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Expressions of CD274 (PD-L1) and CD47 rReceptors on the sSurface of bBlast cCells in AML pPatients

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Abstract

Acute myeloid leukemia (AML) is a genetically heterogeneous clonal disease defined by the proliferation and accumulation of immature hematopoietic cells in the bone marrow and blood. This study aimed to evaluate the CD47 and CD274 (PD-L1) expression in Iraqi AML patients and its role in the disease and evasion of malignant cells from immune system. The study was conducted on 85 patients diagnosed with leukemia, 25 of them were excluded as they had taken chemotherapy for less than a week and because the proportion of tumor cells was less than 20%. Virtually, it was conducted on 60 patients with AML and 20 healthy individuals as a control group. The expression of CD47 and CD274 (PD-L1) was evaluated using flow cytometry in the peripheral blood of AML samples only. Also MMP-2 level in serum was evaluated via ELISA in both AML and control samples. The expression of CD47 and CD274 in AML patients was found to be high. Analysis of CD274 (PDL1) expression in blast populations showed a significant increase in the proportion of CD274 (PD-L1) positive blast cells in isolated peripheral blood samples (66.3050 ± 1.94522). Also, the CD47 expression on blast cells in AML was recorded as significantly high (64.9333±2.05873). For MMP-2 (ng/ml) level in serum, the mean level non-significantly increased in AML as compared to the control group (102.677±8.719; 92.191±5.161) respectively.

Keywords: AML, CD47, PDL-1, CD274, Flow Cytomete

التعبير المناعي لمستقبلات الموت المبرمج (PD-L1) والمعلم ٤٧ على اسطح الخلايا الارومية لدى مرضى ابيضاض الدم النقياني الحاد

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الخلاصه

ابيضاض الدم النخاعي الحاد AML هو مرض وراثي غير متجانس يتم تشخيصه من خلال وجود وتراكم الخلايا الارومية غير الناضجة في نخاع العظام والدم المحيطي. هدفت هذه الدراسة إلى تقييم تعبير CD47 و CD274 في مرضى AML العراقيين ودوره في المرض وتجنب الجهاز المناعي.. اجريت الدراسة

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على 85 مريض مشخصين باصابتهم بابيضاض الدم، تم استبعاد 25 منهم (كانو قد تعاطو العقار الكيمياوي لمدة اقل من اسبوع) لان نسبة الخلايا الارومية كانت اقل من 20 %. فعليا، قد اجريت الدراسة على 60 مريضًا مصابًا بابيضاض الدم النقوي الحاد و 20 فردًا سليمًا كمجموعة سيطرة. تم تقييم التعبير عن CD47 و CD274 (PD-L1) باستخدام قياس التدفق الخلوي في عينات الدم المحيطي للمرضى فقط، وكذلك تقييم مستوى MMP-2 في المصل بواسطة ELISA لكل من المرضى ومجموعة السيطرة. كان مستوى المستقبلات CD47 و CD274 في مرضى AML مرتفعًا. كان تعبير CD274 (PD-L1) في الخلايا الارومية عاليا في عينات الدم المحيطية المعزولة (66.3050 ± 1.94522). كما كان تعبير CD47 على الخلايا الارومية في المرضى مرتفعًا بشكل ملحوظ (64.9333 ± 2.05873). اما بالنسبة لمستوى MMP-2(نانوغرام / مل) في مصل الدم ، فقد ارتفع ارتفاعا غير معنوي في المرضى مقارنة بمجموعة السيطرة (102.677 ± 8.719 ؛ 92.191 ± 5.161) على الترتيب.

