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Estimation of Beta Two Microglobulins, Fetuin-A, Resistin Serum Level in Iraqi Multiple Myeloma Patients

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Abstract

Multiple myeloma is hematological disease produces many complications in the bone, kidney, neural and other complications. The study aims to measure serum biomolecules like fetuin-A and resistin and determined the possibility to use these biomarkers as disease predictor. blood samples were isolated from 58 patients and 24 sex and age-matched control, serum then isolated, and proper ELISA kit then used to a determined level of β2 microglobulin, resistin, and fetuin-A. The result demonstrated significant increase in β2 microglobulin, fetuin-A and resistin in patients compare to control (1.347±0.714 vs. 0.913±0.253), p = 0.000, (14.003±10.352 vs. 9.259±4.264), p= 0.005, (1.967±3.595 vs. 0.604±0.622), p = 0.009, respectively. These differences give the possibility to use these biomolecules as a predictor in multiple myeloma.

Keywords: Multiple myeloma,Fetuin-A,Resistin.

مقارنة بين مستوى الفيتون أي والريزستين بين المرضى بمرض الورم النخاعي والاصحاء

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الخلاصة

الورم النخاعي المتعدد هو احد امراض الدم الذي يسبب مضاعفات في العظم، الكلة، مضاعفات عصبيه وأخرى، الهدف من الدراسة تحديد مستوى بعض الجزيئات الاحيائية في مصل الدم التي هي فيتوين أي ،ريسزتين وتحديد احتمالية استخدام هذه الجزيئات كمتنبئ للمرض . عينات دم عزلت من ٥٨ مريضا و٢٤ شخص صحيح متطابقين من حيث الجنس والعمر. بعدها عزل المصل وباستخدام كتات الالايزه خاصة لتحديد مستوى بيتا٢ مايكروكلوبيولين، فيتوين أي، ريزستين. النتائج أظهرت زيادة محسوسة في مستوى بيتا ٢ مايكروكلوبيولين في المرضى مقارنة بمجموعة السيطرة (١,٣٤٧±٠,٧١٤ ضد ٠,٩١٣±٠,٢٥٣) قيمة p= ٠,٠٠٠، فيتوين أي اظهر زيادة محسوسة مقارنة بمجموعة السيطرة (١٠,٥٣٢±١٤,٠٠٣ ضد ٤,٢٦٤±٩,٢٥٩) قيمة p=٠,٠٠٥، ريسزتين اظهر زيادة محسوسة مقارنة بمجموعة السيطرة (١,٩٦٧±٣,٥٩٥ ضد ٠,٦٢٢±٠,٦٠٤) قيمة p= ٠,٠٠٠، هذه النتائج تطينا احتمالية الاستفادة من هذه الجزيئات الاحيائية كمتنبئ لمرض الورم النخاعي المتعدد.

الكلمات المفتاحية: مرض الورم النخاعي ، الفيتون أي ، الريزستين .

Introduction

Multiple myeloma is a malignant disease characterized by a defect in plasma cells that result in a change in different organs including bone, kidney, and others [(1)].

The disease starts as monoclonal gammopathy of undetermined significance (MGUS) [(2)], which is developed to smoldering (asymptomatic) myeloma and finally becomes overt (symptomatic) myeloma [(3)].

The bone disorder is the most common complication in the MM, the damage that occurs in the bone result from stimulation of osteoclast formation and activation that occurs in the area of the bone that is closed to myeloma cell. Besides, to increase the bone resorption, there is a decrease in bone formation have been reported and this attributed to the suppression effect of myeloma cell on osteoblast cell and so inhibit bone formation [(4)].

The first common cause of the renal disorder is due to light chain immunoglobulin (LCI) [(5)], normally the monoclonal light chain reaches into proximal tubule after glomerular filtration that undergoes endocytosis in that cell by a specific scavenger receptor and then degrades in the lysosome [(6)]. However, in MM patients the excess production of (LCI) exceeds the catabolic capacity of the tubule to metabolize this protein within the tubular fluid result in accumulation of this protein with Tamm-Horsfall protein (loop of Henle glycoprotein) and form cast that cause a tubular obstruction in the distal area and result in increased intraluminal pressure and decrees in glomerular filtration lead to renal function impairment [(7)]. Infection common among MM patients, one of the important reason is to reduce antibody response and abnormality in complement and granulocyte function, the infections represent one of the most important causes of death in those patients [(8,9)].

