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Evaluation of Beta2-Microglobulin as a Possible Biomarker for Assessment of Disease Activity in Systemic Lupus Erythematosus and Chronic Kidney Disease

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Abstract: This study presents a prospective analysis to investigate the pivotal role of beta2-microglobulin (β2m) as a serum marker that may potentially help in assessment of disease activity in systemic lupus erythematosus (SLE) and chronic kidney disease (CKD) patients. This study involved a total of 70 patients; 30 patients with SLE and 40 patients with CKD compared to 42 healthy volunteers as control. Levels of β2m, anti-nuclear antibodies (ANA), anti-double strand DNA (anti-dsDNA), C-reactive protein (CRP), urea and creatinine were measured. β2m mean level of male patients was significantly ($P < 0.0001$ and $P < 0.05$) higher than females patients with SLE in stage 1 and 2 respectively. In contrast, CKD females showed a higher significant ($P < 0.05$) β2m level regards to males in stage 4. In SLE, ANA and β2m levels in males revealed the highest relation with SLE stages among other markers. In CKD, serum urea, CRP concentration and β2m levels in male patients exhibited higher relation with disease stages than female patients. β2m estimation may be a useful tracing marker but is correlated with gender being more related with males than females.

Key words: C-Reactive Protein · Serum Creatinine · Anti-Double Strand DNA · Anti-Nuclear Antibodies

INTRODUCTION

Autoimmune diseases are manifestations of immune system malfunction that leads to self- recognition. SLE is a vigorous example of autoimmune diseases that affects various body parts [1]. The onset of SLE can go for long periods undetected thus unresolved complications may evolve.

When SLE is suspected, testing for anti-nuclear antibodies (ANA) is commonly done, but it must be taken in consideration that ANA positivity may be highly misleading due its highly positive prevalence in the general population [2]. A vast array of IgG and IgM autoantibodies that are directed against one or more nuclear components, double stranded (ds) DNA and single stranded (ss) DNA being the most frequent targets are usually detected in serum of SLE patients. Anti-dsDNA antibody is the most prominent marker of lupus diseases, so it is used to monitor disease activity whereas anti-ssDNA is considered to be non specific [3].

Chronic kidney disease (CKD) is caused by the impairment in kidney function that may be due to a disease that attacks any part of the renal system. Also, it may be associated with diabetes and hypertension [4]. SLE also could be responsible for CKD. Creatinine, an amino acid with a molecular mass of 113 Da and urea, a small molecule of only 60 Da are frequently analyzed to detect renal malfunction, but these could be misleading if assayed uncoupled with other biomarkers [5, 6]. It must be taken into consideration that serum creatinine changes may be due to many other factors such as gender, muscle mass, age and drugs [7]. CRP has been suggested as a major marker of various inflammatory conditions [8], may be used as a direct measure for intensity of disease. Serial CRP measurement could provide a prognostic tool for the level of infection, effect of treatment and early detection of relapse [9]. Clinical studies recognized that CRP, apart from being an inflammatory marker, may take part as an independent prognostic indicator of cardiovascular, tumor and autoimmune diseases. CRP is considered a non-specific inflammatory marker, could be more valid as a prognostic indicator rather than a diagnostic one [10].

It is of vital importance to find a labile prognostic biomarker to evaluate the progression of a disease before and/or after treatment. β2 is the light chain of HLA class I and found on all nucleated somatic cells [11] has been

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investigated as a prognostic biomarker for various diseases [12]. It was used as a serum marker of tumor progression in lymphoid malignancies [13]. In addition, serum elevated levels of β2m were detected in patients of a low glomerular filtration rate and in patients of some rheumatic and autoimmune diseases [14]. Elevation of β2m levels in various body fluids (serum, tears, urine….), was directly related with lymphocyte turnover increase [15].

The present study was aimed to investigate the pivotal role of β2m as a serum marker that may potentially help in assessment of disease activity in comparison to the level of CRP and the main diagnostic tests used for SLE and CKD. This is to mark which of them is more indicative in tracing disease activity.