Introduction

As the name suggests, acute myeloid leukemia (AML) is an aggressive form of blood cancer characterized by impaired differentiation of hematopoietic stem cells, leading to uncontrolled clonal development of undeveloped myeloid progenitor cells and blasts in the bone marrow, peripheral blood and other tissues [1][2]. Among adults, AML is the most common cause of acute leukemia, while juvenile leukemia is the second most common form [1][3]. Not only should the age of patients be considered, but also the presence of cytogenetic or molecular genetic defects [3]. In most AML cases, the first line of treatment is chemotherapy. While this may result in a full recovery, most patients will eventually relapse and succumb to the disease. Hence, new therapeutic approaches are required [2][3]. In a recent study conducted in 2020, Al Nakeeb and Al- Rubaye found that it is possible to infer the serum expression levels of miR-142-3p, miR-223-3p and miR-146-3p which can act as useful indicators for early detection of pediatric acute leukemia [4]. However, AML blasts stimulate various immune evasion mechanisms in order to leak from host immune reactions and bypass immune mediated rejection [5]. Immune evasion relies heavily on the orientation of various co-inhibitory receptors on T cells, such as cytotoxic T-lymphocyte associated protein 4 (CTLA4), programmed cell death protein 1 (PD-1), T-cell immunoglobulin and mucin-domain containing-3 (Tim-3), T cell immunoreceptor with Ig and ITIM domains (TIGIT), lymphocyte-activation gene 3 (LAG-3) and their interaction with their specific ligands [5][6]. More than 60 years ago, Burnet and Thomas proposed that the immune system acts as a protector in identifying and eliminating cancer [7]. Cancer cells, on the other hand, are able to avoid the immune system response in a variety of ways. Tumor escape from immune system responses has been identified as one of the emerging hallmarks of cancer in previous research over the past 15 years [8]. Therefore, the term "cancer immune-editing" was coined to describe the immune system response to cancer during its various stages [9]. There are three stages of this dynamic mechanism in which the immune system not only protects against the development of cancers but also outlines the personality of evolving cancers [10][11]. As a result, researchers were able to reduce the incidence of spontaneous leukemia, lymphomas, breast and lung cancers. According to these findings, at least certain types of tumors require a functioning immune system to be controlled [12]. A number of tumor immune-evasion mechanisms were documented in the past, including down-regulation of MHC I and II expression, metabolic alternates such as consumption of essential amino acids included arginase-2 and indoleamine 2, 3-dioxygenase-1 [13][14], poor tumor cell co-stimulation lead to T cell energy [15], secretion of immune suppressant cytokines and enzymes [16] and expansion and/or indwelling of T cells [17][18]. Pathways that lead to tumor development, invasion and metastasis have a number of connections in the tumor microenvironment. Patients with hematological malignancies benefit from immune surveillance because T cells identify tumor associated antigens and activate tumor cell apoptosis [19]. The immune surveillance of hematological malignancies is also enhanced by NK cells [20]. Due to their origins in primary and secondary lymphoid tissues, hematological malignant cells are immunogenic and immunosuppressive [21]. Many studies have shown that AML cells are prone to being attacked by innate and adaptive immunity. They are susceptible to T-cell recognition because they express both MHC I and II. Some AML subtypes use immune evasion mechanisms to prevent the initiation of an anti-tumor immune response, while others do not [5][22]. PD-L1, Gal-9 and CD155 upregulation on AML cells are few of the essential strategies used to evade the host immune response [23][24][25]. Patients with AML will also eventually exhaust their effector T cells, which can stimulate an antitumor response. Enhanced expression of co-inhibitory particles such as CTLA-4, PD-1, Tim-3, TIGIT and LAG-3 is a characteristic of T cell enervation [24]. Blockade of co-inhibitory molecules in AML can allow for a more efficient anti-leukemic immune response [26].

SUBJECTS AND METHODS:

Study population and design

This prospective cross-sectional study was conducted on Iraqi patients with AML in the Hematology center, Baghdad Teaching Hospital, Medical City. The study was conducted on 85 patients diagnosed with leukemia, 25 of them were excluded as they had taken chemotherapy for less than a week and because the proportion of tumor cells was less than 20%. Virtually, it included 60 patients diagnosed with acute myeloid leukemia, and 20 healthy individuals as a control group with matching age and sex.

Samples collection:

Blood samples:

Blood collected aseptically by venipuncture was divided into two parts, one part was put into a sterile blood collection tube with dipotassium ethylene diamine tetra-acetic acid (K2EDTA) vacutainer and the other part was put in a gel tube to obtain serum.

Flow cytometri analysis of membrane molecule expression:

Membrane molecule CD (Cluster of differentiation) markers expression was analyzed by flow cytometry (FCM) using PE, APC and FITC conjugated anti-CD274 (PD-L1), CD47 and CD45 (CD45 used as gating marker) monoclonal antibodies (Elabscience Biotechnology Inc, United States) as following: fresh peripheral blood was placed in a 12x75mm tube with the appropriate amount of fluorochrome conjugated monoclonal antibody and left for 15-30 minutes in the dark at room temperature (between 20°C and 25°C) with a gentle vortex. Then, 2ml of 1X BD FACS Lysing solution was added to the mixture and left for 10 minutes in dark at room temperature with a gentle vortex. The tubes were centrifuged at 500xg for five minutes. The supernatant was then removed. 2 to 3ml BD cell wash buffer was added and centrifuged at 500xg for 5 minutes, before removing the supernatant. The previous step was repeated one more time. For the final step, 0.5 ml of the 1% solution of paraformaldehyde was added and mixed. The data was analyzed on a flow cytometer using BD FACSC Canto with FACSDIVA software in the flow cytometer [27].

Matrix Metaloprotinase-2 (MMP-2) measurement:

To measure MMP-2, the sandwich ELISA kit, provided by Al-Shkairate establishment of medical supply (Jordan), was used. It was necessary to use a recombinant human protein to create the standard curve of MMP-2. The test was carried out in accordance with the manufacturer's instructions. The concentration of MMP-2 was calculated from a standard curve.

Ethical approval:

All investigations were performed in accordance with the Al-Anbar University Medical Research Ethics Clearance Committee guidelines, Iraq. The local ethics committee approved the study protocol and informed consent was taken from all subjects.

