Comparison of oxidative stress, carotid intima-media thickness in patients with lupus nephritis and normal population

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Abstract: Introduction: The risk of atherosclerosis is described to be increased in systemic lupus erythematosus (SLE). Mortalities and morbidities due to the accelerated atherosclerosis are elevated in these patients. Early diagnosis of this premature atherosclerosis can help to better management of vascular complications of this disease. Carotid intima media thickness (CIMT) appeared to be useful in assessment of vascular atherosclerosis. Also biomarkers of oxidative stress are appeared to be in relation with premature atherosclerosis especially in autoimmune disorders like SLE. This study aims at evaluating relation of oxidative stress biomarkers with CIMT in lupus nephritis patients and in normal population. **Methods & Materials**: In an analytic-descriptive setting, 60 persons (including 30 patients with documented lupus nephritis as the case group and 30 healthy persons as the control counterparts) were recruited in Tabriz Imam Reza Hospital during a 12- month period. Color Doppler ultrasound of carotid for CIMT was done in the two groups and also serum levels of Malondialdehyde (MDA) and Hemocysteine as biomarkers of oxidative stress were measured and other conventional risk factors of atherosclerosis also evaluated in the both groups. The results were compared between the two groups. **Results**: 30 patients with mean age of 30.4±8.65 years including 28 (93.3%) females and 2 (6.66%) males compared with 30 healthy persons as control group with mean age of 28.8±4.61 years including 29 (96.66%) female and one (3.33%) male. There was no difference according sex and age between two groups. There was statistically significant difference in MDA and Hemocysteine levels and CIMT between patients and healthy controls (P=0.001). In comparing between stages of lupus nephritis and level of oxidative stress biomarkers and CIMT, strongest relation was with stage 4 lupus nephritis. Multivariate analysis of several risk factors of atherosclerosis that appeared to be meaningful in the two group in this study, like creatinine, hemoglobin, albumin, CRP, ESR, MDA, Hemocysteine, showed that only increased level of Hemocysteine was statistically important in CIMT measurement and development of atherosclerosis. **Conclusion**: This study showed that atherosclerosis is more prevalent in lupus nephritis patients. And appeared that more severe disease is associated with more CIMT measures and more increase in oxidative stress biomarkers. We prominently concluded that level of Hemocysteine is strongly related with development of atherosclerosis.

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1. Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease that involves different body systems. Changes in the function of the immune system and chronic inflation are the characteristics of this disease (Roman, 2003). This disease is often developed in young women and about 60-80% of patients experience renal involvement in the course of their lives. Renal involvement may be asymptomatic or may be in the form of the nephrotic syndrome or renal failure (Asanuma, 2003; Fauci, 2012).

Lupus nephritis is the most common cause of morbidity and mortality in these patients. Today, it is known that the high rates of morbidity and mortality are the result of cardiovascular problems in lupus patients and incidence of cardiovascular conditions in lupus patients is 5 times the incidence of these problems in normal people (Asanuma, 2003). Lupus is the major cause of death in women with autoimmune diseases (Tang, 2005).

Premature vascular disease in lupus patients is caused by premature atherosclerosis in these patients (Ward, 1999).

Although the immune system disorders play a significant role in development of vascular atherosclerosis in lupus patients, the main cause of increased cardiovascular incidents shall be investigated through further etiological studies. Due to the highly progressive nature of vascular injury in

such patients, it is necessary to carry out more studies to determine the mechanism of vascular injuries (Kaplan, 2009).

According to many studies, increased oxidative stress is one of the prominent characteristics of autoimmune diseases, especially lupus. Oxidative stress, which is caused by ineffectiveness and ORS, contributes to the development of SLE and its complications (Moroni, 2010).

Renal involvement is one of the factors that influence the prognosis of lupus (especially proliferative lupus).

In addition to the traditional factors of premature atherosclerosis (such as hypoalbuminemia, hyperlipidemia, hypertension and diabetes mellitus), factors contributing to the development of lupus and renal function also intensify vascular atherosclerosis and make general prognosis of the disease harder (Sherer, 2010).

Previous studies investigated the relationship between oxidative stress and premature atherosclerosis in normal people and patients with renal involvement, but the relationships between renal involvement, oxidative stress, and atherosclerosis are not known in lupus patients (Nakamura, 2005).

The objective of the present study was, therefore, to study the level of oxidative stress in patients with lupus nephritis and the effect of oxidative stress on CIMT as a criterion for the diagnosis of premature atherosclerosis.

A comparison was also drawn between the results obtained from patients with lupus nephritis and normal people.

