

Circulating B-cell activating Factor in Multiple Myeloma Patients and its Correlations with Serum Levels of β 2-Microglobulin and Interleukin-6

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Abstract

Background: Multiple myeloma (MM) is characterized by clonal proliferation of malignant plasma cells within the bone marrow. In most patients, monoclonal immunoglobulin heavy chains or light chains are produced and are associated with organ dysfunction. The growth factor B-cell activating factor (BAFF) plays an important role in the pathogenesis of multiple myeloma due to its ability to promote B-cell survival, expansion, and differentiation.

Objective: to measure the circulatory level of B-cell activating factor in multiple myeloma patients in relapsed and remission states and explore its possible correlations with the clinical staging, β 2-microglobulin, and interleukin-6.

Methods: This cross-sectional study was performed on 60 multiple myeloma patients with 30 in remission and 30 in relapse, as well as 20 healthy individuals serving as a control group. The study was conducted in the Hematology department of Baghdad Teaching Hospital in the Medical City Complex from January to September 2020. The enzyme-linked immunosorbent assay technique was used to measure plasma levels of B-cell activating factor, β 2-microglobulin, and interleukin-6.

Results: The B-cell activating factor levels were significantly higher in multiple myeloma patients compared to the control, and in relapsed patients compared to those in remission. The level of B-cell activating factor increased with advanced disease, in both remission and relapsed states. There are positive correlations with β 2-microglobulin and interleukin-6 levels in both relapsed and remission states, respectively.

Conclusion: Increased B-cell activating factor level in relapse more than remission states is a useful biomarker of disease activity in multiple myeloma and has positive correlations with β 2-microglobulin and interleukin-6 levels.

Keywords: B-cell activating factor; β 2-microglobulin; Clinical stage; Interleukin -6; Multiple myeloma.

Introduction:

Multiple myeloma (MM) is a hematologic malignancy that almost always produces a monoclonal immunoglobulin detected in the serum and/or urine. Interaction between neoplastic plasma cells and the microenvironment components is essential in the pathogenesis of MM. Cytokines, adhesion molecules, and the extracellular matrix play a crucial role in the interplay between clonal plasma cells and the microenvironment stromal cells, leading to the proliferation, progression, and survival of neoplastic cells (1).

Advancements in novel therapies, such as targeted agents and combination therapies, have contributed to an increase in survival rates for patients with MM and led to improved treatment outcomes. However, most patients eventually relapse, even those who achieve remission (2). B-cell activating factor (BAFF) is a 285-amino-acid cytokine, a member of

the TNF family (3). The BAFF and its receptors (BAFF-R, TACI, and BCMA) play an important role in the development and survival of B lymphocytes (4,5). BAFF is one of the main survival factors for healthy and MM plasma cells through direct activation of the NF- κ B pathway (6). The low baseline BAFF expression in MM patients is associated with longer median progression-free survival than those with high BAFF expression (7). The β 2-microglobulin (β 2M) is a valid independent predictor of survival in patients with MM; its level is directly correlated with tumor burden (8). Increased levels indicate a higher intrinsic kinetic activity of tumor cells, including DNA and RNA. This information is crucial for staging, assessing disease severity, evaluating response to chemotherapy, and determining prognosis. Interleukin-6 (IL-6), via activating the JAK-STAT signalling pathway, promotes the angiogenesis, survival, and proliferation of MM cells. Consequently, it can take part in the development of drug resistance in MM

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(10). IL-6 is elevated in MM patients with advanced disease 11.

The study aimed to measure BAFF plasma levels in MM patients in relapse and remission states and explore possible correlations with clinical staging, β 2M, and IL-6.

Patients, materials, and methods

This cross-sectional study involved 60 treated patients diagnosed with MM and 20 age- and sex-matched healthy controls. Thirty MM patients were in remission, defined by achieving either complete remission (which is indicated by negative immunofixation in both serum and urine and having fewer than 5% bone marrow plasma cells) or a very good partial response (characterized by at least 90% decrease in serum M-protein, and urine M-protein levels being less than 100 mg per 24 hours). Thirty patients were in a relapse state (indicated by the reappearance of M-protein by immunofixation in patients who had previously achieved complete remission or progressed from partial remission and minimal response) (12).

