Study of Some Biomarkers Changes in Patients with Lupus Nephritis and Their Correlation with Disease Activity and Progression

***Walaa A. Keshk*[♣], *Nema A. Soliman*[♣] *and Noha S Esheba* [*]**
Departments of Medical Biochemistry[♣] and Internal Medicine [*],
Faculty of Medicine, Tanta University

ABSTRACT

Background: *Systemic lupus erythematosus (SLE) is a chronic multisystem autoimmune disease characterized by complex clinical manifestations and chronic inflammatory processes with failure of immunoregulatory mechanisms. Kidney is one of the most commonly affected organs. Considering the morbidity associated with SLE and particularly with lupus nephritis (LN), it is important to identify novel biomarkers of disease activity which could aid in the detection and assessment of flares and degree of activity. At present, activity of SLE is assessed based on clinical symptoms and biochemical parameters such as autoantibody (e.g antinuclear antibody (ANA)) and serum complement. However, these biomarkers are not specific for evaluating renal activity. Renal biopsy is the gold standard for assessment of kidney damage and disease activity, but its usage is restricted as it is an invasive procedure.* ***Aim of the work:*** *In the present study, plasma level of advanced oxidation protein products (AOPPs) and peripheral blood mononuclear cells nuclear factor-κB (PBMCs NF-κB) level as well as serum levels of fetuin-A, 25-hydroxyvitamin D (25OHD), calcium (Ca), inorganic phosphate were studied as novel biomarkers of LN activity and progression.* ***Methods:*** *The study included 30 SLE female patients, 15 without nephritis (group II) and 15 with nephritis (group III), in addition to 15 age-matched healthy controls (group I). Overnight fasting blood was collected from all subjects for measurement of plasma AOPPs level, PBMCs NF-κB level and serum fetuin-A level, 25OHD level, Ca and inorganic phosphate levels as well as calculation of calcification risk index (CRI).* ***Results****: Plasma AOPPs, PBMCs NF-κB, serum inorganic phosphate levels and CRI were significantly higher in SLE patients (group II) than age-matched healthy controls (group I) ($p<0.05$) with the highest level in patients with LN (group III) meanwhile, serum fetuin-A and 25OHD levels were significantly lower in SLE patients than age-matched healthy controls ($p<0.05$) with the lowest level in LN patient group. In addition plasma AOPPs, PBMCs NF-κB levels and CRI showed significantly positive correlation meanwhile, fetuin-A and 25OHD levels showed significantly negative correlation with serum creatinine, 24 hours urinary albumin, erythrocyte sedimentation rate (ESR), C reactive protein (CRP), ANA, anti double stranded DNA (Anti- dsDNA) antibodies levels and SLE disease activity index (SLEDAI).* ***Conclusions:*** *In SLE patients, high PBMCs NF-κB and plasma AOPPs levels as well as CRI while low levels of fetuin-A and 25OHD were related to disease activity and progression.*

Key words: *Systemic lupus erythematosus (SLE), lupus nephritis (LN), Advanced oxidation protein products (AOPPs), Peripheral blood mononuclear cells nuclear factor-κB (PBMCs NF-κB), Fetuin-A, 25-hydroxyvitamin D (25OHD), Calcification risk index (CRI)*

INTRODUCTION

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disorder with high morbidity and mortality. SLE characterized by chronic inflammation, abnormal immune system response with failure of immunoregulatory mechanisms[(1)]. SLE affects predominantly women of child-bearing age with multi-organ involvement and is characterized by variable clinical features, including glomerulonephritis, arthritis, and neuropsychiatric disease[(2)]. Kidney is one of the most commonly affected organs and lupus nephritis (LN) represents one of the most serious manifestations of SLE contributes to high mortality [(3)].

Increased oxidative stress contributes to the pathogenesis of some diseases as chronic kidney disease and autoimmune diseases[(4)]. Advanced oxidation protein products (AOPPs) are dityrosine-containing cross-linking proteins, formed by the reaction between chlorinated oxidants and proteins and derived from oxidation-modified albumin, fibrinogen, and lipoproteins. AOPPs have a role in induction of macrophage activation and apoptosis[(5)].

Patients with SLE demonstrate defective clearance of apoptotic cells which allows deposition of immune complexes that can stimulate B and T cells, causing tissue damage[(6)]. Nuclear factor kappa B (NF-κB), is a heterodimeric complex composed of two subunits termed p50 and p65 and is a key transcription factor involved in the regulation of immune responses and apoptosis [(7,8)] .

Calciphylaxis and calcinosis are soft-tissue calcification associated with SLE morbidity and mortality[(9)]. Fetuin-A (α_2–Heremans-Schmid glycoprotein AHSG) is glycoprotein predominantly synthesized in liver [(10)]. It is generally recognized as a calcification inhibitor rather than negative inflammatory marker, which facilitates the formation of soluble calciprotein particles and limits the formation of hydroxyapatite crystals[(10)].

