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Immunohistochemical Analysis of B-Cell Maturation Antigen (BCMA) as a Novel Marker in Correlation with Its Serum Level to Predict Multiple Myeloma Patients' Outcome

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: Multiple myeloma (MM) is a neoplastic disorder characterized by clonal proliferation of malignant plasma cells in the bone marrow (BM) producing monoclonal proteins and inducing specific organ and tissue damage. This study aimed to evaluate the expression and serum level of B cell maturation antigen (BCMA) and its prognostic value in newly diagnosed multiple myeloma patients.
Methods: This prospective study was carried out on 60 patients who were classified into two equal groups including group I (MM patients) which was newly diagnosed as MM and group II (healthy Control) which healthy individuals served as control.
Results: There was a statistically significant increase in MM patients than control group levels were increased in MM patients. Plasma cell percentage in BM aspiration was a statistically significant increase in MM patients. As regarding serum BCMA, there was a statistically significant increase in MM patients than the control group. There was a significantly positive correlation

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between serum BCMA and its surface expression with plasma cells in BM, CD138 expression in a biopsy, creatinine, B2 microglobulin, LDH, ESR, and total calcium. There was no correlation between BCMA level and age, hemoglobin, WBCs, and platelets count. Serum BCMA and BCMA expression showed a significant correlation with the clinical status of the patients' group, patients with complete response showed a lower level of serum BCMA and lower expression of surface BCMA and longer OS and DFS while patients with failure of CR relapsed group showed a higher level of serum BCMA and higher expression of surface BCMA and shorter OS and DFS.

Conclusions: Important role of BCMA expression and its serum level in the diagnosis of multiple myeloma, as the BCMA level in serum significantly elevated in MM patients compared with the control group. Moreover, serum BCMA level and its surface expression are positively correlated with plasma cell percentage in BM aspirate, CD 138 expression in BM biopsy, M protein, and B2 macroglobulin.

Keywords: Immunohistochemical analysis; B-cell maturation antigen; novel marker; correlation; serum level; multiple Myeloma.

1. INTRODUCTION

Multiple myeloma (MM) is a neoplastic disorder characterized by clonal proliferation of malignant plasma cells in the bone marrow (BM) producing monoclonal proteins and inducing specific organ and tissue damage [1,2].

The field of myeloma therapy has shown enormous progress in the past decade and as a result median overall survival has increased to approximately 6 years [3]. However, MM is still considered an incurable disease and most patients will eventually experience a fatal relapse. This is due to the persistence of chemotherapy-resistant myeloma cells in the bone marrow [4,5], even after the destruction of the bulk of tumor cells [6,7].

Immunotherapeutic approaches could potentially play an important role in the global treatment concept for myeloma, targeting residual disease after effective initial therapy. However, an essential first step would be to identify target antigens expressed on the bulk of tumor cells as well as the chemotherapy-resistant and myeloma-propagating cells in the patients' Bone Marrow [8].

B-cell maturation antigen (BCMA), a member of the tumor necrosis factor receptor family, is another protein whose function has been implicated in B-cell malignancies. It is expressed on the cell surface of mature and malignant B lymphocytes [9] and is known to bind B-lymphocyte stimulator (BLyS), also known as B cell-activating factor (BAFF), a protein that plays a significant role in the growth and survival of MM cells [9].

The restricted expression of BCMA to PC and its role in the survival and growth of MM cells makes it a good potential target for immunotherapeutic strategies including monoclonal antibodies [10].

This study aimed to evaluate the expression and serum level of B cell maturation antigen (BCMA) and its prognostic value in newly diagnosed multiple myeloma patients.

2. PATIENTS AND METHODS

This prospective study was carried out in the Clinical Pathology Department of the National Cancer Institute (NCI), Cairo University, and Clinical Pathology Department in Tanta University hospital during the period between 2018 and 2020. After informed written consent from patients or their guardians, 30 consecutive patients with multiple myeloma were included in the study.

2.1 Sample Inclusions and Exclusions

Patients with any other malignancies, patients with autoimmune diseases such as systemic lupus erythematosus and rheumatoid arthritis, patients with chronic inflammatory disorders such as irritable bowel disease and ulcerative colitis, and patients with HIV infection were excluded.

The studied groups were subjected to the following: Detailed history Including age, sex, and symptoms of bone pain as back pain, weakness, numbness, or dysesthesias in the extremities, symptoms of anemia as fatigue, dizziness, and headache and symptoms of hypercalcemia as confusion, somnolence, bone pain, constipation, nausea, and thirst.