Statistical Analysis:

Numbers were presented as minimum and maximum values, as well as a mean and median, and as a standard deviation (SD). For comparisons between groups, the Mann-Whitney U test was used. Nonparametric version of Student's t-test. Resulting qualitative data was presented in the form of frequency and percentage. The chi-square (x^2) test was used to compare and analyze qualitative variables. Using Spearman's correlation coefficient, a significant correlation between the expression of CD47, PDL-1 and MMP-2 was determined. Results were considered statistically significant when the p value of two-sided test was ≤0.05, IBM® SPSS® Statistics Version 23 for Microsoft Windows, SPSS Inc was used to analyze the data.

Results:

Baseline characteristics of included patients:

The present study was conducted on 60 patients with AML and 20 healthy individuals samples collected from the Hematology center, Baghdad Teaching Hospital, Medical City. Out of 60 participants, 24 were males and 36 females, with 20 controls10 were males and 10 females. The mean age of patients was 45.8 years, ranging from 13 to 70 years and control age mean 32.4 years which ranged from 18 to 55 years. According to duration, the patients were divided into 3 groups, 39(65%) newly diagnosed with AML (non-treated), 17(28.33%) were diagnosed in duration between one month to one year and 4(6.67%) were diagnosed for more than one year (Table 1).

Table 1-Characteristics of AML patients and healthy control groups

Characteristics	**Category**	**AML**		**Control**	
Gender	Male	N	%	N	%
		24	40%	10	50%
	Female	36	60%	10	50%
Age	Mean ± SD	39.375±16.75 (13-70)		32.4±11.75 (18-55)	
Duration	New	39 (65%)		-	
	1m-1y	17 (28.33%)			
	>1y	4 (6.67%)			
Treatment	Non treated	39 (65%)		-	
	Treated	21 (35%)			

Flow Cytometry Data:

Assessment of Blast Cells Markers Expression in AML Patients:

At different treatment time points, leukemic (blast) cells from freshly isolated peripheral blood samples of different AML patients were examined for PD-L1 and CD47 expression. The standardized fluorescence intensity (SFI) was used as a readout because the mean fluorescence intensity showed a large range (MFI).

Frequency of CD274 (PDL-1) gene expression:

In the beginning, the constitutive blast cell surface PD-L1 protein expression was assessed with flow cytometer on AML patients. Analysis of PDL1 expression in blast populations showed high level in the proportion of PDL1-positive blast cells in isolated peripheral blood samples (66.3050%) (Figure 1). When comparing the SFI of PD-L1 with freshly isolated blast cells from male patients with blast cells isolated from female patients, no significant differences was observed (66.5958%±3.54757; 66.1111%±2.26158) respectively. For duration the first group (non-treated newly diagnosed) PDL-1 expression was (63.9821%±2.20432), the second group (duration between 1 month to 1 year) PDL-1 expression between (70.8882%±3.80426) and for the last group, more than one year, PDL-1 was (69.4750%±11.72479) (Figures 1, 2; Table 2).

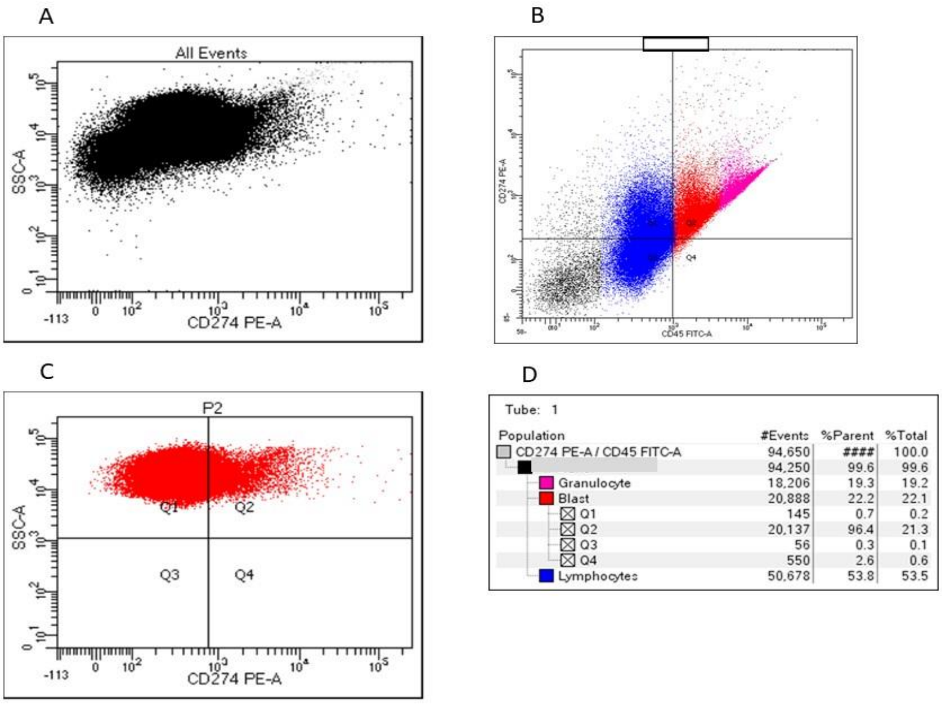

Figure 1-This figure shows a positive case of CD274 expression analysis. Dot plot to identify CD274 + blast cells

