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Evaluation of angiopoietin-2 level in patients with multiple myeloma at presentation and in remission state

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Abstract:

BACKGROUND: Angiopoietin-2 (ANG-2) regulates angiogenesis and enhances the formation of new vessels in tumors by boosting the effect of vascular endothelial growth factor as part of dynamic neovascularization. ANG-2 is a marker of disease progression and therapy response in multiple myeloma (MM).

OBJECTIVES: The study aimed to assess the level of ANG-2 in MM patients at diagnosis and in remission state and elaborate on its correlation with interleukin-6 (IL-6) and beta-2 microglobulin (B2M) levels.

PATIENTS, MATERIALS, AND METHODS: Sixty MM patients; 20 newly diagnosed (ND), and 40 patients in remission were included. Twenty healthy individuals were included as a control group. Plasma levels of ANG-2, B2M, and IL-6 were tested by enzyme-linked immunosorbent assay.

RESULTS: There are significant statistical differences between ND patients and those in remission in hemoglobin, neutrophil count, blood urea, serum creatinine, glomerular filtration rate, B2M, IL6, and ANG-2 (P = 0.001, 0.033, 0.005, 0.001, 0.001, 0.001, 0.004, and 0.001, respectively). ANG-2 showed significant positive correlations with B2M (P = 0.001) and IL-6 (P = 0.012).

CONCLUSION: The low ANG-2 level in the remission group with an insignificant difference from that in the control group with a high level in the untreated patients renders it a useful indicator for treatment response follow-up in MM. The positive correlation of ANG-2 with B2M and IL-6 reflects the active angiogenesis with a high tumor burden and disease progression.

Keywords:

Angiopoietin-2, complete remission, multiple myeloma

Introduction

The clonal plasma cells in multiple myeloma (MM) secrete complete and/or partial immunoglobulins.[1] The growth of a tumor depends on the process of angiogenesis triggered by angiogenic factors produced in the microenvironment of neoplastic cells or released by the neoplastic cells.[2] Angiopoietin (ANG)-1 is important to the formation and integrity of mature blood vessels, whereas ANG-2 blocks ANG-1-dependent activation. It enhances tumor angiogenesis by activating the vascular endothelial growth factor (VEGF) at the beginning of the neovascularization process.[3] ANG-2 can be used as a good marker of angiogenesis and as a significant therapeutic target.[4] The study aimed to compare the level of ANG-2 in newly diagnosed (ND) MM patients and in remission state and elaborate on its correlation with interleukin (IL)-6 and beta-2 microglobulin (B2M) levels.

Patients, Materials, and Methods

This cross-sectional study included 60 MM patients divided into two groups: 20 ND

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and 40 patients in complete remission (CR) or in the very good partial remission (VGPR) states.[5] Patients were selected as follows:

1. Untreated ND patients diagnosed as MM depending on the following criteria:[6]
   - The presence of clonal plasma cells >10% in the bone marrow (BM)
   - The presence of monoclonal globulins in the serum and/or urine
   - Any one or more of the following: hemoglobin level less than 10 g/dL, serum creatinine of more than 2 mg/dL, serum calcium of more than 11 mg/dL, the presence of osteolytic lesions, and magnetic resonance imaging studies showing more than one focal lesion.
2. MM patients in CR, assessed by negative immunofixation
3. MM patients in VGPR showing >90% decrease in serum M-protein.

Patients were excluded from the study if have other BM diseases or neoplasms, liver impairment, or severe infectious diseases.

This study was approved by review ethical committee for Iraqi council of medical specializations and written informed consent was obtained from each patients prior to enrollment into he study.