The study was conducted in the hematology department of Baghdad Teaching Hospital in The Medical City Complex from January to September 2020. Patients were classified into three stages according to the International Staging System, a simple and reliable risk stratification system that utilizes serum β 2M and albumin levels (13). The enzyme-linked immunosorbent assay (ELISA) sandwich technique was applied to measure plasma levels of BAFF using a human BAFF/BLYS/TNFSF13B immunoassay kit (R&D, Quantikine, USA) and β 2M using a human β 2M ELISA kit (AESKULISA, Germany), and IL-6 using a human IL-6 immunoassay kit (R&D, Quantikine, USA).

Research approval was granted by the IRB of the Iraqi Board for Medical Specializations (Path73 on 18/12/2019). The research was conducted in accordance with the Declaration of Helsinki. Informed consent was obtained from all study participants.

Statistical analysis. Data were analyzed using the Statistical Package for Social Sciences (SPSS version 23) computer software program. The descriptive variables were presented as frequencies and tables. Continuous variables were expressed as

mean \pm standard deviation, and categorical variables as numbers and percentages. Student-*t* test and ANOVA test were used to examine the association between categorical variables. The Pearson correlation (*r*) test was used to assess the association between two continuous variables. An *r* value between 0-0.19 is considered “very weak”, 0.20-0.39 “weak”, 0.40-0.59 “moderate”, 0.60-0.79 “strong”, and 0.80-1.0 “very strong”. A *P*-value below or equal to 0.05 was considered to be statistically significant.

Results

The mean age (\pm SD) was 58.10 ± 8.57 years for MM patients and 58.7 ± 9.17 years for controls. The study comprised 34 male patients (56.7%) and 26 female patients (43.3%), resulting in a male-to-female ratio of 1.3:1. The control group consisted of 50% females and 50% males.

The mean BAFF level in MM patients is significantly higher than in the control group, with a *P*-value of 0.001 (Table 1). The BAFF levels were found to be significantly higher in patients experiencing relapse compared to those with remission, as well as in comparison to the control group (*p* = 0.004 and <0.001, respectively). Additionally, BAFF levels in remission patients were significantly higher than in the control group, with a *p*-value of 0.008 (Table 2). The mean BAFF level significantly increases with disease progression in patients experiencing relapse or remission, with *p*-values of 0.0001 for each (Table 3).

The mean β 2M level is significantly higher in patients with MM during both relapse and remission compared to control subjects (*p*-values of <0.001 and 0.003, respectively). Furthermore, it is higher in patients during relapse than in remission, with a *p*-value of 0.005 (Table 4).

The mean IL-6 level is statistically higher in patients in relapse and remission than in the controls (*p* <0.001 and 0.003, respectively). It is significantly higher in patients during relapse than remission, with a *p*-value of <0.001 (Table 5).

The BAFF level shows very strong positive correlations with β 2M and IL-6 in relapse patients (*r* = 0.86 and 0.83, respectively). In patients during remission, a weak positive correlation with β 2M and a moderate positive correlation with IL-6 were demonstrated (*r* = 0.39 and 0.44, respectively) (Table 6).

Table 1: BAFF level in treated MM patients and the control group

Parameter	Patients N= 60	Control N=20	<i>P</i> -value*
BAFF (pg/mL)	Mean \pm SD	1618 \pm 921.5	872.9 \pm 205.8
	Minimum-maximum	400-4400	635-1400

*Student t-test

Table 2: BAFF level in MM patients during relapse and remission, and the control group

Parameter		Relapse N= 30	Remission N=30	Control N=20
BAFF (pg/mL)	Mean±SD	1955±1045	1281±632	872.9±205.8
	Minimum-maximum	713-4400	400-2764	635-1400
P-value*				
Relapse-Remission		Relapse-Control		Remission-Control
0.004		<0.001		0.008

*Student t-test.

Table 3: BAFF level according to the stage in relapse and remission MM patients

BAFF (pg/mL)		Stages of MM			P-value*
		Stage I	Stage II	Stage III	
Relapse	Mean±SD	919.8±162	1428.9±346.3	3000.4±818.5	0.0001
Remission	Mean±SD	791.4±230.5	1070.1±498.3	2021.2±222.7	0.0001

*ANOVA test

Table 4: β2M level in MM patients during relapse and remission, and the control group

Parameter		Relapse N= 30	Remission N=30	Control N= 20
β2M (μg/mL)	Mean±SD	7.6±6.9	3.73±2.6	2.05±0.38
	Minimum-maximum	2.19-24.7	1.8-14.2	1.5-2.8
P-values*				
Relapse-Remission		Relapse-Control		Remission-Control
0.005		<0.001		0.003

*Student t-test.