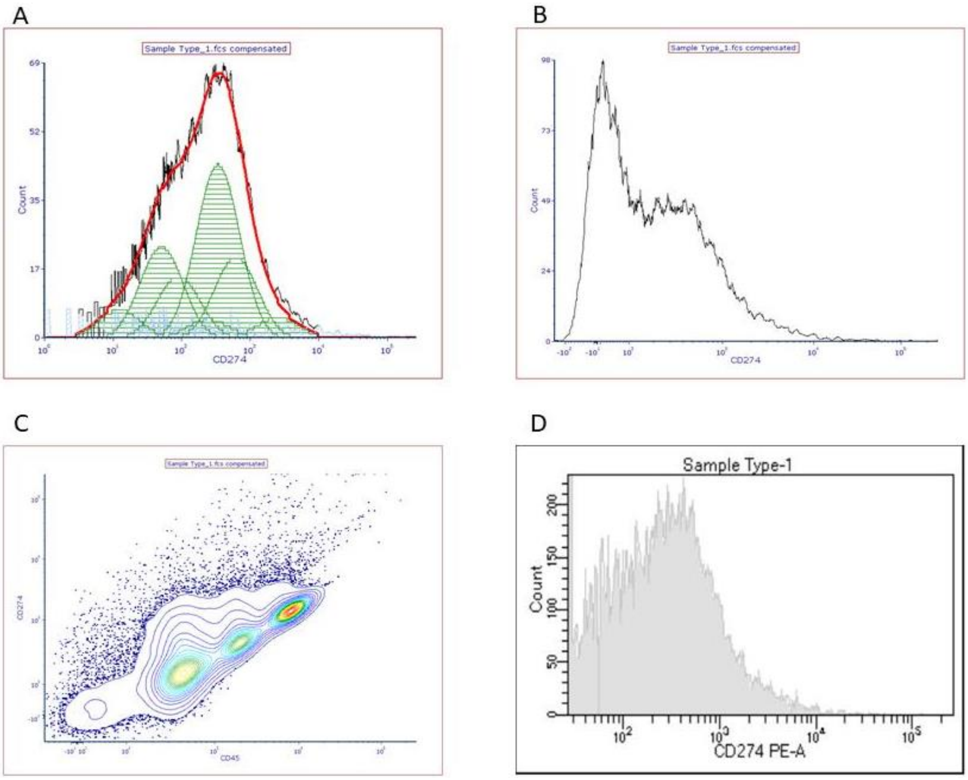

Figure 2 - This figure shows Flow cytometeric CD274 (as a prognostic marker) analysis showed different samples of AML patients.

Frequency of CD47 gene expression

The percentage of CD47 expression on blast cells in AML was high (64.9333%). For CD47 expression percentage on blast cells the (mean ±SD) non-significantly increased in females (65.9278%±2.35296) as compared to males (63.4417%±3.79237). For the time period, it has been noted that the expression increases non-significantly as the disease progresses in time when comparing the newly diagnosed group with the patients whose duration ranges from one month to one year, as well as with the patients who have been infected for more than one year (63.9487%±2.40444; 66.5529%±4.44172; 67.6500%±8.93350) respectively (Figures 3, 4; Table 2).

