# Markers of Bone Turnover in the Evaluation of Diagnosis and Prognosis of Multiple Myeloma in a Sample of Iraqi Patients

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## Abstract

Subjects /This study is aimed to evaluate the bone resorption marker CTX, B-CTX, and bone formation marker Osteocalcin in relation to the clinical presentation as well as to investigate the evidences of the osteoporotic processes by assessing osteoclast bone markers in the different stages of MM patients and comparing these markers- in relation to standard prognostic markers in sample of MM Iraqi patients. – in addition to interpretation of soluble CD138 in relation to prognosis.

Methods/Sixty-five MM (males=41, females=24) patients distributed to different hematology centers in Iraq were enrolled in this cross-sectional study.Their age range was 39-81 years, twelve of them were newly diagnosed and the others were under treatment and distributed all on three stages from the disease according to the international staging system (ISS) : Group A – Stage I (n=21 patients, age mean 57.14±12.25 years), Group B – Stage II (n= 22 patients, age mean of 56.45±11.33 years), and Group C-Stage III (n=22 patients, age mean 60.59±11.55 years). Seven milliliters of venous blood samples were taken from each patient just prior to starting the chemotherapy for the measurement of blood hemoglobin (Hb), serum Creatinine, , Calcium , Beat 2 Microglubulin Osteocalcin (OC), Total and Beta C-terminal telopeptide (CTX, BCTX), Parathyroid hormone (PTH), Syndecan-1 (CD138), and both kappa & lambda Free light chain (FLCκ, FLCλ).

Results/Anemia was a general feature of all patients, with a gradual rise (from stage 1 to 3) in the concentrations of creatinine and B2M P=<0.001, calcium P=0.01 and, all other studied markers CTX, BCTX, CD138, FLCκ and FLCλ has significant elevation in comparison among studied groups P=<0.001 with the exception of Osteocalcin ,which showed a general reduction.

Conclusion/Multiple Myeloma patients have increased in bone remodeling throughout the disease course with progressive increment in relation to disease stage --. Plasma cells in MM leads to disturbance in expression and secretion of CD138 in association with disease advancement.

***Key words*** */ Multiple Myeloma , bone turnover and C-terminal telopeptide (CTX) .*

## Introduction

Clonal dissemination from malignant plasma cells in the microenvironnement of the bone marrow with the appearance , in blood and urine, of monoclonal protein, are important features of multiple myeloma (MM) which are usually associated with organ dysfunction , extensive lytic bone lesions and osteoporosis [(1)] . Patients with MM usually complain of fatigue , bone pain , easy bruisability, bleeding and recurrent infections which are manifested by hypercalcemia, lytic bone lesions, hyperviscosity, thrombocytopenia , hypo-gammaglobulinemia and many others [(2,3)] .

The myeloma cells are synergized by bone microenvironment which include fibroblasts, osteoblasts and osteoclasts [(4)] .Increased rate of bone resorption and remodeling with a morphological assessment of plasma cells are important criteria for the evaluation of symptomatic myeloma [(5)] . Precipitation of light chains

casts within the distal and collecting tubules- as a result of increased production of M- protein could be a cause of development of what is called myeloma kidney together with hypercalcemia, free light chain proteinuria, lack of hydration, hyperuricemia, and nephrotoxic medications and consequent recurrent bacterial infections [6,7] .

Hypercalcemia can hasten and disturb renal insufficiency [8] .

Of the prognostic markers β2 microglobulin its raised concentrations has been consistently reported in conditions characterized by lymphocyte activation and (or) proliferation. It is eliminated via the kidneys [9] .

Circulating serum free light chain immunoglobulins are among the prognostic factors .These comprise two types : Kappa ( K ) and Lambda (λ)The ratio between the two types is increased in plasma cell tumors [10] .

