



Study the activity of Tryptase And Beta 2- Microglobulin Levels In the Sera of Patients With Chronic Renal Failure And Rheumatoid Arthritis

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ABSTRACT

This work was mainly designed to study the levels of Mast Cell Tryptase & Beta 2-Microglobulin in serum of patients with chronic renal failure (CRF), which they were in the end stage of renal disease (ESDR) depending on hemodialysis (HD) and chronic renal failure patients with Rh.arthritis called (renal Osteodystrophy). Blood samples were collected from Kirkuk General Hospital, with their ages between (20-70) years. This study included (135) blood samples including (85) sample for patients with chronic kidney failure and (25) samples for patients with kidney failure and Rh.arthritis in addition to (25) samples from subjects apparently healthy as control group. Results were obtain A highly significant increase ($p < 0.001$) in the levels of tryptase and Beta2 -Microglobulin in serum of patients with Chronic renal failure and renal osteodystrophy disease comparwith normal individuals

Keywords: Chronic renal failure & Rheumatoid Arthritis, Tryptase, Beta 2-Microglobulin

دراسة فعالية مستويات أنزيم التربتيز وبيتا ٢ مايكروكلوبيولين في مصل

المرضى المصابين بالفشل الكلوي المزمن والتهاب المفاصل الرثوي

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الملخص

صمم هذا البحث بشكل رئيسي لدراسة مستويات أنزيم التربتيز وبيتا 2 مايكروكلوبيولين في مصل المرضى المصابين بالفشل الكلوي المزمن والتهاب المفاصل الرثوي المسمى بالمرض العظمي الكلوي. تم جمع عينات الدم من مستشفى كركوك العام ، الذين تتراوح أعمارهم بين (20-70) سنة، وشملت الدراسة (135) عينة، (85) عينة لمرضى الفشل الكلوي المزمن و (25) عينة لمرضى الفشل الكلوي والمصابين بالتهاب المفاصل الرثوي ، و (٢٥) عينة للأصحاء استخدمت كمجموعة سيطرة للمقارنة وأظهرت النتائج وجود ارتفاع معنوي عالي ($p < 0.001$) في تركيز أنزيم التربتيز و $\beta 2M$ في مصل دم مرضى الفشل الكلوي المزمن ومرضى مع التهاب المفاصل (مرضى العظمي الكلوي) مقارنة مع مجموعة السيطرة (الأصحاء) .

الكلمات الدالة: الفشل الكلوي المزمن، التهاب المفاصل الرثوي ، تربتيز، بيتا ٢-مايكروكلوبيولين

1.Introduction

Chronic kidney disease (CKD) describes abnormal kidney function and/or structure. It is common, frequently unrecognised and often exists together with other conditions (such as cardiovascular disease and diabetes). Moderate to severe CKD is also associated with an increased risk of other significant adverse outcomes such as acute kidney injury, falls, frailty and mortality. The risk of developing CKD increases with age [1].

Rheumatoid Arthritis (RA) is an autoimmune disorder that can cause aching and swelling in joints[2].

Tryptase Mast cells are granulated effector cells of the immune system and are believed to play a role in inflammation, tumour angiogenesis, immune regulation, and tissue repair. Tryptases are active at neutral pH, and their production helps distinguish mast cells from other leucocytes [3,4].

Tryptases (EC 3.4.21.59) are tetrameric serine protease enzymes secreted by human mast cells and have a molecular weight of ~ 134kDa (monomer ~ 26-35kDa) [5]. Tryptases account for ~ 25% of the total protein content of mast cell granules, and are stored in their active form prior to release during mast cell degranulation . However, their actions appear to be restricted to the extracellular milieu[6]. The arrangement of tryptase's catalytically active subunits produces a small oval central pore (size~50×30Å), resulting in restricted accessibility for substrates and inhibitors[7]. The tryptase monomer is arranged with six externally exposed domains, which interact with its external environment, including its neighbouring monomers, when in the tetramer formation[8]. These domains are tryptase's surface loops, and are named the "37-loop", the "60-loop", the "70- to 80- loop", the "97-loop", the "147-loop", and the "173-loop" . As these loops surround the active site, any changes in these loops can potentially alter the substrate specificity of tryptase^[8,9] In addition, tryptase contains a catalytic triad (His-57, Asp-102, Ser-195 (chymotrypsin numbering1)), which is essential for its proteolytic activity[9].

