Study of IL-35 levels in Multiple Myeloma patients and its Relation with Immunoglobulins

Zeinab M. M. Al-Rubaei[1], Bushra H. ALI [1], Nawal M. Jawad Al-Shamma'a[1]
[1] Chemistry Department , College of Education for Pure Sciences -Ibn – Al-Haitham , Baghdad University, Baghdad ,Iraq
alizeinabah@yahoo.com,

ABSTRACT

Multiple myeloma (MM) is a malignant disorder characterized by the proliferation of plasma cellsand the second most common hematological standing next to lymphoma. Multiple myeloma patients commonly present with defects in numbers and function of various   immune cells including dendritic cells ,B cells,Tcells and natural kill cells[23].Interleukin- 35 (IL-35) is a novel anti-inflammatory cytokine suppressing the immune response through the expansion of regulatory T cells and suppression of Th17 cell development.The present study aimedto investigate the possible role of IL-35 in pathogenesis of MM and its relation withimmunoglobulins such as IgA,IgG and IgM.Forty Iraqis patients with multiple myeloma(G1,G2) and twenty healthy individuals as a control group (G1)were enrolled in this study.Whole blood used for determination of hemoglobin. Serum samples were used for determination of albumin,total protein using standard procedures of the biochemistry laboratory of hospital. Also immunoglobulin's (IgA,IgG,IgM) and IL-35 were determined in serum.The results revealed a significant increasing in urine total protein ,ESR,creatinine ,uric acid ,BUN, and TP in patients groups(G2,G3) comparing to control group(G1) while a   significant decrease in albumin and Hb were found in patients groups comparing to control .Also a significant differences was observed in calcium concentration in G3 compared to G2 while there are no differences was observed between G2 and G1 . The results shown highly significant increase in IgA,IgG, IgM and IL-35,in patients groups comparing to control group. Also a significant differences were noticed in G3 comparing to G2. A highly significant positive correlation between IL-35 and IgA was noted in G2 and G3. Also a highly significant positive correlation between IL-35 and IgG levels was noticed in G2 and G3. Moreover a significant negative correlation founded between IL-35 andIgM in G2 and G3 .A conclusion could be drown from this study for the first time that MM patients showed increasing in IL-35 levels so, it may be considered as a novel cytokine involved in the pathogenesis of MM.

Keyword:IL-35,Multiple Myeloma,Immunoglobulines.

INTRODUCTION

Multiple myeloma(MM) is a monoclonal post –germinal center tumor that has phenotypic features of plasmablasts / plasma cells and usually localize at multiple sites in the bone marrow and is the second most common hematological standing next to lymphoma[1[. Multiple myelomais clinically characterized by $\geq$ 10% of plasma cell infiltration in the bone marrow and presence of hyperglycemia ,renal disorder ,anemia, and bone lytic lesions[2].

Interleukin (IL)-35 is a novel heterodimeric cytokine in the IL-12 family that is comprised of an IL-12p35 subunit and an IL-12p40 related protein subunit [3,4]. IL-35 functions through IL-35R and has a potent immune-suppressive activity. Although IL-35 was demonstrated to be produced by regulatory T cells, gene-expression analysis revealed that it is likely to have a wider distribution, including expression in cancer cells[5-7].Some cytokines of the IL-12 superfamily are predominantly produced by antigen presenting cells in response to microbial or host immune stimuli and are involved in the regulation of immune responses against infections and tumor development[8,9] .

Other clinical study support the concept that some members of IL-12 may represent a novel, promising therapeutic agent for patients affected by different oncologic diseases including potentially MM ,based upon is documented multifunctional activity[10,11].

Recently, several research groups analyzed Treg cells in multiple myeloma. So far, Treg cells data in multiple myeloma are conflicting. Study from Prabhala et al and Gupta et al [12,13] reported decreased frequency of peripheral blood Treg cells in multiple myeloma when compared to control group . Both studies confirmed that FoxP3 expression was reduced in myeloma patients. Most studies in myeloma agree that Treg cells efficiently suppress both autologous and allogeneic responder cells (CD4+CD25-) similarly to healthy subjects[14-16].Synonymous with differential expression, the IL-12 cytokine family also exhibits distinct functional differences. While IL-12, IL-23, and IL-27 share the common feature of inducing interferon-γ (IFNγ) production and promoting T-helper 1 (Th1) cell differentiation and proliferation, they act differentially on subsets of T cells and with different kinetics. In contrast, IL-35 appears to function solely in an anti-inflammatory fashion by inhibiting T-cell proliferation and perhaps other parameters [17,18].

