



Serum levels of interleukin-10 and its receptor in the multiple myeloma type I patients

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Abstract

Background & Aims: Multiple myeloma is a malignancy of plasma cells that is associated with high morbidity rate. Inflammation is a common feature in malignancy and plays a pivotal role in the cancer pathogenesis. Interleukin-10 is a cytokine with anti-inflammatory properties. The aim of this study was to evaluate the importance of interleukin-10 and its receptor in multiple myeloma in order to find their pathogenic roles and diagnostic value.

Materials & Methods: In this case-control study, 30 multiple myeloma patients and 40 healthy subjects were enrolled. Serum IL-10 and IL-10R1 levels were determined using ELISA method. Data analysis was performed by SPSS software version 23 including descriptive analysis, mean comparison and correlation tests.

Results: The mean IL-10 levels were 50.93 ± 8.76 and 52.56 ± 10.47 pg/ml in control and patient groups, respectively. The serum IL-10R levels of multiple myeloma patients were significantly lower than those of the healthy control ($p = 0.041$), where mean serum levels were 2.51 ± 0.86 and 2.1 ± 0.76 pg/ml, respectively. We found a positive correlation between IL-10 and IL-10R levels ($p = 0.042$).

Conclusion: These results demonstrated that IL-10R, but not IL-10, is increased in the patient's serum and they are proposed as a new diagnostic biomarker. In other words, downregulation of the IL-10 receptor and attenuation of IL-10's bio-effect are associated with further inflammation in multiple myeloma type I patients.

Keywords: Biomarker, Interleukin 10, Interleukin 10 Receptor, Multiple Myeloma

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Introduction

Multiple myeloma is a malignancy of plasma cells that is characterized by the presence of neoplastic plasma cells in the bone marrow and elevated monoclonal paraprotein levels (1). Multiple myeloma

is the second most common hematologic malignancy and is associated with a high morbidity rate due to its end-organ failure (2). The evaluation of serum and urine proteins by capillary, electrophoresis, and bone marrow biopsy analysis, are the main diagnostic tools.

However, the pathogenesis and molecular mechanisms of multiple myeloma are not well understood (3). Inflammation is a common feature in malignancy and plays a pivotal role in the cancer pathogenesis (4). The role of inflammation and pro-inflammatory cytokines in multiple myeloma were reported frequently (5). Bone marrow stromal cells produce pro-inflammatory cytokines by autocrine and paracrine mechanism, which predict the duration of disease-free survival. Furthermore, cytokines have been proposed as biomarkers for the diagnosis of multiple myeloma, patient's prognosis, and tools for immunotherapy (6). The serum levels of TNF- α , IL-6, IL-6R, and IL-1 β are increased in multiple myeloma patients (7-11). Interleukin-10 (IL-10) is a cytokine with immunomodulatory and potent anti-inflammatory properties that is produced by activated T cells, macrophages, dendritic cells, and B-lymphocytes (12, 13). This cytokine belongs to class II cytokine family and plays a critical role in blood malignancy and other diseases (14-16). The biological function of IL-10 is mediated by the IL-10 receptor (IL-10R), which has two isoforms, including IL-10R1 and IL-10R2. The IL-10R1 is a cell membrane associated receptor with a transmembrane and extracellular domain that has higher affinity to IL-10 than IL-10R2 (17, 18). These receptors are expressed in several cell lines. Activation of IL-10R activates JAK/STAT signaling pathway as a downstream mediator. The interaction of IL-10 and receptors leads to the overexpression of anti-inflammatory cytokines and induces cell proliferation in CD8+ T and natural killer cells (19).

To the best of our knowledge, there is currently no comprehensive study on the relationship between IL-10 and its receptor with multiple myeloma. The aim of this case-control study was to determine the serum levels of IL-10 and IL-10R in multiple myeloma

patients and healthy subjects in order to describe the role of this cytokine and its receptor in the pathogenesis and their predictive value as biomarkers.

Materials & Methods

Sampling:

In this case-control study, inclusion criteria was patients with a confirmed multiple myeloma and 30 patients were enrolled. For the control group, 40 age- and sex-matched subjects were selected. Exclusion criteria were subjects who had inflammatory disorders, infections and immunodeficiency, or who had received NSAIDs or anti-inflammatory drugs. Blood samples were obtained and sera were separated by centrifugation at room temperature and were stored at -70°C refrigerator.