MATERIALS AND METHODS

Study Population: All participants were selected from Ahmed Maher Teaching Hospital. This study was approved by the ethics committee of the General Organization for Teaching Hospitals and Institutes (HAM00061) and the patients were included in the study after giving their informed consent. This cross-sectional study included a total of 70 patients: 40 patients with CKD and 30 patients with SLE compared to control group of 42 healthy volunteers (15 males and 27 females) that are free from diseases. CKD patients are those with a kidney damage or GFR <60 ml/min/1.73 m2 for ≤ 3 month [16]. CKD patients were classified into 3 stages according to calculation of GFR; stage 3 (30–59 ml/min/1.73 m^2), stage 4 (15–29 ml/min/1.73 m^2) and stage 5 (<15 ml/min/1.73 m^2). Inclusion criteria: SLE patients were those with normal or elevated kidney function and have used corticosteroids for treatment. Patients with renal CKD were free of autoimmune diseases. Patients with SLE or CKD were free of hepatitis B, hepatitis C and were selected after testing by qualitative detection of antibodies to hepatitis C virus and hepatitis B surface antigen (HBsAg) by ELISA using Axiom diagnostic kit (Axiom, GmbH-Worms, Germany). Exclusion criteria: Patients were positive for hepatitis B or C and on hemodialysis were excluded.

Estimation of Systemic Lupus Erythematosus (SLE): ANA were detected by ELISA using Quanta Lite from INOVA Diagnostics kit-USA [17]. Diluted serum (1:40) samples, ANA low positive, ANA high positive and negative controls were added for incubation. Enzyme labeled anti-human IgG conjugate was added and incubated. After washing away any unbound enzyme labeled anti-human IgG, the remaining enzyme activity was measured by adding a chromogenic substrate and measuring the intensity of the color spectrophotometricaly at 450 nm. Results were estimated as negative (< 20 units), moderate positive (ranged from 20-60 units) and positive (> 60 units).

Detection of anti-dsDNA was performed by using Quanta Lite from INOVA Diagnostics kit-USA [18]. Each serum's sample was diluted 1:101, calibrator, positive and negative control samples were added to the wells containing a highly purified calf thymus dsDNA. After incubation, unbound sample was washed away and an enzyme labeled anti-human IgG conjugate was added to each well for incubation. The enzyme activity was measured by adding a TMB chromogenic substrate and the absorbance of each well was read at 450 nm. Negative range is 0-200 IU/ml, equivocal range is 201-300 IU/ml, moderate positive range is 301-800 IU/ml and strong positive is to be more than or equal to 801 IU/ml.

Estimation of Renal Function: Measurement of serum creatinine was performed based on the reaction of creatinine with sodium picrate (colormetric-kinetic kit) as described by Jaffe method [19]. Creatinine values were 0.6-1.1 mg/dl for women and 0.7-1.4 mg/dl for women. Serum urea was quantitatively determined using Diamond Diagnostics kit, Cairo, Egypt. Normal serum level of women and men range was 15-45 mg/dl. GFR was calculated according to Cockroft and Gault [20] formula:

$$\text{The GFR (ml/min/1.73 m}^2\text{)} = \frac{(140 - \text{age}) \times \text{body weight}}{\text{value of serum creatinine} \times 72} \times (\text{if females, } 0.85)$$

Measurement of β2-Microglobulin; Serum β2m level was determined quantitatively by using DRG kit (DRG international, USA). Serum samples were diluted 1:101 and then were incubated with mouse monoclonal anti-β2m antibody. Addition and incubation of 200 µl of enzyme conjugate (horseradish peroxidase) was then followed with addition of 100 µl of TMB. Finally, the reaction was stopped by adding 100 µl of stop solution and the absorbance was read at 450 nm with an ELISA reader. Normal values of β2m in serum were expected to be 0-2.0 µg/ml.

Measurement of C-Reactive Protein: CRP was measured according to nephlometric methods of analysis involving the reaction of CRP with the antibody bound to latex particles forming insoluble complexes. The measurement

of CRP was done using Minineph™ Human CRP kit (The binding sit group Ltd, Birmingham, UK). Sera were diluted to 1:40 and 400 µl of minineph CRP buffer (0.099% sodium azide) and 40 µl of minineph reagent (monospecific antiserum contains 0.099% sodium azide, 0.1% EACA, 0.01% benzamidine and 0.05% ProClinTM) were added to 20 µl of diluted sample. CRP concentration was measured using Minineph™nephlometry apparatus. Concentrations of CRP obtained less than 3.8 mg/l were investigated as normal levels.