Plasma C- reactive protein (CRP) may rise rapidly after an acute inflammatory stimulus, mostly due to increased synthesis by the liver [11] , and Can serve as an important indicator from survival and post-treatment follow-up in cancer patients [12] .Other recent prognostic criteria could be considered for MM as syndican-1 (CD138 ) , osteocalcin , and Beta C-Terminal Telopeptide (BCTX) are still debated.

## Subjects and Methods

Hospital-Based cross-sectional research was conducted over eleven months from May 2018 to June 2019. A total of 65 Multiple Myeloma (MM) patients participated in the study who were subjected to physical examination and diagnosed by hematologists with Multiple myeloma from both genders (based on the Diagnostic criteria of the International Myeloma Working Group - IMWG). They were distributed to different Departments of hematology from Al-Imamain Al-Kadhimain Medical City, Al-Yarmuk Hospital, Baghdad Medical City teaching laboratories, Mirjan teaching Hospital in Hilla, and center of hematology and oncology in Basra.

The age range was 39 – 81 years . A group ( 12 ) of newly diagnosed patients emerged. The 65 patients were grouped into 3 stages according to the international staging system (ISS) : Group A – Stage I (n=21 patients, age mean 57.14±12.25 years), Group B – Stage II (n= 22 patients, age mean of 56.45±11.33 years), and Group C-Stage III (n=22 patients, age mean 60.59±11.55 years).

Full data were collected from each patient using a preformed questionnaire. Initial laboratory results were recorded from tests performed for the patients. Serum urea, creatinine, and calcium were determined by spectrophotometric methods. Moreover, a complete blood count and ESR, serum Immuno- fixation Electrophoresis, Imaging bone surveys, bone marrow aspirate, and bone marrow biopsy results were registered for each patient.

Patients excluded from the study were those who had Liver disease, active infections (human immune-deficiency virus, HIV, Hepatitis B, or C), and pregnant or breast-feeding women.For each patient the following laboratory tests were done: Free light chains test Kappa (FLC-k) & Lambda (FLC-l), B2Microglubulin (B2-MG), Osteocalcin (OC), C-terminal telopeptide (CTX), Beta C-terminal telopeptide (BCTX), Parathyroid hormone (PTH) and Syndecan-1(CD138). All tests were done by the ELISA technique.

The Practical part was performed in the Department of Chemistry and Biochemistry, College of Medicine / Al Nahrain University Research Laboratories.Test kits were purchased from Cusabio-China for PTH, CD 138, and B2MG, and from Melsin-China for OC & CTX, and from the Bio Vender-Czech Republic for free light chain (Kappa & Lambda).The study approved by IRB at College of Medicine/Al Nahrain University.

## Results

The most prevalent presenting features were anemia (43.08%) followed by back pain (29.23%) and renal complications (13.85%) as in table 1.

A gradual increase in serum B2MG, calcium and creatine with plasma cells is present from stages I to III (P<0-001), but no marked differences in Hb % and immunofixation results (table-1)

For FLCκ, and κ/λ ratio, stage I and II showed a comparable level of these markers with no significant differences, but both stages had significantly lower levels than stage III, (P=<0.001). as in table

Serum parathyroid hormone, CD138 and total FLC, show comparable levels between stage II and stage III, while both stages were significantly higher than stage I (P=<0.001).

There was a gradual increase in the β-CTX marker from stage I to stage III. (P=<0.001). as in table

There is a gradual reduction in serum osteocalcin from stage 1 to III (P< 0.001).