Multiple human tryptases have been identified, including α , β , δ , γ and ϵ [10] , however, uncertainty exists as to whether all forms are functional. The genes that encode human tryptases are located in a cluster within a 2.5Mb region, on the short arm of chromosome 16, at position 16 p13.3[7] . All known human tryptase genes have a six exon/five intron organisation, which is approximately 1.8kb long. This gene architecture differs from that of other mast cell or leucocyte serine proteases. Tryptases contain a 30-amino acid prepropeptide

followed by a 245-amino acid catalytic domain. Although the 5L regulatory region is similar to other serine proteases, it is unique due to its separation from the initiator Met codon by the first intron .Tryptases have been found in several mammals (e.g. human, dog, mouse, rat, gerbil, sheep and cow)[6].Although closely related in primary structure, α and β -tryptase differ markedly in their biochemical properties. β -Tryptase isolated from mast cells or produced in recombinant form is a tetramer composed of four identical catalytic subunits[11]. The quantity of catalytically active tryptase per mast cell (10–35 pg) is dramatically higher than the levels of proteases found in other granulocytes. What regulates tryptase activity after its release in vivo is uncertain, because the tetrameric enzyme resists inhibition by biologic inhibitors of serine proteases. Regulation might occur, in part, when basic proteins, such as ant thrombin III, dissociate the enzyme from heparin, but this is slow and incomplete, providing an unsatisfactory mechanism for tightly regulating catalytic activity. Another possibility for regulation arises from observations that b-tryptase degrades fibrinogen approximately 50-fold faster at pH 6 than at pH 7.4 [12,13].

Although a number of substrates have been identified for tryptases in vitro, their true biological roles and targets are still unclear. However, they are reported to induce micro vascular leakage and inflammatory cell accumulation, and regulate mast cell activation. They are therefore important mediators of inflammation[7]. And have a prominent role in diseases such as asthma, inflammatory bowel disease, and rheumatoid arthritis[14]. In addition, tryptase activity can be inhibited by synthetic protease inhibitors, and several such therapeutic approaches have shown clinical efficacy in the treatment of asthma and ulcerative colitis[15].

Human mature β -tryptase is stored in the mast cells granules and released upon activation while α -tryptase is apparently processed only to the proenzyme stage and is constitutively secreted along with β protryptase. In healthy individuals, only α -tryptase can be detected whereas β -tryptase is undetectable. However, significant elevations of circulating β -tryptase levels were observed in patients with allergic diseases[16].

The common clinical sign of allergic hypersensitivity reactions in skin is edema, which caused by increases in vascular permeability[17].It is reported that tryptase may contribute to vascular permeability by the direct or indirect generation of bradykinin from kininogens. Mast cell tryptase increases intracellular Ca^{2+} , leading to elevation of paracellular permeability of colonocytes. Intradermal injection of tryptase or mast cell secretagogue compound 48/80 in rats can induce the immediate cutaneous reaction and increase dermal micro vascular

permeability, which can be inhibited by potent and specific tryptase inhibitor nafamost at or synthetic tryptase inhibitor[18].

- inflammation

Tryptase is a mediator of inflammation best known for its role in anaphylactic allergic reactions; it is the major mast cell protein constituent. Mast cells are able to initiate, amplify and coordinate various immune responses. These cells are usually poorly represented at kidney level, and their proliferation is related to progressive renal dysfunction and a poorer prognosis in different nephropathies, such as diabetic nephropathy, hypertensive nephropathy, chronic transplant rejection, tubule interstitial nephropathies and primary glomerulopathies[19,20]. Thus, renal interstitial mast cell infiltration could play a role in fibrosis and renal failure progression, as occurs with other inflammatory cells such as macrophages. Despite mast cell involvement in various nephropathies, there are no plasma tryptase measurements available from a population with renal failure on conservative treatment. In hemodialysis (HD) patients, elevated tryptase levels have been reported on few occasions[21].