25-hydroxivitamin D (25OHD) is one of sterols with a critical role in calcium, phosphorus metabolism, although vitamin D is consumed in food, dietary intake alone is often insufficient, supplying only 20% of the body's requirements[(11)]. The main source of vitamin D is the conversion of 7-dehydrocholesterol to pre-vitamin D_3 in the skin, by means of solar ultraviolet B (UVB) radiation. The fully active form, calcitriol (1,25(OH)2D), is synthesized in the kidneys[(12)]. Vitamin D deficiency has a role in the development of neoplasm, cardiovascular disease, increased susceptibility to infections and autoimmune diseases. 25OHD is an immunomodulatory hormone through its inhibitory effect on T lymphocytes, B lymphocytes and

dendrite cells as well as it can alter the cytokine production profile [13].

The aim of the current study is to evaluate the relationship between AOPPs, NF-κB, fetuin-A and 25OHD level as well as calculated calcification risk index (CRI) and LN, and to evaluate their possible clinical significance in disease activity and progression.

PATIENTS & METHODS

Patients:

The current work was carried out in Medical Biochemistry Department in accordance to the guidelines of the Ethical Committee of Medical Research, Faculty of Medicine, Tanta University, Egypt. An informed written consent was obtained from every patient and control subject. This current study was carried out on 45 premenopausal females. They were further subdivided into 3 groups. **Group I** (n=15) healthy volunteers were recruited as controls, **group II** (n=15) age matched SLE patients with no laboratory or clinical manifestations of nephritis and **group III** (n=15) age matched LN patients who had survived one or more manifestations of nephritis. All patients were selected from the Inpatients and Outpatients Clinic of Internal Medicine Department, Tanta University Hospital, Egypt and they fulfilled four or more of the American College of Rheumatology revised criteria for diagnosis of SLE [14]. **The SLE Disease Activity Index (SLEDAI)** was assessed according to **Bombardier et al. (1992)** [15]. SLEDAI assessment for renal involvement was used to assess kidney disease activity. The score consists of the four kidney-related parameters: hematuria, pyuria, proteinuria, and urinary casts. Scores for the renal SLEDAI can range from 0 (inactive renal disease) to a maximum of 16. SLEDAI score of ≥4 was taken as an indicator of active LN[15].

All individuals of the study were subjected to full history taking, thorough clinical examination and those with a history of diabetes mellitus, coronary artery disease (CAD), malignancy, concurrent infection, other connective tissue disorders such as rheumatoid arthritis, liver or other kidney diseases and oral prednisolone greater than 10 mg/day were excluded from the study.

Sample preparation:

Overnight fasting venous blood samples were aseptically collected. Part was collected in plane tubes, centrifuged and the recovered serum aliquots were stored at -80°C for analysis of fetuin-A and 25OHD levels. For PBMCs NF-κB level and plasma AOPPs levels, another part of the blood sample was collected on plastic tubes containing K_2EDTA at a final concentration of 1.2 mg EDTA/ml and was used for plasma and PBMCs separation. Blood was added slowly along the sides of the tube containing Ficoll hypaque (Sigma Aldrich) and centrifuged at 400 x g for 20 minutes. PBMCs (white interphase layer), plasma (upper layer) and red blood cells (bottom layer) were carefully separated. Plasma sample was frozen at -80°C until used for measurement of AOPPs level. The PBMCs were washed with phosphate buffer saline

(PBS) pH 7.4 three times. PBMCs were lysed with lysis buffer containing 0.2% Triton X-100, 0.25 M sucrose, 10 mM EDTA and 1 mM $CaCl_2$ (Sigma Aldrich)[16]. The lysate was centrifuged at 1600 x g for 10 minutes and supernatant was recovered. Samples were then kept at -80°C until the time of assay for PBMCs NF- κB level.

Routine laboratory investigations were done and included urine analysis especially for 24 hours proteinuria using commercial kits supplied by Diamond Diagnostic, Egypt and RBCs casts, complete blood count, ESR-1 by Westergreen method, Fasting blood sugar (FBS) by oxidase method using commercial kit supplied by Biodiagnostic., Egypt, C reactive protein (CRP), serum albumin, serum creatinine all were estimated using commercial kits (Randox Laboratories Ltd., Diamond Diagnostic, Egypt). Antinuclear antibody (ANA) by commercial ELISA kit (Kallestad HEp-2 Kit., Biorad), anti double stranded DNA antibodies (anti-dsDNA) by commercial ELISA kit (Inova Diagnostics Inc., San Diego, USA) and renal biopsy for diagnosis of LN.