Statistical Analysis: The present data were analyzed by using SPSS statistical package version 20. Two-way analysis of variance was used to analyze the effects of stages of the disease, sex and their interaction on the studied parameters. In addition, the post-anoval hoc test (least significant difference, LSD) was applied to compare between each two groups. Regression analysis and correlation coefficient were used to fit the relationship between the studied variables. The data of the present study were expressed as means±standard error of mean (SEM).

RESULTS

Systemic Lupus Erythematosus (SLE): Demographic and clinical characteristics of 5 male and 25 female patients with SLE compared to 15 male and 27 female controls are depicted in Table 1. The mean age of female and male SLE patients showed no significant difference between them. The mean age of SLE female patients was significantly lower than female controls. In female patients, the mean level of ANA, anti-dsDNA, CRP and β2m levels showed a significant increase regards to female controls, male patients also exhibited a significant elevation for all markers except CRP as compared to male controls. Moreover, β2m levels of male patients revealed a significant increase compared with female patients. However, a non-significant difference in the mean levels of ANA, anti-dsDNA and CRP was observed between male and female patients (Table 1). The statistical effects of SLE disease stages, sex and their interaction on the levels of ANA, anti-dsDNA, CRP and β2m are represented in Table 2. Levels of ANA, anti-dsDNA and β2m were significantly affected by stages of the SLE disease except CRP, while they were unaffected by sex and its interaction with disease stages except for β2m level.

In male SLE patients, ANA levels in stage 2 revealed the highest percentage of change (766.27%) whereas stage 1 revealed the highest percentage of change (529.96%) in females in comparison with controls (Table 3). ANA mean levels of stages 1 and 2 for males and females were significantly increased compared to their corresponding healthy controls. In male SLE patients, anti-dsDNA level in stage 2 revealed the highest percentage of change (64.19%) while female patients of stage 1 revealed the highest percentage of change (273.55%). CRP concentration of female SLE patients at stage 1 exhibited the highest percentage of change regards to healthy controls. In both male and female SLE patients, β2m levels of stage 2 revealed the highest percentage of change. In addition, mean levels of β2m in both stages 1 and 2 of male and female patients implied a significant rise versus their corresponding healthy controls. In male patients, β2m levels in both stages 1 and 2 showed a significant increase in comparison to the same stages in female patients (Table 3).

Table 1: Demographic and clinical data of control and patients of systemic lupus erythematous (SLE)

	Males		Females	
	Control	Patients	Control	Patients
Number	15	5	27	25
Age (range, years)	24-55	23-44	20-54	20-44
(mean±SEM)	33±2.46	32.2±4.21	33.6±1.82	28.24±1.26‖
ANA (units)	18.68±1.94	144.91±16.95‖	18.99±1.18	116.22±7.43‖
Anti-dsDNA (IU/ml)	137.87±12.99	267.41±3.11‖	133.77±10.28	446.50±81.70‖
CRP (mg/l)	2.69±0.167	3.46±0.024	2.32±0.139	6.01±0.963‖
β2m (µg/ml)	1.99±0.134	16.72±1.46‖	1.85±0.074	6.89±0.675‖*

Data are represented as mean±standard error of mean (SEM).

ANA: anti-nuclear antibodies. Anti-dsDNA: anti double strand DNA. CRP: C-reactive protein, β2m:Beta2-microglobulin.

*: ($P < 0.0001$) significant difference in comparison to male patients.

‖: ($P < 0.0001$) significant difference in comparison to its corresponding control.

‖: ($P < 0.05$) significant difference in comparison to its corresponding control.

Table 2: Two-way ANOVA to test the effects of the stages of SLE, sex and their interaction on the concentration of CRP, β2m, ANA and anti-dsDNA in serum of SLE patients