- obesity

Evidence derived from human data supports an association of mast cells and obesity since obese subjects had higher serum tryptase levels and an increased number of mast cells stained with tryptase than lean individuals in white adipose tissue[22,23,24]. In the same setting, tryptase has also been associated with older age, fasting glucose, total- and LDL-cholesterol and fasting triglycerides, and several studies have established an association of blood tryptase levels with atherosclerotic plaque instability[25].

Beta-2 microglobulin(β 2M): is a non-glycosylated, a low molecular weight protein (11600 Da) protein that is freely filtered by glomeruli, reabsorbed by renal tubule and destroyed , found on the surface of lymphocytes and other nucleated cells. Free molecules are also detectable in the plasma as products of cell turn over, particularly from lymphocytes. The serum concentrations of β 2M closely depend on renal function because the kidneys are the main site of clearance. The amount of serum β 2M is very low in the healthy individuals. Its levels increase in case of inflammatory, immunologic and neoplastic events[26]. The β 2M is a major component in dialysis-related amyloidosis, a disabling disease affecting long-term dialysis patients. Several studies have used β 2M as a biomarker in diabetic patients, but little



is known about the advantages of using β 2M over other commonly used parameters, such as serum creatinine and BUN for detection of the kidney disease[27]. Clinically the appearance of significant amount of this protein in urine is one of the earliest sign of almost all renal diseases. Serum creatinine is affected by factors other than GFR, in particular muscle mass and meat intake. β 2 Macroglobulin is released at constant rate in normal subjects readily filters through the glomerular capillary wall, over 99.9% being reabsorbed and catabolised in proximal tubules with virtually no return of the filtered protein to the circulation. β 2M is therefore theoretically a highly suitable biomarker of renal dysfunction[28].

Aim of the Study: Estimate the levels of tryptase and Beta-2 microglobulin in relations with kidney and arthritis patients.

2. Materials and Methods

This study was conducted in Kirkuk General Hospital on 110 patients who had kidney failure and Rheumatoid arthritis in addition to 25 blood samples from healthy people starting from September 2013 to July 2015, The clinical status of patients have been diagnosed by doctors specialized in artificial kidney Department the questionnaires of patients have been filled in for every patients their ages ranged from 20 to 70 years has been rated the samples studied by group, as follows:

Patients and control were divided into three groups:

Group 1: It included a total of 85 patients (50 males and 35 females); their ages ranged from 20-70 years for females and males. All of them are suffering from kidney Failure diseases.

Group 2: It included a total of 25 patients (10 males and 15 females); their ages ranged from 20-70 years for males and females. They were suffering from Renal Failure with Arthritis inflammation .

Group 3 : This group was include 25 apparently healthy subjects as control group (12 males and 13 females), and their ages ranged from 20to 70 years .

The Tryptase & Beta 2 microglobulin assay employs the quantitative sandwich enzyme immunoassay technique suppliers by cusabio and DRG-USA Companies [29,30].

STATISTICAL ANALYSIS : The Data have been analyzed by Using computer system(mini tab) according to the chart analysis (T- test and F-test). The Arithmetic mean were compared by using the (Duncan Test) .The level of probability $P < 0.001$ =highly significant $P < 0.05$ = significant, $P > 0.05$ = non-significant,

3. Results and discussion

3.1 Estimation of Serum tryptase activity

The current study showed highly significant increase ($P \leq 0.001$) in the activity of serum tryptase in patients with chronic renal failure & chronic renal failure with arthritis as shown in Table (1) and Fig. (1) , where the results were (33.5 ± 3.0) and (34.7 ± 5.6) compared with control groups (14.6 ± 2.2) .