**Table 1: - Association of common laboratory findings with disease staging**

| Variables | Stage I (n=21) | Stage II (n=22) | Stage III (n=22) | P-value |
|---|---|---|---|---|
| Hb (g/dl) | 10.15±0.34 | 10.08±1.17 | 9.86±0.63 | 0.453 |
| Plasma cell% | 24.95±10.84a | 37.09±10.45b | 51.59±9.29c | <0.001 |
| Creatinine (mg/dl) | 0.942±0.33a | 2.04±0.53b | 2.91±0.59c | <0.001 |
| Calcium (mg/dl) | 10.11±0.87a | 10.51±1.46a | 11.35±1.53b | 0.01 |
| β2-M (mg/L) | 3.57±0.95a | 5.92±0.29b | 8.34±0.77c | <0.001 |
| Immuno- Fix.<br>IgG-K<br>IgG-L<br>IgA-K<br>IgA-L | <br>12(57.14%)<br>5(23.81%)<br>3(14.29%)<br>1(4.76%) | <br>7(31.82%)<br>9(40.91%).<br>3(13.46%)<br>3(13.46%) | <br>15(68.18%)<br>2(9.09%)<br>4(18.18%)<br>1(4.55%) | <br><br><br><br>0.165 |

a, b and c different small letters indicate significant differences

**Table 2: Association of specific laboratory investigation with disease staging**

| Variables | Stage I (n=21) | Stage II (n=22) | Stage III (n=22) | P-value |
|---|---|---|---|---|
| PTH (pg/ml) | 137.14±25.87a | 160.54±28.49b | 165.95±26.25b | 0.002 |
| Osteocalcin (ng/ml) | 12.43±5.78a | 10.60±6.35a | 6.22±4.08b | 0.001 |
| CTX (ng/ml) | 18.24±5.80a | 24.08±5.02ab | 29.65±6.99b | <0.001 |
| BCTX (ng/ml) | 5074.3±1742a | 6824.5±1507b | 8497.7±2099c | <0.001 |
| CD138 (ng/ml) | 246.8±115.5a | 443.6±183.8b | 495.6±160.5b | <0.001 |
| FLCκ (ng/ml) | 15.19±9.83a | 15.60±14.51a | 53.35±25.31b | <0.001 |
| FLCλ (ng/ml) | 17.19±10.60a | 51.35±39.30b | 22.10±24.69a | <0.001 |
| κ/λ ratio | 1.33±0.99a | 1.02±1.266a | 4.95±6.62b | 0.002 |
| Total FLC (ng/ml) | 32.30±8.98a | 66.96±32.12b | 75.45±26.38b | <0.001 |

a, b, c different small letters indicate significant differences

The Receiver operating characteristic (ROC) curve was used to evaluate the diagnostic value for the detection of new MM cases. The patients were divided into two groups regardless of staging: newly diagnosed (12 cases) and longstanding (53 cases) the results revealed:

For osteocalcin, the area under the curve (AUC) was 0.553 (95%CI= 0.35-0.756), p= 0.565. The sensitivity and specificity of the test at osteocalcin cut off = 7.4 mg/dl were 0.604 and 0.587 respectively.

For CD138 the AUC was 0.748, 95%CI= 0.629-0.891, p= 0.008. The sensitivity and specificity of the test at CD138 cut off= 330 ng/ml was 0.68 and 0.677 respectively.

better results were obtained from total FLC, FLCκ or FLCλ:

For FLCκ, the AUC= 0.809, 95%CI= 0.64-0.971, p=0.001. The sensitivity and specificity of the test at FLCκ cut off= 9.5 ng/ml was 1.0 and 0.677 respectively.

For FLCλ, the AUC= 0.816, 95%CI= 0.7-0.932, p=0.001. The sensitivity and specificity of the test at FLCλ cut off= 8.3 ng/ml was 0.717 and 0.677 respectively.

while total FLC was the best; the AUC was 1.0, 95%= 1.0-1.0, p<0.001. The sensitivity and specificity of the test at total FLC cut off= 28.35 ng/ml were 1.0 for both.

Beta-2-microglobulin was found to be significantly decreased in longstanding patients compared with the newly diagnosed, and hence it was analyzed with a separated ROC curve. The AUC was 0.601, 95%CI= 0.45-0.751, p=0.279. The sensitivity and specificity of the test at β2-microglobulin cut off= 5.55 mg/L were 0.883 and 0.66 respectively.

