

# Levels of Some Cytokines in Iraqi Patients with Multiple Myeloma

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## Abstract

**Introduction:** Multiple myeloma is hematological disease characterize by abnormal functioning plasma cell result in deterioration in different organs include bone and kidneys in addition to other complications. The study aimed to measure some cytokines in the serum and determined if there is possibility to use this cytokines as predictor for the multiple myeloma.

**Materials and Method:** Blood samples was drowned from 58 patients and 24 sex and age match control, serum then isolated and proper ELISA kit then used to determined level of  $\beta$ 2 microglobulin, interleukins-13, 19, and 38.

**Result:** The result demonstrated significant increase in  $\beta$ 2 microglobulin in patients compare to control (1.347+0.714 vs. 0.913+0.253),  $p = 0.00$ , interleukin-13 significant increase in patients group compare to control group (204.56+189.84 vs. 106.17+52.07),  $p=0.001$ , interleukin-19 non-significant increase in patients group compare to control group (22.46+42.09 vs. 11.252+8.574),  $p=0.211$ , interleukin-38 significant increase in patients group compare to controls (60.087+54.131 vs. 26.791+21.382),  $p= 0.00$ .

**Discussion:** The results of this study indicate that IL-13 and 38 possible to use as predictor for the disease.

**Keywords:** Health, Iraqi patients; myeloma; cytokines.

## Introduction

Multiple myeloma (MM) is malignant disease effect plasma cell, this accompany with a number of changes include elevation in calcium levels, renal insufficiency, bone lesion and metabolic disorder in addition to the abnormal protein in the urine contributed to renal disorder<sup>(1)</sup>.

The bone disorder is the most common complication in the MM, the damage that occur in the bone result from stimulation of osteoclast formation. In addition, there is

decrease in the bone formation have been reported and this attributed to the suppression effect of myeloma cell on osteoblast cell<sup>(2)</sup>.

Other MM complications is renal disorder that occur due to light chain immunoglobulin (LCI) accumulation<sup>(3)</sup>, Anemia is common hematological complication and account (60% to 80%) untreated active MM patients and its usually normochromic and normocytic type<sup>(4)</sup>.

Interleukin 13 is a Cytokine involve in allergy and inflammation, they produce pathological effect when produce in high dose, however, in considerable dose useful against intestinal helminthic parasite<sup>(5)</sup>.

Interleukin 19 (IL-19) is one of interleukin 10 (IL-10) family that produce from activated monocyte, T and B cell to lesser extent, non-immune cell like keratinocytes and foetal membranes<sup>(6, 7)</sup>.

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Interleukin-19 receptors abundant in the skin, lung and reproductive organ tissue, and seen to be important in production T helper 2 (TH 2) cytokine and induce expression of IL-6, IL-8 and IL-10 in monocyte. IL 19 elevated in the asthma and a number of the disease include type1 DM, aging, vascular disease and rheumatoid arthritis<sup>(8)</sup>.

Interleukin 38 one of interleukin family 1 that include 11 type and play important role in the inflammation, it have specific receptor called IL-1 receptor-related protein 2 (IL-1Rrp2, IL-36R) these receptor considered as interleukin36 (IL36) receptor antagonist.

Interleukin 38 considered anti-inflammatory agent through inhibit the effect of IL 36 in that receptor<sup>(9)</sup>.

Beta2 microglobulin is a polypeptide presented in the serum its origin is the cell membrane of all nucleated cell in which found in tight junction with major histocompatibility complex 1(MHC1): elevated its level reflected increase intrinsic kinetic activity of tumor cell including DNA and RNA kinetic, therefore, it's important for staging, determined disease severity, response to chemotherapy and prognosis<sup>(10)</sup>.

**This study was aimed to:** Determined level of some cytokines in the serum of the patients with multiple myeloma.

Study the possibility of the serum level of these cytokines to be used as predictor of multiple myeloma disease.

