Level of Some Biomarkers of Bone Remodeling in Treated Multiple Myeloma Patients and Compered with New Diagnostic Multiple Myeloma Patients

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Abstract

This study was conducted to evaluate the role of some biomarkers of bone remodeling and osteoclast activation/function in treated myeloma patients and to compare results with new diagnostic multiple myeloma(MM) patients who did not receive any treatment . during the period from March (2019) to end November (2019). The study involved measuring serum level of RANKL, Osteoprotegerin (OPG) , RANKL/OPG ratio, Interleukin 6 (IL-6), C-terminal cross-linking telopeptide of type I collagen (CTX type –I) , tartrate-resistant acid phosphatase isoform-5b (TRACP-5b) as markers of bone resorption in 47 treated myeloma patients and the results of these parameters comparing with 13 new diagnosed patients and (30) age- and sex-matched healthy controls groups. The statistical analysis of results showed that the serum level of (RANKL, RANKL/OPG ratio, IL-6, CTX type –I and TRACP-5b) decreased in the treated myeloma patients compared to the new diagnosis patients and the level OPG increased in treated myeloma patients.

The results also showed a significant rise ($p \leq 0.05$) serum level of (RANKL, RANKL/OPG ratio, IL-6, CTX type –I and TRACP-5b) and reduce level OPG in myeloma patients (treated and new diagnosis) in general compared to healthy control individuals . In conclusion, RANKL, OPG and IL-6 play significant roles in MM pathophysiology, as regulators of bone turnover The study showed that an increase leads to an increase in bone resorption, and it appears that chemotherapy reduces resorption .

Keywords: *Multiple Myeloma, Bone Remodeling, Biomarkers.*

Introduction

Multiple myeloma is a type of malignant cancer of plasma cells that normally develops and found in the bone marrow and this disease is part of a group of disorders known as "plasma cell dyscrasias"[1]. Plasma cell are playing important roles in immune system through produce humoral factors which is called antibodies[2].

A hallmark feature of Multiple myeloma is Myeloma bone disease (MBD); up to 80% of patients present with osteolytic bone lesions (bone disease) at diagnosis and leading to pathophysiological features referred to as skeletal-related events (SREs), which contribute to a reduced quality of life and associated with rise mortality and morbidity[3].

MBD occurs due to the interactions between malignant plasma cells (MPCs) and cells in the bone marrow microenvironment (BMME), leading to accelerated overall bone loss and the formation of focal osteolytic lesions**.**

Although the mechanisms responsible for the development of a myeloma bone disease still not clear, several studies have begun highlights on cytokines and growth factors produced by myeloma cells or by the stromal cells of bone[3]. As a result of the interaction between them, leads to an increase in the formation and activity of the osteoclast and adherence of myeloma cells to bone marrow stromal cells hence an increase production of cytokines, Among these cytokine is (RANKL), a member of the tumor necrosis factor (TNF) gene family produce by stromal cells causes activation of the cellular receptor RANK on osteoclasts by its ligand, RANKL, differentiation, proliferation, and survival of osteoclasts is enhanced, osteoclast fusion

and activation is promoted, and osteoclastic apoptosis is suppressed, leading to a dramatic increase of the number and activity of osteoclasts.[4] In addition, production of osteoprotegerin (OPG), a soluble decoy receptor of RANKL produced by marrow stromal cells, is suppressed through the above interactions and has been found to be reduced in patients with MM [5]. Studies In vitro show that IL-6 trans-signaling promotes osteoclastogenesis by increasing RANKL expression in osteoblasts and T cells (Wong et al,2006) this effect is dependent on the JAK/STAT-3 signaling pathway (Duplomb *et al*,2008) IL-6 transsignaling also increases RANKL expression by synovial fibroblasts to support osteoclastogenesis in the inflamed joint., however, under some circumstances IL-6 can actually inhibit osteoclastogenesis from human CD14+ precursors in vitro [6].

Bio markers of bone turnover, C-terminal cross-linking telopeptide of type I collagen CTX type –I tartrate resistant acid phosphatase isoform 5b (TRACP-5b), provide information on bone dynamics that in turn may reflect myeloma disease activity in bone [7]. Several studies have shown bone markers to be elevated in myeloma patients and reflect the extent of bone disease, while in some of them bone resorption markers correlate with survival. These markers may also be helpful in knowledge respond to bisphosphonate treatment, and monitoring the effectiveness of bisphosphonate therapy in the management of myeloma bone disease [3].

The purpose of this study was to evaluate serum levels of OPG, RANKL . the ratio sRANKL/OPG, CTX type –I, and (TRACP-5b) at diagnosis and to estimate the effect of treatment on their circulating levels in patients with MM.

