

## Original Research Article

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

### 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 apparently healthy individuals served as control.

**Results:** There was statistically significant increase in MM patients than control group levels were increased in MM patients. Plasma cell percentage in BM aspiration was statistically significant increase in MM patients. As regarding serum BCMA, there was statistically significant increase in MM patients than control group. There was a significantly positive correlation between serum BCMA and its surface expression with plasma cell in BM, CD138 expression in 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 significant correlation with clinical status of patients group, patients with complete response showed 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 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 the multiple myeloma, as the BCMA level in serum significantly elevated in MM patients compared with 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

UNDER PEER REVIEW

## **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 <sup>[1, 2]</sup>.

The field of myeloma therapy shown enormous progress in the past decade and as a result median overall survival has increased to approximately 6 years <sup>[3]</sup>. 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 <sup>[4, 5]</sup>, even after destruction of the bulk of tumor cells <sup>[6, 7]</sup>.

Immunotherapeutic approaches could potentially play an important role in the global treatment concept for myeloma, targeting residual disease after an 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 <sup>[8]</sup>.

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 lymphocyte <sup>[9]</sup>, and is known to bind B-lymphocyte stimulator (BLyS), also known as B cell activating factor (BAFF), a protein which plays a significant role in the growth and survival of MM cells <sup>[9]</sup>.

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 <sup>[10]</sup>.

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.

## **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.

Patients with any other malignancies, patients with autoimmune diseases such as systemic lupus erythromatosis 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 including 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 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 diagnosis of abnormal plasma cells (CD38, CD138 and CD56) and to confirm diagnosis of monoclonal plasma cells (anti-kappa and anti-lambda monoclonal antibodies). Immunohistochemistry of BM biopsy samples to confirm 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  $\geq 10\%$  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 [ $> 177$   $\mu$ mol/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  $\geq 10\%$ . Abnormal serum free light chain (FLC) ratio  $\geq 100$  (involved kappa) or  $< 0.01$  (involved lambda). One or more focal lesions  $> 5$  mm on MRI scans <sup>[11]</sup>.

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 -20°C 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 an 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 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 the 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 sample were positively correlated.

Assay procedure: Standard dilution: This test kit supply one original Standard reagent that was diluted into 5 standards of different concentrations by serial dilution. Procedures: Blank well: no sample or standard were 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 60 minutes at 37°C. 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 for 3 times. Chromogen solution A 50µl was added, then chromogen solution B 50µl to each well. Gently mixed, incubated for 10 min at 37 °C away from light. Stop: Stop Solution 50µl was added into each well to stop the reaction (the blue changed into yellow immediately). Final measurement: 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).

Sampling of BM aspirate: Patient was positioned in prone or side-lying position. Area for aspiration was exposed and 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 alcohol swab using concentric motion beginning in the center. This step was repeated twice with new swab. Then area was allowed to dry. Exact point for aspiration was located and area between thumb and index finger was outlined. 2-3 ml Xylocaine 2% was injected perpendicularly subcutaneously and into periosteum. Then lidocaine was left for 2-3 minutes to take effect. Then bone marrow needle was prepared, assuring stylet moves freely. Skin was stretched taut over puncture site; crest was kept between thumb and index finger of one hand. Bone marrow needle was held with stylet in place, skin was punctured and advance 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 quick suctioned and obtained.

### **Statistical analysis**

Data were fed to the computer using IBM SPSS software package version 20.0. Qualitative data were described using number and percent. The distributions of quantitative variables were tested for normality using 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 was 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 population were done using independent t-test, while more than two population were analyzed F-test (ANOVA) to be used and Post Hoc test (Scheffe). Correlations between two quantitative variables were assessed using Pearson coefficient. Significance test results are quoted as two-tailed probabilities. Significance of the obtained results was judged at the 5% level.

### **Results:**

Patients' characteristics (age and sex) were discussed in the following table. [Table 1]

**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 <sup>2</sup>	1.111		
	P-value	0.292		

Laboratory data of the studied groups were discussed in the following table. [Table 2]

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

		Range	Mean ± S. D	t. test	p. value
HB	MM patients	6 – 14.1	9.96 ± 2.11	26.918	<b>0.001*</b>
	Control group	12 – 14	12.8 ± 1.10		
Platelet count	MM patients	32×10 <sup>3</sup> – 498×10 <sup>3</sup>	221.3×10 <sup>3</sup> ± 110.1×10 <sup>3</sup>	0.298	0.587
	Control group	150×10 <sup>3</sup> – 350×10 <sup>3</sup>	233.5×10 <sup>3</sup> ± 55.1×10 <sup>3</sup>		
WBC count	MM patients	1600 – 19000	6793.33 ± 3827.75	0.017	0.897
	Control group	4500 – 10600	5600.33 ± 2586.85		
Ionized calcium	MM patients	4.84 – 6.8	5.72 ± 0.58	50.489	<b>0.001*</b>
	Control group	4.7 – 5.3	4.94 ± 0.16		
Total calcium	MM patients	11 – 13.8	12.39 ± 0.92	118.600	<b>0.001*</b>
	Control group	8.5 – 11	9.88 ± 0.86		
LDH	MM patients	240 – 1250	625.43 ± 286.00	98.963	<b>0.001*</b>
	Control group	70 – 150	105.83 ± 24.92		