Clinical examination: includes Pallor, purpuric eruptions, pathological fracture, inability to move,

recurrent infection, and renal impairment. Bone scan to demonstrate the presence of bone lesions. Laboratories Investigations including Routine investigations: Complete blood count was done on Sysmex XS-500i with a thorough examination of peripheral blood smears stained with Giemsa stain. Serum calcium was done on Beckman Coulter (AU 480). ESR. Serum creatinine was done on Beckman Coulter (AU 480). Serum LDH was done on Beckman Coulter (AU 480). B2 microglobulin was done on Biosystems BTS-350.

Diagnosis of MM was done by: Morphological examination of peripheral blood and/or bone marrow aspirate smears. Immunophenotyping of plasma cells in BM aspirate samples using Becton Dickinson (BD) FACSCalibur to confirm the diagnosis of abnormal plasma cells (CD38, CD138, and CD56) and to confirm the diagnosis of monoclonal plasma cells (anti-kappa and anti-lambda monoclonal antibodies). Immunohistochemistry of BM biopsy samples to confirm the diagnosis of abnormal plasma cells (CD138 and monoclonal light chain).

Diagnosis of multiple myeloma was based on ***(National Comprehensive Cancer Network, 2018):*** Bone marrow clonal plasma cells ≥10% or bony or extramedullary plasmacytoma (confirmed by biopsy). One or more myeloma-defining events include the following: Serum calcium level >0.25 mmol/L (>1 mg/dL) higher than the upper limit of normal or >2.75 mmol/L (>11 mg/dL). Renal insufficiency (creatinine >2 mg/dL [>177 μmol/L] or creatinine clearance < 40 mL/min). Anemia (hemoglobin < 10 g/dL or hemoglobin >2 g/dL below the lower limit of normal). One or more osteolytic bone lesions on skeletal radiography, CT, or PET-CT. Clonal bone marrow plasma cells ≥10%. Abnormal serum free light chain (FLC) ratio ≥100 (involved kappa) or < 0.01 (involved lambda). One or more focal lesions >5 mm on MRI scans [11].

Peripheral blood sample: Sampling and preparation: 5 ml of venous blood was withdrawn from each subject under complete aseptic condition, and divided into 1 ml blood was added to EDTA tube for assay of CBC, and 4 ml blood was collected on a dry vacutainer for the performance of the lab assays. Blood sample in the dry vacutainer was left to clot, then centrifuged at 3000 r.p.m for 10-20 min. Serum was separated into two separate aliquots. The first aliquot was used for lab analysis of serum calcium, serum creatinine, serum B2-microglobulin, and serum LDH, the second aliquot was immediately frozen at -20ºC for analysis of serum BCMA level by ELISA. Assessment of BCMA level by ELISA: BCMA level was estimated by Enzyme-linked immune-sorbent assay (ELISA) kit by SunRed Company made in Germany supplied by Bio-Gene and Egypt. (Catalogue No.: SRB-T-82892).

Principle of the Assay: The kit uses a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA) to assay the level of Human B-Cell Maturation Antigen (TNFRSF17/ BCMA /CD269) in samples. Add B-Cell Maturation Antigen (TNFRSF17/ BCMA/ CD269) to monoclonal antibody Enzyme well which is pre-coated with Human B-Cell Maturation Antigen (TNFRSF17/BCMA/CD269) monoclonal antibody, incubation then, add B-Cell Maturation Antigen (TNFRSF17/ BCMA/CD269) antibodies labeled with biotin, and combined with Streptavidin-HRP to form an immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into blue, and at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the Human Substance B-Cell Maturation Antigen (TNFRSF17/ BCMA/CD269) of the sample were positively correlated.

Assay procedure: Standard dilution: This test kit supplies one original Standard reagent that was diluted into 5 standards of different concentrations by serial dilution. Procedures: Blank well: no sample or standard was added. Standard wells: 50μl standard were added then, Streptavidin-HRP 50μl (since the standard already has combined biotin antibody, it is not necessary to add the antibody). Test wells: 40μl from each sample were added, and then we added both human apelin antibody 10 μl and Streptavidin-HRP 50 μl. Then sealed the sealing membrane, gently shaken & incubated for 60 minutes at 37ºC. The washing solution was prepared: diluted 30 times with distilled water as standby. The membrane was removed carefully, and the liquid was drained. The remaining water was shaken away and washing was repeated 3 times. Chromogen solution A 50μl was added, then chromogen solution B 50 μl to each well. Gently mixed, incubated for 10 min at 37ºC away from light. Stop: Stop Solution 50 μl was added to each well to stop the reaction (the blue changed into yellow immediately). Final measurement: the blank well was taken as zero

& the optical density (OD) was measured under 450 nm wavelength within 15min after the stop solution was added.