Subjects and Method

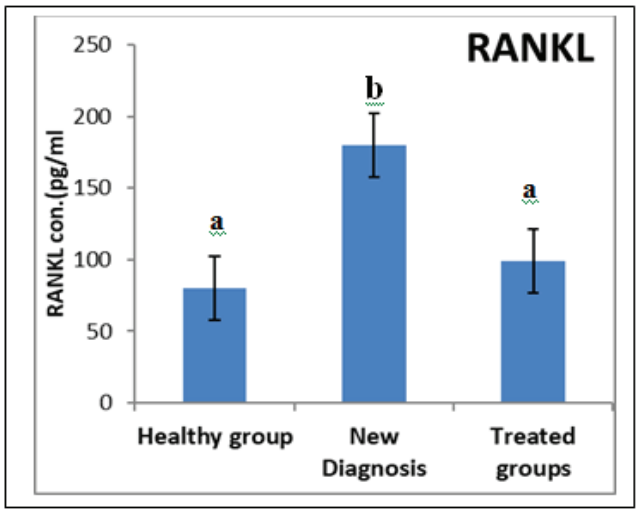
1. **Patients and healthy:** The study subjects comprised of 60 patients suffering from multiple myeloma, 47 patients received drugs of multiple myeloma (28 male and 19 female) and 13 new diagnosis (7 male and 6 female) , age (mean ± SD) = 63.28 ±6.68 (ranging from 45 to 80 years),All patients were suffered from MM and were referred to the Hematology Consultation Clinic in each of the teaching hospitals at Baghdad governorate (medical city), Babil governorate (Marjan city) for diagnosis and/or treatment Those MM cases then have been diagnosed by a specialized haematologist. Diagnosis was based on bone marrow aspiration, biopsies reports and other diagnostic criteria included complete blood counts (CBC), serum protein electrophoresis and renal function (urea and creatinine) treated patients received first-line bortezomib- or lenalidomide-based chemotherapy . The healthy group included 30 individual (15 male, 15 female) age (mean± S.D) = (55.96 ± 4.7) (range 45 to 62), not suffering from any disease, served as a control group and this group matched with patient group. All subjects in this study were taken consent before participation in this study.
2. **Biomarkers analysis:** Venous blood samples were drawn from patients and control subjects by using disposable syringes . Five ml of blood was obtained from each subject, pushed slowly into disposable gel containing tubes, allowed to clot at room temperature for 15 minutes and then centrifuged at 3000 rpm for approximately 10-15. minutes, after that sera was obtained[14] and stored at -20˚C until used. Quantitative detection of RANKL, OPG,CTX type-I and IL-6 in serum was done according to the industrial company (Bioassay Technology Laboratory (China), that depended on the technique of the quantitative sandwich enzyme immunoassay (ELISA) and (TRAP – 5b) was assayed in serum according to the industrial company Express Biotech International (USA).that also depended on the technique (ELISA).
3. **Statistical Analysis:** Analysis of data was made by using Statistical Package for Social Science (SPSS) system/version 23 Results expressed as mean ± Standard division S.D . The analysis of variance (ANOVA),the independent sample T- test, and correlate bivariate were used for this purpose.