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Beta2 microglobulin is a polypeptide presented in the serum its origin is the cell membrane of all nucleated cell in which found in tight junction with major histocompatibility complex 1(MHC1) [10], elevated its level reflected increase the intrinsic kinetic activity of tumor cell including DNA and RNA kinetic [11], therefore, it is important for staging, determining disease severity, response to chemotherapy and prognosis.

Fetuin-A is a multifunctional plasma protein secreted mainly from the liver to the bloodstream, fetuin-A consider as an inhibitor of insulin they act through inhibit tyrosine kinase insulin receptor and thereby participate in insulin resistance and metabolic syndrome and even type 2 D.M.[12,13].

Fetuin-A also has a role in calcium salt precipitation and calcification in the bone and teeth, also reported behaving anti adipokines action through antagonize adiponectin action and thereby affect the fatty acid uptake by adipocyte and precipitate atherosclerosis [12,14].

Fetuin-A is a negative acute phase reactant and therefore its level going to be decreased in inflammatory condition [12].

One study showed the role of fetuin-A in the insulin resistance state as a result of increase free fatty acid in the circulation and they concluded FFA increase proinflammatory cytokine release from adipose tissue through enhancing expression of fetuin-A from the liver that in turn activated toll-like receptor 4 (TLR 4) in the adipose tissue to release these pro-inflammatory cytokines [14].

The normal value of fetuin a 450-600 µg/ml and the difference in the level is genetically determined and the gender difference does not influence it's level [12].

Resistin is one of 114 amino acid Adipocytokines that secreted primarily from macrophage and monocyte in human and from adipocyte in mice [15], play important role in regulating energy homeostasis and triglyceride storage and mobilization through not a well mechanism, resistin augmented type 2 DM by enhancing insulin resistance and enhancing the inflammation and its role in the obesity [16]

A study showed increase resistin level with increasing obesity in the body [17-19], in the inflammatory process, resistin has shown to increase transcription event of several proinflammatory cytokines and thereby increase their levels, examples of these cytokines are interleukin-1 (IL-1), interleukin-6 (IL-6), interleukin-12 (IL-12), and tumor necrosis factor-α (TNF-α) [20-22].

The study aimed to measure the level of fetuin-A and resistin in the serum of patients with multiple myeloma and determined the possibility to use these markers as a predictor of the disease.

Subject, Materials, and methods

The study was conducted in Baghdad city in (Baghdad hospital in the medical city and hematological center) from October 2018 to May 2019 were (58) patients diagnosed to have multiple myeloma and most of them regularly visit the hospital to receive chemotherapy, from the total number of the patients, (36) was male and (22) was female.

The control subjects were randomly selected which were healthy, the control was age, sex, body mass index (BMI) matching to patients group.

Disposable syringe and needles used for blood collection, venous blood sample about six ml collected from patients and healthy volunteers in a plain tube, blood sample were centrifuge at 2000 rpm for 5 minutes to obtain serum.

The serum was divide into an Eppendorf tube and freeze in -20 C^{o} until all serum collected to measure the biomolecules by ELISA technique using ELISA kit.

Determination of serum level of β2-Microglobulin

Serum β2- Microglobulin is determined through the use of a commercial kit from Demediect by sandwich ELISA method. In which highly purified anti-human-beta-2-microglobulin antibodies are bound to microwells. Beta-2-microglobulin, if present in diluted serum, plasma, or urine, binds to the respective antibody, Washing of the microwells removes unspecific components, Horseradish peroxidase (HRP) conjugated anti-human beta-2-microglobulin immunologically detects the bound patient beta-2- microglobulin forming a conjugate/beta-2-microglobulin / antibody complex. Washing of the microwells removes unbound conjugate. An enzyme substrate in the presence of bound conjugate hydrolyzes to form a blue color. The addition of an acid stops the reaction forming a yellow product. The intensity of this yellow color is measured photometrically at 450 nm. The amount of color is directly proportional to the concentration of beta-2-microglobulin present in the original sample [23]. Reference range of β2 microglobulin (1-2 microgram/milliliter) [24].