Paraffin-embedded bone marrow biopsy sections and bone marrow aspirate: Sampling of BM biopsy: The patient was placed in the lateral decubitus position, with the top leg flexed and the lower leg straight. Posterior superior iliac spine (PSIS) was palpated then the skin was sterilized with topical antiseptics. The skin, subcutaneous tissue, and periosteum of the marked area were infiltrated with a local anesthetic (Xylocaine 2%). After a brief delay (3-5 min) a small 3-4 mm skin incision was made with a scalpel blade (size: 15).

A sampling of BM aspirate: The patient was positioned in the prone or side-lying position. The area for aspiration was exposed and the posterior superior iliac crest was located. Bone marrow site was swabbed with povidone-iodine swab stick, applying some friction and working in a circular motion beginning in the center and moving outward. Repeated twice with new swabs. Povidone-iodine was allowed to dry. It was removed with an alcohol swab using concentric motion beginning in the center. This step was repeated twice with a new swab. Then the area was allowed to dry. The exact point for aspiration was located and the area between the thumb and index finger was outlined. 2-3 ml Xylocaine 2% was injected perpendicularly subcutaneously and into the periosteum. Then lidocaine was left for 2-3 minutes to take effect. Then bone marrow needle was prepared, assuring the stylet moved freely. The skin was stretched taut over the puncture site; crest was kept between thumb and index finger of one hand. Bone marrow needle was held with stylet in place, the skin was punctured and advanced through subcutaneous tissue, periosteum, and into marrow cavity using a steady, controlled pressure with a twisting motion. When the needle was firmly in place and a slight give in pressure is felt, the cavity had been entered. Then stylet was removed and the plain 10- or 20-ml plastic syringe was quickly attached to the needle hub. Needle hub was applied strongly and approximately 0.5 ml marrow was quickly suctioned and obtained.

2.2 Statistical Analysis

Data were fed to the computer using IBM SPSS software package version 20.0. Qualitative data were described using numbers and percentages. The distributions of quantitative variables were tested for normality using the Shapiro-Wilk test and D'Agstino test, also Histogram and QQ plot were used for vision test. If it reveals normal data distribution, parametric tests were applied. If the data were abnormally distributed, non-parametric tests were used. Quantitative data were described using mean and standard deviation for normally distributed data. For normally distributed data, comparison between two independent populations was done using an independent t-test, while more than two populations were analyzed F-test (ANOVA) to be used and Post Hoc test (Scheffe). Correlations between two quantitative variables were assessed using the Pearson coefficient. Significance test results are quoted as two-tailed probabilities. The significance of the obtained results was judged at the 5% level.

3. RESULTS

Patients' characteristics (age and sex) were discussed in the following table [Table].

Table 1. Comparison between MM patients and Control group regarding age and sex

Age			**MM patients (Group 1)**	**Control group (Group II)**	
Range			35 **–** 82	30– 70	
Mean ± SD			53.63**±**10.56	57.13**±**7.95	
T. test			2.104		
P. value			0.152		
Sex			**MM patients**	**Control group**	**Total**
Male		**N**	16	20	36
		%	53.3%	66.7%	60.0%
Female		**N**	14	10	24
		%	46.7%	33.3%	40.0%
Total		**N**	30	30	60
		%	100.0%	100.0%	100.0%
Chi-square	X^2		1.111		
	P-value		0.292		

Table 2. Comparison between MM patients and Control group regarding laboratory data

		Range	Mean ± S. D	t. test	p. value
	MM patients	6 – 14.1	9.96 ± 2.11	26.918	**0.001***
HB	Control group	12 – 14	12.8 ± 1.10		
Platelet count	MM patients	32×10^3 – 498×10^3	$221.3\times10^3\pm 110.1\times10^3$	0.298	0.587
	Control group	150×10^3– 350×10^3	$233.5\times10^3 \pm 55.1\times10^3$		
WBC	MM patients	1600 – 19000	6793.33 ± 3827.75	0.017	0.897
count	Control group	4500 – 10600	5600.33 ± 2586.85		
Ionized	MM patients	4.84 – 6.8	5.72 ± 0.58	50.489	**0.001***
calcium	Control group	4.7 – 5.3	4.94 ± 0.16		
Total	MM patients	11 – 13.8	12.39 ± 0.92	118.600	**0.001***
calcium	Control group	8.5 – 11	9.88 ± 0.86		
LDH	MM patients	240 – 1250	625.43 ± 286.00	98.963	**0.001***
	Control group	70 – 150	105.83 ± 24.92		
ESR	MM patients	12 – 130	56.20 ± 30.66	75.398	**0.001***
	Control group	5 – 10	1.96 ± 7.50		
B2	MM patients	3.3 – 7.2	5.34 ± 0.99	447.676	**0.001***
macroglobulin	Control group	0.04 – 2	0.87 ± 0.60		
Serum	MM patients	1.2 – 4.5	2.39 ± 0.92	87.507	**0.001***
creatinine	Control group	0.5 – 1.2	0.79 ± 0.19		

**: statistically significant as P value < 0.05, HB: Hemoglobin, LDH: Lactate dehydrogenase, ESR: erythrocyte sedimentation rate*

Laboratory data of the studied groups were discussed in the following table [Table].

