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RESEARCH ARTICLE

CLINICAL EXPRESSION OF Th17 RELATED CYTOKINES IN ACUTE MYELOID LEUKEMIA PATIENTS: A PROSPECTIVE DESCRIPTIVE STUDY

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Abstract

Background: Acute myeloid leukemia (AML) is a hematological neoplasm that affects adults of all ages and accounts for 1-2% of malignancies worldwide. T- cell function is significantly impaired in AML patients. Increasing evidence states that a recently identified subtype of CD4 + T cells called T helper 17(Th17) is associated with the pathogenesis of AML.

Aim: This study aims to estimate the concentration of Th 17 related cytokines IL-23 and IL-17 in the plasma samples diagnosed with AML and to assess their clinical value in the prognosis of AML.

Methods: This study includes 31 patients who were newly diagnosed with AML. Similarly, 21 healthy subjects were selected for normal control group. After two courses of induction chemotherapy 22 patients achieved complete remission (CR), whereas 9 patients failed to achieve CR. IL-23 and IL-17 plasma levels were measured using ELISA kits.

Results: The IL-23 and IL-17 plasma levels in the newly diagnosed group were significantly higher than the CR and control group. Similarly, after two courses of standard chemotherapy, the IL-23 and IL-17 expression levels in the non-remission (NR) group were also much higher than in the CR and control group. No significant differences in the expression of IL-23 and IL-17 between CR and control groups were found. In AML patients, a significant positive correlation was found between IL-23 and IL-17.

Conclusion: The IL-23 and IL-17 plasma levels correlate with the progression of AML, which may express clinical significance in the prognosis and therapeutic evaluation of AML.

Introduction:-

AML is a rapidly progressive hematopoietic malignancy associated with myeloid precursor cell proliferation in blood and bone marrow with impairment of normal hematopoiesis [1,2]. It is seen in 80% of acute leukemic adult patients and comprises 1-2 % of all cancer cases [3]. The incidence increases with age and a median age of onset is 70 years [4]. The prior history of related hematologic disorders constitutes the major risk factors, and myelodysplastic syndrome (MDS), being the most common [5]. The underlying mechanism in AML is characterized

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by the arrest of myeloid cells at their early stage of development and maturation [6]. Although the impairment in the immunological pathways is involved in the pathogenesis and progression of AML, the detailed mechanism is still unclear. CD4+ T cells have a vital role in the initiation and maintenance of immune responses against targeted leukemic cells [7,8]. Recent studies stated that in AML patients there is a significant elevation of Th17 cells in blood plasma samples as compared to healthy subjects [9,10]. These new and unique types of CD4+ T cells are synthesized directly from the naive CD4+ T cells. They have an impact on the pathogenesis and prognosis of various autoimmune and inflammatory diseases, as well as tumors [11]. However, the precise mechanism of these Th17 cells in AML remains poorly understood. Various cytokines as TGF-β, IL-6, IL-17, IL-21, IL-22, and IL-23 have certain relationships with Th17 cells [12]. In this research study, we estimated the concentrations of Th17 related cytokines IL-23 and IL-17 in plasma samples of AML patients and expected to determine their clinical significance in the prognosis and management protocol.

Material and Methods:-

Participant's characteristics

This research was design as a prospective descriptive study. 31 adult patients newly diagnosed with AML at the Hematology department of The First Teaching Hospital of China Three Gorges University from September 2020 to October 2021 were included in this study. Simultaneously 21 healthy volunteers were studied as a control group. Out of 31 patients, 18 were males and 13 were females, aged between 21 to 71 years with a median age of 49 years. Among 21 subjects of the control group, 12 were males and 9 females, aged between 21 to 65 years with a median age of 47 years. All patients had undergone two courses of standard chemotherapy with DA regimen. 8 patients in the M3 subgroup were also treated with All-Trans Retinoic Acid (ATRA). Of these, 22 patients achieved CR, whereas the remaining nine patients failed to achieve CR. The diagnosis was established according to the 2016 WHO classification system [13]. CR is defined as <5% blast cells without Auer rods, neutrophil count >1.0 ×109/L, platelets greater >100 ×109/L, independence of RBC transfusions, and absent of extramedullary disease. NR is the presence of blast cells >20%.

The exclusion criteria were patients with the presence of other neoplasm or hematological diseases, history of autoimmune diseases or hypersensitivity reactions, and organ transplantation. Both written and informed consent was obtained from all participants. The First Teaching Hospital of China Three Gorges University ethical committee approved this study.

Determination of plasma IL-23 and IL-17 levels

After centrifuging the peripheral blood of participants, the plasma samples were collected and stored at the temperature of -80°C until use. The concentrations of IL-23 and IL-17 in plasma were assessed by using commercial ELISA kits (Shanghai YL Biotech Co., LTD, China), according to manufacturer's protocols.

Statistical analysis

All the data were computed as Mean ±SD. Data analysis was performed by using SPSS

Software (version 24, SPSS Inc., USA). The measured values of groups were compared using the ANOVA test and Pearson's correlation test to determine the correlation coefficient. P values <0.05 were designed to be statistically significant.

