Atherosclerosis Biomarkers Among Egyptian Patients with Systemic Lupus Erythematosus: Population Based Study

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Introduction

The association between atherosclerosis and SLE was first suggested in a case report by Aravanis and colleagues 1964 [1]. Attention was first drawn to the increased risk of atherosclerosis and, in particular, death due to atherosclerosis in patients with SLE by Murray Urowitz and his colleagues in 1976 [2]. Atherosclerotic disease is common in SLE and is the result of multiple pathogenic mechanisms that include traditional risk factors as well as SLE-related factors [3].

Microparticles (MPs) are defined as membrane vesicles released by various cell types (platelets, endothelial cells, monocytes) in circulation after cell activation or apoptosis [4]. All blood cells produce MP, the greatest amount being released by platelets, platelet MP. Endothelial MP (EMP) represents a smaller population of MP in plasma, but has been associated with cardiovascular disease, mainly endothelial dysfunction. EMP includes CD31, CD51, CD54, CD62E, CD105, CD106, CD144, CD146, E-selectina and VE-caderina. Several studies identify plasma levels of EMPs as a surrogate marker of vascular function [5]. Patel and Celermajer even stated that Endothelial dysfunction is believed one of the most important initial steps of the atherosclerosis process [6].

Tushuzien stated that Endothelial cells shed fragments of their plasma membrane known as endothelial microparticles (EMPs) [7]. The protein compositions of endothelial microparticles depend on the stimuli that trigger their release. Endothelial microparticles carry endothelial proteins such as vascular endothelial cadherin, platelet endothelial cell adhesion molecule-1, intercellular cell adhesion molecule (ICAM)-1, endoglin, E-selectin and integrins.

VCAM-1 is the first adhesion molecule expressed before atherosclerotic plaque development [8]. Endoglin (CD105) expression was demonstrated in atherosclerotic vessels predominantly in endothelial cells and smooth muscle cells in various types of blood vessels in mice and humans, suggesting its participation in atherogenesis [9].

The aim of this study was to assess and evaluates cIMT as well as EMPs (VCAM-1 and CD105) among SLE patients and its correlation to SLE related risk factors.

Methods

Clinical and laboratory data were collected from 60 patients with SLE who were selected randomly from the Rheumatology and Immunology outpatient clinic of El-Maadi Armed Forces Hospital and also 30 randomly selected apparently healthy age and sex matched...
controls. All SLE patients diagnosed according to 1997 update of the 1982 ACR revised criteria for classification of SLE [10]. Exclusion criteria included hypertension, diabetes mellitus as well as smoking. All patients gave written informed consent and the recommendations of the WHO and of the Declaration of Helsinki were followed in terms of protecting the rights and well-being of the people studied.

Carotid arteries ultrasound was performed for all patients and controls at El-Maadi Armed Forces Hospital Radiology department. Sonographers scanned the right and the left common carotid artery as well as carotid bulb. For each location, the sonographer imaged the vessel in multiple planes and then focused on the interfaces required to measure IMT and also on any areas of focal plaque. We documented the mean of six IMT measurements at the far wall of the CCA over a 1 cm long segment, 1 to 2 cm proximal to the carotid bifurcation for both sides.

We compared the power of the left and right IMT values and documented a mean IMT of the CCA as the mean of the 12 measurements among both sides. Examination was performed by the same operator with an ultrasound scanner (Siemens SONOLINE G40, Siemens Medical Solutions USA, Inc., Mountain View, CA, USA) with 7-MHz linear transducer and a transducer aperture of 38 mm. There are three different definitions of pIMT: a European version (pIMT>0.9), a German version (men 40 to 70 years old, pIMT > 1.0 mm; women 40 to 54 years old, pIMT>0.85 mm and 55 to 70 years old, pIMT>1.0 mm), and an Atherosclerosis Risk in Communities (ARIC) version, defined by the 90th percentile for different age and sex groups out of ARIC cohort [11]. We applied the European version where the IMT was considered “normal” when less than 0.9 mm, “thickened” when the IMT was equal to or more than 0.9 mm and when the thickness was more than 1.3 mm indicative of atherosclerotic plaque.

Vascular cell adhesion molecule-1 (VCAM-1) estimation was done by ELISA using Human sVCAM-1/CD106 immunoassay lot 329262 manufactured and distributed by R&D systems (USA).

Endoglin (CD105) human conjugated to FITC. Identification and enumeration was done using flow cytometer BD facscalibur, Becton Dickinson four color readers.

Full lipid profile (HDL-C, LDL-C, total cholesterol, total triglycerides) was performed on automated chemistry analyser (Siemens, Dimension EXL200, Germany). Anti-dsDNA ELISA was done manually using INOVA kits while anti-cardiolipin IgM and IgG ELISA using Bioflash kits and Bioflash analyser (USA), in addition to, basic laboratory investigations.