## Subject, Material and Method

The study was conducted in Baghdad city in (Baghdad hospital/medical city and hematological center)from October 2018 to May 2019 where (58) patients diagnosed to be have multiple myeloma and most of them regularly visit the hospital to receive the chemotherapy. From the total number of the patients, (36) was male and (22) was female.

The control subjects were randomly selected which were apparently healthy, the control were age, sex, body mass index (BMI) matching to patients group.

Disposable syringe and needles used for blood collection, venous blood sample about six ml collected from patients and healthy volunteers in plain tube, blood sample were centrifuge at 2000 rpm for 5 minute to obtain serum.

The serum were divide in Eppendorf tubes and freeze in -20 C° until all serum collected to measure the biomolecules by ELISA.

Serum  $\beta$ 2- Microglobulin determined through use commercial kit from Demediet by sandwich ELISA method.<sup>(11)</sup> Reference range of  $\beta$ 2 microglobulin (1-2 microgram/milliliter)<sup>(12)</sup>.

Serum IL-13 and IL-19 determined using commercial kit obtain from CUSBIO, using sandwich ELISA method,<sup>(11)</sup>.

Serum IL-38 determined using commercial kit obtain from My BioSource, using sandwich ELISA method<sup>(11)</sup>.

The results were express as mean (+/-) standard error of the mean. The statistical analysis was perform using statistical package for social science (SPSS 23), independent student (T) test use to test the degree of significance difference between the patients and control, the p value less than 0.05 considered statistically significant.

## Results

**Serum levels of biomolecules:** Table (1) showed serum level of  $\beta$ 2 microglobulin, IL-13, 19 and 38in controls and patients group.

**Table (1) Serum level  $\beta$ 2 microglobulin, IL-13, 19 and 38 of in patients and controls group**

Parameters	Patients group (n=58)	Controls group (n=24)	Degree of significance
B2 microglobulin ( $\mu$ g/ml)	1.347 $\pm$ 0.714	0.913 $\pm$ 0.253*	0.000
Interleukin 13 (pg/ml)	204.56 $\pm$ 189.84	106.17 $\pm$ 52.07*	P=0.001
Interleukin 19(pg/ml)	22.46 $\pm$ 42.09	11.252 $\pm$ 8.574	P=0.211
Interleukin 38(pg/ml)	60.087 $\pm$ 54.131	26.791 $\pm$ 21.382*	P=0.00

The results express in term of (mean  $\pm$  standard deviation of the mean), n=number of the subject, (\*) = significance difference, (p<0.05) compared to control group.

## Discussion

The study showed level of  $\beta$ 2 microglobulin significance increase in patients group compare to control group despite most of the patients received MM therapy, this indicate the disease is active.

Interleukin 13 significant increase compared to control group, T helper 2 (Th2) cell one of the immune cell that secreted IL-13, so activation of Th2 cell enhance it to secrete the cytokines that include IL-13, Th2 derived from T helper 0 cell (Th0) and required to IL-4 for this development<sup>(13)</sup>, IL-4 secreted from number of cell like natural killer (NKC), Basophil, or even CD4 cell themselves<sup>(14)</sup>.

Many studies showed that total CD4 cell significant reduce in the number among MM patients and CD4/CD8 ratio going to be reduce<sup>(15, 16)</sup>, besides that, T helper 2 in most studies reported to be either decrease or not significance change in MM in contrast to other CD 4 cell like T helper 1 cell and T regulatory cell<sup>(16,17)</sup>, so, from all above it is possible to suggest that Th2 cell have no any role in increase IL-13 in this study.

Eosinophil cell is large factory that secreted many type of biomolecules include the IL-13<sup>(18)</sup>, so, high level of IL-13 in this study can possibly attributed to activation of eosinophil.

The role of eosinophil in MM have been studied and showed that eosinophil found in association with plasma cell in bone marrow and secrete factors that necessary to plasma cell survival in B.M.<sup>(19)</sup>.