Results:-

Assessment of expression level of IL-23 and IL-17 in plasma

The level of IL-23 and IL-17 in plasma of newly diagnosed AML group was significantly higher, as compared with CR and control group, $p < 0.05$. After two courses of standard chemotherapy, the expression levels of IL-23 and IL-17 in plasma of the NR group were also higher than the CR and control group, $p < 0.05$. No significant difference found between the CR and the control group, $p > 0.05$. The plasma level results are expressed in **Table 1**.

Table 1:- Plasma levels of IL-23 and IL-17 for different groups（$\bar{x}$ ±SD, pg/ml）.

Groups	N	IL-23	IL-17
Control	21	13.23±0.57	54.30±3.43
Newly Diagnosed	31	20.26±1.82*#	61.92±8.36*#
CR	22	13.37±1.61	53.18±6.71

NR	9	20.48±1.32*#	61.89±6.78*#

*p< 0.05 when compared with control group; #p< 0.05 when compared with CR group

Correlation between IL-23 and IL-17 in AML patients

The Pearson correlation analysis showed a significant positive correlation between IL-23 and IL-17 plasma levels in newly diagnosed AML patients (r=0.654, p =0.0001) **(Fig 1).** There was also a significant positive correlation between IL-23 and IL-17 levels in CR group (r=0.604, p = 0.02) (**Fig 2**) and NR group (r=0.864, p = 0.002) (**Fig 3**). We didn't find any significant correlation between IL-23, IL-17, and other parameters include RBC, WBC, neutrophils，platelets, hemoglobin，hematocrit，LDH and uric acid in newly diagnosed patients.

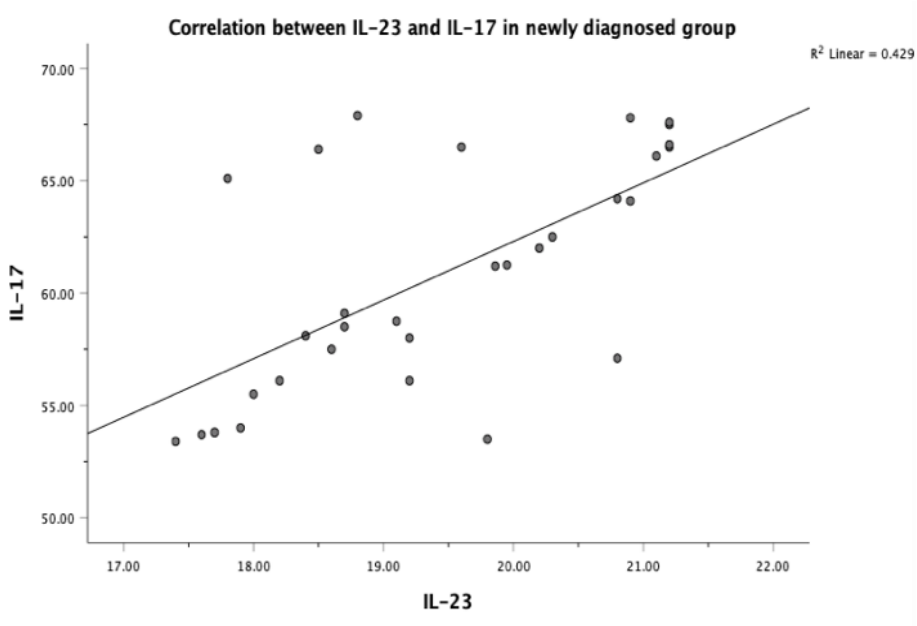

Figure 1:- Correlation between IL-23 and IL-17 in newly diagnosed group.

