake in both AML cell lines and primary AML cells repressed IL-6-induced chemoresistance. Also IL-6 promoted CD36 expression at both the mRNA and protein levels through stat3 signaling. Knockout of CD36 or stat3 repressed IL-6-induced FA uptake and chemoresistance. In conclusion, IL-6 promotes chemoresistance in AML through the stat3/CD36-mediated FA uptake and demonstrating that targeting of CD36 is a promising therapeutic strategy. IL-6 level may be an independent predictor of the success of chemotherapy and the likelihood of the patient achieving remission. These findings may assist clinical decision making and the management of AML patients with abnormally high cytokine levels at the time of diagnosis to reduce their resistance to chemotherapy drugs. Furthermore, the findings may stimulate the development of drugs for treating AML patients with abnormal cytokine levels[27].

## CONCLUSION

IL-6 and IL-10 levels may be an independent predictor and a prognostic markers for the success of chemotherapy and the likelihood of the patient achieving remission, so can be beneficial in the evaluation of the therapy and making them highly applicable to routine clinical laboratories. Also may assist clinical decision making and the management of AML patients with abnormally high cytokine levels at the time of diagnosis to reduce their resistance to chemotherapy drugs. Furthermore, the findings may stimulate the development of drugs for treating AML patients with abnormal cytokine levels that open a new avenue in the study of tumor immunotherapy.

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**Conflict of interest disclosure:**

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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