Biochemical Assessment included:-

1. **Plasma AOPPs** was assayed immediately by spectrophotometric method according to **Witko-Sarsat et al (1998)** [18].
2. **Peripheral mononuclear cells NF-κB level** (PBMCs NF-κB level) was determined by commercial ELISA kit (Uscn Life Science Inc.) E91824Hu.
3. **Serum Fetuin-A level** was measured by a commercial ELISA kit (OmniKine™) Catalog Number OK-0329.
4. **Serum 25OHD level** was measured by radioimmunoassay (RIA), (Nichols Institute Diagnostics, San Clemente, CA, USA). Values of 25OHD ≤20 ng/ml (50 nmol/L) were considered 25OHD deficient; values ranging from 21 to 29 ng/ml (52 to 72 nmol/L) were considered 25OHD insufficiency, and levels ≥30 ng/ml (75 nmol/L) were considered sufficient for 25OHD [17].
5. **Serum Ca** was measured by QuantiChrom™ Assay Kit (DICA-500).
6. **Serum inorganic phosphate** was measured by CHRONOLAB phosphomolybdate UV kit (101-0458).
7. **Calculation of calcification risk index (**CRI) = Ca X P/Fetuin-A. (Where: Ca, P and fetuin-A are serum levels of Ca, inorganic phosphate and fetuin-A, respectively)[19].

Statistical analysis:

Statistical presentation and analysis of the present study was conducted, using the mean, standard deviation and Person correlation test (r) by SPSS version.16. . Analysis of variance [ANOVA] tests and Tukey's test was used to determine the significance between more than 2 groups: According to the computer program SPSS for Windows. P value < 0.05 was considered significant.

RESULTS

Table (1) shows statistical comparison between descriptive

parameters among all studied groups. There were statistically insignificant differences in values of age and disease duration, however there were statistically significant differences in values of serum creatinine, 24 hours urinary albumin and ESR-1 among all studied groups (F value was 3.147, 21.121, and 10.37 respectively, P<0.05). Using multiple comparisons test (Tukey's test), the values were markedly higher in LN group (group III) and SLE group (group II) compared to healthy control group (group I) with statistically higher level in LN group (P < 0.05). There were statistically significant differences in values of CRP (mg/L), ANA (IU/ml), Anti-dsDNA (IU/ml) and SLEDAI score between LN patient group (group III) and SLE group (group II) (p < 0.05) with statistically higher level in LN group.

Table 1: Statistical comparison between descriptive parameters among all studied groups

Parameters/Group	Group I n=15	Group II n=15	Group III n=15	ANOVA test		Tukey's test		
				F	P	P1	P2	P3
Age (years)	31.1±7.9	35.1±10.3	29.1±12.6	0.735	0.231	0.352	0.639	0.441
Duration of diagnosis of SLE (month)	Not applicable (NA)	17±6	12±3	-	-	-	-	0.085
Serum Creatinine (mg/dl)	0.83+0.08	0.92±0.09	2.93±0.63	3.147	0.049*	0.152	0.036*	0.031*
24 hours urinary albumin (mg/24 hours)	64.25±3.36	95.41±7.52	6352.5±952.3	21.12	0.001*	0.036*	0.001*	0.001*
ESR-1 (mm/hour) (Mean ±SD)	9.5±3.2	61.47±8.63	82.41±10.72	10.36	0.002*	0.001*	0.001*	0.042*
CRP (mg/L)	-ve	23.36±5.63	49.63±12.25	12.51	0.001*	-	-	0.030*
ANA (IU/ml)	-ve	23.63±6.52	43.18±5.96	-	-	-	-	0.012*
Anti- dsDNA (IU/ml)	-ve	76.52±12.41	101.4±10.30	-	-	-	-	0.028*
SLEDAI score	NA	1.65+0.23	5.96+2.10	-	-	-	-	0.006*

*Significant at P-value < 0.05** *P1 comparison between group I and II*
P2 comparison between group I and III *P3 comparison between group II and III*

Table (2) and figure (1) (a,b,c,d and e) show comparative statistics of different biochemical studied parameters among all studied groups. There were statistically significant differences in values of plasma AOPPs (μmol/L), PBMCs NF-κB level (ng/ml) and CRI among all studied groups (F values were 10.36, 9.634, and 11.61 respectively, P < 0.05). Using multiple comparisons test (Tukey's test), the values were markedly higher in group III and group II when compared to group I with statistically higher values in group III. There were statistically significant lower levels of serum fetuin-A (pg/ml), and 25OHD (ng/ml) between group III and group II with statistically lower level in group III.

Also, there were statistically insignificant differences in values of serum Ca (mg/dl) and serum inorganic phosphate (mg/dl) between all studied groups, by using multiple comparisons test (Tukey's test), there were statistically significant differences between group I and III and between group II and III with statistically significant lower serum Ca and higher inorganic phosphate levels in group III than group I and group II. The significant differences in levels of previous parameters reflect their strong relation to LN disease activity and progression.