Factor	Sum of Squares	Df	Mean Square	$F_{calculated}$	Significance level
Stages					
ANA	131732.661	2	65866.331	111.058	P < 0.001
Anti-dsDNA	422373.280	2	211186.640	3.414	P < 0.05
CRP	46.544	2	23.272	2.702	P > 0.05
β2m	1139.898	2	569.949	131.394	P < 0.001
Sex					
ANA	610.609	1	610.609	1.030	P > 0.05
Anti-dsDNA	63083.491	1	63083.491	1.020	P > 0.05
CRP	9.972	1	9.972	1.158	P > 0.05
β2m	128.503	1	128.503	29.625	P < 0.001
Stages and Sex interaction					
ANA	656.834	1	656.834	1.107	P > 0.05
Anti-DNA	69197.308	1	69197.308	1.119	P > 0.05
CRP	17.913	1	17.913	2.079	P > 0.05
β2m	119.898	1	119.898	27.641	P < 0.001
Error					
ANA	39736.471	67	593.082		
Anti-dsDNA	4144679.220	67	61860.884		
CRP	577.162	67	8.614		
β2m	290.626	67	4.338		

P > 0.05: insignificant; P < 0.05, 0.01, 0.001 significant effect at α=0.05, 0.01 and 0.001 respectively. df: degree of freedom. ANA: anti nuclear antibodies, anti-dsDNA: anti double strand DNA, CRP: C-reactive protein, β2m:Beta2-microglobulin

Table 3: The level of ANA, anti-dsDNA, CRP and β2m in serum of male and female controls and different stages of systemic lupus erythematous (SLE) patients

		Stages of SLE patients	
ANA (units)	Control	Stage1(normal kidney function)	Stage2 (higher kidney function)
Male	18.68±1.94	119.55±42.44$^{\blacksquare}$	161.82±0.09$^{\blacksquare}$
% of change		+539.98%	+ 766.27%
Female	18.99±1.18	119.63±8.14$^{\blacksquare}$	102.6018.41$^{\blacksquare}$
% of change		+ 529.96%	+ 440.28%
Anti-dsDNA(IU/ml)			
Male	137.87±12.99	216.39±33.92	226.37±85.15
% of change		+ 56.95%	+ 64.19%
Female	133.77±10.28	499.71±97.78$^{\blacksquare}$	233.66±66.65
%of change		+ 273.55%	+ 74.67%
CRP (mg/l)			
Male	2.69±0.167	3.45±0.050	3.46±0.033
% of change		+ 28.25%	+ 28.62%
Female	2.32±0.139	6.64±1.16$^{\blacksquare}$	3.51±0.323
% of change		+ 186.20%	+ 51.29%
β2m ((µg/ml)			
Male	1.99±0.134	14.94±0.160$^{\blacksquare}$	17.91±2.32$^{\blacksquare}$
% of change		+650.75%	+800%
Female	1.85±0.074	5.90±0.579$^{\blacksquare}$**	10.87±1.53$^{\blacksquare}$*
% of change		+ 218.91%	+ 487.56%

Data represented as mean±standard error of mean (SEM).%of change: percentage of change in relation to healthy control.

$^{\blacksquare}$: (P < 0.0001) significant difference in comparison to the corresponding control.