Table 5: IL-6 level in MM patients during relapse and remission, and the control group

Parameter		Relapse N= 30	Remission N=30	Control N=20
IL-6 (pg/mL)	Mean±SD	17.7±17	4.2±4.1	2.2±0.84
	Minimum-maximum	1.43-51.7	0.49-19.37	1.3-4.5
P-value*				
Relapse-Remission		Relapse-Control		Remission-Control
<0.001		<0.001		0.025

*Student t-test.

Table 6: Pearson correlation of BAFF level with the disease activity markers, β2M and IL-6, in MM patients during relapse and remission

BAFF (pg/mL)		Relapse		Remission	
		r*	P-value	r*	P-value
β2M (μg/mL)		0.86	0.00001	0.39	0.031
IL-6 (pg/mL)		0.83	0.0001	0.44	0.014

*r, correlation coefficient.

Discussion

The clonal expansion of plasma cells in the bone marrow, accompanied by the production of monoclonal immunoglobulins, leads to organ dysfunction, suppression of normal hematopoietic cell formation, and bone lesions (4,15).

In this study, the mean age of MM patients was 58.10 ±8.57 years, and slightly higher in males; this result agreed with another Iraqi study (9).

In treated MM patients, plasma levels of BAFF were found to be significantly higher compared to those in the control group. This finding was further supported by a study done in China (16), showing that BAFF mRNA expression was significantly elevated in patients in the initial treatment group, non-remission group, and remission group compared to the control group. The BAFF levels in relapsed

MM patients were significantly higher than in the control group. Similar findings were reported by other studies done in New Delhi (17,18). However, BAFF levels decreased in MM patients in a remission state. Comparable findings were reported by studies done in Greece³ and Poland⁶, where BAFF levels decreased significantly after treatment. The comparison of BAFF levels in relapse MM patients with patients in remission state showed a significant difference. This result was supported by a Chinese study (19) reporting that BAFF mRNA expression in relapsed/refractory patients was higher than that in patients after treatment in the plateau stage and control. Further studies done in New Delhi (17,18) reported that the BAFF level was higher in relapse than in the newly diagnosed MM patients;

this may reflect different biological mechanisms in relapse (more aggressive disease). However, other studies reported that BAFF levels increased in newly-diagnosed MM patients and decreased after effective treatment, such as that reported by studies done in Poland (20) and Greece (21).

In this study, MM patients were found to have higher levels of BAFF in advanced disease stages. This finding agreed with two studies done in Greece (3,21). The elevated BAFF level in advanced MM disease may be attributed to the expansion of the myeloma clone.

The β 2M level is a useful marker in MM and reflects tumor burden. In this study, the level of β 2M in MM patients was statistically higher than in the control group. Similar results were reported by other Iraqi studies (15). This study showed that β 2M levels decreased in response to chemotherapy, aligning with findings found in studies done in Egypt (22) and Greece (21), and the level of β 2M increased in the relapsed state. These observations are comparable to a study conducted in the United States (23). There was a significant difference in β 2M levels between relapsed and remission MM patients in this study, which agreed with the study performed in Sweden (24).

IL-6 is a principal cytokine responsible for the growth of myeloma cells. The IL-6 level in this study was significantly higher in patients than in the controls; this finding is comparable to other studies conducted in Greece (21) and Poland (20). However, these studies included patients who were newly diagnosed with MM. The IL-6 was found to be significantly higher in relapsed MM patients compared to the control group. This finding agreed with the results reported by studies done in New Delhi (17,18).

In this study, despite high plasma levels of IL-6 being observed in patients during a remission state compared to the controls, the levels are much lower than those in patients during relapse. Similar results were reported by studies done in Greece (25).

Plasma levels of BAFF have a positive correlation with β 2M. A finding consistent with a study done by Greece (21). This positive correlation may indicate that BAFF is associated with tumor burden. Plasma levels of BAFF have correlated very strongly with IL-6 in relapsed patients, a similar result reported by Poland (20), who stated a positive correlation between BAFF and IL6 concentration, and also agreed with other studies conducted in Greece (3,21), and Poland (20). However, these studies were done on newly diagnosed MM patients. This may be explained by the fact that IL-6 can induce the BAFF gene and enhance its protein expression level (26).