Regarding serum BCMA level, there was a statistically significant increase in MM patients compared to the control group [Table 3].

As regarding correlation between serum BCMA and types of M band (IgG, IgA, IgM) in IgA isotype group serum BCMA, there was no statistically significant difference between them. Also, in a correlation between serum BCMA and clinical outcome of the MM patients, there was a statistically significant increase of serum BCMA in MM patients with failure of CR & relapsed cases than those with PR and CR [Fig. 1].

There was a significantly positive correlation between serum BCMA level with plasma cell percentage in BM aspirate, CD138 in BM biopsy, M protein, ionized calcium, total calcium, LDH, ESR, B2 macroglobulin serum creatinine, while there was no correlation between serum BCMA level with age, Hb concentration, platelet count and WBC count [Table].

As regarding the correlation of serum BCMA with Overall Survival in multiple myeloma patients, there was no statistically significant difference regarding OS between above and below the median level of serum BCMA. Also, in the correlation of serum BCMA with Disease-free Survival in multiple myeloma patients, there was no statistically significant difference regarding DFS between above and below the median level of serum BCMA [Fig.].

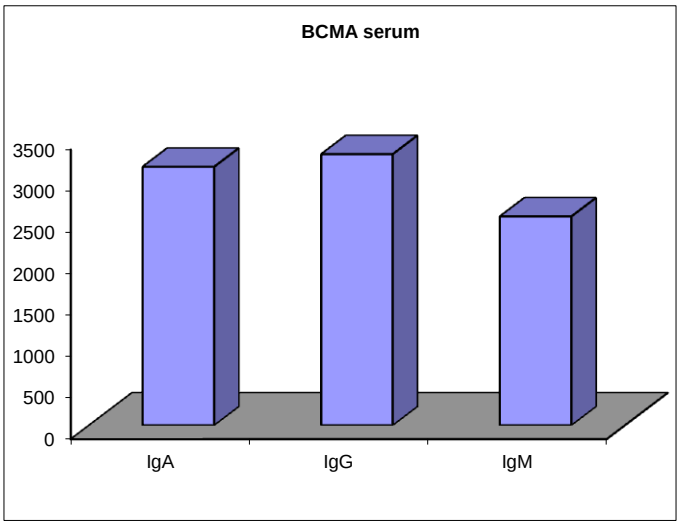
MM patients' subgroups regarding age and sex were compared in the following table [Table].

Laboratory data of MM patients' subgroups were discussed in the following table [Table 6].

Table 3. Comparison between MM patients and Control group regarding serum BCMA level

BCMA	MM patients	Control group
Range	2125.7 **–** 4158.1	86 – 1325.8
Mean ± SD	3182.29**±**588.61	493.98**±**349.30
T. test	462.807	
P. value	**0.001***	

**: statistically significant as P value < 0.05*



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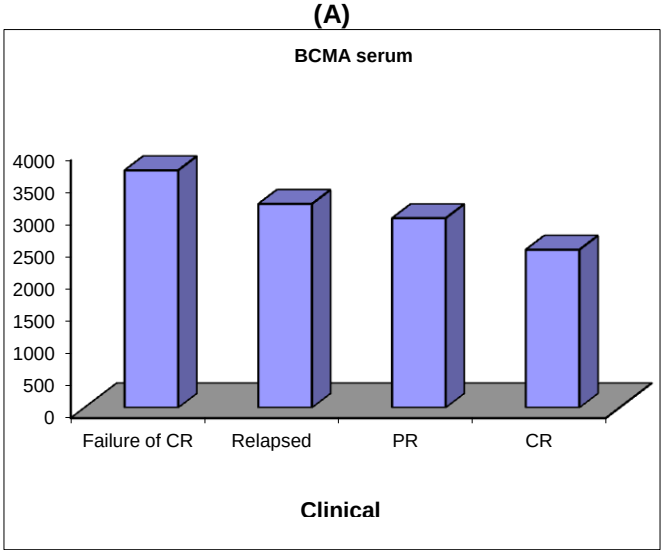