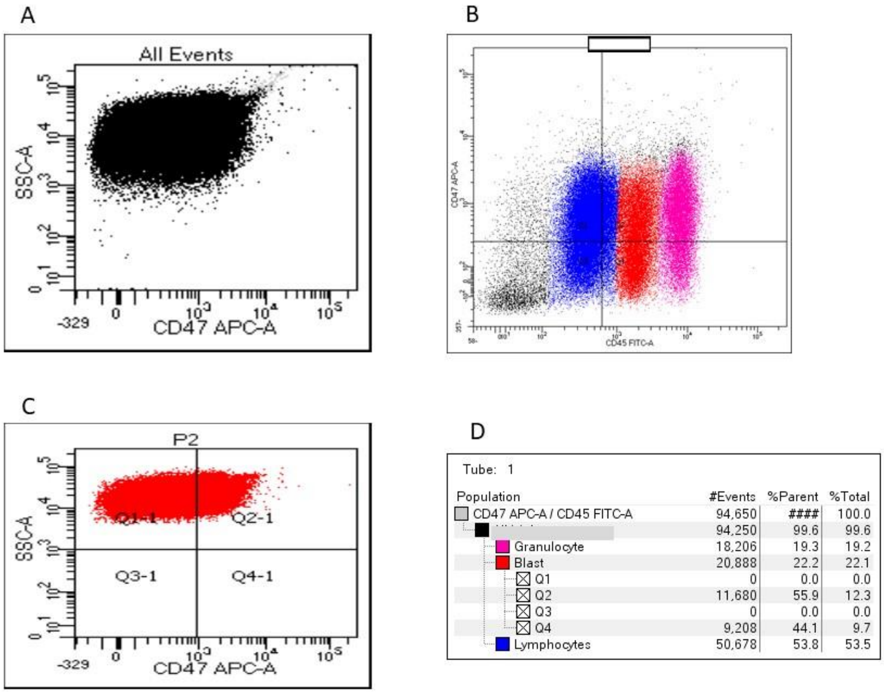

Figure – 3 - This figure shows a positive case of CD47 expression analysis. Dot plot to identify CD47 + blast cells.

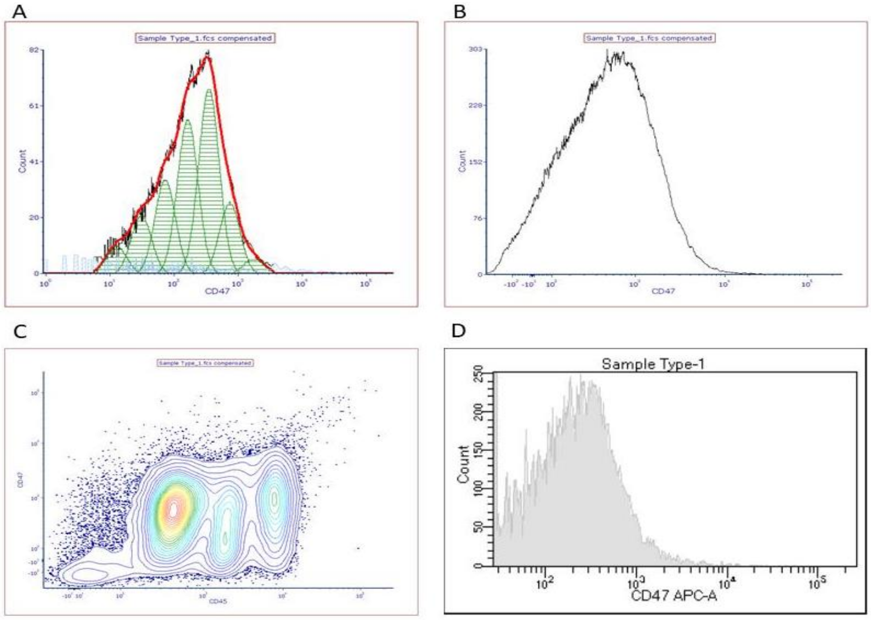

Figure 4 - This figure shows Flow cytometeric CD47 (as a prognostic marker) analysis showed different samples of AML patients.

MMP-2:

In the present study, the mean level of MMP-2 (ng/ml) non-significantly increased in AML as compared to the control group (102.677±8.719; 92.191±5.161) respectively (Figure 5). For the effect of gender, MMP-2 (ng/ml) increased significantly in males (103.865±12.247) as compared to the females (84.857±7.889) in AML patients. When comparing the effect of treatment, it has been found that the concentration of MMP-2 ng/ml increased insignificantly in treated patients as compared to the newly diagnosed patients (95.402±8.751; 92.960±7.070) respectively (Table 2).

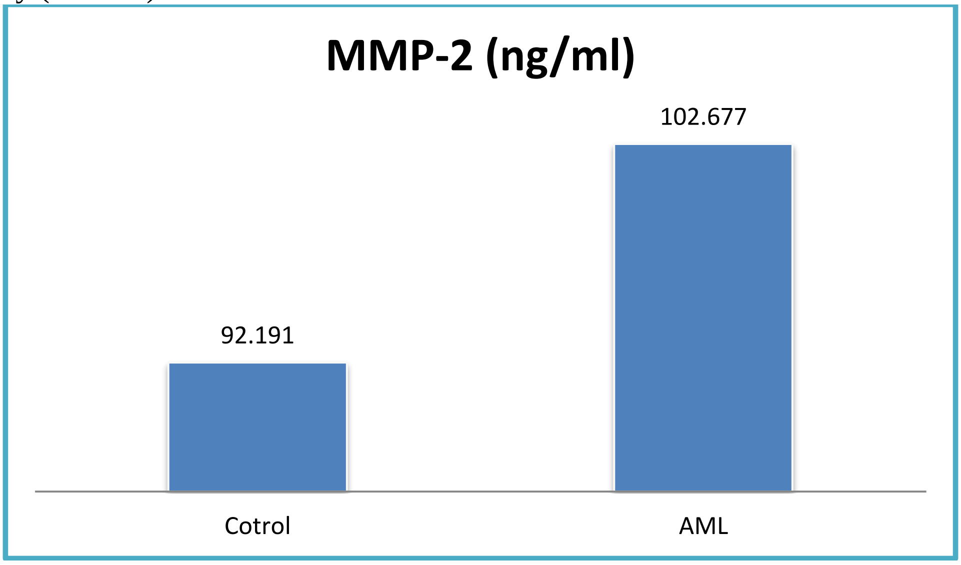

Figure 5 - This figure shows MMP-2 (ng/ml) level between groups

Table 2- It shows the levels of CD274 and CD47 expression on blast cells and MMP-2 levels in different categories.