Conclusion

Higher plasma BAFF levels were observed in MM patients compared to control subjects, and these levels were even higher in relapsed patients than in remission. This suggested that elevated BAFF levels indicate disease activity and progression as the condition advances. BAFF levels correlate positively

with β 2M and IL-6 levels, which were also elevated in MM patients, particularly in those who had relapse compared to those in remission. Future studies on MM patients may explore the impact of BAFF on their survival and evaluate the potential therapeutic use of BAFF inhibitors, which could lead to new insights into the treatment of these patients.

Authors' declaration:

We confirm that all the Tables in the manuscript belong to the current study. The research was conducted in the Hematology Department of Baghdad Teaching Hospital in the Medical City Complex in Baghdad. The project was approved by the IRB of the Scientific Council of Pathology, the Iraqi Board for Medical Specializations according to the code number (Path-73) on (18/12/2019).

Conflict of Interest: None

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Data availability: Upon reasonable request, the corresponding author will make the data sets generated and/or analyzed during the current work available.

Authors' Contributions:

Study conception & design: Wafaa Fadhil Sahib & Haithem Ahmed Al-Rubaie. Literature search: Wafaa Fadhil Sahib. Data acquisition & Data analysis: Wafaa Fadhil Sahib & Haithem Ahmed Al-Rubaie. Manuscript preparation, editing & review: Wafaa Fadhil Sahib & Haithem Ahmed Al-Rubaie

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عامل تنشيط الخلايا البائية (BAFF) لدى مرضى ورم النقي المتعدد وارتباطه مع مستويات بيتا 2 مايكروغلوبولين و إنترلوكين 6 في مصل الدم

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الخلاصة:

يتميز الورم النقوي المتعدد (MM) بالتكاثر النسيلي للخلايا البلازمية الخبيثة داخل نخاع العظم. لدى معظم المرضى، يتم إنتاج سلاسل ثقيلة أو خفيفة من الغلوبولين المناعي وحيد النسيلة، وتؤدي إلى خلل في وظائف الأعضاء. يلعب عامل النمو (عامل تنشيط الخلايا البائية) (BAFF) دوراً مهماً في التسبب في الورم النقوي المتعدد نظراً لقدرته على تعزيز بقاء الخلايا البائية وتوسعها وتمايزها.

هدفت الدراسة: قياس مستوى BAFF في الدورة الدموية لدى مرضى الورم النقوي المتعدد في حالات الانتكاس وفي حالة الإبراء، واستكشاف ارتباطاته المحتملة بالتصنيف السريري، و $\beta 2$ -ميكروغلوبولين، وإنترلوكين-6.

المرضى، المواد، وطرائق العمل: أجريت هذه الدراسة المقطعية على 60 مريضاً مصاباً بالورم النقوي المتعدد، 30 منهم في حالة الإبراء و30 في حالة انتكاس، فضلاً عن 20 فرداً سليماً كمجموعة ضابطة. استعملت تقنية اختبار الممتز المناعي المرتبط بالإنزيم (ELISA) لقياس مستويات BAFF، و $\beta 2$ -ميكروغلوبولين، وإنترلوكين-6 في البلازما.

النتائج: كانت مستويات BAFF أعلى بشكل ملحوظ لدى مرضى الورم النقوي المتعدد مقارنة بالمجموعة الضابطة، ولدى المرضى المنتكسين مقارنة بالمرضى في حالة الإبراء. ارتفع مستوى BAFF مع تقدم المرض، في كل من حالتي الإبراء والانتكاس). هناك ارتباطات إيجابية مع مستويات $\beta 2$ -ميكروغلوبولين وإنترلوكين-6 في كل من حالتي الانتكاس والإبراء، على التوالي.

الاستنتاج: يعد ارتفاع مستوى BAFF في حالات الانتكاس مقارنة بحالة الإبراء مؤشراً حيوياً مفيداً لنشاط المرض في الماييلوما المتعددة، وله ارتباطات إيجابية مع مستويات $\beta 2$ -ميكروغلوبولين وإنترلوكين-6.

الكلمات المفتاحية: الورم النقوي المتعدد؛ BAFF؛ الإنترلوكين-6؛ $\beta 2$ -ميكروغلوبولين؛ المرحلة السريرية.