<b>ESR</b>	MM patients	12 – 130	56.20 ± 30.66	75.398	<b>0.001*</b>
	Control group	5 – 10	7.50 ± 1.96		
<b>B2 macroglobulin</b>	MM patients	3.3 – 7.2	5.34 ± 0.99	447.676	<b>0.001*</b>
	Control group	0.04 – 2	0.87 ± 0.60		
<b>Serum creatinine</b>	MM patients	1.2 – 4.5	2.39 ± 0.92	87.507	<b>0.001*</b>
	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.

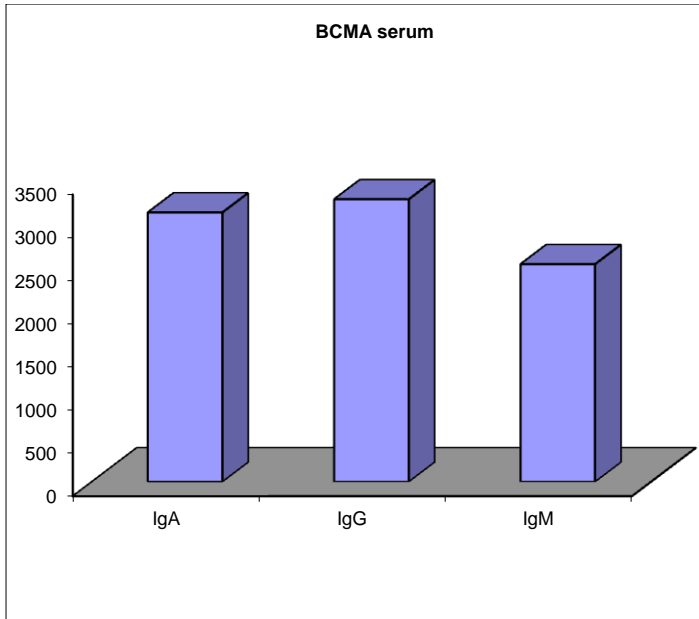
Regarding serum BCMA level, there was statistically significant increase in MM patients compared to Control group. [Table 3]

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

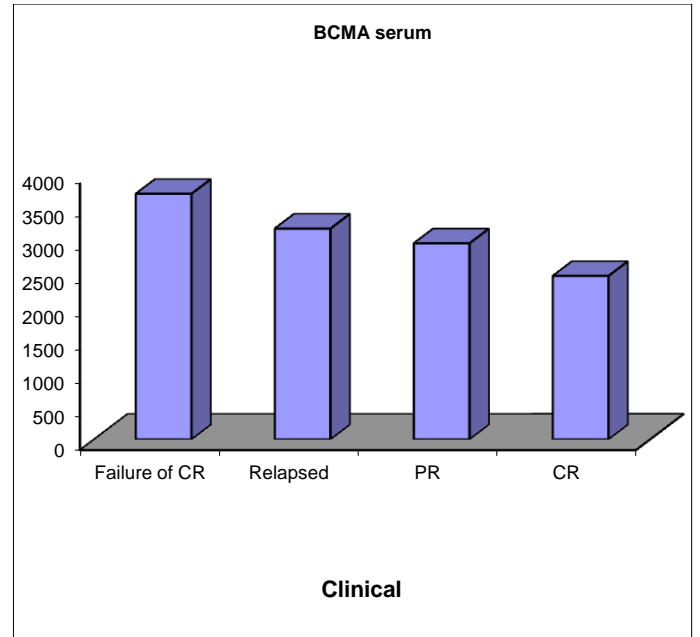
<b>BCMA</b>	<b>MM patients</b>	<b>Control group</b>
<b>Range</b>	2125.7 – 4158.1	86 – 1325.8
<b>Mean ± SD</b>	3182.29±588.61	493.98±349.30
<b>T. test</b>	462.807	
<b>P. value</b>	<b>0.001*</b>	

\*: statistically significant as P value < 0.05

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 correlation between serum BCMA and clinical outcome of the MM patients, there was statistically significant increase of serum BCMA in MM patients with failure of CR & relapsed cases than those with PR and CR. [Figure 1]



(A)



(B)

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

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 4]