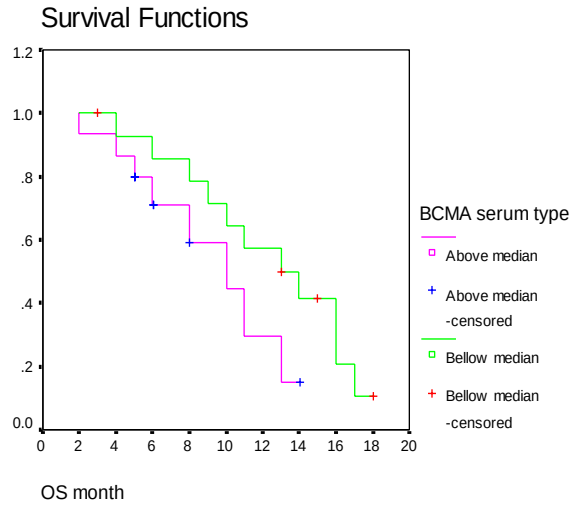
(B)

Fig. 1. Correlation between Serum BCMA and types of M band (IgG, IgA, IgM) (A) and Clinical outcome (B) in MM patients

Table 4. Correlation between serum BCMA level and studied laboratory tests in MM patients

	Serum BCMA	
	R	**P**
Plasma cell in BM aspirate	0.512	**0.001***
CD 138 in BM biopsy	0.467	**0.001***
HB	0.039	0.840
Platelet count	-0.239	0.203
WBC count	-0.206	0.275
M protein (gamma globulin)	0.893	**0.001***
Ionized calcium	0.962	**0.001***
Total calcium	0.885	**0.001***
LDH	0.939	**0.001***
ESR	0.922	**0.001***
B2 microglobulin	0.810	**0.001***
Serum creatinine	0.819	**0.001***

**: statistically significant as P value < 0.05, BM: bone marrow, CD: Celiac disease, LDH: Lactate dehydrogenase, ESR: erythrocyte sedimentation rate*



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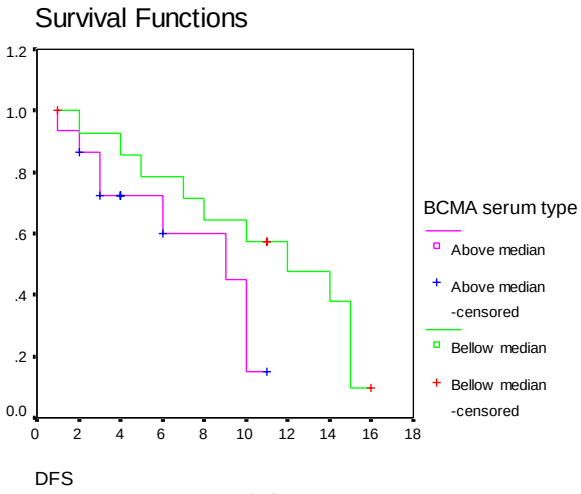


(B)

Fig. 2. Correlation of serum BCMA with Overall Survival in multiple myeloma patients (A) and disease Free Survival in multiple myeloma patients (B)

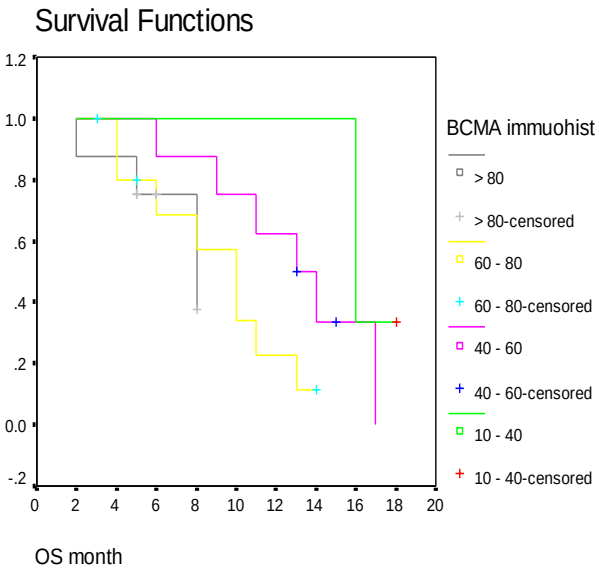
Table 5. comparison between MM patients' subgroups regarding age and sex