Determination of serum level of Fetuin-A:

Serum Fetuin-A determined using a commercial kit obtained from CUSABIO, this assay employs the quantitative sandwich enzyme immunoassay technique.

Antibody specific for Fetuin-A has been pre-coated onto a microplate.

Patients and control samples are pipetted into the wells and any Fetuin-A present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for Fetuin-A is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells, following a wash to remove any unbound avidin-enzyme reagent, a

substrate solution is added to the wells and color develops in proportion to the amount of Fetuin Abound in the initial step. The color development is stopped and the intensity of the color is measured [25], Normal value of fetuin a 450-600 µg/ml [12].

Determination of serum level of Resistin:

Serum Resistin determined using a commercial kit obtain from CUSBIO, this assay employs the quantitative sandwich enzyme immunoassay technique, Antibody specific for resistin has been pre-coated onto a microplate, Standards and samples are pipetted into the wells and any resistin present is bound by the immobilized antibody, After removing any unbound substances, a biotin-conjugated antibody specific for resistin is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of resistin bound in the initial step. The color development is stopped and the intensity of the color is measured [25], the normal value of resistin is 7 to 22 ng/mL [26].

The results were expressed as mean (+/-) standard error of the mean. The statistical analysis was performed using statistical package for social science (SPSS 23), independent student (T) test used to test the degree of significant difference between the patients and control, the p-value less than 0.05 considered statistically significant. The correlation between biomolecules is measured by the Pearson correlation test and also performed by (SPSS 23), the value considered significant when the p-value less than 0.05, the negative sign indicates inverse relation while positive correlation indicates direct relation, the value of zero indicates no relationship between the biomolecules.

The power of correlation considered weak when the value of Pearson correlation lower than 0.4, moderate when the value 0.4-0.7, strong when the value more than 0.7.

Results

Demographic characteristic of the patients and controls group:

The demographic presentation of 58 patients with multiple myeloma and 24 control subjects were elucidated in the Table and Figure (1-3).

Table 1. Demographic presentation of patients and controls group

Character	Multiple myeloma patients N=58 (100%)	Control N=24(100%)
Mean age (year)	56.32	55.43
Mean weight (kg)	76.72	73.84
Male gender	36(62%)	15(62%)
Female gender	22(38%)	9(38%)
Body mass index (kg/m^2)	28.2	25.9
Smoker	0(0.00%)	0(0.00%)
Alcoholism	0(0.00%)	0(0.00%)
Pain in extremity	8(13.79%)	2(8.33%)
Hypertension	16(27.58%)	5(20.83%)
Diabetes type-1-	12(20.68%)	4(16.66%)
Arthritis	5(8.62%)	2(8.33%)
Back pain	4(6.89%)	0(0.00%)
Hypothyroidism	1(1.72%)	0(0.00%)
Angina	2(3.44%)	0(0.00%)
Hyperthyroidism	1(1.72%)	0(0.00%)
Osteoporosis	4(6.89%)	0(0.00%)
Prostatic hyperplasia	1(1.72%)	0(0.00%)
Viral infection	20(34.48%)	2(3.44%)
Anemia	5(8.62%)	0(0.00%)

Serum levels of biomolecules:

Table (2) showed a serum level of serum β2 microglobulin, resistin, and fetuin-A in the controls and patients group.

Table 2. Serum level of β2 microglobulin in patients and control group

parameters	Patients group (n=58)	Control group n=24)	Degree of significance
B2 microglobulin (µg/ml)	1.347 ±0.714	0.913± 0.253*	0.000
Fetuin A (ng/ml)	14.003 ±10.352	9.259 ±4.264*	0.005
Resistin (ng/ml)	1.967±3.595	0.604 ±0.622*	0.009

The results expressed in terms of (mean ± standard deviation of the mean), n=number of the subject, (*) = significance difference, (p<0.05) compared to the control group.

