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Relationship between Fetuin-A and Systemic Lupus Erythematosus as a Predictor Marker for Atherosclerosis

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Abstract: Problem statement: Associations between serum levels of fetuin-A, C3 complement, calcium × phosphate product and calcification risk index and lipid profile in SLE patients were established. However, the mechanism of accelerated atherosclerosis accompanied with SLE remains elusive. We therefore turned to investigate the association between Fetuin-A, disease activity and accelerated atherosclerosis in patients with SLE. **Approach:** Serum blood samples were taken from 100 female SLE patients. All Patient samples were analyzed by ELISA for determination of Fetuin-A level. Calcium, Phosphate, C3 compelement, Lipid profile, Creatinine and urea were measured also in SLE patients compared with healthy control volunteers. **Results:** We found that Serum fetuin-A had been positively associated with carotid arterial stiffness, independent of known atherogenic factors in healthy subjects. Furthermore, Fetuin-A was correlated negatively with IMT, SLEDAI, CRI, CaxP product, Triglycerdies, VLDL and LDL. While it was correlated positively with C3 complement. **Conclusion:** Fetuin-A deficiency accompanied with increasing levels of calcium and phosphate gave an evidence that there was a key role of fetuin-A as a strong inhibitor of Cardio Vascular Calcification (CVC) by formation of a complex called (calciprotein) with calcium and phosphate in blood stream. So, Identification of biologic markers of disease activity associated with atherosclerosis may help to optimize therapy for this important manifestation of systemic autoimmune disease.

Key words: Fetuin-A, atherosclerosis, systemic lupus erythematosus, Cardio Vascular Calcification (CVC), disease activity

INTRODUCTION

SLE is an autoimmune disease in which immune system attacks the body cells and tissues, resulting in inflammation and tissue damage. SLE can affect any part of the body, but often harms the heart, joints, skin, kidney, lungs, blood vessels and central nervous system. (Manson and Rahman, 2006) SLE reflects a general defect in immune regulation that results in hyperactive T cells and B cells. The role of vascular injury in the pathogenesis of SLE is due to circulating immune complexes of autoantibodies and self antigens are deposited in the vascular wall of SLE patients and activate the complement pathway that initiate inflammatory response (Tincani *et al*., 2005).

The cause of SLE remains unknown. Genetic predisposition, hormonal, immunological and environmental factors trigger likely result in the disordered immune response that typifies the disease (Silva and Isenberg, 2001).

It is proposed that atherosclerosis arises as a response of the vascular wall to endothelial injury and this injury is due to endothelial apoptosis. Accelerated Atherosclerotic Vascular Disease (ASVD) is a major problem in SLE and is one of its major causes of death (Urowitz and Gladman, 2007). Coronary Artery Disease (CAD) develops in 6-9% of SLE patients and accounts for up to 36.4% of deaths in SLE. Young and predominantly SLE are unusual group manifesting an extraordinary strong predisposition for the development of early-onset and accelerated atherosclerosis (El-Magadmi *et al*., 2004).

It has been suggested that a combination of traditional risk factors, including hypertension, dyslipidemia and lipid oxidation as well as nontraditional risk factors, such as autoantibodies and inflammation, may contribute to advanced vascular disease in SLE. Therefore, defining the autoimmune mechanisms that promote atherosclerosis is essential to optimize risk reduction and develop targeted

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therapeutics for prevention of Coronary Vascular Disease (CVD) in SLE (Harley *et al*., 2006).

Fetuin-A (Alpha 2-Heremans-Schimd glycoprotein, AHSG), is a glycoprotein secreted by adult liver into the peripheral circulation. This protein is commonly present in the cortical plate of immature cerebral cortex and bone marrow hemopoietic matrix (Kazama *et al*., 2005). Moreover it is considered to be a member of the cystatin superfamily of cysteine protease inhibitors involved in vascular pathology and bone metabolism (Fiore *et al*., 2007). Fetuin-A is linked to a number of cellular functions such as; brain development, bone remodeling and inhibition of soft tissue calcification by formation of a soluble colloidal microsphere of fetuin-calcium-phosphate complex in blood stream and antagonist of Transforming Growth Factor-β (TGF-β) (Reynolds *et al*., 2005; Demetriou *et al*., 1996).