2. Material and Methods

In a descriptive-comparative-analytical study that was carried out in the internal medicine department of Tabriz University of Medical Sciences on SLE patients, the level of oxidative stress in patients with lupus nephritis and its effect on CIMT (as a criterion for the diagnosis of premature atherosclerosis) were studied.

Moreover, a comparison was also made between the results of the aforementioned investigations and results of normal people.

A total of 30 SLE patients and 30 normal people with matching ages and genders were selected and included in the study.The only exclusion criterion was the lack of consent of samples to take part in the study. Samples were also selected randomly.

This research project was conducted for 1 year (2011-2012) in the Imam Reza Research Center of Tabriz City.

The following factors and parameters were measured in all of the patients and members of the control group: the levels of oxidative stress with the MDA and thiol (Hemocysteine) markers; traditional factors of atherosclerosis such as blood pressure, blood sugar, TG, BMI, and cholesterol; level of renal function; the 24-hour stage of urine protein in lupus nephritis; level of serum albumin; the activity of lupus associated with nephritis (Anti DSDNA, C_3, C_4); the type of drugs in use (which are identified through blood sampling); and CIMT(which is carried out through G-S ultrasonography).

The common carotid artery was identified using the Medison (v10) device and the highest level of IMT was also measured 2 cm from the forking section of the common carotid artery.

Normal participants were voluntarily exposed to ultrasonography for the purpose of CIMT as well as urinary and serum analyses mentioned above. About 3 CC of the blood of participants was obtained to examine the levels of oxidative stress and MDA.

Statistical Analysis:

The collected data were analyzed by SPSS-17 statistical software. The collected data were expressed as percentage and mean ± SD. Continuous (quantitative) variables were compared by Independent samples and Paired t test. Categorical (qualitative) variables were compared by contingency tables and Chi-square test or Fisher's exact test. P-value ≤ 0.05 was considered statistically significant.

3. Results

In this study, the levels of oxidative stress and CIMT were measured and examined in 30 patients with lupus nephritis (LN) at an average age of 30.4±8.65 years as well as 30 healthy individuals at an average age of 28.8±4.61 years. The following results were obtained.

93.3% of patients with LN and 96.7% of patients in the control group were female. The age and gender of the two groups also matched.

In this study, 14 patients (44.46%) were diagnosed with stage-III LN, 13 patients (43.33%) had stage-IV LN and 3 patients (10%) were diagnosed with stage-V LN.

Experimental and CIMT results of the patients in the two groups are presented in Table (1). According to this table, the average levels of MDA, Hemocysteine, Right intima-media thickness, and Left intima-media thickness, BUN, Urine Protein (24 h), ESR, and CRP in patients with LN were significantly higher than the patients in the control group.

Table 1: Laboratory finding of patient between two groups

	Group		P
	Case	Control	
MDA	4.08 ± 1.39	1.76 ± 0.74	<0.001
Hemocysteine	23.50 ± 15.86	2.71 ± 1.57	<0.001
R_IMT	0.74 ± 0.14	0.58 ± 0.11	<0.001
L_IMT	0.74 ± 0.17	0.61 ± 0.12	<0.001
TG	196.43 ±81.58	220.10 ± 45.60	0.17
LDL	125.63 ± 36.29	128 ±19.61	0.77
BMI	24.23 ± 2.89	25.36 2.28	0.98
FBS	86.26 ± 7.41	83.10 ± 7.92	0.11
BUN	46.40 ± 12.60	25.73 ± 4.72	<0.001
Urine Protein (24 h)	1780.36 ± 15.25	65.76 ± 45.28	<0.001
ESR	52.10 ± 19.94	14.10 ± 6.84	<0.001
CRP	1.33 ± 0.99	0.33 ± 0.54	<0.001
Alb	3.1 ± 0.57	5.31 ± 0.45	<0.001
Hb	11.90 ± 0.88	13.12 ± 0.56	<0.001
Hct	32.93 ± 6.24	42.10 ± 2.45	<0.001
C3	39.46 ± 17.15	130.96 ± 18.13	<0.001
C4	8.76 ± 4.51	29.4 ± 6.76	<0.001
Ch50	53.78 ± 22.24	195.86 ± 49.93	<0.001

Table 2: Evaluation of studied based on LN stages and normal persons

	Group				P
	LN stage			Control	
	III	IV	V	Normal	
Hemocysteine	11.71 ± 6.32	36.36 ± 14.39	22.80 ± 9.72	2.71 ± 1.57	<0.001
MDA	3.55 ± 1.57	4.77 ± 0.93	3.53 ± 1.10	1.76 ± 0.73	<0.001
Right IMT	0.63 ± 0.10	0.85 ± 0.11	0.73 ± 0.15	0.58 ± 0.11	<0.001
Left IMT	0.63 ± 0.11	0.87 ± 0.14	0.70 ± 0.20	0.61 ± 0.12	<0.001

The average levels of Alb, Hb, Hct, C3, C4, and Ch50 in patients with LN were also significantly lower than patients in the control group.