*: (P < 0.05) significant difference in comparison to the corresponding male

**: (P < 0.0001) significant difference in comparison to the corresponding male

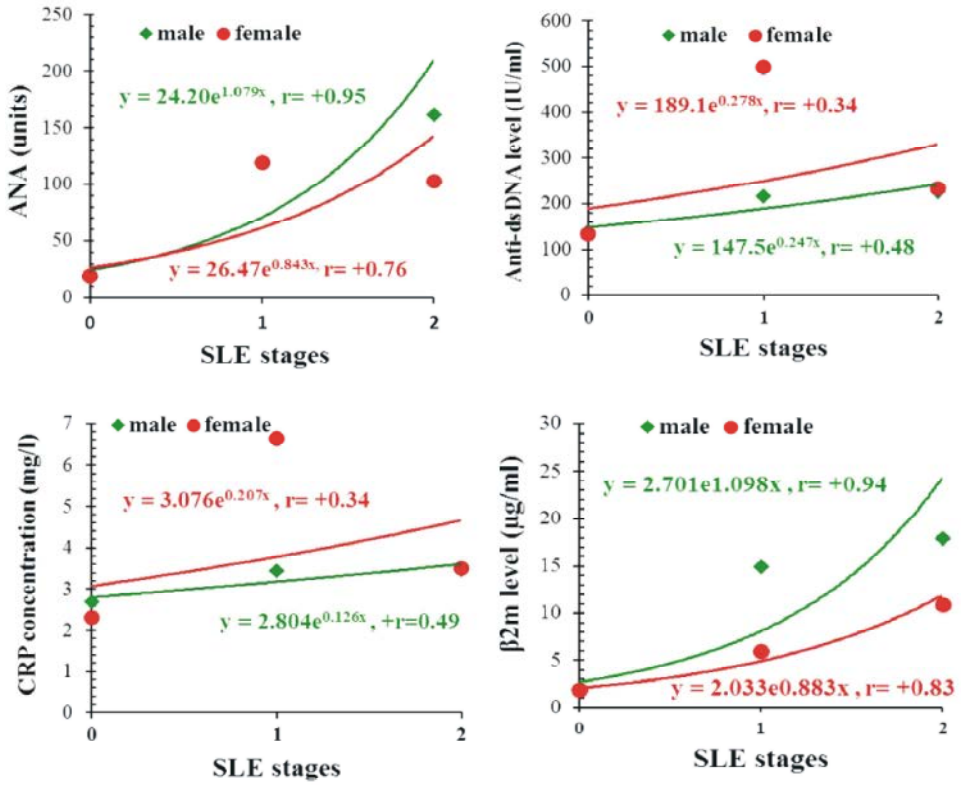

Fig. 1: Relation between stages of systemic lupus erythematosus (SLE) and levels of ANA, anti-dsDNA, CRP and β2m in male and female patients with SLE. r: correlation coefficient.

Table 4: Demographic and clinical data of control and chronic kidney disease (CKD) patients

	Males		Females	
	Control	Patients	Control	Patients
Number	15	17	27	23
Age				
(range, years)	24-55	21-68	20-54	20-70
(mean±SEM)	33±2.46	49.70±3.25[ǀ]	33.62±1.82	52.3±3.67[ǀ]
Creatinine	0.99±0.049	6.97±0.929[ǀ]	0.93±0.031	5.36±0.589[ǀ]
Urea (mg/dl)	29.8 6±1.90	238.94±13.81[ǀ]	29.37±1.27	192.11±14.81[ǀ*]
CRP (mg/l)	2.69±0.167	70.2±6.87[ǀ]	2.32±0.139	72.44±19.74[ǀ]
β2m (µg/ml)	1.99±0.134	13.44±0.806[ǀ]	1.85±0.074	16.47±0.804[ǀ*]

Data are represented as mean±standard error of mean (SEM).

ANA: anti-nuclear antibodies, anti-dsDNA: anti-double strand DNA. CRP: C-reactive protein, β2m:Beta2-microglobulin.

*: ($P < 0.05$) significant difference in comparison to corresponding male patients.

ǀ: ($P < 0.0001$) significant difference in comparison to its corresponding control.

Serum levels of ANA, anti-dsDNA, β2m and CRP concentration were positively correlated with SLE stages in both male and female patients (Figure 1). Additionally, serum levels of ANA, anti-dsDNA, β2m and CRP concentration for male patients showed higher correlation with stages of SLE (r= + 0.95, r= + 0.48, r= + 0.94 and r= +0.49) than female patients (r= + 0.76, r= + 0.34, r= + 0.83 and r= + 0.34) respectively.

Chronic Kidney Disease: The CKD patients enrolled 17 male and 23 female patients with a significant mean of age compared to their corresponding controls. The mean levels of creatinine, urea, CRP and β2m either of male or female patients were significantly higher than their corresponding controls (Table 4). In patients, males demonstrated a higher significant urea level compared to females, however the other markers showed

Table 5: Two-way ANOVA to test the effects of the stages of chronic kidney disease (CKD), sex and their interaction on the concentration of CRP, β2m, creatinine and urea in serum of patients with CKD

Factor	Sum of Squares	df	Mean Square	$F_{calculated}$	Significance level
Stages					
Creatinine	728.054	3	242.685	101.443	P < 0.001
Urea	756166.794	3	252055.598	395.304	P < 0.001
CRP	157949.135	3	52649.712	265.038	P < 0.001
β2m	3287.073	3	1095.691	174.895	P < 0.001
Sex					
Creatinine	10.373	2	5.186	2.168	P > 0.05
Urea	14291.175	2	7145.587	11.207	P < 0.001
CRP	10332.850	2	5166.425	26.008	P < 0.001
β2m	62.056	2	31.028	4.953	P < 0.05
Stages and Sex interaction					
Creatinine	12.828	3	4.276	1.787	P > 0.05
Urea	9366.205	3	3122.068	4.896	P < 0.01
CRP	62389.575	3	20796.525	104.689	P < 0.001
β2m	83.119	3	27.706	4.423	P < 0.01
Error					
Creatinine	174.640	73	2.392		
Urea	46546.550	73	637.624		
CRP	14501.453	73	198.650		
β2m	457.334	73	6.265		