Table 2: Comparative statistics of different biochemical studied parameters among all studied groups

Parameters/Group	Group I n=15	Group II n=15	Group III n=15	ANOVA test		Tukey's test		
				f	P	P1	P2	P3
Plasma AOPPs (µmol/L)	22.52±5.36	56.85±8.63	74.52±12.98	10.36	0.002*	0.001*	0.001*	0.001*
PBMCs NF-κB level (ng/ml)	6.93±2.94	45.63±9.35	80.63±13.61	.634	0.001*	0.001*	0.001*	0.001*
Serum fetuin-A level (pg/ml)	0.95±0.10	0.61+0.21	0.42±0.11	6.219	0.006*	0.007*	0.003*	0.037*
25OHD level (ng/ml)	34.28±2.41	22.85±4.09	14.20±1.52	7.002	0.006*	0.020*	0.019*	0.017*
Serum Ca (mg/dl)	10.18 ± 1.47	8.3±1. 06	7.79 ± 1.38	0.963	0.135	0.528	0.038*	0.027*
Serum inorganic phosphate (mg/dl)	3.52±0.41	4.63 ± 0.91	5.62± 0.51	2.159	0.063	0.159	0.032*	0.015*
CRI	34.49±5.44	37±4.55	73. 7±15.36	11.610	0.002*	0.236	0.001*	0.001*

*Significant at P-value < 0.05 ** *P1 comparison between group I and II*
P2 comparison between group I and III *P3 comparison between group II and III*

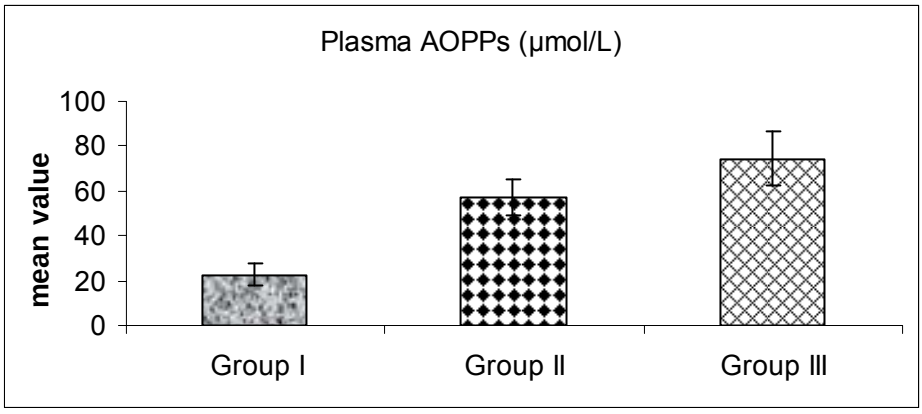

Fig. (1a): Comparison of Plasma AOPPs (µmol/L) among all studied groups using ANOVA test

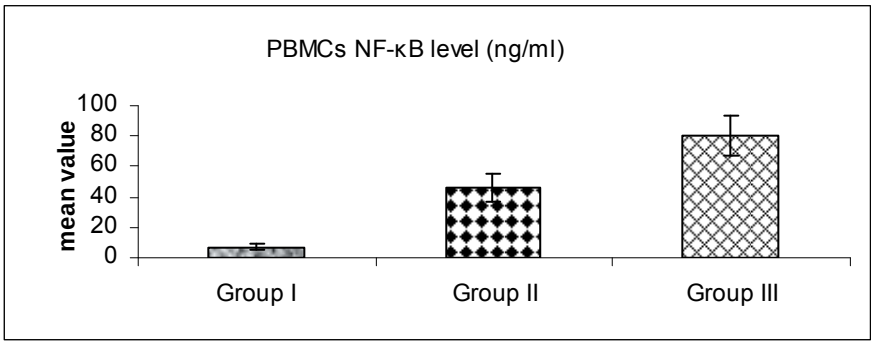

Fig. (1b): Comparison of PBMCs NF-κB level (ng/ml) among all studied groups using ANOVA test

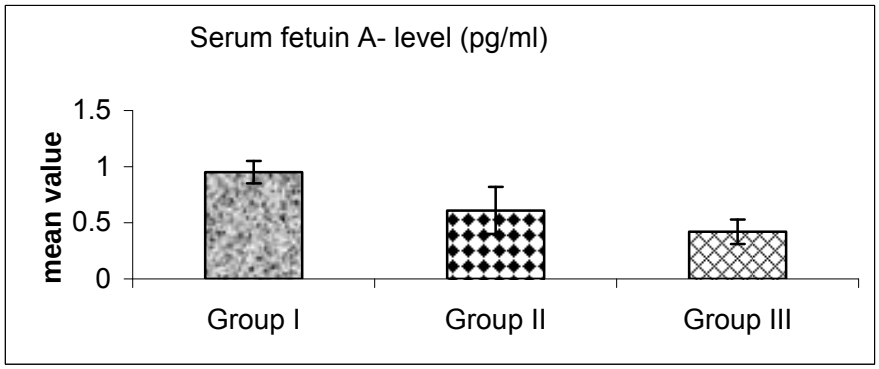

Fig. (1c): Comparison of serum fetuin-A level (pg/ml) among all studied groups using ANOVA test