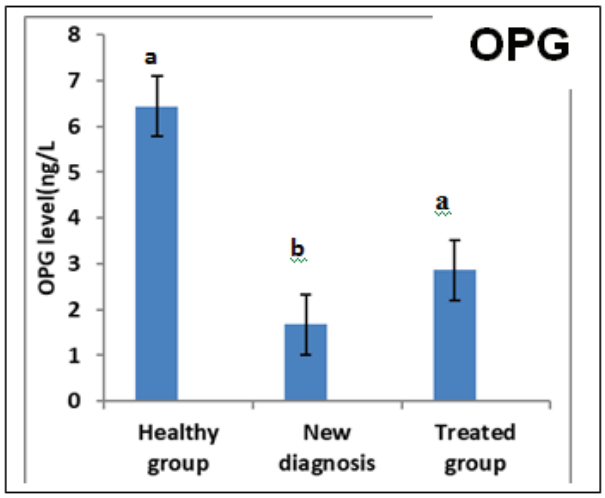
Results

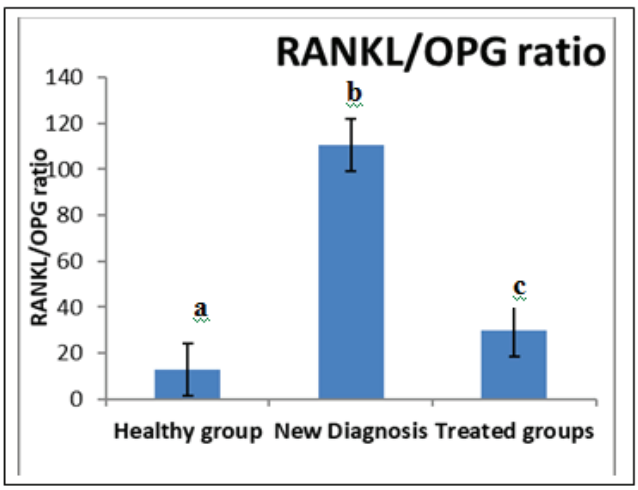
General features and clinical characteristics of patients and healthy controls are reported in Table 1. In a figure(1) shows the results of the statistical analysis of the biomarkers that were included in this work, it was found that there was a significant increase ($p \leq 0.05$) in the level RANKL, RANKL/OPG ratio, IL-6 and enzymatic markers (TRACP-5b and CTX- type I) of myeloma patients groups when compared with healthy control individuals. Also the study showed the presence of a significant decrease ($p \geq 0.05$) in the level of (OPG) when compared with healthy people. As The results showed the presence difference with statistically indication of the level (enzymatic marker (TRACP-5b) in treated patients when compared with new diagnosed patients While the difference was not significant in, RANKL, RANKL/OPG and CTX type –I .

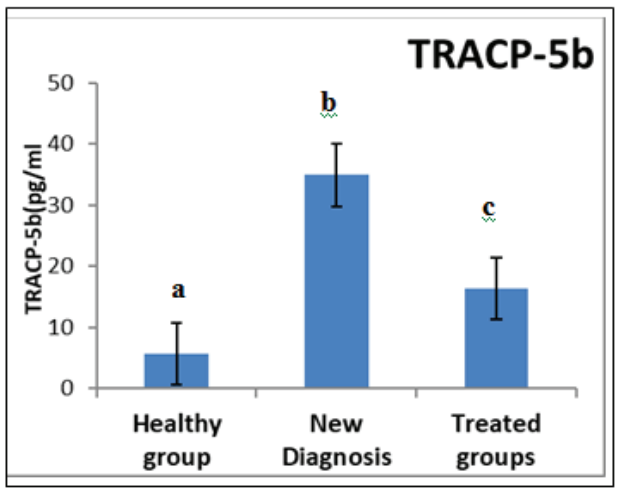
Table (1): Demographic data and clinical characters of MM patients(treated and new diagnostic) and controls Mean ± S.D.

Subjects Variables	MM patients treated	MM patients New diagnosis	Healthy controls
Age	62.34 ±5.68	64.23±6.23	55.96 ± 4.7
Gender (male/female)	(28/19)	(7/6)	(15/15)
RBC counts (10^6/ml)	2.614±0.429	2.250±0.496	5.68±8.34
WBC counts(10^6/ml)	3.79±0.439	3.216±0.381	9.806±0.814
Platelets count(10^3/ml)	210±18.104	187.142±17.43	248±35.09
Hb levels (g/dl)	9.214±0.508	6.90±0.701	12.82±0.944
ESR(mm/hour)	45.32±9.67	87.23±9.67	18.25±3.34
Urea (mg/dl)	41.971±5.455	87.357±40.148	38.43±24.24
Creatinine (mg/dl)	1.703±0.532	3.442±1.180	0.98±1.205
Calcium (mg/dl	10.571±663	12.412±2.027	
Albumin(g/L)3	30.282±1.458	27.671±3.055	36.106±1.656
Total protein(g/L)	79.428±10.361	110.571±9.253	69.33±2.768
Bence Jones protein in urine	30 (positive) 17 (negative)	(13) Positive (0) negative	(0) positive (30)Negative
Beta 2 microglobulin (g/L)	12.56±9.3	14.563±11.793	0.615±0.351









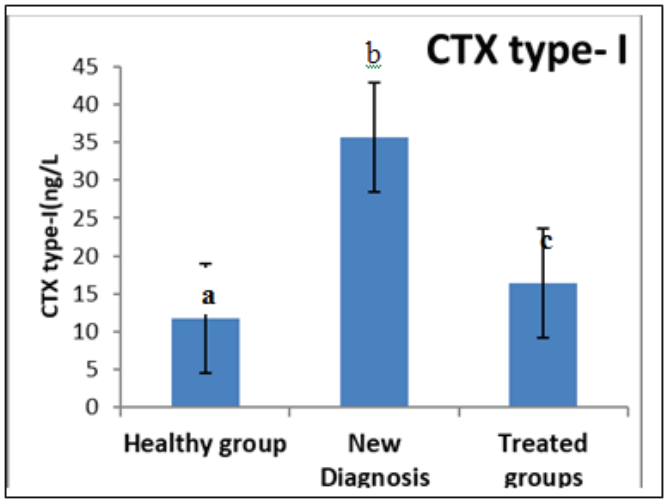


Figure (1) : Levels of biomarkers (RANKL,OPG, RANKL/OPG ratio, IL-6, TRACP-5b and CTX type –I) in study groups , Mean ± S.D. level of significant at (p≤0.05). Different letters refer to significant difference between groups . Similar letters refer to non significant difference.