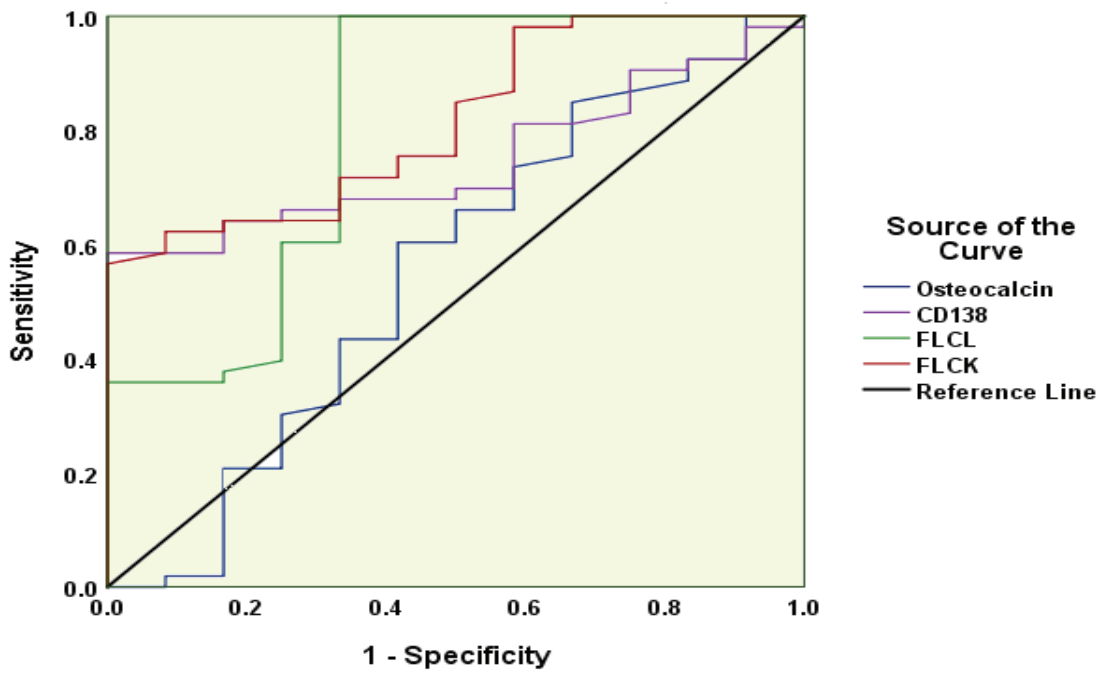


**Fig. 1: Receiver Operating Curve for Some Selected Markers in The Context of Discrimination Between Newly Diagnosed and Longstanding Multiple Myeloma**