ELISA Assay:

Serum IL-10 and IL-10R1 levels were determined using ELISA kits (bioassay technology laboratory Shanghai). The ELISA assays were performed according to the manufacturers' instructions.

Statistical Analysis:

Data analysis was performed using SPSS software version 23. Descriptive analysis was performed and represented as charts, tables, and figures. Parametric and non-parametric statistical tests were used to compare the mean of variables between the case and control groups. The findings were expressed as mean \pm SD, and $p < 0.05$ was considered as statistically significant.

Results

Serum IL-10 Levels:

The data analysis results of IL-10 are shown in Table 1 and the Figure 1A depicted IL-10 levels. The mean IL-10 levels did not show a significant difference between the patients and control groups.

Table 1. Serum IL-10 levels in patients and control groups

IL-10(pg/ml)	Minimum	Maximum	Mean	SD	Range	p value
Control	37.30	86.60	50.93	8.76	49.30	
Multiple myeloma	23.30	71.90	52.56	10.47	48.60	0.481

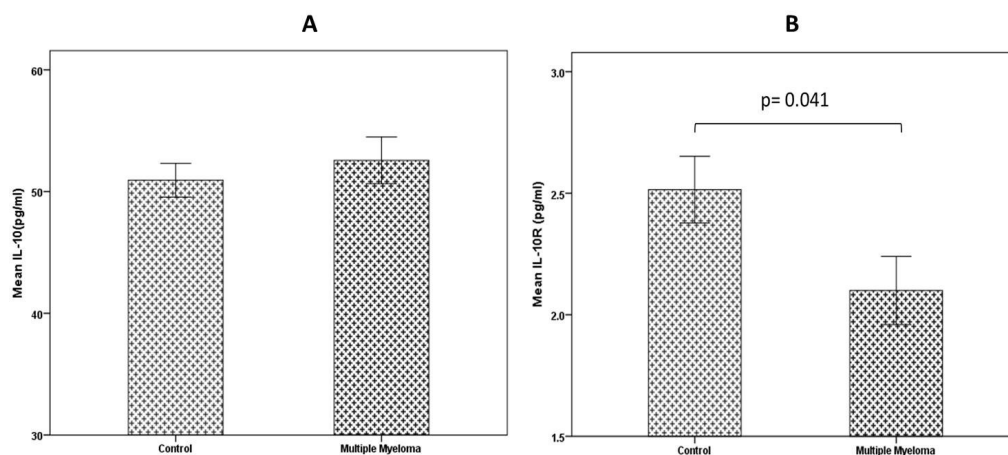


Fig. 1. A: Comparison of serum IL-10 levels in multiple myeloma and normal groups. There was no significant difference, B: Serum IL-10R levels in multiple myeloma was lower than normal groups significantly ($p = 0.041$).

IL-10R Levels:

The descriptive analysis results of IL-10R are shown in Table 2 and the Figure 1B depicted IL-10R levels. The mean IL-10R levels showed significant

difference between the patients and control groups (0.041). The mean IL-10R levels were lower in multiple myeloma patients.

Table 2. Serum IL-10R levels in patients and control groups

IL-10R(pg/ml)	Minimum	Maximum	Mean	SD	Range	p value
Control	1.2	5.6	2.51	0.86	4.40	0.041
Multiple myeloma	1	4.40	2.1	0.76	3.40	

Correlation Analysis:

There was a significant positive correlation between IL-10 and IL-10R in patients, healthy subjects, and in

the total population. Figure 2 showed this correlation analysis between IL-10 and IL-10R levels.