Characteristic	category	CD274%	CD47%	MMP-2 (ng/ml)
Disease	AML	64.9333±1.94522	66.3050±2.05873	102.677±8.719
	Control	-	-	92.191±5.161
Gender	Male	66.5958±3.54757	63.4417±3.79237	103.865±12.247
	Female	66.1111±2.26158	65.9278±2.35296	84.857±7.889
Duration	Newly diagnosed	63.9821±2.20432	63.9487±2.40444	92.792±6.662
	1m-1y	70.8882±3.80426	66.5529±4.44172	97.398±6.24
	>1y	69.4750±5.72479	67.6500±5.93350	114.096±6.1694
Treatment	Non treated	64.8876±2.233	65.87±2.546	92.960±7.070
	Treated	69.665±5.875	66.653±4.45	95.402±8.751

Discussion

Acute Myeloid Leukemia AML patients in Iraq were assessed for CD47 and CD274 expression and MMP-2 levels to determine their role in the disease pathogenesis and immune system escape. In order to achieve the study objective, flow cytometry was used to measure the expression of CD47 and CD274 on AML cells, as well as the MMP-2 level in serum. The standardized fluorescence intensity (SFI) was used as a readout because the mean fluorescence intensity showed a large range (MFI). Patients in complete remission and those who relapsed, showed higher PD-L1 expression in this study. These findings were in agreement with those of a previous study that analyzed PD-L1 expression on blast cells isolated from bone marrow aspirates and peripheral blood samples of different AML patients at different treatment time points, and observed minor PD-L1 upregulation after IFN-c exposure [28]. It is possible that the recently proposed paradigm shift on PD-L1 and immune inflammatory response could explain these seemingly contradictory observations. Oncogene-driven tumor immune evasion may well be mediated by PD-L1 expression, which has been proposed to be up-regulated on cancer cells [29][30][31][32]. PD-L1 is widely known to be present in a wide variety of tumors, which include solid and hematological tumors [33][34].

Upon allo-HSCT [35] and hypomethylating entities [36]; along with azaticidine and decitabine; and at the time of relapse, PD-L1 increased levels is common in AML. When looked at the blast cells from AML patients, it has been found that the expression of PD-L1 differed from one patient to another. Oncogene PD-L1/CD274 expression in tumor sites has been identified as a potential biomarker of response to the treatment [37]. Age, stage and histotype had no effect on the prognosis of PD-L1/CD274 expression in lung cancer [38]. Cancer cells normally express PD-L1, which suppresses PD-1-positive T-cell function, thereby enhancing the effectiveness of chemotherapy [39]. Recently, Tamura *et al*. [40] found that interleukin-6-induced PD-L1 expression was found on myeloma cells, which is another mediator of inflammation. As AML developes, higher PD-1 expression on blast cells, which results in stronger immune suppression could contribute to the disease progression [41]. In most cases, PD-L1 expression on animal AML blast cells is inconclusive. AML de novo cases expressing PD-L1 were found in less than half of the cases studied by Tamura *et al*. [40], while Kozako *et al*. [42] found PD-L1 expression in far more and over half of the cases studied. Patients with French-American-British (FAB) M5 type AML; the studied PD-L1 expression on bone marrow cases found to be increased as the disease progressed [43]. PD-L1 was also found to be limited upon AML prognosis but managed to improve on such relapses in another study. According to Berthon *et al*., PD-L1 expression on AML blast cells is controlled at diagnosis, but increases dramatically as the disease progresses or after systemic chemotherapy [44].

According to the previous studies, CD274 inhibits the antitumor effect of immune cells, including such cytotoxic T cells. In a murine form of chronic lymphoblastic leukemia, CD274 preventing antibodies have been shown to decrease tumor size and restore cytotoxic effects of CD8+ T cells [45]. In the same way, other studies have shown that anti-CD274 antibodies can extend the life of mouse model with acute myeloid leukemia [46][23]. It has also been noted that CD274 stimulates leukemogenesis by sustaining Cyclin D2 levels. Further research is needed regarding how CD274 regulates Cyclin D2. Breast cancers can benefit from doxorubicin ability to suppress the expression of CD274 on cell membranes and enhance its nucleus translocator [47]. CD274 overexpression stimulates pancreatic tumor cell proliferation by restricting many cell cycle-related genetic traits and the JNK phosphorylation method [48].