BCMA immunohistochemistry		**Range**		**Mean ± S. D**		**F. test**	**p. value**
	Score 2 (10 -40%)	40 – 60		48.33 ± 10.41			
Age	**Score3(40 – 60%)**	26 – 82		52.50 ± 18.21		0.167	0.917
	Score4(60 – 80%)	30 – 63		49.36 ± 9.22			
	Score5 (> 80%)	32 – 63		48.63 ± 9.49			
Sex		**Score 2 (10 – 40%)**	**Score 3 (40-60%)**	**Score 4 (60 – 80%)**	**Score 5 (> 80%)**	**Total**	
Male	**N**	1	5	7	3	16	
	%	33.3%	62.5%	63.6%	37.5%	53.3%	
Female	**N**	2	3	4	5	14	
	%	66.7%	37.5%	36.4%	62.5%	46.7%	
Total	**N**	3	8	11	8	30	
	%	100.0%	100.0%	100.0%	100.0%	100.0%	
Chi-square	**X^2**	2.027					
	P-value	0.567					

Table 6. Comparison between MM patient's subgroups as regarding laboratory data

BCMA immunohistochemistry		Range	Mean ± S. D	F. test	p. value
Percent of Plasma cell in BM aspirate	**Score 2(10 – 40%)**	1 – 4	2.33 ± 1.53		0.001*
	Score 3(40 – 60%)	2 – 10	4.38 ± 2.56	12.13	
	Score 4 (60 – 80%)	5 – 37	18.55 ± 12.72		
	Score 5 (> 80%)	6 – 73	42.00 ± 21.27		
CD 138 expression in BM biopsy	**Score 2 (10 – 40%)**	15 – 40	23.33 ± 14.43		0.001*
	Score 3 (40 – 60%)	12 – 70	25.38 ± 18.84	22.11	
	Score 4 (60 – 80%)	30 – 80	63.64 ± 15.67		
	Score 5 (> 80%)	70 – 90	76.88 ± 7.0		
HB	**Score 2 (10 – 40%)**	8.4 – 14.1	11.25 ± 1.60		
	Score 3 (40 – 60%)	8.5 – 12.3	9.86 ± 1.60	3.23	0.039*
	Score 4 (60 – 80%)	6 – 11.8	8.80 ± 2.08		
	Score 5 (> 80%)	6.4 – 12.2	8.57 ± 3.17		
Platelet count	**Score 2 (10 – 40%)**	55000– 301000	160333.33 ± 126749.10		
	Score 3 (40 – 60%)	32000– 498000	256000.00 ± 132107.10	0.739	0.539
	Score 4 (60 – 80%)	57000– 379000	231909.09 ± 108580.34		
	Score 5 (> 80%)	55000– 324000	194875.00 ± 85974.14		
WBC count	**Score 2 (10 – 40%)**	2400 – 6900	5233.3 ± 2466.44		
	Score 3 (40 – 60%)	1600 – 7800	5425.0 ± 1996.96	2.666	0.069
	Score 4 (60 – 80%)	3600 – 19000	9190.9 ± 5133.90		
	Score 5 (> 80%)	7600	5450.0 ± 1744.38		
BCMA immunohistochemistry		**Range**	**Mean ± S. D**	**F. test**	**p. value**
M protein gamma globulin	**Score 2 (10 – 40%)**	2.5 – 3	2.73 ± 0.25	15.83	0.001*
	Score 3 (40 – 60%)	3.3 – 6.2	4.30 ± 0.98		
	Score 4 (60 – 80%)	3.2 – 6.4	5.25 ± 1.01		
	Score 5 (> 80%)	5 – 7.5	6.55 ± 0.79		
Ionized calcium	**Score 2 (10 – 40%)**	4.84 – 4.88	4.86 ± 0.02	24.94	0.001*
	Score 3 (40 – 60%)	4.9 – 6	5.31 ± 0.36		
	Score 4 (60 – 80%)	5.2 – 6.2	5.75 ± 0.34		
	Score 5 (> 80%)	6.04 – 6.8	6.40 ± 0.26		
Total calcium	**Score 2 (10 – 40%)**	11 – 11.8	11.50 ± 0.44	12.02	0.001*
	Score 3 (40 – 60%)	11.1 – 12.9	11.65 ± 0.54		
	Score 4 (60 – 80%)	11.2 – 13.6	12.46 ± 0.85		
	Score 5 (> 80%)	13 – 13.8	13.36 ± 0.32		
LDH	**Score 2 (10 – 40%)**	240 – 280	262.00 ± 20.30	20.54	0.001*
	Score 3 (40 – 60%)	284 – 777	401.63 ± 162.50		
	Score 4 (60 – 80%)	330 – 865	648.09 ± 193.04		
	Score 5 (> 80%)	800 – 1250	954.38 ± 144.14		
ESR	**Score 2 (10 – 40%)**	20 – 30	26.67 ± 5.77	21.52	0.001*
	Score 3 (40 – 60%)	12 – 63	31.50 ± 15.84		
	Score 4 (60 – 80%)	22 - 75	54.18 ± 15.58		
	Score 5 (> 80%)	65 – 130	94.75 ± 22.59		
B2 microglobulin	**Score 2 (10 – 40%)**	4.2 – 4.5	4.37 ± 0.15	11.93	0.001*
	Score 3 (40 – 60%)	3.3 – 5.7	4.60 ± 0.74		
	Score 4 (60 – 80%)	3.9 – 6.2	5.37 ± 0.69		
	Score 5 (> 80%)	5.3 – 7.2	6.41 ± 0.68		
Serum creatinine	**Score 2 (10 – 40%)**	1.2 – 1.6	1.37 ± 0.21	10.32	0.001*
	Score 3 (40 – 60%)	1.5 – 2.2	1.79 ± 0.26		
	Score 4 (60 – 80%)	1.5 – 4.5	2.42 ± 0.87		
	Score 5 (> 80%)	2.4 – 4.2	3.34 ± 0.65		