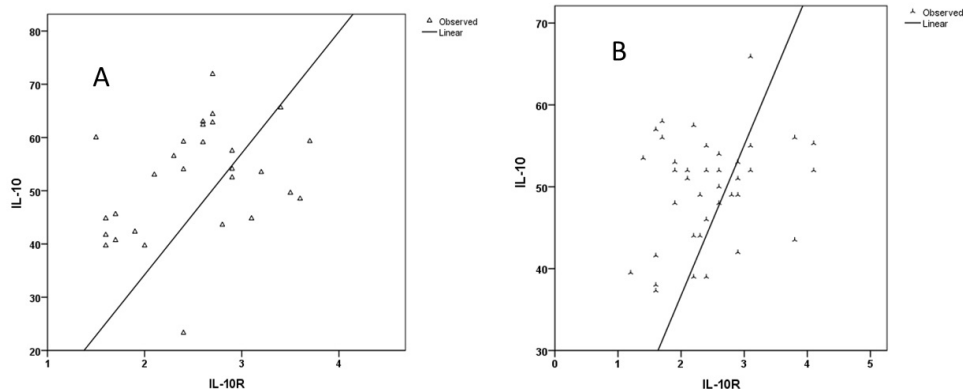


Fig. 2. Correlation between serum IL-10 and IL-10R levels in patients (A) and healthy control (B)

Discussion

About two centuries ago, multiple myeloma was characterized, but the molecular mechanisms of pathogenesis remain unclear (20, 21). Current knowledge supports the pathological role of inflammation in multiple myeloma. Pro-inflammatory cytokines have double-edge effects on multiple myeloma, which increase cancer progression by induction of cell growth and apoptosis inhibition and anticancer effect by CD4+ T cells and natural killer cells (22).

In our study, IL-10 levels showed no significant difference between patients and healthy control. However, a previous study by Pappa et al. showed increased levels of IL-10 in multiple myeloma (23). This inconsistency probably may be due to the patient's disease stage. Wang et al. showed that upregulation of IL-10 is associated with poor prognosis (24). So, in the patient population where IL-10 levels remain unchanged, there may be a good prognosis that may be due to ethnicity and genetic properties (25).

In the multiple myeloma patients, IL-6 induces cell proliferation and cancer progression via IL-6R in bone marrow stromal cells (26).

The promoter sequences regulate the gene expression by modulation of transcription factors interaction. Mazur et al. showed that IL-10 promoter gene polymorphism does not associate with the susceptibility for multiple myeloma, which is in agreement with our finding (27). However, the evaluation of serum IL-10 levels in multiple myeloma patients with poor and good prognoses showed that this cytokine is associated with better survival and outcome (24). In Wang et al.'s study, the mean IL-10 was 201.96 pg/ml, which was four times higher than our report (24). Because race and ethnicity affect the cytokine levels, and studied populations are different, we interpret this difference as racial variation (28).

The biological effects of ligands depend on ligands concentration and receptors levels (29). Therefore, we conclude that the biological effects of IL-10 in multiple myeloma patients are reduced due to the downregulation of receptors (30-32). We observed that

IL-10R down-regulated in multiple myeloma patients and probably be a good biomarker for diagnosis. The reductions of biological effects of an anti-inflammatory cytokine lead to inflammation, that would trigger the cancer progression. There are reports showing that IL-2R and IL-6R, the pro-inflammatory cytokines receptors, were unregulated in multiple myeloma (6). These findings are in agreement with our findings because IL-10R is an anti-inflammatory signaling (33). Besides, Kasamatsu et al. found an association between IL-10R gene polymorphism and multiple myeloma (34). However, another study found no significant changes in IL-10R gene expression (35).

Here, we report a positive correlation between IL-10 and IL-10R. Urbańska et al. showed that there is a strong correlation between IL-10 and IL-6 receptor (36). We have no insight into the inducing effects of IL-10 on its receptors and other cytokines levels and their receptors regulation, and need more studies (36). This study is the first report that measured the IL-10R levels in multiple myeloma patients type I. Shekarriz et al found a significant increase in the IL-10 of multiple myeloma patients where it was correlated with disease stage and markers of disease activity (37).

At the molecular level, toll-like receptors are the main upstream receptors which induce the IL-10 production in myeloma and other cell lines (38, 39). Because toll-like receptors are active in multiple myeloma, absence of changes in the IL-10 levels in the type I may be due to the stage of the disease or suppression of genes by other mechanisms such as microRNAs (40). Several studies showed that microRNAs have obvious effects on both the expression of IL-10 and multiple myeloma progression (41).

In the downstream of IL-10 receptors, STAT3 transcription factor is located, which expresses the anti-inflammatory gene (such as Bcl-xl). On the other hand, STAT3 is considered as a target for the treatment of multiple myeloma (42). Therefore, the manipulation and regulation of IL-10 and its receptors could be

considered as targets for the treatment of the disease and need more scrutiny.

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Conflict of interest

No conflict of interest declaration between the authors.

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