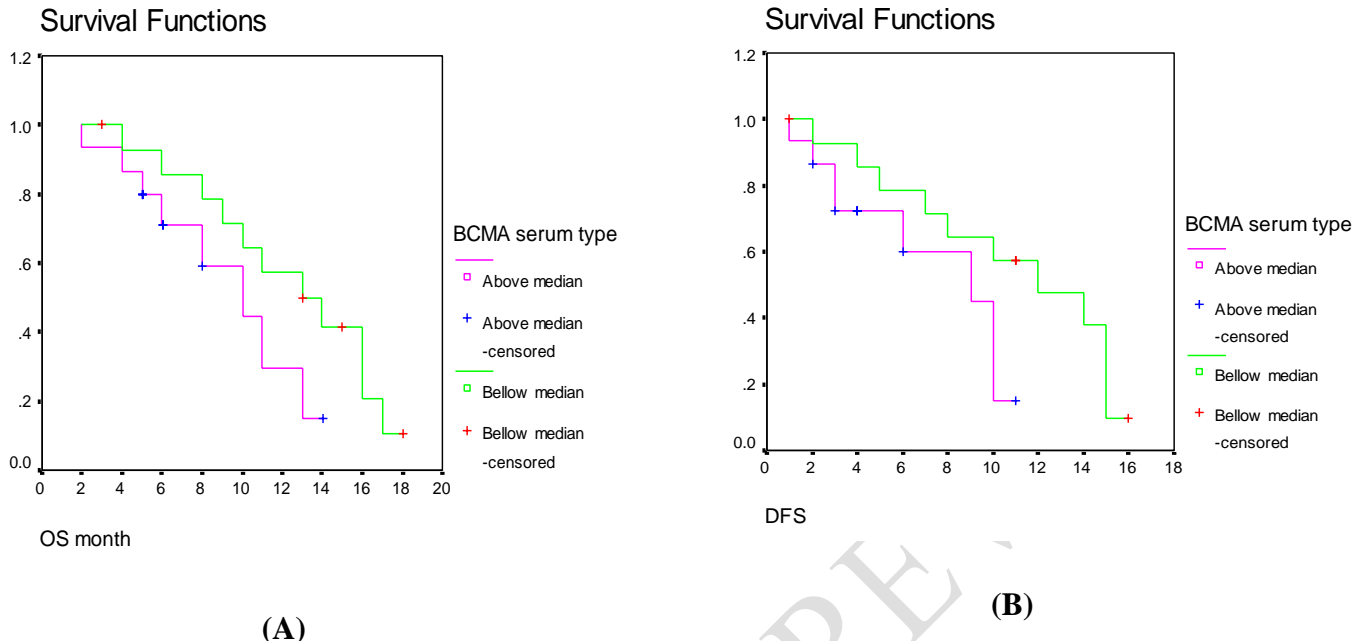
**Table 4: Correlation between serum BCMA level and studied laboratory tests in MM patients**

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

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

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

Free Survival in multiple myeloma patients, there was no statistically significant difference as regarding DFS between above and below the median level of serum BCMA. [Figure 2]



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

MM patients’ subgroups regarding age and sex were compared in the following table. [Table 5]

**Table 5: comparison between MM patients’ subgroups regarding age and sex**

BCMA immunohistochemistry		Range		Mean ± S. D		F. test	p. value	
Age	Score 2 (10 - 40%)	40 – 60		48.33 ± 10.41		0.167	0.917	
	Score3(40 – 60%)	26 – 82		52.50 ± 18.21				
	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 <sup>2</sup>	2.027						
	P-value	0.567						