P > 0.05: insignificant; P < 0.05, 0.01, 0.001 significant effect at α=0.05, 0.01 and 0.001 respectively.

df: degree of freedom. CRP: C-reactive protein, β2m:Beta2-microglobulin

Table 6: The level of creatinine, urea, CRP and β2m in serum of male and female controls and different stages of chronic kidney disease (CKD) patients

		Stages of CKD patients		
Creatinine(mg/dl)	Controls	Stage 3	Stage 4	Stage 5
Male	0.99±0.049	2.35±0.150	4.78±0.472‖ ‖	9.00±1.166‖
% of change		+ 137.37%	+ 382.82%	+ 809.09%
Female	0.93±0.031	2.175±0.295‖ ‖	3.06±0.418‖*	7.411±0.523‖
% of change		+ 133.33%	+ 229.03%	+ 696.88%
Urea(mg/dl)				
Male	29.86±1.90	127.50±11.50‖	220.60±25.86‖	270.40±4.49‖
%change		+ 326.99%	+ 638.78%	+ 805.55%
Female	29.37±1.27	83.65±8.05‖*	164.16±10.53‖	238.38±13.23‖
% of change		+184.81%	+ 458.93%	+ 711.64%
CRP(mg/l)				
Male	2.69±0.167	72.80±23.10‖	41.46±5.99‖	84.04±7.47‖
% of change		+ 2606.31%	+ 1441.26%	+ 3024.16%
Female	2.32±0.139	266.92±10.92‖**	4.28±0.343***	44.05±6.332‖**
% of change		+ 11405.17%	+ 84.48%	+ 1798.70%
β2m(μg/ml)				
Male	1.99±0.134	15.86±1.15‖	11.33±1.10‖	14.02±1.11‖
% of change		+ 696.98%	+ 469.34%	+ 604.52%
Female	1.85±0.075	17.51±2.22‖	17.42±2.143‖*	15.72±0.852‖
% of change		+ 846.48%	+ 841.62%	+ 749.72%

Data represented as mean±standard error of mean (SEM).%of change: percentage of change in relation to control.

‖‖: (P < 0.05) significant difference in comparison to the corresponding control.

‖ : (P < 0.0001) significant difference in comparison to the corresponding control.

*: (P < 0.05) significant difference in comparison to the corresponding male.