Table (2) shows the results of Hemocysteine, MDA, Right intima-media thickness, and Left intima-media thickness for patients with LN based different stages of LN. No significant difference was seen between the levels of TG, LDL, FBS and BMI of patients of the two groups.

4. Discussions

The role of inflammatory diseases is also of concern in the development and progress of atherosclerosis (Tzoulaki, 2005). In this study the levels of biomarkers such as CRP and ESR were increased considerably in patients with lupus nephritis. Previous studies introduced chronic anemia as one of the risk factors of atherosclerosis and cardiovascular diseases (Sarnak, 2002).

In this study, the level of hemoglobin in lupus patients was significantly different than the level of hemoglobin in the control group members.

The level of complement in patients with active lupus also decline (Jasin, 1979). The same finding was obtained in the present study with SLE patients.

Higher levels of oxidative stress markers were seen in patients with a more active lupus (Wang, 2010).

In this study, the effects of different disease stages were studied in terms of renal biopsy pathology and oxidative stress markers.

The relationship of all the three stages of lupus with levels of Hemocysteine and MDA was found to be significant. In Stage 4 of lupus the two relationships were more considerable. The increase in Hemocysteine was significantly related to all of the three stages of lupus nephritis, but no significant relationship was observed between MDA and Hemocysteine in stages 3 and 5. There was a significant relationship between levels of Hemocysteine in stages 4 and 5 but no significant relationship was observed between levels of MDA in stages 4 and 5. Moreover, no significant relationship was observed between levels of Hemocysteine and MDA in stages 3 and 5. Research results revealed that even in patients with active lupus there are

considerable statistical differences between the increased levels of these oxidative stress markers in all stages.

In a study by Cacciapaglia in 2009 analysis of premature diagnosis of carotid artery through ultrasound techniques was found to be necessary for the assessment of sub-clinical atherosclerosis in SLE patients. In this study, CIMT was considered a useful parameter for the assessment of vascular injuries (Cacciapaglia, 2009). In the present study analysis of the relationship between different stages of the disease and CIMT revealed an increase in the carotid intima-media thickness in the patients group as compared to the healthy individual in the control group. The most significant relationship was observed in stage 4 of lupus. The thickness of carotid as well as its average thickness was measured. In this study it was found out that there was a significant relationship between the mean right and left CIMT results in stages 3 and 4. This finding indicated that even in patients with active lupus there are significant differences between CIMT results obtained from different stages of lupus. In this study, similar to the levels of MDA and Hemocysteine no significant relationship was observed between CIMT results of stages 3 and 5. Therefore, the level and extent of inflammation and progress of atherosclerosis were probably higher in stage 4 than stages 3 and 5. The highest progress of atherosclerosis was seen in stage 4 of lupus nephritis.

In this study all of the risk factors of atherosclerosis were studied. Of the aforementioned risk factors, the levels of creatinine, albumin, MDA, CRP, ESR and Hemocysteine were statistically different for the patient and control groups. Other risk factors of atherosclerosis such as HTN, LPF, BS, job, age, and gender did not show a considerable statistical difference. Analysis of the risk factors with significantly different results for the two groups revealed that only Hemocysteine was significantly related to CIMT results because no considerable relationship existed between other risk factors and development of atherosclerosis.

Results of the present study indicated that Hemocysteine, as one of the markers of oxidative stress, has a prominent and significant relationship with the onset of atherosclerosis in the patients.

Conclusion

According to the evidence result from this study, atherosclerosis is more common in lupus patients. Patients in Stage 4 of lupus nephritis demonstrate more severe signs of CIMT and atherosclerosis. This finding shows that a more severe disease is associated with a more severe and more mature form of atherosclerosis.

In addition, findings of this study proved the role of oxidative stress biomarkers in the increase in CIMT and development of atherosclerosis. Among the factors influencing the onset of atherosclerosis in such patients, the role of oxidative stress markers (especially Hemocysteine) was more prominent.

Suggestions

Future studies shall be carried out with larger samples and longer follow ups of lupus patients to further investigate the role of oxidative stress (especially Hemocysteine) in such patients. With interventional studies, longer follow ups, and examination of the effect of treatment of these risk factors on the clinical condition of patients it is possible to help lupus patients and minimize the complications caused by their disease.

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