In pathological aspect, Tina *et al.* Showed eosinophil also act to enhance myeloma cell proliferation and considered distinct pathway involve in myeloma cell development beside the proliferation development produce by stroma cell pathway<sup>(20)</sup>.

Basophil is another cell that secreted IL-13<sup>(21)</sup>, however, few studies that determined the relation between basophil and MM, the only information available indicated the basophil stimulate and secrete IL-13 in response to IgE secretion<sup>(22)</sup>, the same relation it's possible to be found in case IgE myeloma, however IgE myeloma considered as one of unusual myeloma

that include also IgD and IgM which is totally represent no more than 10% of all MM cases<sup>(23)</sup>.

Mast cell also synthesis and release IL-13 in addition to other mediators<sup>(24)</sup>, many studies noted the role of mast cell in MM pathogenesis especially in the angiogenesis and osteolytic disorder through secretion of its mediators<sup>(25, 26)</sup>.

Mast cell can be activated by IgG which is one of the most anti body secreted by myeloma cell also could be considered one of the biomolecules that stimulate mast cell to secrete IL-13<sup>(27)</sup>.

Additionally, interaction between Mesenchymal stem cell and MC cell lead to higher secretion of stem cell factor (SCF)<sup>(28)</sup> which is in turn stimulate mast cell to secrete IL-13<sup>(29)</sup>, all of these factor strongly suggest the mast cell stimulation as one of main cause of increase IL-13 in this study.

Natural Killer cell (NKC) is another immune cell that secrete IL-13, however, involvement of these cell in IL-13 level elevation in MM it's unlikely, since these cell showed inverse relationship with stage of myeloma, this support by the study that demonstrated normal or arise in the levels of NKC in the early or treatment stage of MM<sup>(30)</sup>, while in advance and active disease going to depleted<sup>(31)</sup>.

The study demonstrated non-significant difference in IL-19 between control and patients group.

T helper 2 cell one of the immune cell secreted IL-19, T cell in all type (CD4 and CD8) have been reported either decrease or not change in MM<sup>(15,16)</sup>, so, T cell unable to increase IL-19 secretion in MM and this agree with this study.

Although IL-19 secrete also from B cell, its effect on IL-19 level is negligible since the studies showed the secretion of this cytokine from B cell is very low and irregular<sup>(32,33)</sup>.

Monocyte is other immune cell that secrete IL-19, number of the studies evaluate the level of monocyte in MM, one of them showed deterioration in monocyte function including chemotactic function in patients with MM<sup>(34)</sup>, other demonstrated deterioration in monocyte that showed improvement in monocyte enhance dendritic cell activity upon immunotherapy<sup>(35)</sup>, so give the possibility to suggest that activation of monocyte to secrete and increase IL-19 is unlikely and this agree with this study.

The study demonstrated significant increase in IL-38 level compare to the control. Interleukin 38 release from apoptotic cell like in lung or breast cancer<sup>(36)</sup>, IL-38 act to decrease IL-6 secretion from macrophage that play significant role in the MC growth and proliferation, so, increase IL-38 secretion can explain as a compensatory mechanism to fight MC development<sup>(37)</sup>.

Myeloma cell also report to increase apoptosis of immune cell, and this considered as way to explain increase in IL-38 level since these IL release from apoptotic cell as mention before<sup>(38)</sup>. Interleukin 38 have been report to be secrete from B cell<sup>(39)</sup>.

### Conclusion

Multiple myeloma produce change in the number of cytokines, either proinflammatory and anti-inflammatory both can be effected. Elevation of IL-13 is possible in the patients with MM and this possibility attributed to the stimulation of number of immune cell that known to secrete IL-13 in MM like eosinophil, mast cell. Possibility to use IL-13 and IL-38 as predictor for MM disease.

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

**Conflict of Interest:** The authors declare that they have no conflict of interest.

**Funding:** Self-funding

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