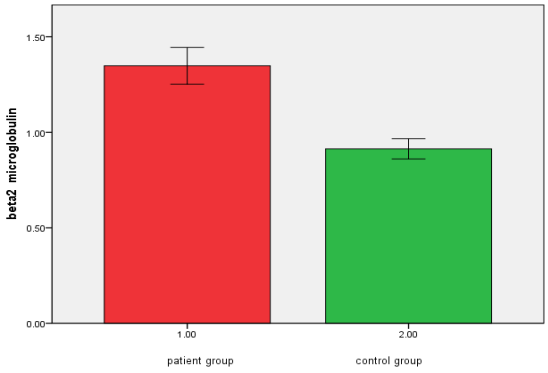

Figure 1. Serum β2 microglobulin levels in patients and control groups.
Significance difference between patients and control groups (p<0.05), β2 microglobulin levels express as (µg/ml).

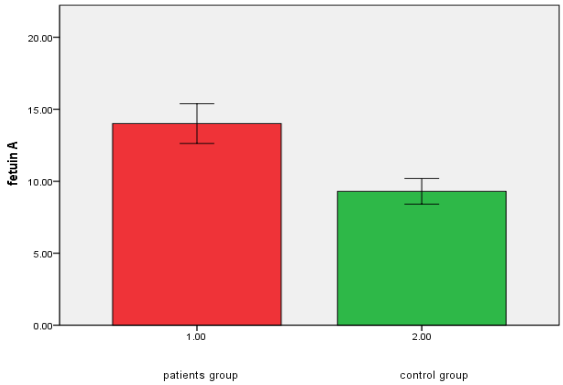

Figure 2. Serum fetuin-A levels in patients and control groups.
Non-significance difference between patients and control groups (p>0.05), fetuin-A levels express as (ng/ml).

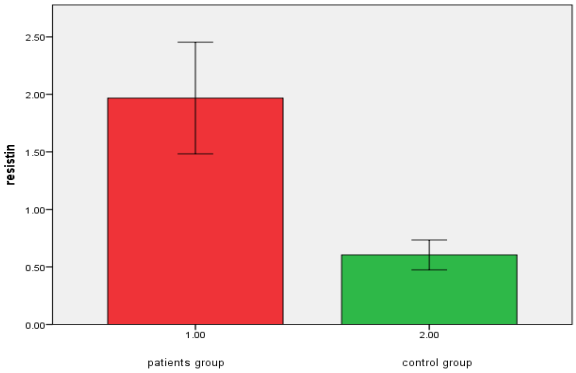

Figure 3. Serum resistin levels in patients and control groups.
Non-significance difference between patients and control groups (p>0.05), resistin levels express as (ng/ml).

Correlation between biomolecules:

Table (3) showed no correlation that has been obtaining between the biomolecules in the study using the Pearson correlation.

Table 3. The correlation between the biomolecules

	β 2 microglobulin	Fetuin-A	Resistin
β 2 microglobulin		**PC= 0.145 Sig= 0.286**	**PC= -0.131 Sig= 0.340**
Fetuin-A	**PC= 0.145 Sig= 0.286**		**PC= 0.145 Sig= 0.286**
Resistin	**PC= -0.131 Sig= 0.340**	**PC= -0.083 Sig= 0.546**	

PC= Pearson correlation, sig= significance 2 tail, *= significant difference.

Discussion

In this study, 58 patients were randomly selected with an average age of those patients about 56 years while most references mention the median age at diagnosis of MM approximately 66-70 years [27-29], however, only 12 patients from 58 (20%) in this study have age more than 65 years and many of them diagnosed to behave MM before several years. To make an explanation for these difference, we suppose that age is not the only risk factor for MM although it is considered an important risk factor, another factor has an important role in the development of the disease, include gender since the male more prone to develop MM by about 50% than female, and this agreement with the present study since from 58 patients was randomly selected, 36 (62% of the total patients) was male [30].

Other factor contributed to MM development include family history, exposure to the radiation or petroleum industries that also have been considerably noted among the patients in the present study, other risk factor is obesity and this also agrees with the study since the mean BMI (kg/m^2) in this study is 28 that consider as overweight [31].

Although bone pain is common in the patients with MM, few patients in this study suffer from pain (Arthritis 5 (8.62%), Back pain 4 (6.89%), Pain in extremity 8(13.79%)) this may be attributed to the use the treatment that improves patients case.