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

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

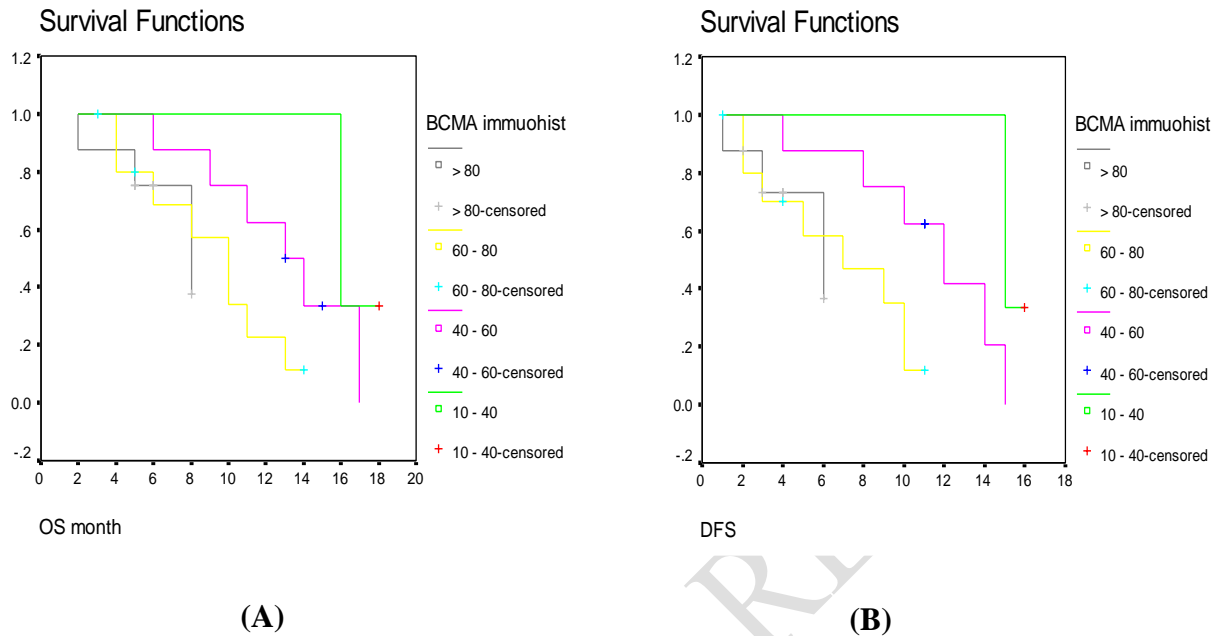
BCMA immunohistochemistry		Range	Mean ± S. D	F. test	P. value
Percent of Plasma cell in BM aspirate	Score 2(10 – 40%)	1 – 4	2.33 ± 1.53	12.13	0.001*
	Score 3(40 – 60%)	2 – 10	4.38 ± 2.56		
	Score 4 (60 – 80%)	5 – 37	18.55 ± 12.72		
	Score 5 (> 80%)	6 – 73	42.00 ± 21.27		
CD 138 expression in BM biopsy	Score 2 (10 – 40%)	15 – 40	23.33 ± 14.43	22.11	0.001*
	Score 3 (40 – 60%)	12 – 70	25.38 ± 18.84		
	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	3.23	0.039*
	Score 3 (40 – 60%)	8.5 – 12.3	9.86 ± 1.60		
	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	0.739	0.539
	Score 3 (40 – 60%)	32000 – 498000	256000.00 ± 132107.10		
	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	2.666	0.069
	Score 3 (40 – 60%)	1600 – 7800	5425.0 ± 1996.96		
	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.

As regarding correlation of BCMA expression by immunohistochemistry with Overall Survival in multiple myeloma patients, there was statistically significant difference as

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



**Figure 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).**

## 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 study by **Hussain et al.** [12] and **Diwan et al.** [13].

In the present study total calcium in patient group was significantly elevated, and this result agrees with **kyle RA and Gertz MA** [14] and with **Mirrakhimov AE** [15] who founded that hypercalcaemia is common in patients with advanced cancer stages. The primary cause of the 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 the most of patients and this agrees with **Diwan et al. (2014)**,<sup>[13]</sup> who reported the same results, that mainly due to increased immunoglobulins. **Alexandrakis et al.**<sup>[17]</sup> 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 patient group. This result agrees with **Barlogie et al.**<sup>[18]</sup> who suggested that high level of serum LDH and calcium decreased the early survival of MM patients. **Chen et al.**<sup>[19]</sup>, reported that high  $\beta$ 2-MG, high serum LDH, and low serum albumin levels were poor prognostic factors for early mortality.

In this study,  $\beta$ 2microglobulin was found to be significantly elevated in patient group. This result agrees with **Ghermezi et al.**<sup>[20]</sup> and **Hussain et al.**<sup>[12]</sup> who reported that  $\beta$ 2 microglobulin was raised in most of MM patients and confirmed that  $\beta$ 2microglobulin 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 patient group. This result agrees with **Hussain et al.**<sup>[12]</sup> who reported the same result. **Audard et al.**<sup>[21]</sup> 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 MM patient group showed that BCMA expression by IHC was evaluated by semi-quantitative system of (0%,0-10%,10-40%,40-60%,60-80% and >80%). Most of patients in the current study showed high BCMA expression (>80% and 60-80%) then patients with intermediate expression (40-60%) and finally patients with low expression 10-40%. **Seckinger et al.**<sup>[22]</sup> 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<sup>[23]</sup>.

In this study correlation between BCMA expression and serum BCMA showed significantly positive correlation, as 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,  $\beta$ 2microglobulin and serum creatinine.

As regarding 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 significant correlation with clinical status of patients group, patients with complete response showed lower level of serum BCMA and 10-40% 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 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 median. OS was also longer for patients with sBCMA levels below the median than those with sBCMA above median.

As regarding BCMA expression by IHC, in the present study there was significant correlation with overall survival (OS) and disease-free survival (DFS). Patients with 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 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 being applicable 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.

### **Conclusions:**

Important role of BCMA expression and its serum level in the diagnosis of the multiple myeloma, as the BCMA level in serum significantly elevated in MM patients compared with 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.

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