Table (1) : Activity of tryptase in patients with CRF & CRF with Arthritis compared with control group.

Parameters Groups	Mean± SD		
	G1(n=85) CRF	G2(n=25) (CRF+Arthritis)	G3(n=25) Control
Tryptase(ng/ml)	33.5±3.0	34.7±5.6	14.6± 2.2

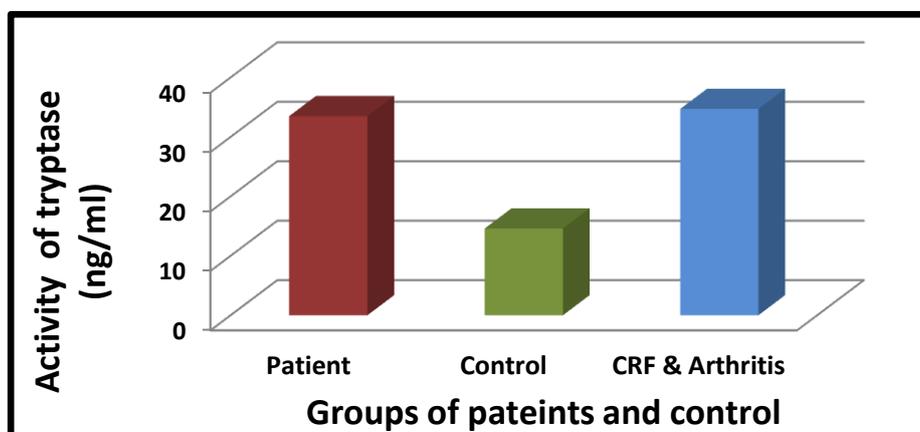


Fig. (1): The activity of tryptase in the Serum of patients with CRF & CRF with Arthritis compared to control group.

When determination the activity of tryptase according to age groups, the results showed significant increase in the age group of (40-50) years and the result were (44.07 ± 9.05) , compared to the other age group as shown in Table (2) and Fig. (2) .

Table (2): The Activity of tryptase in the Serum of patients with chronic renal failure according to age groups.

Age(Years) Parameter	Mean± SD Tryptase(ng/ml)
20-30 (n=13)	27.18 ± 3.76
30-40 (n=8)	22.33±4.16
40-50 (n=18)	44.07± 9.05
50-60 (n=21)	31.14± 3.30
60-70 (n=25)	34.58± 5.09

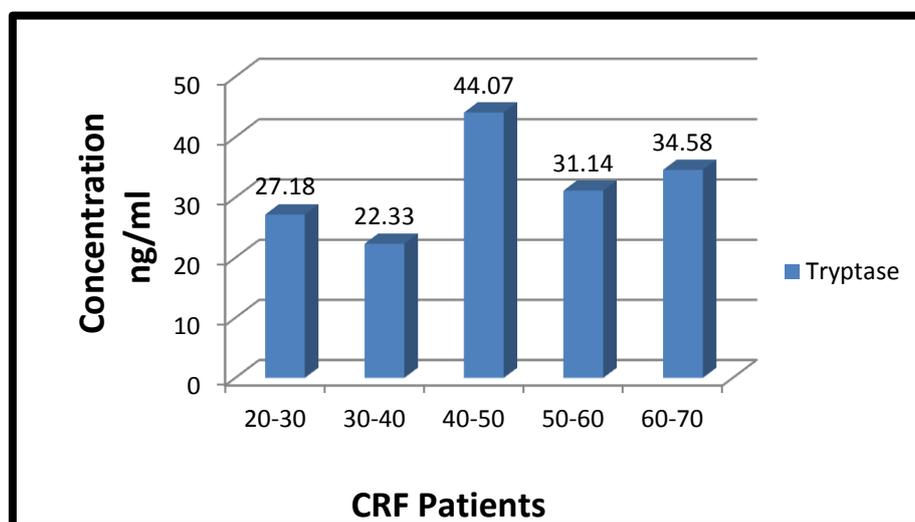


Fig. (2): The Activity of tryptase in the Serum of patients with chronic renal failure according to age groups.