The aimed of the present study is to investigate the possible role of IL-35 in pathogenesis of MM and its relation withimmunoglobulins(IgA,IgG and IgM).

MATERIALS AND METHODS

Fasting blood samples were obtained from forty Iraqis patients with multiple myeloma and twenty healthy individuals as a control group (G1) which enrolled in this study .Patients were divided into two stages(G2,G3) according to Durie-Salmon staging system [19]which attended Baghdad Teaching hospital.Whole blood used for determination of heamoglobin. Serum samples were used for determination of albumin ,total protein using standard procedures of the biochemistry laboratory of hospital.Immunoglobulins (IgA,IgG,IgM) determined by Riaimmunodiffusion (RID) method [20] using kit from LTA s.r.l. (Italy).

 Serum IL-35 levels were measured using specific enzyme-linked immunosorbent assay (ELISA) kit (CUSABIO Human IL-35 for *in vitro* quantitative measurement) ,according to the manufactures protocol.

The data was expressed as mean ± SD. The comparison between patients group and control group were analyzed by using student t-test. Pearson,s correlation coefficient was used to examine between IL-35 and immunoglobulins in patients group. P-value of < 0.001   and   < 0.05 were considered highly significant and significant respectively.

RESULTS AND DISCUSSION

Descriptive and diagnostic parameters for the three studied groups(C,G1,G2) are shown in table (1). The results revealed a significant increasing in urine total protein ,ESR,creatinine ,uric acid ,BUN, and TP in patients groups(G2,G3) comparing to control group(G1) while a   significant decrease in albumin and Hb were found in patients groups comparing to control .Also a significant differences was observed in calcinm concentration in G3 compared to G2 while there are no differences was observed between G2 and G1 .

Table (1): Descriptive and diagnostic parameters for the three studied groups

| Groups \ Parameters	G1 n=20	G2 n=20	G3 n=3	P-value	P*-value	P**-value
Age(year)	58.5 ± 6.4	56.5 8.4	58.4 8.3	NS	NS	NS
Urine total protein (mg/24 hours)	150	2400, M protein κ	3000, M protein κ	HS	HS	S
Amyloid	Negative	Positive	Positive	-------	-------	-------
Monoclonal band	--------	Mband, IgGκ κ in in serum	MbandIgG κ in serum and urine	-------	-------	-------
ESR (mm in 1st hr)	12 ± 3.4	50 ± 15.9	64 ± 20.33	HS	HS	S
Hb(g/dl)	13.6 ±0.86	9.1 ± 2.3	7.66 ± 2.12	HS	HS	S
Calcium(mmol/L)	2.3 ±0.54	2.4 ± 0.6	5.3 ± 1.54	NS	S	S
Creatinine(μmol/L)	62 ± 18.1	88.4 ± 25.6	105.2± 30.1	S	HS	HS
Uric acid(μmol/L)	299 ± 68	433 ± 125	533 ± 152	HS	HS	S
BUN(mmol/L)	5 ± 1.45	7.1 ±2.5	8.9± 2.41	S	HS	S
TP(g/dl)	7.5± 0.4	8.5 ± 2.4	9.9 ± 3.1	S	S	S
Albumin(g/dl)	5.25 ± 1.45	4.33 ± 1.26	3.25 ± 1.11	S	HS	NS

 S Significant (P < 0.05),HS highly significant(P < 0.001), NS no significant(P≥ 0.05)

The results in table (2) shown the levels of IgA,IgG, IgM and IL-35. The results revealed highly significant increase in IgA,IgG,IgM and IL-35,in patients groups comparing to control group. Also a significant differences were noticed in G3 comparing to G2.

Table 2: levels of   IgA,IgG, IgM and IL-35 in all studied groups.

Groups Parameters	G1 n=20	G2 n=20	G3 n=20	P-value	P*-value	P**-value
IgA(mg/dl)	173.85 15.12	204.15 49.63	352.2 72.69	HS	HS	S
IgG(mg/dl)	270.2 52.57	305.45 52.44	410.45 60.23	S	HS	S
IgM(mg/dl)	149.9 8.28	270.95 42.1	349 40.37	HS	HS	S
IL-35(pg/ml)	23.21 2.12	45.87 2.9	69.9569.95 4.28	HS	HS	HS