Systemic Lupus Erythematosus Disease Activity Index (SLEDAI) was assessed using a combination of the clinical history, physical examination, organ specific functional tests, and serologic studies [12].

Statistical analysis was done to all data collected from patients and controls included in this study. Data was analyzed by Microsoft Office 2010 (excel) and Statistical Package for Social Science (SPSS) version 20. Parametric data was expressed as mean ± SD, and non-parametric data was expressed as number and percentage of the total. Comparing the mean ± SD of 2 groups was done using unpaired student’s t test.

Results

Table 1 demonstrates a comparison between SLE patients and normal controls as regard cIMT, VCAM-1, CD105 and lipid profile. Results revealed highly significant increase of cIMT, VCAM-1, CD105, total cholesterol and LDL-C in SLE patients as compared to normal controls (P<0.001) but no significant difference as regard total triglycerides and HDL-C (P>0.05).

Table 2 compares between anti-dsDNA positive and anti-dsDNA negative SLE patients. Results revealed significant increase of cIMT in anti-dsDNA positive group (P<0.05) and no significant difference as regard VCAM-1 and CD105 (P>0.05).

Table 3 demonstrates a comparison between two subgroups; ACL positive and ACL negative SLE patients. There was no significant difference as regard cIMT, VCAM-1 and CD105 (P>0.05) between both subgroups.
SLEDAI>5) revealed no significant difference as regard VCAM-1, CD105 and cIMT (P>0.05).

| Parameters | Anti-dsDNA -ve patients (n=20) mean ± SD | Anti-dsDNA +ve patients (n=40) mean ± SD | t-value | P   | S  |
|------------|----------------------------------------|----------------------------------------|---------|-----|----|
| cIMT (mm)  | 0.593 ± 0.121                          | 0.690 ± 0.153                          | 2.689   | 0.010 | S  |
| VCAM-1 (ng/ml) | 1052.4 ± 415.89                        | 1092.6 ± 647.23                        | -0.291  | 0.772 | NS |
| CD105 (count/ml) | 64,954 ± 22,246.12                    | 56,766 ± 20,881.27                    | 1.371   | 0.179 | NS |

Table 2: Comparison between anti-dsDNA positive and negative patients in SLE group as regard cIMT and EMPs (VCAM-1/CD105).

| Parameters | ACL -ve patients (n=50) mean ± SD | ACL +ve patients (n=10) mean ± SD | t-value | P   | S  |
|------------|----------------------------------|----------------------------------|---------|-----|----|
| cIMT (mm)  | 0.66 ± 0.16                      | 0.65 ± 0.12                      | 0.208   | 0.838 | NS |
| VCAM-1 (ng/ml) | 1062.3 ± 569.19                  | 1163.8 ± 639.83                  | -0.466  | 0.649 | NS |
| CD105 (count/ml) | 58,091 ± 20,120.24              | 66,517 ± 27,636.2               | -0.917  | 0.379 | NS |

Table 3: Comparison between Anti-cardiolipin +ve or –ve SLE patients as regard cIMT and EMPs (VCAM-1/CD105).

Discussion

The IMT measurement in the carotid artery in its extracranial part can serve as an early marker of atherosclerotic changes and a risk factor for atherosclerotic organic complications and this very thickening of the complex triggers a long process of building the atherosclerotic plaque, whose fracturing in the future activates a cardiovascular incident [13].

According to the concept of parallel atherosclerosis development, early atherosclerotic changes observed in the lumen of peripheral arteries are a reflection of a generalized atherosclerotic process in other vessels and for that reason, measurement of the intima-media complex is presently a recognized evaluation method both of the initial and advanced atherosclerotic changes and also a control method of the applied pharmacotherapy efficiency [14].
In the present study, our results revealed highly significant increase as regard cIMT in SLE patients as compared to normal controls (P<0.001) (0.657 ± 0.149 and 0.56 ± 0.11, respectively) and this observation suggests a faster atherosclerotic process in patients with SLE. Our results were supported by Colombo study which showed that patients with SLE have an increased mean cIMT value compared with a healthy control [15]. This is in agreement with Cacciapaglia who stated that patients with SLE presented a higher mean IMT of the common carotid artery than healthy subjects (0.7 ± 0.2 mm vs. 0.5 ± 0.1 mm, P<0.0001) [16]. In favor of our results, additional two meta-analyses and reviews published by Au and Tyrrell which revealed a higher cIMT compared with healthy controls in SLE patients [17,18]. In disagreement with our work, Gallelli and his colleges (2009) who stated that the serum concentration of VCAM-1 in SLE patients than healthy controls [31]. On the other hand, Skeoch and his colleges who stated that in patients with SLE some non-traditional risk factors for atherosclerosis were identified, the most important of which was the cumulative prednisone dose [20].