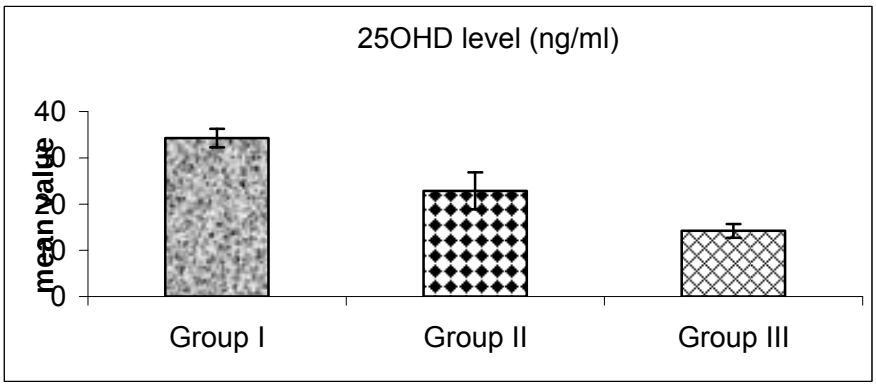

Fig. (1d): Comparison of serum 25OHD level (ng/ml) among all studied groups using ANOVA test

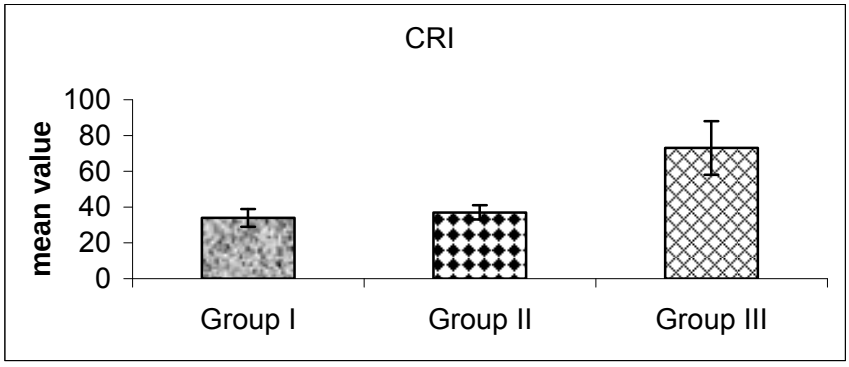

Fig. (1e): Comparison of CRI among all studied groups using ANOVA test

Table (3) shows person correlation between PBMCs NF-κB (ng/ml) plasma AOPPs μmol/L levels and CRI with serum levels of 25OHD (ng/ml) and fetuin-A (pg/ml) in patient groups (group II and III), negative correlations were found between PBMCs NF-κB (ng/ml), plasma AOPPs (μmol/L) levels and CRI with serum levels of both 25OHD (ng/ml) and fetuin-A (pg/ml) in patient groups (r value was -0.362, -0.425, -0.419, -0.377, -0.296 and -0.536 respectively, $P < 0.05$).

Table 3: **Correlation between PBMCs NF-κB (ng/ml), plasma AOPPs levels (μmol/L) and CRI with serum levels of 25OHD (ng/ml) and fetuin-A (pg/ml) in patient groups**

	Peripheral lymphocyte NF-κB level (ng/ml)		**Plasma AOPPs μmol/L**		**CRI**	
	r	**P**	**r**	**p**	**r**	**p**
25OHD level (ng/ml)	-0.362	0.009*	-0.419	0.020*	-0.296	0.007*
Serum fetuin-A level (pg/ml)	-0.425	0.017*	-0.377	0.006*	-0.536	0.002*

*Significant at P-value < 0.05 **

Table (4) shows person correlation between PBMCs NF-κB level (ng/ml) plasma AOPPs μmol/L CRI, serum 25OHD level (ng/ml) and serum fetuin-A level (pg/ml) with some laboratory parameters in patient groups. Statistically significant positive correlation were found between PBMCs NF-κB level (ng/ml), plasma AOPPs (μmol/L), CRI and serum creatinine (mg/dl), 24 hours urinary albumin (mg/24 hours), ESR-1 (mm/hour), CRP (mg/L), ANA (IU/ml), Anti-dsDNA antibodies (IU/ml), SLEDAI score. Meanwhile, serum levels of 25OHD (ng/ml), fetuin-A (pg/ml) showed statistically significant negative correlation with the above studied parameters. These results reflect the relationship of studied parameters to LN disease activity and progression.