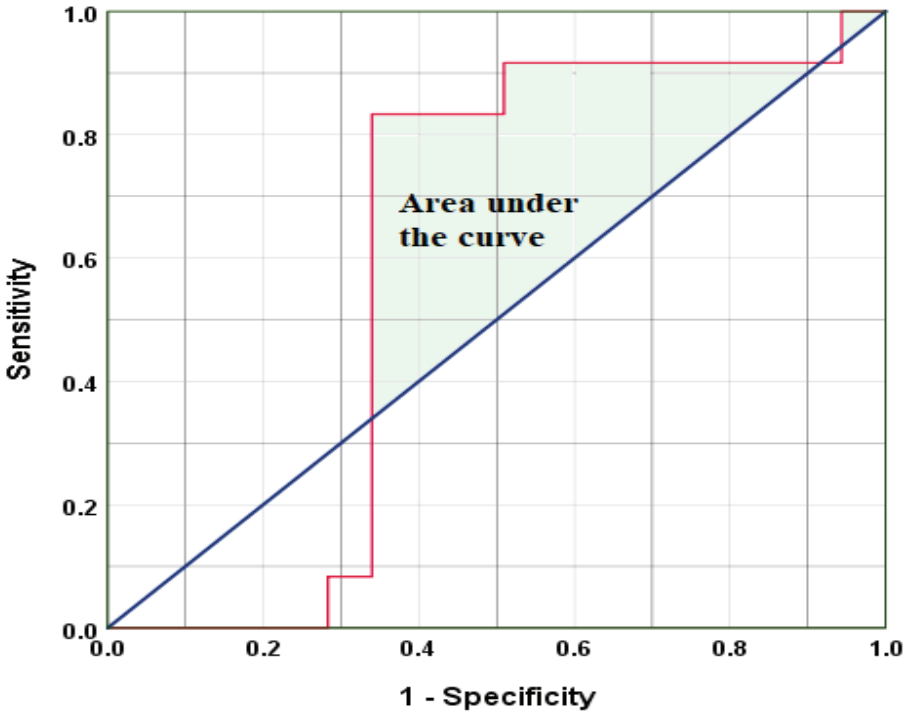


**Fig.2: Receiver Operating Curve For B2-Microglobulin in The Context of Discrimination Between Newly Diagnosed and Longstanding Multiple Myeloma**

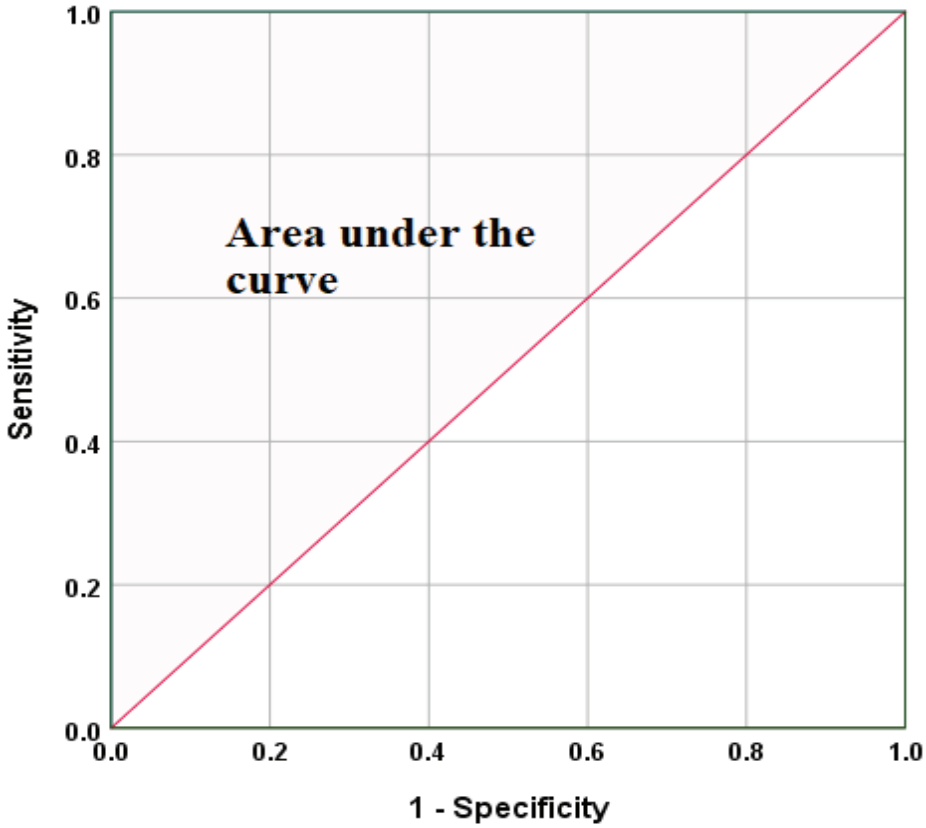


**Fig.3: Receiver Operating Curve for Total Free Light Chains in The Context of Discrimination Between Newly Diagnosed & Longstanding Multiple Myeloma**

## Discussion

The distribution of MM patients among the three stages of the disease is more or less, the same which makes the comparsion more convenient.

The hypercalcemia shown by the present patients could be attributed to the raise in the osteoclast-activating factor cytokines, Receptor Activator of Nuclear Factor Kappa-B Ligand (RANKL) is a member of the TNF ligand/receptor superfamily [13].