**: statistically significant as P value < 0.05, HB: Hemoglobin, LDH: Lactate dehydrogenase, ESR: erythrocyte sedimentation rate.*



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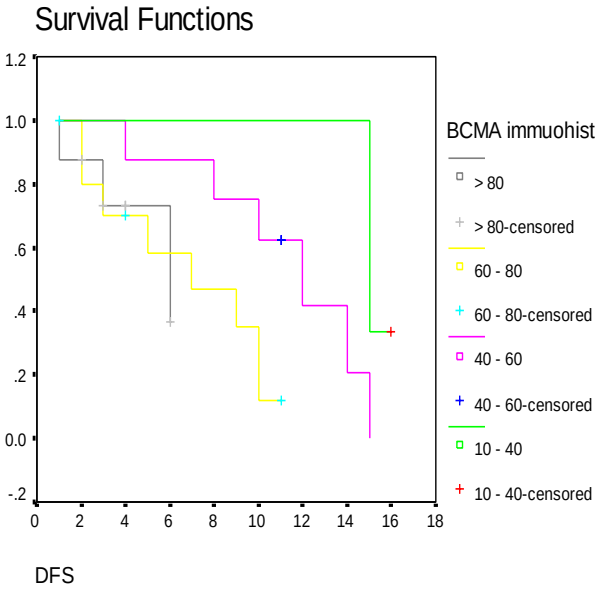

(B)

Fig. 3. Correlation of BCMA expression by immunohistochemistry with Overall Survival in multiple myeloma patients (A) and with Disease-Free Survival in multiple myeloma patients (B)

As regarding the correlation of BCMA expression by immunohistochemistry with Overall Survival in multiple myeloma patients, there was a statistically significant difference regarding OS between the 4 subgroups. Also, regarding the correlation of BCMA expression by immunohistochemistry with Disease-free Survival in multiple myeloma patients, there was a statistically significant difference regarding DFS between the 4 subgroups [Fig. 3].

4. DISCUSSION

Multiple myeloma (MM) is a neoplastic disorder characterized by clonal proliferation of malignant plasma cells in the bone marrow (BM) producing monoclonal proteins and inducing specific organ and tissue damage [2].

As regarding leucocytic and platelet counts, there was no statistically significant difference between

the MM patients and control group that was similar to the study by Hussain et al. [12] and Diwan et al. [13].

In the present study, total calcium in the patient group was significantly elevated, and this result agrees with kyle RA and Gertz MA [14] and with Mirrakhimov AE [15] who found hypercalcemia is common in patients with advanced cancer stages. The primary cause of hypercalcemia is widespread tumor-induced bone destruction. This is primarily due to increased osteoclastic bone resorption caused by potent cytokines expressed or secreted locally by the myeloma cells or over-expressed by other cells in the local microenvironment [16].

In the present study, ESR was seen to be significantly elevated in most patients and this agrees with Diwan et al. [13] who reported the same results, that mainly due to increased immunoglobulins. Alexandrakis et al. [17] reported that ESR is a good prognostic marker for myeloma with higher values of ESR associated with a more advanced cancer stage. National Institute for Health and Care Excellence guidelines currently recommend the use of ESR when myeloma is suspected.

In the current study, LDH was significantly elevated in the patient group. This result agrees with Barlogie et al. [18] who suggested that a high level of serum LDH and calcium decreased the early survival of MM patients. Chen et al. [19], reported that high β2-MG, high serum LDH, and low serum albumin levels were poor prognostic factors for early mortality.