Cardiovascular risk scores are not adequate for risk stratification in women with SLE. Measurement of coronary calcification may add information to identify asymptomatic women with lupus who might benefit from aggressive preventive measures. An elevated incidence of Cardiovascular Calcification (CVC) is observed in Hemodialysis (HD) patients. Fetuin-A is an important inhibitor of CVC. Reduced fetuin-A levels associated with inflammation and increased CVC (Cozzolino *et al*., 2006; Selim *et al*., 2006).

MATERIALS AND METHODS

Patients: 100 adult female patients (15-45 years) with systemic lupus erythematosus were admitted to the Internal Medicine Department of Medical Research Institute hospital, Alexandria University. All patients signed informed consent form for participation in the study. Confirm the diagnosis. All patients signed informed consent form for participation in the study. Presence of simultaneous infectious or inflammatory condition, malignant disease or any other condition that could significantly influence the course and outcome of disease were excluded.

Control group: 50 healthy volunteers in the control group were matched for sex and age without any chronic or acute illness. All included control subjects signed the informed consent form.

Blood sampling: Venous blood samples were collected from normal control subjects and patient groups. Immediately blood sera after draw were separated by centrifugation and stored until analysis at -20°C. Full history and clinical examination, with meticulous focus on the duration and activity of SLE. Were carried out for all patients. The inclusion requirements for patients' recruitment were: diagnosis of SLE according to the American College of Rheumatology (ACR) criteria and the absence of any concomitant CVC traditional risk factors (e.g., hypertension, diabetes and smoking)' Disease Activity was assessed the time of enrollment in the study using the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI). Comprehensive medication histories were obtained through interviews with the patients. The use of corticosteroid therapy was categorized as current, former, or none and quantified in terms of the average daily dose over the preceding five years of the study.

Biochemical parameters:

- Serum Fetuin-A level was measured by a commercially available ELISA kit (Epitope Diagnostics, Inc) KT 800
- Serum intracellular Calcium was measured by QuantiChrom^{TM} Assay Kit (DICA-500)
- Serum Inorganic Phosphorous was measured by CHRONOLAB phophomolybdate UV kit (101-0458)
- Lipid Profile (Total cholesterol, Triglycerides, VLDL, LDL and HDL) were measured by RANDOX UV kits on (Beckman DU-640 UV/Vis Spectrophotometer)
- Serum Creatinine was measured by QuantiChrom^{TM} Assay Kit (DICT-500)
- Serum Urea was measured by QuantiChrom^{TM} Assay kit (DIUR-500)
- Serum C3 complement was measured by AssayMax Human C3 complement ELISA kit (EC2101-1)
- Carotid Ultrasonography reports for the measurement of Intima-Media Thickness (IMT) and the presence of atherosclerotic plaque
- Calculation of Calcification Risk Index (CRI)

The gathered data were further used to calculate two index scores for each patient under study. The first is the 'Calcification Risk Index' (CRI), given by the formula CRI = Ca X P/Fetuin-A. (where: Ca, P and Fetuin-A are serum levels of calcium, inorganic phosphate and Fetuin-4, respectively).

Statistical analysis were performed using: The SPSS-10 package software Student t-test was used to compare the means of the different parameters between each two groups. The correlation between the studied clinical and biochemical parameters was calculated using Pearson's correlation coefficient.

Table 1: Mean values of different biochemical studied parameters in control and SLE patient groups

Biochemical Studied parameters	Control group	Patient group
Fetuin-A (g/l)	0.7±0.07	0.5±0.13
Calcium (mg/dl)	8.06±0.25	8.94±0.37
Phosphate (mg/dl)	2.97±0.20	3.77±0.46
Calcium × Phosphate product (mg^2/dl^2)	23.92±1.91	33.58±3.68
CRI	34.49±5.44	73. 73±25.43
C3 Complement(g/l)	129.70±32.20	82.80±14.25
Total Cholesterol (mg/dl)	154.30±25.65	154.5±36.54
Triglycerides (mg/dl)	73.60±18.37	127.25±53.41
HDL (mg/dl)	42.70±10.40	27.70±7.14
VLDL (mg/dl)	13.37±5.56	25.45±10.68
LDL (mg/dl)	93.70±18.25	106.50±31.01
Creatinine (mg/dl)	0.87±0.07	0.99±0.13
Urea (mg/dl)	10.17±1.73	28.90±7.59