While when estimation the Activity of tryptase according to gender groups there were no significant differences between them (33.4 ± 4.2), (33.5 ± 4.5) in males and females respectively, as shown in the Table (3) and Fig. (3) .

Table (3): The Activity of tryptase in the Serum of patients with chronic renal failure depending on gender .

Sex	Mean± SD
Parameter	Tryptase(ng/ml)
Male(n=50)	33.4± 4.2
Female(n=35)	33.5±4.5

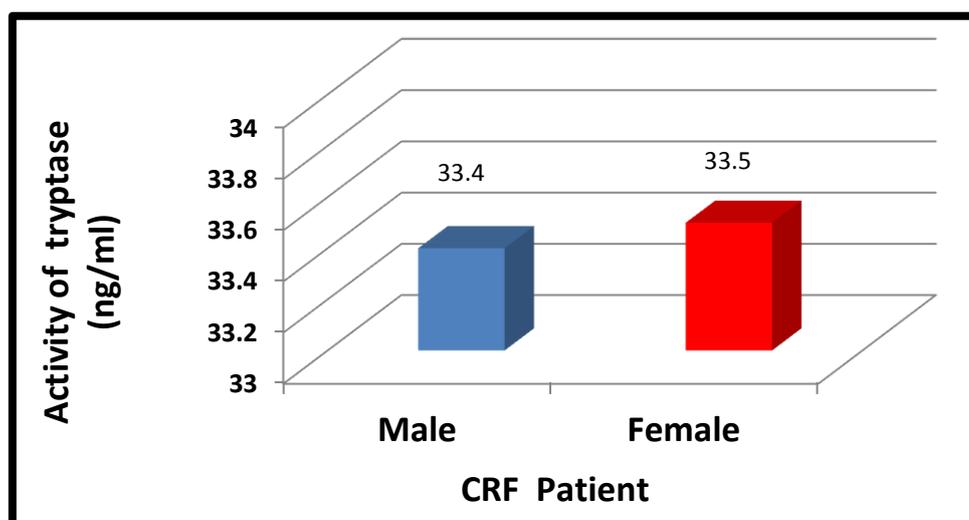


Fig. (3): The Activity of tryptase in the Serum of patients with chronic Renal failure according to gender.

The pathogenesis of high tryptase activity in renal disease is unclear. It is unknown whether the presence of renal failure may cause abnormalities in tryptase clearance or metabolism[31].Tryptase is a neutral serine protease with a molecular weight of 134 kDa, making it difficult to eliminate from the plasma via the kidney. Tryptase main regulatory mechanism seems to be dependent on its dissociation from heparin at the local level. Heparin is released together with tryptase from mast cell granules, which allows for stabilization of

tryptase . Some basic substances have a greater affinity for heparin than does tryptase, and can therefore displace heparin from tryptase and leads to its rapid inactivation. Anti thrombin III is one of the substances with the greatest affinity for heparin. Low levels of anti thrombin III have been found in uremia tryptase stabilization could be prolonged in renal failure via this mechanism as it relates to anti thrombin III or other substances with competitive activity for heparin[32].

It has been known that tryptase is a trypsin-like serine protease, which is stored in the secretory granules of mast cells. When mast cells are triggered, e.g. during allergic conditions, tryptase is released together with other inflammatory mediators. Accordingly, tryptase has been implicated in many diseases linked to inflammation, such as asthma, sudden infant death syndrome, arthritis, multiple sclerosis/experimental autoimmune encephalomyelitis, psoriasis fibrosis and atopic dermatitis[33]. In patients with RA, the number of mast cells and tryptase protein levels increase in synovial fluids[34]. Tryptase has an anti-apoptotic effect on rheumatoid arthritis synovial fibroblasts (RASFs) via the activation of Rho, which promotes proliferation of rheumatoid arthritis synovial fibroblasts and hyperplasia of synovial tissues in RA patients. These results suggest that tryptase from mast cells acts as an effector molecule in inflammatory diseases such as RA[35].