**: (P < 0.01) significant difference in comparison to the corresponding male.

***: (P < 0.0001) significant difference in comparison to the corresponding male.

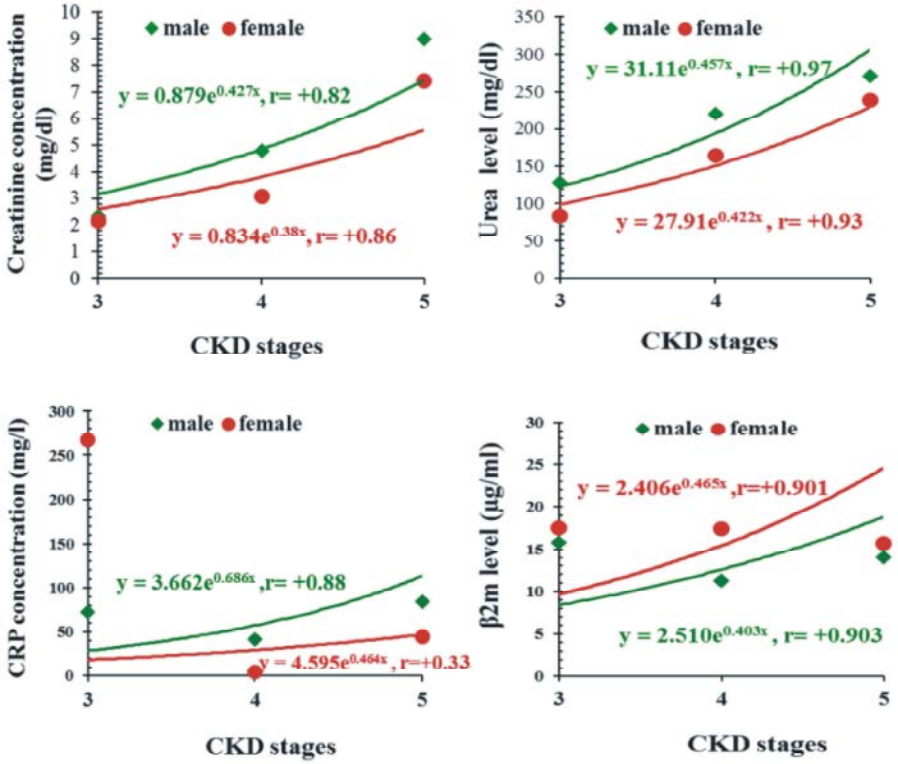

Fig. 2: Relation between stages of chronic kidney disease (CKD) and levels of creatinine, urea, CRP and β2 in male and female patients with CKD. r: correlation coefficient.

non-significant difference. As illustrated in Table 5, all serological parameters including creatinine, urea, CRP and β2m were significantly affected by stages of CKD disease however urea, CRP and β2m were affected by sex. Interaction of disease stages with sex significantly affected on urea, CRP and β2m.

In both males and females, serum creatinine levels of patients in stage 5 revealed the highest percentage of change while stage 3 revealed the lowest percentage of change (Table 6). Serum creatinine level of male patients in stages 4 and 5 was significantly enhanced compared to male healthy controls. In females, creatinine mean level of stages 3, 4 and 5 were significantly increased compared to healthy controls. Moreover, mean level of creatinine of female patients in stage 4 showed a significant decline in comparison to the same stage in male patients (Table 6). In both males and females, serum urea levels for stage 5 revealed the highest percentage of change regards to their corresponding controls. Serum urea levels in stages 3, 4 and 5 of both male and female patients were significantly elevated in comparison to their corresponding healthy controls. Serum urea level of female patients in stage 3 was significantly decreased as compared to the same stage in male patients (Table 6). Serum CRP concentration of stage 5 in male patients and stage 1 in female patients exhibited the highest percentage of change. Females in stage 3 employed a significant rise in CRP concentration while stage 4 and 5 showed a significant decrease in regards to male patients. In both male and female patients, serum β2m levels of stage 3 recorded the highest percentage of change compared to healthy controls. Serum β2m levels of male and female patients in stages 3, 4 and 5 were significantly raised compared to their corresponding controls. In stage 4 of female CKD patients, β2m levels were significantly increased as compared to the same stage in male patients (Table 6).

Serum creatinine, CRP concentrations, urea and β2m levels were positively correlated with kidney disease stages in male and female patients (Figure 2). Additionally, serum urea and CRP concentration for male patients exhibited higher positive correlation with stages of CKD (r= +0.97 and r= +0.88 and) than female patients (r= +0.93 and r= +0.33) respectively. However, serum creatinine levels of female patients showed higher correlation with kidney disease stages (r= +0.86) than male patients (r= +0.82). Serum β2m levels appeared nearly the same correlation in male and female patients.

DISCUSSION

Autoimmune diseases are due to factors that tend to attack self antigens which are contrary to the proposed function of the immune response. SLE is an autoimmune disease that causes damage to various parts of the body. The most indicative criteria for the diagnosis of SLE is the level of ANA as almost 95% of the patients recorded a profound elevated level during the course of the disease. For early diagnosis, ANA level could not be completely depended on as many patients were negative for ANA testing in early disease [21].

Another serological marker that was used for the diagnosis and prognosis of SLE disease was measuring the level of anti-dsDNA antibodies [22]. This could be attributed to the fact that the level of anti-dsDNA was elevated in active disease stages and was associated with progression of lupus nephritis [23], but negative results could be recorded in early stages of the disease, or after medical treatments as well as in clinical remission. Thus, not all SLE patients will be positive for anti-dsDNA [24]. In SLE disease, our results demonstrated that females showed a higher% of change in stages 1 and 2 than males compared with controls. It is deduced that females were much more affected by SLE disease stages. Additionally, mean levels of anti-dsDNA of only female patients in stage 1 showed a significant elevation compared to controls. A number of studies have shown that the increase of anti-dsDNA antibodies titers have been used as a guide for treatment of lupus patients with conventional therapy before flares are clinically apparent [22, 23]. The increase in anti-dsDNA antibodies, as shown by testing at regular intervals, is considered to indicate an increased risk of disease exacerbation [23]. In the present study, anti-dsDNA titers of female and male patients showed less correlation (r= +0.34 and r= +0.48) respectively with the disease activity of SLE that could be due to the variation in measuring intervals.