RESULTS

The basic clinical and biochemical parameters of the patients with SLE included in the study are given in Table 1. We found a significant positive correlation between age and fetuin-A (g/l) [r = 0.209, p<0.001]. In addition, significant negative correlations were found between fetuin-A (g/l), IMT (mm) and SLEDAI [r = -0.898 p<0.001, r = -0.685 p<0.01] respectively. Moreover, significant negative correlation was found between fetuin-A (g/l) and CRI [r = -0.901, p<0.001]. Also, a significant negative correlation between fetuin-A (g/l) and Calcium x Phosphate product (mg^2/dL^2) [r = -0.056, p<0.001]. On the other hand, significant positive correlation was observed between C3 complement (g/l) and fetuin-A (g/l) [r= 0.780, p <0.001]. Significant negative correlations were found between fetuin-A (g/l) with triglycerides (mg/dL), LDL (mg/dL) and VLDL (mg/dL) [r = -0.291 p<0.001, r = -0.057 p < 0.001 and r = -0.291 p<0.001] respectively.

DISCUSSION

In the present study, IMT was significantly higher in SLE group compared to control (p<0.001) and a significant positive correlation was observed between SLEDAI and IMT[r = 0.479, p = 0.033], while a significant negative correlation was found between IMT and C3 complement [r = -0.790, p<0.001]. Also, there was a significant negative correlation between C3 complement and SLEDAI [r = -0.345, p<0.001]. These findings are in accordance with Roman *et al.* (2003) who reported that, the prevalence of atherosclerosis is significantly increased among patients with lupus and this increase is not attributable only to traditional risk factors for cardiovascular disease. The activity of the disease presented as increase of SLEDAI and lower levels of C3 complement had additional factors to atherosclerosis in SLE patients in addition to traditional risks (Roman *et al*., 2003).

Thomas *et al.* (2002) reported that many patients with SLE are likely to have asymptomatic atheromatous vascular disease. In the study of 175 women, 40% had focal plaques identified in their carotid arteries by B-mode ultrasound (Manzi *et al*., 1999), which are often associated with atheroma in other vascular beds, especially the coronary arteries (Frcs *et al*., 1994; Chambless *et al*., 1997). The high risk of coronary artery disease complications associated with SLE after adjustment for conventional risk factors had led to the suggestion that SLE itself is an independent cardiovascular risk factor (Urowitz and Gladman, 2000).

Fetuin-A acts as a binder of basic calcium-phosphate allowing stabilization of their level in serum and preventing its precipitation. Also, fetuin-A promote phagocytosis of excess calcium so it was considered as a potent circulating inhibitor of vascular calcification (Kuznair *et al*., 2008). Fetuin-A is a negative acute phase reactant protein with decreased level after an inflammatory insult (Coen *et al*., 2006). In the current study, a significant positive correlation was found between Age and Fetuin-A [r = 0.209, p<0.01] in SLE patient group. These results are in agreement with Marhaug *et al.* (2008) who showed that fetuin-A levels in all groups of children and the adolescent were much lower than described previously in adults and there was a significant positive correlation between age and fetuin-A level (Marhaug *et al*., 2008). In the present study fetuin-A was significantly decrease in SLE group compared to control (p<0.001) and negative correlations were found between fetuin-A and IMT, SLEDAI, CRI (r = -0.898 p<0.001, r = -0.683 p<0.001 and r = -0.901 p<0.001) respectively.

Ziolkowska *et al*. (2008) reported an inverse association between IMT and fetuin-A levels among patients with Chronic Kidney Disease (CKD). While in patients with End-Stage Renal Disease (ESRD), a positive correlation of IMT with phosphate level and age was observed (Ziolkowska *et al*., 2008). The mechanisms of action of fetuin-A are less understood. However, confocal microscopy and electron

microscopy-immunogold labeling with fetuin-A suggest that the uptake of the serum protein fetuin-A by vascular smooth muscle cells is a key event in the inhibition of vesicle-mediated calcification (Proudfoot and Shanahan, 2006).