In this study, β2microglobulin was found to be significantly elevated in the patient group. This result agrees with Ghermezi et al. [20] and Hussain et al. [12] who reported that β2 microglobulin was raised in most MM patients and confirmed that β2microglobulin was a highly significant prognostic factor. It also reflects tumor burden and renal impairment.

In the current study, serum creatinine level was significantly elevated in the patient group. This result agrees with Hussain et al. [12] who reported the same result. Audard et al. [21] also found that creatinine level is elevated in MM patients which may be due to multiple pathogenic mechanisms that cause kidney injury, some of which are the result of nephrotoxic monoclonal Ig and some of which are independent of paraprotein deposition monoclonal IgM, which is a much larger molecule than IgG, can produce hyperviscosity associated renal impairment and additionally form deposits that occlude glomerular capillaries.

In this study, Bone marrow biopsy of the studied samples of the MM patient group showed that BCMA expression by IHC was evaluated by a semi-quantitative system of (0%,0-10%,10-40%,40-60%,60-80%, and >80%). Most of the patients in the current study showed high BCMA expression (>80% and 60-80%) than patients with intermediate expression (40-60%) and finally patients with low expression 10-40%. Seckinger et al. [22] evaluated BCMA expression in a large population of newly diagnosed and relapsed MM patients, showing selective expression on malignant PCs from most patients. Consistent with these observations, significantly higher levels of membrane-bound BCMA expression were found in MM bone marrow mononuclear cells (BMMCs) obtained from patients than in BMMCs from healthy donors [23].

In this study correlation between BCMA expression and serum BCMA showed a significantly positive correlation, as a group of patients with BCMA expression >80% showed higher levels of serum BCMA. These results agree with a study conducted by Lee et al. [24] who reported the same results. Serum BCMA founded to be significantly correlated with plasma cell percentage in BM aspirate, CD138 expression in BM biopsy, hemoglobin level, M protein, total calcium level, LDH, ESR, β2microglobulin, and serum creatinine.

As regarding the response of patients to treatment and clinical status, patients were grouped into complete response (CR), partial response (PR), relapsed (R), and failure of complete response. Serum BCMA and BCMA expression showed a significant correlation with the clinical status of the patients' group, patients with complete response showed lower level of serum BCMAand10-40% expression of surface BCMA while patients with failure of CR relapsed group showed higher level of serum BCMA and 60-80% or>80% expression of surface BCMA (P-value =0.001). Ghermezi et al. [20] stated that a strong correlation between patients' current clinical status and sBCMA level was also identified. Specifically, patients who were in CR displayed lower sBCMA levels than those who were in minor response or partial response those

with the non-responsive disease had the highest levels of sBCMA. Sanchez et al. [25] showed that sBCMA levels correlated with current clinical status among MM patients when comparing patients with a complete response (CR) showing lower levels versus those with a partial response (PR).

In the current study, the ability of sBCMA to predict clinical outcomes, DFS and OS, was also examined. Patients with lower sBCMA levels at the time of starting initial or a new therapy were both found to have longer DFS than those with higher sBCMA levels above the median. OS was also longer for patients with sBCMA levels below the median than those with sBCMA above the median.

As regarding BCMA expression by IHC, in the present study, there was a significant correlation between overall survival (OS) and disease-free survival (DFS). Patients with the highest levels of BCMA expression >80% showed shorter OS and shorter DFS while patients with lowest levels of BCMA expression showed longer OS and DFS. Lee et al. [24] reported the same results. Salem et al. [26] suggested that objective measurement of BCMA expression in myeloma cases was important as it may have an impact on the treatment response and may help in patient stratification for dose adjustment. IHC was the method of choice for patients with minimal bone marrow involvement. IHC was primarily successful in cases with significant myeloma infiltration of the bone marrow but does have the advantage of not requiring fresh tissue and applying to evaluation of previous biopsies. In contrast with CD138, BCMA is readily identified in delayed and frozen MM samples. Shah et al. [27] stated that B-cell maturation antigen (BCMA) is preferentially expressed by mature B lymphocytes, and its overexpression and activation are associated with MM in humans, supporting its potential utility as a therapeutic target for MM. Moreover, the use of BCMA as a biomarker for MM is supported by its prognostic value, correlation with clinical status, and its ability to be used in traditionally difficult-to-monitor patient populations.

5. CONCLUSIONS

The important role of BCMA expression and its serum level in the diagnosis of multiple myeloma, as the BCMA level in serum significantly elevated in MM patients compared with the control group. Moreover, serum BCMA level and its surface expression are positively correlated with plasma cell percentage in BM aspirate, CD 138 expression in BM biopsy, M protein, and B2 macroglobulin.

CONSENT

As per international standard or university standard, patients' written consent has been collected and preserved by the author(s).

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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