Ahighly significant positive correlation between IL-35 and IgA was noted in G2(r=0.408,p<0.001) and G3 (r= 0.405 , p<0.0001) (figures 2A,2B) .
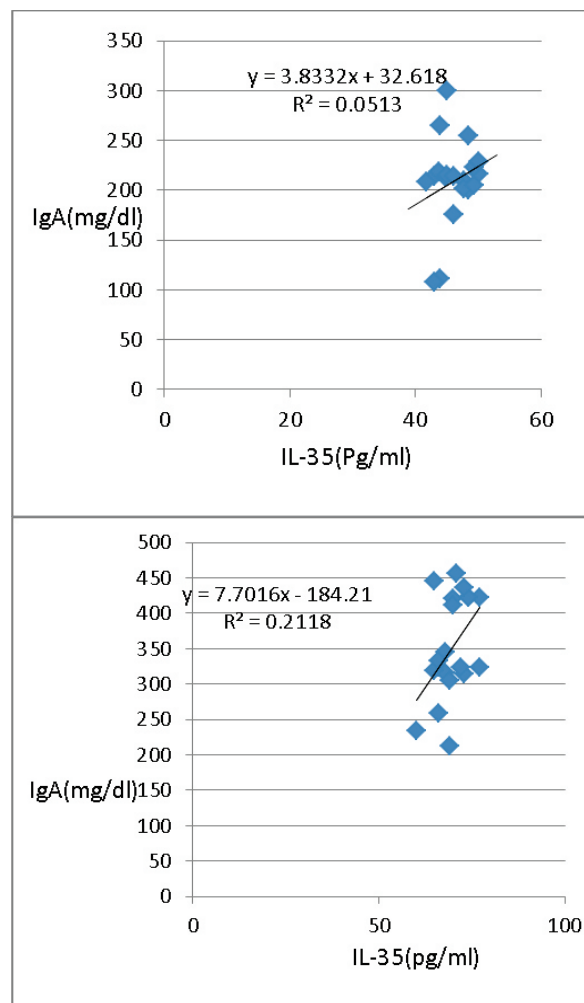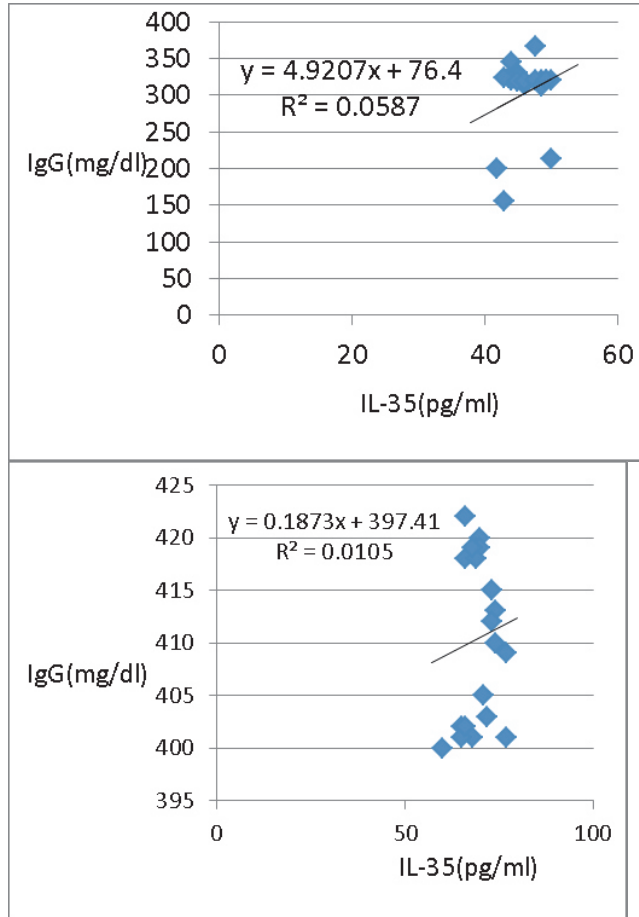

1A

1B

Figure 1: Correlation between serum levels of IL-35 and IgA in G2(1 A) and G3 (1B)of MM patients