Table 4: Correlation of PBMCs NF-κB level (ng/ml) Plasma AOPPs (µmol/L), CRI, serum 25OHD level (ng/ml) and serum fetuin-A level (pg/ml) level with some laboratory parameters in patients groups

	Peripheral lymphocyte NF-κB level (ng/ml)		Plasma AOPPs µmol/L		CRI		25OHD level (ng/ml)		Serum fetuin-A level (pg/ml)	
	r	p	r	p	r	p	r	p	r.	p. value
Serum creatinine (mg/dl)	0.352	٠.٠١٤*	٠.٤١٧	٠.٠٠٦*	٠.٤٢٨	٠.٠٠٦*	-0.263	٠.٠٣٧*	-0.550	٠.٠٠٣*
24 hours urinary albumin (mg/24 hours)	0.285	٠.٠٢٠*	٠.٥٢٨	٠.٠٠١*	٠.٢٩٩	٠.٠٣٥*	-0.635	٠.٠٠١*	-0.418	٠.٠٠٩*
ESR-1 (mm/hour)	0.417	٠.٠٠٩*	٠.٣٠٧	٠.٠٢٥*	٠.٣٦٣	٠.٠١٥*	-0.410	٠.٠٢٨*	-0.352	٠.٠٢٨*
CRP (mg/L)	0.562	٠.٠٠١*	٠.٤١٥	٠.٠٠٧*	٠.٣١٢	٠.٠٢٤*	-0.320	٠.٠٣٩*	-0.295	٠.٠٤٢*
ANA (IU/ml)	0.314	٠.٠٣٤*	٠.٢٧٥	٠.٠٤٢*	٠.٢٧٤	٠.٠٣٠*	-0.2٩٩	٠.٠٢٨*	-0.305	٠.٠٣٦*
Anti- dsDNA (IU/ml)	0.411	٠.٠١٨*	٠.٤١٩	٠.٠٢٩*	٠.٣٢٠	٠.٠٢٨*	-0.٣٣٩	٠.٠١٧*	-0.418	٠.٠٠٥*
SLEDAI score	٠.٣٣٩	٠.٠١٠*	٠.٣١١	٠.٠٣١*	٠.٤١٩	٠.٠٠٨*	-0.466	٠.٠٠٤*	-0.٤١٨	٠.٠١٢*

Significant at P-value < 0.05

DISCUSSION

Systemic lupus erythematosus is a chronic inflammatory autoimmune disease characterized by defective clearance of apoptotic cells with release of nuclear and cytoplasmic auto-antigens followed by chronic inflammation, auto-antibody production, immune complex formation and deposition in target organs such as the kidney leading to organ damage [(20)]. Nephritis is a major cause of morbidity and mortality in patients with lupus which may arise as a consequence of inflammation, cytokine, chemokine and adhesion molecule production with further influx of more inflammatory cells, tissue injury and fibrosis. End-stage renal disease in lupus is secondary to loss of glomerular and tubular function as a result of renal cell death and fibrosis[(21)]. Lymphocytes and their soluble mediators participate at all levels in disease pathogenesis and relapse [(22)].

Excessive generation of reactive oxygen species (ROS), have the potential to initiate lipids, proteins and DNA modification and damage with generation of auto-antigens which can elicit autoimmune responses by stimulating T and B lymphocytes [(23)]. Advanced oxidation protein products represent a class of uremic toxins and/or pro-inflammatory mediators which contain larger amount of dityrosine, carbonyl groups and cross-linked bonds, they derived from oxidation modified albumin, fibrinogen, and lipoproteins[(24)]. AOPPs act as inflammatory mediators that result in excessive inflammatory

response, through increased plasma levels of tumor necrosis factor-α (TNF-α), enhanced neutrophils, monocytes and T-lymphocytes as well as macrophage invasion, smooth muscle cell (SMC) proliferation and excessive stimulation of dendritic cells with more oxidative stress [25].

The current study revealed that patient groups had significantly higher level of AOPPs than control group with the highest level in LN patient, a finding in accordance with that of **Lozovoy et al.** [26]. That finding may reflect a state of inflammation and oxidative stress associated with SLE that is aggravated with nephritis. Significant higher plasma AOPPs level may be explained on the basis of SLE association with inflammatory cell infiltration as neutrophil. Neutrophils are rich in myeloperoxidase (MPO) enzyme which is the main factor for production of chlorinated oxidants that oxidize protein leading to the formation of AOPPs which have a role in induction of macrophage activation and apoptosis [27].