Patients were stratified into three stages based on B2M level as follows: stage I, <3.5 mg/L; stage II, 3.5 to <5.5 mg/L; and stage III, ≥5.5 mg/L.[7]

The blood samples from the patients and the twenty healthy individuals in the control group were centrifuged and the plasma was separated and stored at −80°C for up to 2 months for measuring the levels of the study markers by enzyme-linked immunosorbent assay (ELISA) using ANG-2 and IL-6 human immunoassay, Quantikine ELISA kit (R and D System, Inc. Minneapolis, Minnesota, USA), and β-2-microglobulin ELISA kit (AESKULISA 3801, Wendelsheim, Germany). The hemoglobin level, absolute neutrophil count, platelet count, blood urea, serum creatinine, and serum calcium were taken from patients' records. The glomerular filtration rate (GFR) was calculated by the equation of modification of diet in renal disease.[8]

Statistical analysis was done using Statistical Package for Social Sciences (SPSS) version 25 (IBM Corporation, Armonk, North Castle, New York, United States). The data were presented as mean, standard deviation, and ranges. Categorical data were presented by frequencies and percentages. The independent *t*-test and a two-tailed analysis of variance were used to show the difference between continuous variables accordingly. *Post hoc* test (LSD) was run to confirm the differences between groups in ANG-2, IL6, and B2M levels. Pearson correlation was used for continuous variables. A level of significance was considered at a $P < 0.05$.

Results

Distribution of patients group according to clinical information and treatment protocol

The patients' mean age was 61.71 ± 10.1 years (range: 40–87 years). The age of the highest proportion of participants was ≥60 years in 65% of the ND group, 62.5% of the remission group, and 60% of the control group. The proportion of females was slightly higher than males in ND and control groups, while in the remission group, it was equal.

In this study, 60% of the ND group patients were diagnosed with stage III of MM, while 55% of the remission group patients were at that time in stage I. In the remission group, there were 40 patients, 21 (52.5%) in CR, and 19 (47.5%) in VGPR.

Hematological and biochemical parameters

The means of hemoglobin level and neutrophil count were significantly lower in the ND group than that in the remission group, while the means of blood urea and serum creatinine were significantly higher in the ND group than that in the remission group. The mean GFR is significantly lower in the ND group than that in the remission group [Table 1]. However, no statistically significant differences were found between ND and remission groups regarding platelet count and serum calcium ($P \geq 0.05$).

Beta-2 microglobulin and interleukin-6

The mean levels of IL-6 and B2M were significantly higher in ND patients than in remission and control groups [Table 2]. The comparison of the mean levels between remission and control groups showed a statistically significant difference in B2M while an insignificant difference for IL-6.

Table 1: Comparison of hematological and biochemical parameters between newly diagnosed multiple myeloma and remission groups

Parameters	MM patients groups (mean±SD)		*P**
	ND	Remission	
Hemoglobin (g/dL)	8.83±1.9	10.94±2.1	0.001
Platelet (×10⁹/L)	241.15±133.6	169.7±65.0	0.262
Neutrophil (×10⁹/L)	3.425±2.657	4.230±2.417	0.033
Blood urea (mg/dL)	42.15±15.7	30.44±10.80	0.005
Serum creatinine (mg/dL)	2.054±0.6	1.0±0.5	0.001
GFR (mL/min/1.73 m²)	40.45±20.7	79.85±31.4	0.001
Serum calcium (mg/dL)	9.6±2.3	8.84±1.6	0.2

*Independent *t*-test. GFR=Glomerular filtration rate, ND=Newly diagnosed

Comparison of angiopoietin-2 level between the study groups

The mean level of ANG-2 was higher in ND patients than in those in remission and control groups with a statistically significant difference [Table 3]. The differences were confirmed using the *post hoc* tests (LSD) and showed that the mean level of ANG-2 was significantly higher in the ND group than that in the remission group and control group ($P = 0.001$, both). The difference in the mean levels of ANG-2 between the remission and control groups was insignificant ($P = 0.332$).

Correlation of Angiopoitin-2 with interleukin-6 and beta-2 microglobulin levels in 60 multiple myeloma patients

ANG-2 level showed significant positive correlations with B2M ($r = 0.52$, $P = 0.001$) and with IL-6 ($r = 0.284$, $P = 0.012$), as shown in Figures 1 and 2.