Discussion

The microenvironment of the bone is the primary facilitator of the myeloma where MM plasma cells (PCs) typically reside in and spread through the hematopoietic bone marrow. As result accumulation of myeloma cells in the bone marrow is increased osteoclast activity resulting in lytic bone disease in 80% of patients (Yaccoby *et al*,2016). In normal physiological conditions, osteoblasts perfume essential role in recruitment of osteoclast precursors and induction of osteoclast formation via production of osteoclastogenic cytokines and chemokines (Kohli and Kohli, 2016). Myeloma bone disease patients is characterized by reduced number of osteoblasts on bone surfaces adjacent to myeloma cells (Terpos *et al*, 2019), this explain that myeloma cells may play a direct role in increasing osteoclast activity through production of key osteoclastogenic factors. while recent studies showen that myeloma plasma cell stimulate indirectly the formation of osteoclast through disruption of the balance of osteoprotegerin and the receptor activator of nuclear factor-B ligand (RANKL) in the bone marrow (Raji *et al*, 2019), other studies suggested that myeloma cells express RANKL or macrophage inflammatory protein-1 and can directly induce differentiation of OCosteoclast progenitors into osteoclast [7]. This may explain how myeloma cells induce osteoclastogenesis in bone areas highly infiltrated by myeloma cells with reduced number of osteogenic cells. However,. In our work we test level of RANKL, OPG, TRACP-5b and CTX type –I as markers of bone resorption To study the relationship between myeloma cells and osteoclast in myeloma patients and knowledge effect the treatment on these biomarkers , we established that levels of RANKL rise significantly in myeloma patient when compared with healthy control groups as result of binding RANKL its signaling receptor RANK - a tumor necrosis factor receptor (TNFR) family member - on the surface of osteoclast precursor cells, leading to the fusion of these cells into multinucleated cells which then differentiate into mature osteoclasts So it plays an important role in skeletal-related events in patients multiple myeloma [8]. many Previous studies have demonstrated that the (IL-6)/signal transducer and activator of transcription 3 (STAT3) plays a central role in osseous metabolism and remodeling by signaling pathway in osteoclasts and osteoblasts [9], our results shown elevated in Il-6 concentration in MM patients this perhaps this cytokine implicated in bone metabolism and is principally secreted by myeloid precursor cells and myeloma cell as inflammatory factors in addition IL-6 enhances osteoclast differentiation, whereas it sustains MM cell survival .

On the other hand, the results of the current study showed a clear decrease in the level of OPG in myeloma patients compared with the healthy group, this finding, agreeing with its mention Seidel and his colleagues 2013, that OPG binds to heparan sulfate chains of syndecan-1 expressed on the surface of myeloma cells. Syndecan-1 is shown to be involved in internalization of heparan sulfate-binding molecules, but whether OPG is eliminated by this route is unknown. In later stages

of the disease, bone formation and osteoblast function are impaired, (Bataille *et al*, 1989)which gives an explanation to the reduced OPG levels in patients with overt bone destruction. while there was an insignificant increase in the treated myeloma patients group compared with the new diagnosed myeloma patients This is due to the role bisphosphonates impair osteoclastogenesis and disrupt osteoclast activity.

To have a better picture of bone formation and bone destruction in MM patients, we checked the levels of serum CTX -I and TRACP-5b a marker of bone resorption that has been demonstrated useful to evaluate osteoclast activity in MM patients [5] In our work level of serum CTX –I and TRACP-5b of MM patients are significantly elevated compared with healthy control groups , despite the reduction of serum CTX levels after bortezomib treatment when compared with new diagnosis myeloma patients . This observation . correspond into Giulian *et al* (2007) [12]. indicate that bortezomib increases osteoblast differentiation in human mesenchymal cells without affecting the number of osteoblast progenitors and the viability of mature osteoblasts. In vivo and in vitro observations support the hypothesis that both direct and indirect effects on bone formation process could occur during bortezomib treatment.

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Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved and all experiments were carried out in accordance with approved guidelines.

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