Inflammatory cytokines like TNF-α and ROS are signals for the activation of NF-κB which is a family of transcription factors that contribute to the transcriptional regulation of several genes required for the immune response and is a heterodimeric complex typically composed of two subunits p50 and p65. NF-κB remains inactive by binding to the inhibitory protein-kappa B (IκB) in the cytoplasm of many cell types as lymphocytes and monocytes[28,29]. IκB kinases (IKK) phosphorylate IκB exposing it to proteasomal degradation with subsequent release of free NF-κB; IKK activity is strongly induced by TNF-α, interleukin1-beta (IL-1ß), B or T lymphocytes activation and oxidative stress [30]. Free NF-κB translocates to the nucleus, where it activates the transcription of genes involved in the inflammatory response, apoptosis and cell proliferation angiogenesis**.** Inflammatory cell infiltration and inflammatory cytokines cause glomerular and tubular injury, which may play a role in the pathogenesis of LN[31].

Activation of NF-κB has an important role in maturation of dendritic cell, the professional antigen-presenting cells, capable of promoting either immunity or tolerance to specific antigens through induction of effector T-cell responses. Therefore, inhibition of NF-κB activation has been proposed as a strategy to maintain dendritic cell in an immature state to promote immune tolerance [32].

The present study revealed that patient groups had significantly higher PBMCs NF-κB level than control group with the highest level in LN patient group, a finding in accordance with that of **Zheng et al.** and **Kalergis et al.**[33,34]. This result can be explained by state of chronic inflammation and oxidative stress associated with SLE which activates NF-κB mediated apoptosis of renal cells, so NF-κB may play an important role in mediating chronic renal injury, especially tubulointerstitial lesions that may be manifested clinically as progressive renal insufficiency and this run hand in hand with the results of the current study.

Systemic lupus erythematosus is associated with leukocyte-mediated inflammatory state, tissue damage and bones demineralization. Tissue inflammation is often followed by deposition of type I collagen and may produce an alkaline environment, allowing calcium precipitation and alkaline phosphatase activation[35]. Calciphylaxis and calcinosis is the deposition of Ca and phosphate salts in soft-tissue and is associated with a number of metabolic and inflammatory disorders. So, controlling the SLE activity would help arrest or reverse soft tissue calcification [36,37].

Fetuin-A is a negative hepatic acute-phase glycoprotein belonging to the subgroup 3 of the cystatin superfamily, which also includes fetuin-B, histidine-rich glycoprotein, and kininogen[38]. Fetuin-A is involved in the regulation of calcified matrix metabolism through inhibition of ectopic Ca/phosphate ion precipitation and vascular calcification, it is responsible for approximately half of the precipitation inhibitory properties in the extracellular space through formation of protein-mineral complexes, called calciprotein particles (CPPs)[39,40]. Fetuin-A is downregulated during an inflammatory process and has a role in attenuation of apoptosis, dystrophic calcification in atherosclerosis and downregulates vascular profibrotic activities [41].

In the present study, serum fetuin-A was measured in SLE patients with and without nephritis in a trial to clarify its potential contribution to disease activity and progression. This current study revealed that patient groups had a significantly lower serum fetuin-A level than control group with the lowest level in LN patient group, a finding in agreement with those of **Ketteler et al.[42] and Slough et al.[43]**. This result may be explained by: firstly, SLE associated inflammation which cause down regulation of fetuin-A gene expression, secondly, significant urinary loss of fetuin-A due to disease associated proteinuria and lastly, the polymorphism of fetuin-A gene may decrease its level [44].

Vitamin D is an essential hormone for bone/ mineral homeostasis, regulates the growth and differentiation of multiple cell types, and has an immunoregulatory and anti-inflammatory properties [45]. Cells involved in innate and adaptive immune responses, including macrophages, dendritic cells, T lymphocytes and B lymphocytes express the vitamin D receptor (VDR), and respond to 1,25(OH)2D. 25OHD inhibits T-lymphocyte proliferation, dendritic cell differentiation, cytokine production and B lymphocytes antibody/ autoantibody production and secretion[46,47].

The current study revealed that patient groups had significantly lower serum level of 25OHD level than control group with the lowest level in LN patient group, and the reverse occurs for CRI, a finding in agreement with those of **Kamen et al.[48] Borba et al.[49] and Bonakdar et al.[50]**. This result may be explained by photosensitivity, renal involvement with defect in 1-Hydroxylation which is essential to make the active form of

vitamin D and hydroxychloroquine used in SLE treatment which can lower the conversion of provitamin D_3 to the active vitamin D_3, also elevated inorganic phosphate level inhibits 25OHD conversion to 1,25(OH)2D[51]. The reduction in 1,25(OH)2D decreases intestinal absorption of Ca that causes hypocalcemia. Hyperphosphatemia leads to the development of renal bone disease, extraosseous calcifications of soft tissue and vasculature. Also, inorganic phosphate level more than 6.5 mg/dl and higher CRI is associated with increased risk of death [52].

This current study revealed positive correlation between PBMCs NF-κB level , AOPPs level and CRI with levels of creatinine, 24-hours urinary protein, ANA, anti-dsDNA antibodies, CRP, ESR-1 and SLEDAI, on the other hand an inverse correlation was detected with serum fetuin-A and 25OHD in SLE patient groups included in the present work. On the basis of these observations, it could be suggested that the decrease in the serum levels of fetuin-A and 25OHD and the increase in the levels of PBMCs NF-κB and plasma AOPPs`s as well as CRI occur during the course of the disease activity and progression.