Discussion

MM is a malignancy characterized by the proliferation of monoclonal plasma cells in the BM, with excessive production of monoclonal globulins usually associated with bone destruction, and suppression of normal hematopoietic cells.

In this study, the mean age of all MM patients was comparable with previous Iraqi,[9] Saudi Arabian,[10] and Jordanian studies.[11] MM cases at presentation were slightly more in females, which is comparable with the Iraqi studies of Alwan[12] and Yassin,[13] however, this result disagrees with another Iraqi study[14] and an American study.[15]

The majority of MM patients at the time of presentation were in stage III which is in agreement with Terzi *et al*.[16] and Abu Haleeq *et al*. studies.[17] Previously, MM patients had an average survival of 3 years but with the advancement in diagnosis and the use of combination therapy, many patients obtained CR with more than 10-year overall survival. The percentage of patients with CR was higher than those with VGPR, a finding consistent with the results of Mohammed *et al*.[18] and Terzi *et al*. studies.[16]

The mean hemoglobin concentration in ND patients is comparable to what was reported by other studies of El-Naby *et al*.[19] and Kumar *et al*.,[20] while in patients in remission state, the mean hemoglobin level is comparable with Lee *et al*.[21] and Birgegård *et al*. studies.[22] The mean neutrophil count was lower in ND patients than in patients after remission, although both of them are within the normal range, and those levels are in agreement with Kim *et al*. study,[23] and that may exhibit the improvement of the hematological status of patients after remission.

Renal insufficiency is an important feature in myeloma patients that predict the future outcome and overall survival. In this study, a significant renal impairment was noticed in ND patients compared with patients in remission state. Most ND patients were in stage III and the mean level of GFR was comparable to what was reported in the Omosule study.[24] While for those in remission state, most of the patients were in stage II which may reflect the role of MM therapy in improving and restoring renal functions besides their main action on myelomatous mass itself, and the mean value of GFR was in agreement with other international studies.[25-27]

Table 2: The Mean levels of beta-2 microglobulin and interleukin-6 in the study groups

	ND	Remission	Control	*P**
B2M (μg/mL)				
Mean±SD	7.51±4.0	3.61±1.8	2.21±0.75	0.001
Range	1.7–15.0	1.5–8.2	1.5–4.5	
IL-6 (pg/mL)				
Mean±SD	13.62±18.0	4.69±6.2	2.14±0.66	0.001
Range	2.2–66.2	1.8–10.6	1.3–3.9	
***P*-value**	**Parameters**	**ND and remission**	**ND and control**	**Remission and control**
*P***	B2M	0.001	0.001	0.035
	IL-6	0.004	0.019	0.23

*ANOVA test; ***Post hoc* test. B2M=Beta-2 microglobulin, IL=Interleukin, SD=Standard deviation, ND=Newly diagnosed

Table 3: Comparison of angiopoietin-2 level between study groups

ANG-2 (pg/mL)	Study groups			*P**
	ND	Remission	Control	
Mean±SD	3064.2±3266.1	1205.17±631.9	754.9±175.9	0.001
Range	484–11486	538–3129	538–1165	
	ND and remission	ND and control	Remission and control	
*P***	0.001	0.001	0.332	

*Independent *t*-test, ***Post hoc* test. SD=Standard deviation, ND=Newly diagnosed, ANG=Angiopoietin