3.2 Estimation of Beta 2-Microglobulin concentration

Results in Table (4) and Fig. (4) have shown highly significant increase ($p \leq 0.001$) in the concentration of Beta 2 micro globulin in the serum of patients with chronic renal failure and CRF with arthritis (3.35 ± 1.45) (3.51 ± 0.29) respectively compared with control group (1.69 ± 0.13).

Table (4): The concentration of Beta 2 Micro globulin in the serum of patients with chronic Renal failure & CRF with arthritis compared with control.

Parameters groups	Mean± SD		
	G1(n=85) CRF	G2(n=25) (CRF+Arthritis)	G3(n=25) Control
β2 Microglobulin(μg/ml)	3.35± 1.45	3.51± 0.29	1.69±0.13

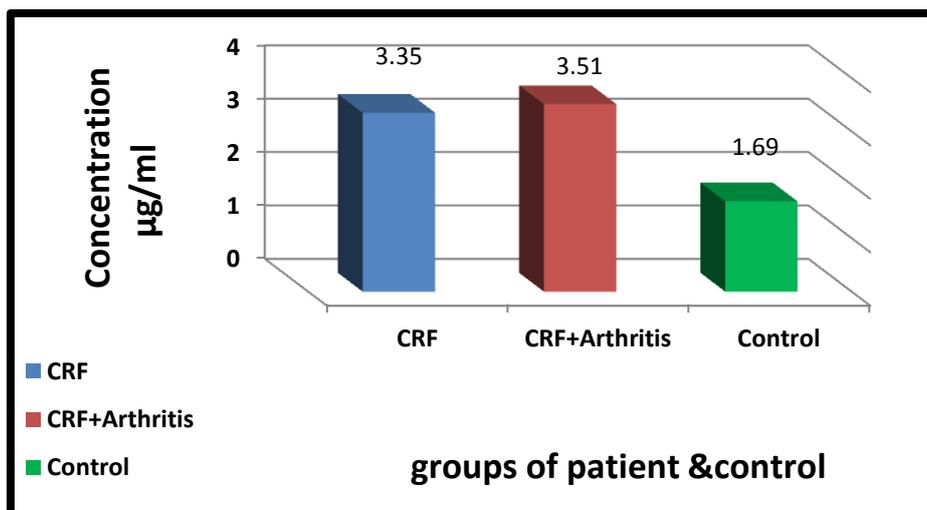


Fig. (4): The concentration of Beta 2 Micro globulin in the Serum of patients with chronic Renal failure & CRF with arthritis compared with control.

Table (5) and Fig. (5) showed no significant differences in Beta 2 micro globulin in age groups (3.195 ± 1.057) (2.913 ± 0.736), (3.379 ± 1.370), (3.660 ± 1.480) (3.457 ± 1.569) respectively for first ,second, third ,fourth ,fifth groups respectively.

Table (5): The concentration of Beta 2-Microglobulin in the serum patients with chronic Renal failure depending to age groups.

Age (Years)	Mean± SD
Parameter	Beta 2 Microglobulin (µg/ml)
20-30 (n=13)	3.195±1.057
30-40 (n=8)	2.913±0.736
40-50 (n=18)	3.379±1.370
50-60 (n=21)	3.457±1.569
60-70 (n=25)	3.660±1.480

