ORIGINAL ARTICLE

Serum Levels of CD28 and CCL17 as a Markers for Immune Status and Progression of Leukemia in Babylon Province-Iraq

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ABSTRACT

Chemokines are important constituent of immunosystem that play a vital roles to regularize the traffic of inflammatory cells and immune responses. Furthermore, many chemokines possess efficient roles on the proliferativeness and invasiveness of cancer cells. Immunity to tumor can be impaired when the regulatory T cells (Treg) population increased. CCL17 achieve a vital role in many cases like fungal, parasitic infection and some malignancies like leukemia. High level of CD28 and low level of CCL17 can indicate good immunity to leukemia. Thirty five leukemic patients and fifteen health person we involved in the study and the serum level of human CD28 and human CCL17 were measured using ELISA kits. The results reveal no increase in the level of CD28 (for patients (3.435±3.901) and for control (2.694±2.258 ng/ml) accompanied with significant higher increase in the level of CCL17 (for patients (394.470±456.554 ng/ml) and for control (134.546±79.836 ng/ml).

The current study conclude that, there is no significant increase in CD28 level as a marker for impaired anti-leukemic T-cell responsiveness combined with higher level of CCL17, in leukemic patients compared with healthy control, which mean also impaired immunity to leukaemia and may implicated in pathological mechanism of leukemia and possibility of using CD28 and anti-CCL17 antibodies to improve immunity among leukemic patients.

Keywords: CCL17, CD28, Leukemia and T-cell.

INTRODUCTION

Leukemia is defined as the accumulations of single, hematopoietic cells in the bloodstream or lymph⁴. Generic cells (hematopoietic stem cells) mature into either myeloid or lymphoid ancestral cells from which all other specialized cells arise². When an infection or damage occur, as a stimuli, it will induce many cells to secret cytokines to regulate the biological and immune responsiveness those stimuli³⁴. Chemokines are heparin-binding proteins with four cysteine residues in the conserved positions⁵⁶. Two intermolecular disulfide binding are formed between the 1st and 3rd cysteines and between the 2nd and 4th cysteines. These bonds lead to establishment of triple-stranded β-sheet structures, while the carboxyl-terminal region forms a α-helix form⁷. Structurally, chemokines can be grouped into 4 subgroups, namely, C, CC, CXC and CX3C. Among CC group the first 2 cysteines are adjacent while in CXC and CX3C they are disjointed by 1st and 3rd amino acids. The C chemokines lacks the 2nd and 4th cysteines⁸.

The CCL17, also called (TARC), is a member of the CC group that is expressed in the thymus, keratinocytes, dendritic colon tissue, endothelial cells and fibroblasts. Likewise, CCL17 is a ligand for CC chemokine receptor 4 (CCR4) and attracts CCR4+ cells. CCR4 is expressed on Th2-type and regulatory T cells (Treg) cells that produce anti-inflammatory cytokines and maintaining immune balance respectively⁹⁰. Many studies have stated the contribution of the CCL17 impairment of anti-tumor immunity in ovarian cancer and lymphoma¹¹¹². T-cell activation and sustainability occur via CD28 who mainly employed in costimulation leading to proliferation of T-cell and production of cytokines.¹³ Many studies showed the roles of CD28+ T-cell as anti-cancer immune cell against many tumors like non-Hodgkin lymphoma, chronic lymphocytic leukemia and Acute Lymphoblastic Leukemia¹⁴¹⁷.

This study aimed to measure CD28 and CCL17 levels in serum to estimate immunity to and progression of leukemia.

MATERIALS AND METHODS

During a period of 10 months (September 2015 until June 2016), Fifty samples were collected. Two enrolled groups of subjects were involved in this study.

Patients: This study includes 35 patients with Leukemia admitted to Marjan Hospital. Patients with
an age range (8-76) years, they were diagnosed by specialist physicians and selected in the current study. Blood and serum samples were taken from every patient and control having thoroughly examined.

Healthy control group: Fifteen of actual healthy persons from various Iraqi populations were arbitrarily involved in the study.

Blood Sampling: The blood was placed in gel tube for thirty minutes, then transferred to plain tube and serum was gottenafter 15 min of centrifugation at 3000 rpm and then the serum collected and kept in the freezer (-20 °C) until it was used for immunoassay

Human cytokines evaluation using ELISA Kit: The ELISA method applies to the in vitro quantitative determination of human cytokines (CD 28 and CCL17) concentrate in serum. The procedure was done as manufacture company leaflet (Eabscience/China).

**Statistical Analysis:** The statistical analysis of this study were performed using T test (P<0.05) using IBM SPSS 23.

**RESULTS AND DISCUSSION**

The worsness and status can be monitored via estimation of some chemokines and cytokines. Serum levels of CD28 and CCL17 were estimated in both 35 leukemic patients and 15 health control. The results reveal that non significant differences (P<0.05) between serum levels of CD28 in patients (3.43±3.901) and control (2.694±2.258 ng/ml). The results of CCL17 reveal significant differences between serum levels of CCL17 in patients (394.47±456.554 ng/ml) and control (134.54±79.836ng/ml) (Table 1).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>No. of samples</th>
<th>Mean ±S.D. (ng/ml)</th>
</tr>
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<tbody>
<tr>
<td>CD28</td>
<td>35</td>
<td>3.43±3.901</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>2.694±2.258</td>
</tr>
<tr>
<td>CCL17</td>
<td>35</td>
<td>394.47±456.554</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>134.54±79.836</td>
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*=significant difference.

Concern CD28 concentration among patients with different malignancies, many studies stated a prominent decrease of CD28 expression among T-cells in patients with B-CLL, peripheral T-cell lymphomas and breast cancer18,19. Down regulation of CD28 or CD28+ T-cell impaire the adaptive immune response or mediate the immune tolerance among malignant patients20. Our results were also in accordance with Boleslawski et al (2011)21 who found that, non significant difference in CD28+ cells between cancer and non-cancer cells.

Our results in accordance with many studies whose stated the increased serum levels of CCL17 in malignancies patients when compared with healthy control. Kumai et al (2015)22 displayed that, levels of CCL17 were suggestively higher in NNKTL than in healthy controls. Also CCL17 has been stated to be expressed massively in some malignancies like B-cell lymphoma and Hodgkin’s lymphoma23,24. Temburni et al (2010)25 found that CCL17 serum level among chronic lymphocytic leukemia(CLL) were significantly increased and impairment of CCR4:CCL17 collaboration in vivo signifies a innovative therapy by inhibitingimmigration of CLL cells on the way to an environment that encourages their survival. Sauer et al. (2013)26 found that CCL17 expressed in high amounts by Hodgkin and Reed/Sternberg (H/RS) cells and Classical Hodgkin lymphoma (cHL) patients27.

**CONCLUSIONS**

The current study conclude that, the elevatedserum CCL17, in leukemic patients compared with healthy control, may implicated in pathological mechanism of leukemia and anti-CCL17 antibodies may improve immunity among leukemic patients.

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