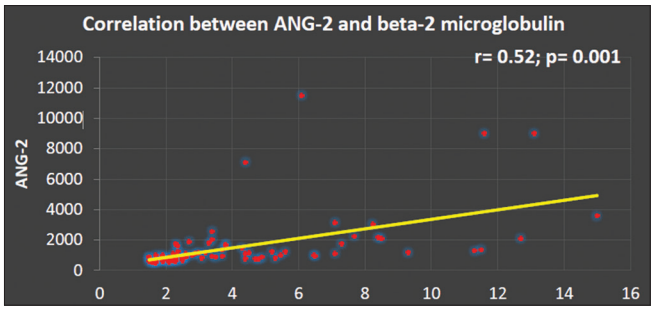

Figure 1: Correlation between the levels of ANG-2 and B2M in the 60 MM patients. ANG-2 = Angiopoietin-2, B2M = Beta-2 microglobulin, MM = Multiple myeloma

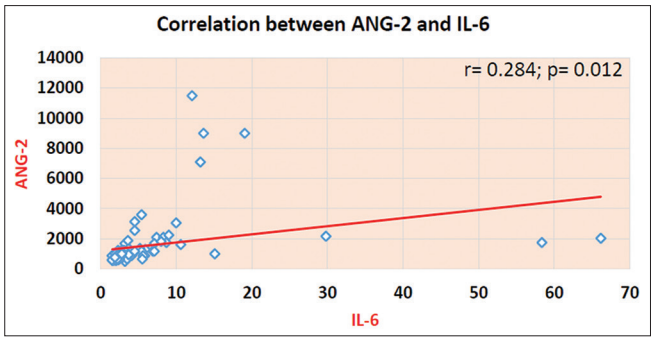

Figure 2: Correlation between the levels of ANG-2 and IL-6 in the 60 MM patients. ANG-2 = Angiopoietin-2, IL = Interleukin, MM = Multiple myeloma

The B2M level is an extremely useful marker in initial stratification and follow-up of MM patients, this study shows a significantly high mean level of B2M in ND patients which is comparable with that reported in the Terzi *et al*. study,[16] but a bit higher than what was reported (5.13 ± 3.53 µg/mL) by a previous Iraqi study[14] and that may be due to most of the patients in this study being in stage III at the time of diagnosis. Patients in remission showed a remarkable decrement in B2M level, which is in agreement with Pappa *et al*. study.[4]

IL-6, through its antiapoptotic activity, acts as a growth factor and thus plays an important role in the regulation of hematopoiesis and the immune system. Overproduction of IL-6 may contribute to the development of malignant diseases such as MM. The mean IL-6 level was higher in ND patients than those in the remission and control groups, which is in agreement with other international studies.[4,28-30]

Many cytokines act in a synergistic manner to regulate the complex process of angiogenesis in tumors. It is well known that patients with MM have increased BM angiogenesis by stimulating vessel formation, several molecules have been involved, and one of them is ANG-2. The higher mean ANG-2 level in MM patients at presentation than those in remission and healthy control individuals, and the insignificant difference in ANG-2 level between remission and control groups is in agreement with other international studies,[4,31,32] and may reveal the implication of ANG-2 in biology, and progression of MM by a mechanism including increment of BM vascular density.

In the BM, VEGF is secreted by stromal and myeloma cells which stimulates the production of IL-6. The latter in turn stimulates the production of VEGF through an autocrine mechanism, therefore, it seems that both IL-6 and ANG-2 molecules possess a pivotal role in the angiogenesis process and tumor progression.[33]

The significant positive correlation of ANG-2 with known prognostic factors reflecting the disease burden was found in this study with B2M, and IL6, this correlation is in agreement with other international studies.[4,31,34] The prognostic significance of soluble ANG-2 in MM lies in the opportunity to block its activity using anti-ANG therapy in the future hopefully to reduce the growth of the neoplastic cells and control the aggressive course of the disease.

Conclusion

In conclusion, the high level of ANG-2 in ND patients and the low level in patients in remission state that shows an insignificant difference from that of healthy individuals renders it a useful marker for follow-up treatment response in MM. The significant positive correlation of ANG-2 with B2M and IL-6 may suggest its role in reflecting the significant degree of angiogenesis with the high tumor burden and disease progression.

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Conflicts of interest
There are no conflicts of interest.

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