CONCLUSION

Excessive production of ROS disturbs redox status and can modulate the expression of inflammatory chemokines through NF-κB leading to inflammatory processes and tissue damage, which was more profound with LN and could reflect disease activity, considering antioxidants as a novel therapeutic strategies are important in SLE patients and patients with LN. The correlations between inflammation mediated changes in the fetuin-A level (an endogenous inhibitor of calcification) and 25OHD deficiency with abnormalities in calcium-phosphate metabolism represented in CRI are associated with disease activity in SLE patients in the form of renal impairment. Routine screening for 25OHD deficiency and its prompt treatment in patients with SLE is recommended. Also, correlation studies between the studied parameters and kidney function can reflect their role as a novel disease activity monitoring biomarkers.

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دراسة التغير فى بعض الدلائل الكيميائية فى مرضى الذئبة الحمراء المصاحب بالإعتلال الكلوى و علاقته بنشاط وتقدم المرض

ولاء عرفة كشك♣، نعمة علي سليمان♣، نهى السيد عشيبه*

قسمي ♣الكيمياء الحيوية الطبية - *الباطنة العامة -كلية الطب- جامعة طنطا

ازداد معدل الإصابة بالذئبة الحمراء و التى ربما ترتبط بحدوث مضاعفات للكلي. ولذا ، فإن الغرض من هذه الدراسة هو تحديد ما إذا كان عامل النواة كابا-بي، فيوتين أ، فيتامين د و ناتج أكسدة البروتين المتقدم كعوامل خطورة للاصابة بالاعتلال الكلوى لدى مرضى الذئبة الحمراء وكذلك مدى تأثيرهم على نشاط المرض و تقدمه. أجريت هذة الدراسة على ثلاث مجموعات من السيدات : المجموعة الأولى و عددها ١٥ متطوعه وتمثل المجموعة الضابطة، المجموعة الثانية و عددها ١٥ مريضة بالذئبة الحمراء ا و المجموعة الثالثة و عددها ١٥ مريضة بالذئبة الحمراء المصاحب بالإعتلال الكلوى و تتطابق جميع المجموعات فى السن. و تم عمل فحوصات معملية مثل قياس تركيز كل من عامل النواة النواة كابا-بي، فيوتين أ، فيتامين د و منتج تاكسد البروتين المتقدم ، تحليل البول ، نسبة البروتينات فى بول ٢٤ ساعه، صورة الدم ، حمض البوليك ، مستوى الكرياتينين ، سرعة الترسيب ، الأجسام المضادة لنواة خلايا الجسم (ANA) ، الأجسام المضادة للحامض النووى (Anti ds-DNA) والجلوكوزالصائم . و قد تم استبعاد المرضى الذين لديهم تاريخ مرضى بالبوال السكرى ،ارتفاع ضغط الدم ، و مرض الشريان التاجي ،الروماتويد، الأورام و أمراض الكبد. وقد أسفرت نتائج البحث إلى أن تركيزات عامل النواة كابا-بي و منتج تاكسد البروتين المتقدم كانت أعلى بشكل ذو دلاله احصائية فى مرضى الذئبة الحمراء الجهازية عن المجموعة الضابطة كما ان تركيز كل من ، فيوتين أ و فيتامين د كانت اقل بشكل ذو دلاله احصائية فى مرضى الذئبة الحمراء عن المجموعة الضابطة. كما يرتبط إرتفاع تركيزات عامل النواة كابا-بي و منتج تاكسد البروتين المتقدم إرتباطا إيجابيا ذو دلاله احصائية مع مؤشر نشاط الذئبة الحمراء الجهازية و ارتباطه باضطراب وظائف الكلي . كما وجد أن تركيزات عامل النواة كابا-بي و منتج تاكسد البروتين المتقدم ترتبط إرتباطا عكسيا ذو دلاله احصائية مع انخفاض تركيز فيوتين أ، فيتامين د . كما ثبت أيضا أن مرضى الذئبة الحمراء ذوى ارتفاع مستوى الكرياتينين كانت تركيزات عامل النواة كابا-بي و منتج تاكسد البروتين المتقدم أعلى بكثير مقارنة بالمرضى ذوى الكرياتينين الطبيعي التركيز و خلص البحث الى أنه في مرضى الذئبة الحمراء ، ارتفاع مستوى تركيزات عامل النواة كابا-بي و منتج تاكسد البروتين المتقدم و انخفاض مستوى تركيزات فيوتين أ و فيتامين د يعكس النشاط الأساسي لهذا المرض ، ويرتبط مع بعض عوامل خطورة اصابة الكلي.