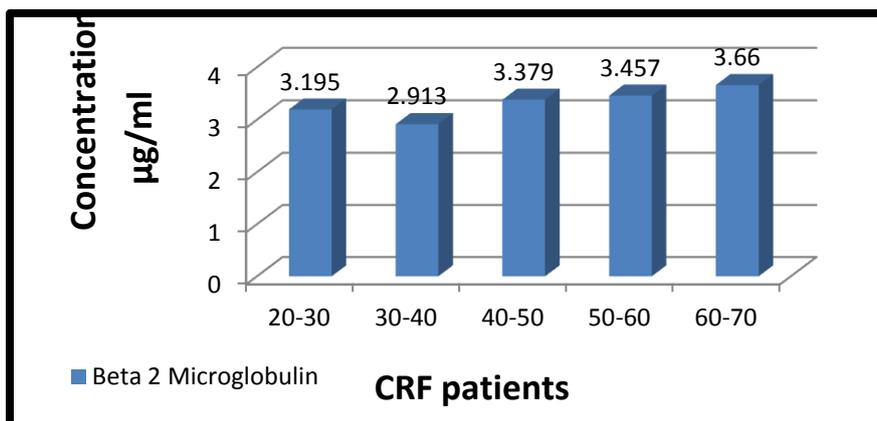


Fig. (5): The concentration of Beta 2 Microglobulin in the Serum patients with chronic Renal failure depending to age groups.

When depending on sex of patient , Table (6) and Fig. (6) showed no significant differences between males and females in the concentration of Beta 2 Micro globulin.

Table (6): The concentration of Beta 2 Micro globulin in the Serum of patients with chronic Renal failure depending on gender.

Sex Parameter	Mean± SD
	Beta 2 Micro globulin (µg/ml)
Males(n=50)	3.40 ± 1.33
Females(n=35)	3.29±1.59

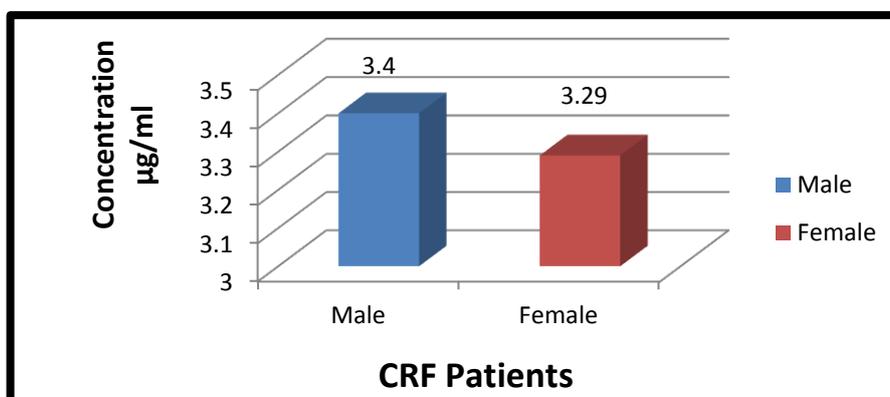


Fig. (6): The concentration of Beta 2 Microglobulin in the serum of patients with chronic Renal failure depending on gender.

The high levels of β_2 Micro globulin in patients could be due to any subclinical acute phase disease, as Beta 2- microglobulin globulin is released in high amount in acute inflammatory conditions. It was depicted that β_2 Micro globulin correlates more closely to glomerular filtration rate in all different levels of renal functions in different groups of patients as compared to creatinine. β_2 microglobulin showed positive correlation with creatinine and blood urea nitrogen in all groups of patients while both Beta 2 microglobulin and creatinine showed inverse correlation to creatinine clearance in both male and female patients of all four different groups. The consistent negative correlation between β_2 Micro globulin and creatinine clearance, indicates the importance of Beta 2- microglobulin in diagnosing renal damage at any level. Measuring β_2 micro-globulin concentrations is a simple and accurate method of detecting minor degrees of renal damage and monitoring the effects of treatment [36].

Since β_2 -microglobulin is produced by lymphocytes whose number is increased in rheumatoid arthritis, the reason for the elevated of Beta 2- microglobulin concentration in active rheumatoid arthritis is obvious. A study showed that patients with active rheumatoid arthritis had significantly higher concentration of serum Beta 2- microglobulin than healthy control which confirmed the results of previous study [37].

4. Conclusions

Human mast cell tryptase and Beta 2- Microglobulin have higher diagnostic validity values in the current study, which may be useful as a diagnostic tool to identify recurrence of the renal Osteodystroph.

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