Cancerous cells and T cells which reduce the activity of CD4+ and CD8+ T cells, made contact with each other through the immune-suppressive PD-L1 / PD1 interaction [49]. While CD274 (PDL-1) expression by tumor tissue has been linked to the presence of cancer-infiltrating lymphocytes, which could have been elaborated in a better immune-therapy stimulated outcome [50][51]. Although CD274 (PDL-1) is highly expressed in cell lines, it can be induced by a variety of stimuli [52]. It is believed that PD-L1/CD274 expression is increased in cancer microhabitats by macrophages, dendritic (DC), myeloid-derived repressor (MDSCs), regulatory T cells and endothelium. It is a well-known fact that the cancer cells often express CD274 (PD-L1), which causes them to repress the immunologic system ability to detect and eliminate them [39]. Antigen presenting cells with CD274 (PDL-1), a co-inhibitory receptor-ligand pair whose primary function is to minimize counterparty tissue damage through which a host immune system might help tumors escape.

Acute myeloid leukemia (AML) has an increased expression of CD47 on blast cells. Consequently, CD47 expression on human AML cell populations and their normal counterparts should be further investigated. CD47 was found to be highly expressed on multiple samples of AML blast cells utilizing flow cytometry. Cells with high levels of CD47 (PDL-1) expression were also included in this group, as were the cells from LSC-enriched portion [53]. Immune escape promoter/checkpoint receptor CD47 is overexpressed in cancer cells, making it a prime candidate for immunotherapeutic interference. Another aspect is the fact that CD47 is unified self-marker found on normal healthy tissue that regulates programmed cell discharge via homeostasis. As a result, CD47 is involved in a variety of physiological processes, including cardiovascular homeostatic regulation, neuronal development, bone remodeling and adaptive natural immune response as well as stem cell regeneration, adhesion, cell motility, proliferative processes and survival [54].

As a prognostic indicator for AML, CD47 has been studied extensively [53], but these studies have focused on cytometry analysis, even though targeting CD47 is a particular effort pathway and may also have broad applicability with a range of cancers [55]. The gene expression data from a previously described investigation of 285 AML patients was analyzed in order to determine CD47 expression throughout morphologic, cytogenetic and molecular specimens of AML [56]. CD47 was found to be highly expressed in all of the study's categories at similar levels. According to Majeti *et al.*, CD47 was overexpressed on AML LSC as compared to normal samples in three independent studies of adult patients with AML [57]. According to a previous research, anti-CD47 antibodies attacking leukemia cell lines directly induce apoptosis. It has also been shown that treating human B-CLL cells with anti-CD47 antibodies promotes caspase independent cell death [58].

CD47 overexpression has been implicated in the pathogenesis of human AML and was predicted to be associated with poorer medical outcomes in a prior study [59]. A previous research of a group of 285 AML patients with different cytogenetic and molecular deficiencies [56] confirmed this hypothesis, showing a significantly higher risk of death in the elevated CD47 expression patients when patients were dichotomized into higher and lower CD47 expression groups. There is a link between overall survival and CD47 expression in a second paper of 242 patients [60] with usual karyotypes (NK-AML). A study by Galli *et al*. [61] found that CD47 staining on marrow leukemia cell populations was semi-quantitatively documented and was related to parameters and renowned elements in AML. There is a strong correlation between CD47 expression and BM blast cell penetration, as well as peripheral blood blasts. CD47 gene expression and overall survival were inversely correlated, with higher expression being associated with lower overall survival. Using leukemic stem cells, it the same results were found by Jaiswal *et al*., [53] and Majeti *et al*., [59] CD47 expression was reported to be linked with a poor outcome in AML patients [62].

An increase throughout CD47 expression was found to be an independent predictor of AML in two studies conducted on adults with AML. Cells with AML leukemia were phagocytosed by monoclonal antibodies against CD47 *in vitro*, which inhibited their growth in mouse models [53][59]. Aside from that, it is expressed by a variety of cell types in the carcinoma microenvironment and is crucial for malignancy metastasis establishment. CD47 overexpression is frequently linked to poor health outcomes. To verify its safety and effectiveness in healing hematological neoplasms, CD47 is currently being researched in many preclinical models as well as clinical trials. AML different types of cells and pathophysiological conditions influence CD47 expression levels. All healthy cells express a low level of CD47 under normal conditions. While stem cells and novel red blood cells migrate, they express higher levels of CD47 to avoid being attacked by macrophages [53][63]. So many cancer cells are also known to express CD47 on their surface in order to evade phagocytosis by macrophages [64].

It has been found that AML had significantly greater MMP-2 levels. MMP-2 in MDS and AML, MMP-9 in AML-M4 and TIMP-1 in AML-M3 showed similar results. As a result, this data suggests that levels of gelatinase increased as the disease progressed. Lin *et al*. found that MMP-2 levels were higher in BM donors than in healthy controls [65]. Previously, a number of researches had found evidence that the MMP-2 excreted by blast cells is correlated with leukemic dissemination[66]. When compared with a control group, Travaglino *et al*. found that MMP-2 levels were significantly higher among MDS patients. There is a possibility that

MMP expression in MDS may be a useful diagnostic and prognostic tool as well as an active target in treatment options [67].