Mori *et al.* (2007) predicted that feutin-A level is associated with carotid arterial stiffness, independent on atherogenic factors in healthy subjects. A negative correlation was found between fetuin-A and activity of SLE disease and CRI this support previous results that; active disease and severe infections are more important causes of death in the early phase of SLE. The pathogenesis and risk factors of this premature atherosclerosis in a disease that mainly affects young women, a group usually free of atherosclerosis, are not fully understood. Coronary vasculitis is not considered to be the underlying mechanism but persistent inflammation, autoimmunity, immune complex deposition and antiphospholipid antibodies are hypothesized to cause intimal damage followed by accelerated atherosclerosis (Knockaert, 2007).

Ix *et al.* (2007) demonstrated an inverse correlation between mitral and aortic valve calcification and serum fetuin-A levels in a cross sectional study of 970 patients with coronary artery disease and without renal disease. While, on the other hand there is no relationship exists between fetuin-A and renal function in patients without significant renal impairment. Genetic polymorphisms affect the level of serum fetuin-a and the risk of vascular calcification in end-stage renal disease patients. So far, this study was the first to relate valvular calcification with serum fetuin-A in patients with normal renal function. Ix *et al*. (2007) in the present study Ca×P product was increased significantly in SLE group compared with control group and there was a significant negative correlation between fetuin-A and Ca×P product. It was documented that the acceleration of aortic wall stiffening in dialysis patients is connected with medial calcification, an active cellular process controlled by calcification inducers and inhibitors. Blacher *et al*. (2001) also, extensive epidemiologic data demonstrated a link between calcium-phosphate metabolism derangements and cardiovascular mortality in dialysis patients. The list of calcification inducers in ESRD patients encompasses hypercalemia, hyperphosphatemia, an elevated Ca × P product, an increased parathyroid hormone level and excessive treatment with vitamin D (Reynolds *et al*., 2005; Fine *et al*., 1993; Spasovski, 2007).

Serum level of C3 complement was measured in the current study as indication of SLE disease activity. Serum level of C3 complement was significantly decreased in SLE patient group compared to control group and correlated negatively with SLEDAI and IMT and positively with fetuin-A in SLE patient. Complement has a big part to play in the autoimmune processes in lupus. In SLE continued complement activity due to immune complexes causes damage via the membrane attack complex, promotion of phagocyte adhesion and activation (releasing as array of inflammatory mediators and cytokines) and promotes blood clot formation (Arason *et al*., 2004). Deficiencies in the earlier parts of the cascade are associated with reduced ability to remove the immune complexes generated in lupus and so are often seen in lupus nephritis and vasculitis. Low concentrations of complement components due to increased catabolism are found in a majority of patients with active and sever SLE. Schur and Sandsson (1986) Along with the interpretation of our results we have to take into account that the identified relations are mainly descriptive and aim at preparing the ground for new hypothesis generation. The nature of the observed relationships as well as possible mechanisms that can direct or influence them remains to be examined.

Data obtained from the current study revealed that serum levels of triglycerides, LDL and VLDL were higher [($p<0.001$, $p = 0.241$, $p<0.001$) respectively] in SLE group compared to control group, while serum level of HDL ($p<0.001$) in patients group was significantly lower compared to control. Also, significant negative correlations were observed between fetuin-A level and each of triglycerides, LDL and VLDL[$r = -0.291$ $p<0.001$, $r = -0.057$ $p<0.001$ and $r = -0.291$ $p<0.001$] levels in SLE patient group respectively.

These results are in consistent with Leong *et al*. (1994) who stated that the 'lupus pattern' is characterized by significant elevated levels of Very Low Density Lipoprotein (VLDL), Triglycerides (TG) and lower levels of High Density Lipoprotein (HDL). Although these levels were within the normal range, they were significantly higher in untreated inactive lupus patients compared to healthy controls (Leong *et al*., 1994).

CONCLUSION

Fetuin-A deficiency accompanied with increasing levels of calcium and phosphate gave an evidence that there was a key role of fetuin-A as a strong inhibitor of Cardio Vascular Calcification (CVC) by formation of a complex called (calciprotein) with calcium and phosphate in blood stream. So, Identification of biologic markers of disease activity associated with atherosclerosis may help to optimize therapy for this important manifestation of systemic autoimmune disease.

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