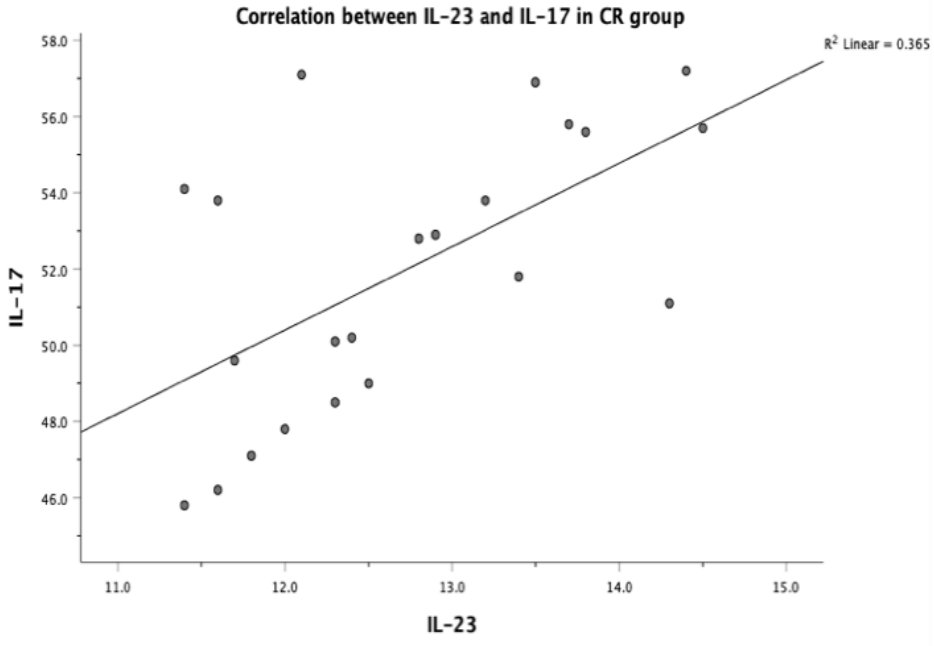

Figure 2:- Correlation between IL-23 and IL-17 in CR group.

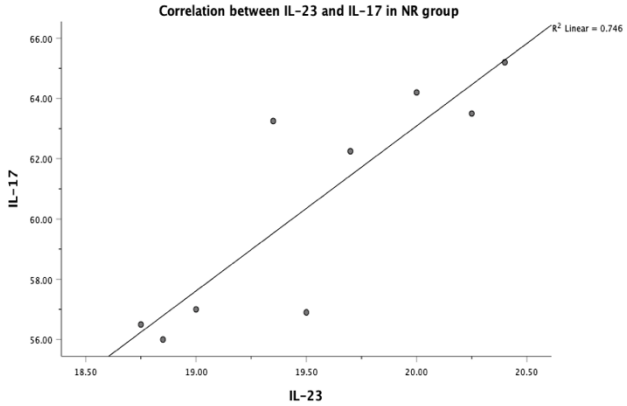

Figure 3:- Correlation between IL-23 and IL-17 in NR group.

Discussion:-

AML, as a lethal hematopoietic stem cell neoplasm, causes severe infection, bleeding, organ invasion, and hyperplasia of white blood cells. The pathophysiology of AML is characterized by heterogeneity and complexity and associated with various immunological abnormalities [14]. There is an interaction between myeloid cells and bone marrow microenvironment which result in the formation of immune-modulatory multiple cytokines that direct T helper cell towards highly pathogenic Th17 cell lineage [15]. Th17 cells and cytokines mediated immunological phenomenon is closely linked to the pathogenesis of various inflammatory and autoimmune diseases [16]. Kryczek et al. [17] have reported an elevation in the frequency of Th17 cells in patients diagnosed with advanced ovarian, renal cell and pancreatic carcinomas. A study by Zhang et al [18] shows that patients with gastrointestinal tumors had a high concentration of Th17 cells in their plasma samples and increased levels of these Th17 cells were related to the clinical stage. Another research also stated significantly elevated frequencies of Th17 cells in plasma and tumor-draining lymph nodes were found in uterine cervical cancer patients [19]. All the above findings indicated that these Th17 cells may exhibit a certain role in the pathogenesis of the solid tumor.

Certain studies have demonstrated IL-23 stimulates pathogenic Th17 cells and release IL-17 during chronic inflammation, and the IL-23/17 pathway has a significant therapeutic evaluation for many diseases [20-22]. Hence, we assumed the increase in these numbers of Th17 cells and related cytokines may be involved in tumor immune escape and contribute to the progression of AML. Our findings clarified that frequencies of Th17 related cytokines were expressed significantly in plasma samples from newly diagnosed AML patients as compared to those from the control group. The concentration levels of IL-23 and IL-17 in plasma of newly diagnosed and NR patients were higher than the control group. We also found that frequencies of Th17 related cytokines were reduced obviously when patients with AML achieved CR after standard induction chemotherapy. No any significant differences found in the expression levels of IL-23 and IL-17 in the CR group as compared to control. Furthermore, our study determined a strong positive correlation between IL-23 and IL-17 expression levels in AML patients. These results suggested that the Th17 cell-related cytokines the plasma levels have high clinical value in the leukemia patients for the therapeutic outcomes evaluation. A recent study revealed that the increase of Th17 cell number and IL-17 level were associated with poor prognosis in AML patients which were consistent with our results.

However, other studies reported that the circulating Th17 cells levels expressed by CD3+ CD8- IL-17A- phenotype, have no differences between healthy controls and with untreated AML patients, chemotherapy-induced cytopenia, and after chemotherapy regeneration [23, 24].

As our study only evaluated the IL-23 and IL-17 plasma levels in AML patients, further research is required to describe the precise involvement of Th17 cells in tumor pathogenesis.

In summary, the current findings indicated that the IL-23 and IL-17 expression levels were generally elevated in patients diagnosed with AML. The variation in these Th17 related cytokines expression level was strongly associated with the outcomes of the disease. Additionally, these Th17 related cytokines can be used as biomarkers to predict the chemotherapy response and prognosis in AML patients.

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Disclosure Statement
The authors have no conflicts of interest to declare.

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