MMP-2 enzyme secretion from BM-MNC has indeed been observed in patients with myeloid leukemia as well as pre-leukemia, but not in normal persons, according to the research. AML patients with 92 percent blasts in the bone marrow had cell sections that contained just about exclusively leukemic blasts upon extra enhancement with Ficoll purification because of the increased levels of MMP-2 in these samples, it is highly likely that both enzymes have been produced by leukemic blast cells. Findings on AML blasts found in peripheral blood of AML patients support that MMP-2 is continuously secreted by these cells [68].

Since MMP was identified in all AML cases from FAB M1 to M5, it appears that MMP production and leak from AML patients' bone marrow blast cells occurred independently of leukemic cellular differentiation grade. HL60, NB4, U937 and THP-1, which are all known to release MMP, completely confirm this [69]. As a result, the ability of AML blast cells to produce MMP-2 continuously *in vivo* may depend on cell differentiation, because this enzyme was not detected in three cases of AML types M1 and M2 but was present in cases of AML-M2–M5. MMP-2 production has been reported to occur only in fully developed macrophages and T-lymphocytes in response to immunological stimuli, with neutrophil granulocytes unable to synthesize this enzyme [70].

In regular leukocyte function, MMP enzymes play a significant role in degrading collagen IV, the main part of the basal lamina in tissues, which is almost always associated with the internal migration and degenerative changes of extracellular matrix (ECM) formations in tissue and blood vessels [71]. According to a research conducted on interleukin-8-induced rhesus monkeys, the participation of the MMP enzyme in haemopoietic primogenitor cell transmission from bone marrow to peripheral blood has been demonstrated [72]. Consequently, it is possible that the premature formation of gelatinases in leukemia cell lines elaborated in their propagation in the marrow. 71 percent of AML patients with MMP-2-positive cell lines in their bone marrow often have leukemic blast cells in their peripheral blood, according to Ries and coworkers [73]. Another 52 percent of patients had MMP-2-negative blasts, albeit at a lower number. It is possible that MMP-2 is generated by the blast cells, but most of the MMP-2 negative patients have CML or MDS, which have low blast numbers in the bone marrow and therefore produce too little MMP-2 for zymographic recognizing but enough for that to join circulation. When leukemic blast cells separated from de novo AML patients were cultured in primary culture, Matsuzaki and Janowska-Wieczorek (78) found that MMP-2 and MMP-9 were constitutively produced. Ries *et al* findings on AML blast cells from bone marrow support this outcome [73]. In an *in vitro* study using reconstructed basal membrane as an immigration boundary, the researchers found that metalloproteinase enzyme inhibitors reduced cellular insensitivity, suggesting that these enzymes may be participating in leukemic cell invasion. Gelatinase (MMP) has been found to have some atypical substrates in addition to its ability to degrade matrix. Metalloproteases, for example, release active tumor necrosis factor (TNF) from its developments formula and remove the Fas ligand from surface of the cell of leukemia [74]. It is also possible that the bone marrow transformation of regulatory proteins could influence the growth of hematopoietic cells [73].

In the absence of activation, MMP enzymes are produced and released as dormant zymogens [75]. BM-MNC conditioned media often contain additional gelatinase (MMP), which is most likely an activated form of MMPs, because MMP activation is based on proteolytic concatenation that results in a decrease in molecular mass. This activity exact mechanism is still unknown. In order for MMPs to remain active outside of the cell, they must maintain a delicate balance between their powerful enzyme and their specific inhibitor. Previously, MMP-2 was found to be a marker of malignant transformation in AML patients with prospective prognostic significance for CML and MDS [73]. There is evidence that leukemic blast cells reveal MMP enzymes in a manner similar to how tumor cells invade and metamorphose. Solid tumor diffusion can be improved by targeting MMP enzymes [76]. A number of cancer models have shown that synthetic MMP enzyme inhibitors, such as with the MMP inhibitors Batimastat and Marimastat, are efficient [77]. Ultimately, in a previous study, Jumaah *et al* concluded that patients with AML clearly suffer from disorders and defects in liver and kidney functions, and some other blood variables, and that there were negative effects on the ALT, AST, ALP as well as the levels of EPO and LDH in the serum of AML patients [78].

Conclusion

This research found a link between CD47 and CD274 expression on AML blasts in the peripheral blood and patient outcome, as evaluated by flow cytometry. Flow cytometry may be a clinically effective, rapid and effective method for assisting in the diagnosis of AML patients, as well as assessing treatment progress and predicting prognosis. Furthermore, CD47 and CD274 have proven to be feasible, dependable and simple way to investigate as a potential indicator in AML prognosis and survival (OS). Elevated MMP-2 levels were observed in blood of AML patients. However, these levels did not associate with any clinic-pathological parameters of patients.

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