It is a major mediator of osteoclast differentiation and activation and is over-expressed in the microenvironment from myeloma marrow. It is antagonized by osteoprotegerin (OPG), which is downregulated in myeloma lesions. Thus, there is a net increase in RANKL and a decrease in OPG in multiple myeloma, leading to osteoclast hyperactivity [14].

Hypercalcemia associates PTH which is reported to increase in MM [15].

The increase in B2 Microglubulin (B2MG) is a common feature of MM which was attributed to a mutation in the B2MG gene which has been shown to result in hypercatabolic hypoproteinemia. Diseases associated with B2M include immunodeficiency 43 and amyloidosis. High serum B2MG values can be thought of as a result of increased "synthesis" whether owing to increased expression, increased HLA turnover, cell proliferation, cell lysis, or some combination of these and not of decreased renal clearance [16,17].

High levels of B2MG are associated with greater tumor burden and renal failure. This is manifested in this study by the high concentration of serum creatinine which was seen to be more prominent in 25% of myeloma patients [18].

Pathologic injury of myeloma kidney is the presence of monoclonal light chains in the tubules in the type of thick, regularly overlaid, cylindrical throws. These casts contain - Tamm-Horsfall protein [19]. Uromodulin, is a protein unique to the kidneys. It coats the epithelium luminal side and is the most abundant in human urine [20].

Osteocalcin is expressed directly by Osteoblasts. Most historical studies have focused on characterizing OC as a fracture indicator and only as a part of bone metabolism [21]. Normally bone homeostasis depends on the coupling of OCs and Osteoblasts. This coupling mechanism is lost in MM leading to an expansion of osteoclastic action with consequent bone resorption and diminished arrangement [22,23].

The increase in CTX and Beta-CTX could be due to the secretion of a mixture of acid and neutral proteases by osteoclasts during bone resorption. This had made them specific markers for the degradation of type1 collagen [24,25].This study shows an increase in the CTX and BCTX levels according to MM stages, the highest being in the third stage. These findings agree with Schiano C, Soricelli A. 2019, In vitro evidence supports a role for CD138 (syndecan-1) in the adherence of plasma cells to the stromal matrix of the bone marrow via an association with type I collagen.

From studies on monoclonal antibodies human syndecan-1 seems to be plasma cell-specific among hematopoietic elements and appears to be involved in the process of carcinogenesis [26].

In people with osteoporosis, bone turnover markers may be useful for evaluating the response to anabolic and antiresorptive therapies, for evaluating therapy compliance or for suggesting potential secondary osteoporosis . A great deal remains to be learned about how bone turnover markers can be used to track the impact of stopping bisphosphonate therapy (e.g., determining a threshold above which restart therapy should be taken). several studies are needed to research the use of bone turnover markers to evaluate the bone safety of new drugs [27].

The ROC test, in this study, shows clearly the differences in the specificity and sensitivity of the different bone biomarkers in MM, the net result of this test reveals that the Total free light chain remained the best by getting sensitivity and specificity of 1 for each.

At last we may say that careful monitoring of Bone markers levels at regular intervals is recommended with the emphasis on the control of parathyroid hormone and reduction of serum levels of bone markers by any possible mean.

Therefore, the bone remodeling markers are pivotal in evaluation of progression of MM patient which indeed may attribute in treatment decision as well as the monitoring of treatment results according to specific therapy.

## Conclusion

Multiple Myeloma patients have increased in bone remodeling throughout the disease course with progressive increment in relation to disease stage --. Plasma cells in MM leads to disturbance in expression and secretion of CD138 in association with disease advancement.

-The ethical clearance of my article taken from the Institutional Ethics Committee in Medical College / fallujah University / Iraq ,this ethical clearance Installed and written in materials and methods of my article .

**The Source of funding** : Self).

**Conflicts of Interest**: Not found

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