The current study showed a significant increase in β2 microglobulin level in the patient's group compare to the control group despite most of the patients received MM therapy, this indicates the disease is active, that mean patients treatment not guarantee to produce improvement, one study showed that half of the patients in the study with MM did not improve after taken chemotherapy and level of β2 microglobulin still high [32].

Serum level of fetuin-A

This study demonstrated a significant correlation between patients and the control group. Fetuin-A knows to have a role in tumor progression through enhance tumor cell attachment, invasion, and motility [33]. Different effects of fetuin-A on bone have been reported but its exact action on bone mineral density not well known [34], fetuin-A known to behave a role in preventing vascular calcification in vascular disease through inhibition of hydroxyl appetite formation [35]. While at the bone they act to inhibit transforming growth factor-beta (TGF-β)/bone morphogenic protein (BMP) activity, both cytokines involved in process of osteogenesis, so suppose that is important in inhibiting the osteogenic process [36].

Besides that, also it is considered as a major non-collagenous protein fraction of mineralizing bone [37], their abundance in mineralize tissue especially in bone indicated for its high affinity to the calcium appetite, a high level of calcium and phosphate in the extracellular matrix available as a result of their transport from serum for bone calcification and synthesis, this high amount mineral subject to calcify out of the collagen fibril of the bone, so, fetuin-A here provide a barrier to prevent extracellular fibrillar mineralization and promote intracellular mineralization of fibrillar collagen through inhibiting calcium phosphate appetite formation extracellularly [38].

This is supported by the fact that fetuin A deficient mice are characterized by increased thickness of cortical bone and this pointed to increase calcification in the extracellular matrix [39].

Because it is clear that MM produce resorption and decalcification of the bone [40-43], one of the possible explanations of fetuin-A elevation in MM is the liberation of abundance fetuin-A from bone tissue to the serum during the decalcification process.

Murat Yuce *et.al*. suggested that decalcification diseases like osteoporosis and osteopenia have a considerable effect on fetuin A levels [44].

Although one study showed a decrease in the level of fetuin-A among post-menopausal osteoporotic patients [24], this could be attributed to the age rather than osteoporosis since age produce physiological change cause decrease in fetuin-A production that in turn produce osteoporosis, this explanation is possible since fetuin-A have been reported as a protein of fetal life and level going to decrease after birth [45], other study showed an inverse relationship between fetuin A level and age [46].

Serum level of resistin

The present study demonstrated a significant correlation between patients and the control group.

Resistin has been noted to be expressed in macrophage peripheral blood mononuclear cells [47] and BM as major tissue that secrete resistin [48], several inflammatory stimuli like TNF-α, IL-6 report to stimulate resistin secretion from macrophage and monocyte [49].

Resistin upon it releases from macrophage and monocyte stimulate further signal include proinflammatory cytokine, several intracellular signals activated by resistin, one of these signals involve PI3K/AKT that activate NF-kβ that stimulate osteoclastogenesis that in turn augmented MC action on BM [50].

On the cytokine level, a possible explanation for the correlation between resistin and MM disease base on previous studies, first IL-6 that stimulates resistin secretion it also stimulates MC as mention before, MC and stroma cell, in turn, upregulate and secret IL-6 [51-53].

Another cytokine involve in resistin secretion, which is TNF-α also reported to secreted from MC and MC stroma cell interaction [54-55], resistin, in turn, have been reported to stimulate IL-6 secretion from different tissue [56,57], so it possible to say there

is direct relationship or synergism between resistin and MC development.

The role of resistin in other malignant disease gives further support for the role of resistin in MM, One study demonstrated a high level of resistin among patients with colorectal cancer and suggest resistin as a risk factor for colorectal cancer [58], another study showed an increase in resistin level in the woman with breast cancer [59].

Another research found a high association between resistin and lung cancer [60],

Conclusions

1- An increase in fetuin A level could be considered as one of the predictors of bone deterioration that occur as a complication of myeloma and useful as a marker to monitor the bone status in the disease prognosis.

2- An increase resistin level indicated its important role in the inflammatory process in multiple myeloma and a treatment strategy directed to antagonize resistin action might be beneficial.

3- Fetuin-A and resistin could be considered as a predictor of MM disease**.**

Recommendations

1- Determination of Fetuin A level at a different age.

2- measure fetuin A level in bone marrow tissue to detect its liberation during the process of bone resorption

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