Also a highly significant positive correlation between IL-35 and IgG levels was noticed in G2 (r= 0.139, p< 0.001) and G3 (r= 0.102,p<0.001) (figure 2A, 2B).
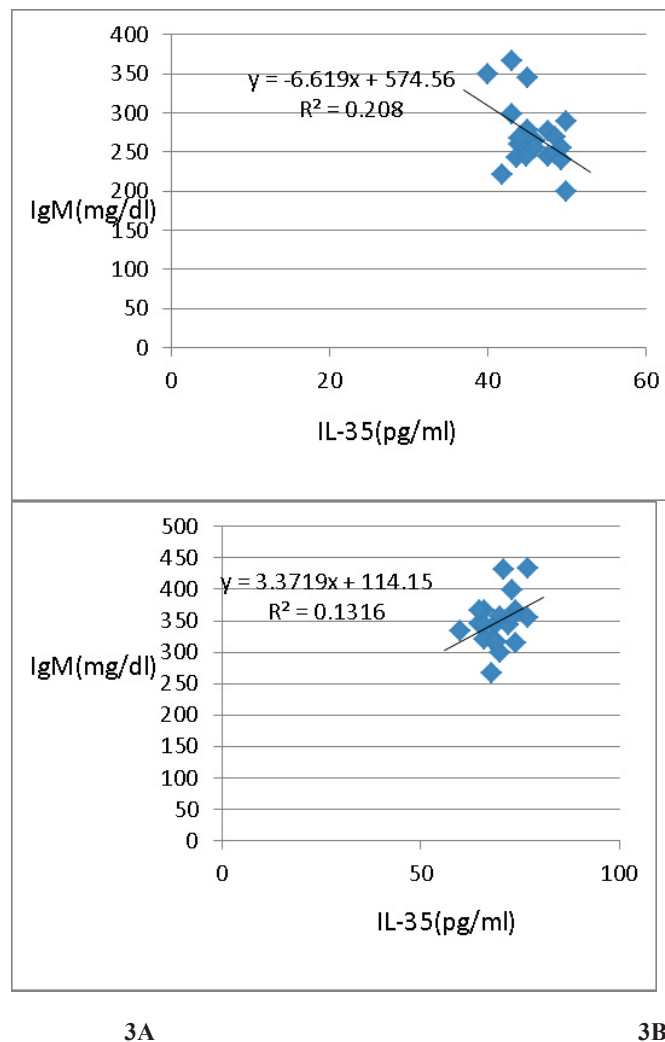

2A   2B

Figure (2) :correlation between serum levels of IL-35 and IgG in G2 (2A)and G3 (2B) ofMM patients.

Moreover a significant negative correlation founded between IL-35 andIgM in G2(r=- 0.456 ,p<0.05)   and G3 (r=0.358 ,p<0.05) (figures 3A,3B).

3A   **3B**

Figure 3: Correlation between serum levels of IL-35 and IgM in G2 (A) and G3 (B) ofMM patients.

Pathogenesis of MM   is complex and dependent on the interactions between tumor cells and their microenvironment[8].Tumors grow within an intricate network of epithelial cells, vascular and lymphatic vessels, cytokines and chemokine's, and infiltrating immune cells. Different types of infiltrating immune cells have different effects on tumor progression, which can vary according to cancer type[21].Multiple myeloma patients commonly present with defects in numbers and function of variousimmune cells including dendritic cells ,B cells,Tcells and natural kill cells[22]. Previous work indicated that, like IL-27,and IL-35 may be an immunomodulation at the feta-maternal border[7].

Most of the studies strongly suggest that myeloma patients Treg cells are functional in suppressing the conventional T cell proliferation,and this suppressive function encourages the immune impairments and dysfunctions. However, Treg cells suppressive function could be appreciated in the case of graft versus host disease where donor cells require engraftment to ensure the anti-tumor effects [18]. All three of the original family members, IL-12, IL-23, and IL-27, were initially described as pro-inflammatory/stimulatory cytokines, promoting T-cell proliferation and cytokine production[23].IL-35 is a novel anti-inflammatory cytokine suppressing the immune response through the expansion of regulatory T cells and suppression of Th17 cell development[24].Another study indicate that IL-27 is important for anti-parasite immunity against *L. major* and

IL-35 suppresses this immunity, the net effect of losing both IL-27 and IL-35 might be susceptibility to the infection, at least during the early phase of infection[25].

The recent study found that both IL-35 subunits were expressed concurrently in most human cancer cell lines compared to normal cell lines. In addition, we found that TNF-α and IFN-γ stimulation led to increased IL-35 expression in human cancer cells. Furthermore, over-expression of IL-35 in human cancer cells suppressed cell growth *in vitro*, induced cell cycle arrest at the G1 phase, and mediated robust apoptosis induced by serum starvation, TNF-α, and IFN-γ stimulation . In conclusion, this results reveal a novel functional role for IL-35 in suppressing cancer activity, inhibiting cancer cell growth, and increasing the apoptosis sensitivity of human cancer cells through the regulation of genes related to the cell cycle and apoptosis[4].

CONCLUSION

The results revealed an increasing in the level of IL-35 in MM patients.Also,the positive relation of MM with immunoglobulins reflect the possible relation of IL-35 with immunesystem.Therefore,further studies are required to investigate the precise effect and the signaling transduction mechanisms of IL-35 in the MM process .

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