SLE being an inflammatory disease, it will induce the production of acute-phase proteins by inflammatory cytokines and other factors. Thus, CRP level and SLE disease relation was thoroughly investigated. Contradictory reports have been cited concerning this relation. Some studies revealed that CRP levels although are high in SLE patients they were over inflamed compared to other disease such as arthritis. This was attributed to the theory which indicated that serum CRP is consumed in SLE by anti-CRP autoantibodies, binding to complement factors and renal excretion. These limiting factors could be the direct cause that CRP levels are not directly proportional to the disease severity [25]. In the present study, the CRP was lower correlated with the stages of SLE disease in males and females (r= +0.49 and r= +0.34) respectively.

It has been suggested by Kim *et al*. [26] that the measurement of β2m level seems to be useful in addition to the laboratory tests that can help in assessment of disease activity of SLE. β2m levels (2.64±0.11) of the SLE patients were higher than the normal controls (2.14±0.04). Similarly, in the present study the β2m levels of female and male SLE patients were significantly increased compared to female and male controls. Therefore, it could be concluded that ANA (r= + 0.95) in male SLE patients was more related to the disease activity than β2m (r= + 0.94), while in females the β2m (r= + 0.83) was more related to disease activity than ANA (r= + 0.76). Thus, β2m serum level was found to be positively correlated to the disease activity in female and males SLE patients with more correlation to gender.

In CKD, the level of creatinine as an endogenous marker usually reflects kidney malfunction as a reduction of GFR. Serum creatinine level could lead to misleading results as its increase is detectable only when GFR decreases more than 50% [27]. Urea level may increase as a result of kidney damage or malfunction, being more marked in males than females [28], in agreement with present study, the urea level showed a higher correlation with the stages of the disease in males and females (r= +0.97 and r= +0.93) respectively than all the other considered parameters (serum creatinine, CRP concentration and β2m level).

There is an association between levels of inflammatory markers and stages of kidney disease [29]. A study by Dashti and colleagues [30] stated that measuring CRP as a marker of inflammation can be helpful in managing renal patients. Similar to their observation, an elevated serum CRP levels exceeding 10 mg/l appeared in most of male and female patients. Fox and coworkers [31] mentioned that CRP is significantly related to CKD as defined by estimated GFR (eGFR) < 60 ml/min/1.73 m^2 in African Americans community. In the present work the CRP concentration also showed a higher correlation (r= +0.88) with males than females (r= +0.33) of CKD.

In the current study, serum β2m levels of male and female patients in stages 3, 4 and 5 were significantly increased compared to their corresponding controls. This was confirmed by data obtained by various previous studies [32, 33]. A study including 44 participants with varying etiologies of kidney disease showed that β2m serum level was strongly correlated with GFR than with

serum creatinine [32]. Thus, it was confirmed that β2m was a useful marker better than serum creatinine in detecting kidney injury. Filler and colleagues [33] were able to detect patients with GFR less than 90 ml/min/1.73 m^2 in 225 children with elevated levels of β2m. Additionally, Shahjahan *et al*. [34] mentioned that β2m is more sensitive and accurate as biomarker for the assessment of renal functions in comparison to serum creatinine and even more correlated to GFR in all stages of kidney functions. In agreement with the previous mentioned reports, our findings revealed that β2m was more correlated with the stages of CKD disease in males and females (r= + 0.903, r= + 0.901) than the creatinine (r= +0.82 and r= +0.86) respectively. β2m and urea levels showed the highest correlation with the stages of the disease in males than females.

CONCLUSION

Finally, these findings suggest that β2m may be used as a useful serological marker for assessment of disease activity in SLE patients especially in males. In CKD, the urea and β2m levels are more related to the stages of kidney disease in both genders more than all the other markers (creatinine and CRP).

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