Atherosclerotic plaque lesions can be found frequently in absence of intima-media thickening in SLE patients [11]. This was the case in our study where the four patients with plaques had normal cIMT.

The mean of the cIMT for SLE patients in our study which was 0.657 mm agrees with Skeeoch who found that the mean of cIMT for SLE patients in their study was 0.633 mm but slightly higher than Roman who found that the mean cIMT for SLE patients was 0.61 mm [23,24].

Petri and Hamper stated that subclinical atherosclerosis detected by cIMT measurement in various lupus populations and has ranged from 8% to 40%, depending on the technique and the patients demographic background, which can explain this variation [25].

Our results demonstrated no correlation between cIMT and SLEDAI. This agrees with Sazliyana who stated that there was no correlation between disease activity measured by SLEDAI and thickened cIMT, since atherosclerosis itself is the result of a chronic insult to the vessel wall, perhaps measuring disease activity at the current study time point is not a good indicator of the overall activity or severity of lupus activity throughout the course of the disease [26]. Our results disagree with Kisiel who stated that IMT was associated with SLEDAI [27]. This could be explained by different technical methods used and/or absence of standardization. Our results also disagree with Nassef who found that there was a statistically significant correlation between IMT and SLEDAI [28].

Also, cIMT of patients with SLE did not correlate with the presence of antiphospholipid syndrome and/or the presence of anticardiolipin antibodies in the present study. Our results agree with Sazliyana who stated that no association was found between thickened cIMT and the presence of antiphospholipid syndrome and/or the presence of anticardiolipin antibodies [26].

Comparative study between patients with normal cIMT and patients with increased cIMT in SLE group revealed significant difference as regard steroid duration (P<0.05) and high significant difference as regard steroid cumulative dose and disease duration (P<0.01). This agrees with Doria and her colleges who stated that in patients with SLE some non-traditional risk factors for atherosclerosis were identified, the most important of which was the cumulative prednisone dose [20].

In our study, VCAM-1 mean ± SD for SLE patients was 1,079.2 ± 577.04 (ng/ml) while that of the control group was 531.87 ± 163.89 (ng/ml) which showed significant increase as compared to normal control group (P<0.001). The mean of VCAM-1 for SLE patients was similar to that reported by Young and his colleges (2008) where it was 1077.4 ng/ml (887.9-1203.3), but they reported higher levels of the mean value for their control group [29]. Our results agree with Robak and his colleges (2009) who stated that the serum concentration of VCAM-1 was detectable in all SLE patients [30]. VCAM-1 was higher in patients with SLE than in the control group. Our results also agree with Kassem who stated that there was significant elevation of VCAM-1 in SLE patients than healthy controls [31]. On the other hand, Skeoch and his colleges found no significant difference in VCAM-1 levels in SLE patients and controls [23]. This could be explained by different assay methods, variation in studied patients as regard age, gender and nationality, also the number of the studied cases can affect the statistical results.

In this study, a comparative study between patients with normal cIMT and patients with increased cIMT in SLE group revealed no significant difference as regard VCAM-1 (P>0.05).

Also, in this study the correlation between VCAM-1 in all 60 patients enrolled in this study revealed no significant correlation with

| Parameters | SLE patients with SLEDAI ≤ 5 (n=23) mean ± SD | SLE patients with SLEDAI >5 (n=37) mean ± SD | t-value | P | S |
|------------|---------------------------------------------|---------------------------------------------|----------|---|---|
| cIMT (mm)  | 0.665 ± 0.139                               | 0.653 ± 0.158                               | 0.322    | 0.748 | NS |
| VCAM-1 (ng/ml) | 1033.8 ± 427.948                           | 1107.4 ± 657.003                            | -0.525   | 0.601 | NS |
| CD105 (count/ml) | 61,632 ± 23,146.874 | 58,167 ± 20,645.533 | 0.587 | 0.56 | NS |

**Table 5:** Comparison between SLE patients with no or mild activity (SLEDAI ≤ 5) and SLE patients with high activity (SLEDAI>5) as regard cIMT and EMP (VCAM-1/CD105).
cIMT. These results might denote that VCAM-1 is not the only player in the atheroma formation in carotid arteries. Our results agree with the study of Roman and his colleagues, who stated that adhesion molecules (VCAM and ICAM) were not associated with the presence or absence of carotid plaque in SLE [24]. But our results disagree with Rubio-Guerra who suggest that systemic levels of ICAM-1 and VCAM-1 are associated with IMT and correlated with the degree of atherosclerosis [32].

Comparative study between SLE patients with no or mild activity (SLEDAI ≤ 5) and SLE patients with high activity (SLEDAI>5) revealed no significant difference as regard VCAM-I, the results were 1033.8 ng/ml and 1107.4 ng/ml, respectively (P>0.05). Our findings agree with Skeoch and his colleges who studied 178 patients and 69 controls with a median age of 53 and 50 years old, respectively [23]. They found no association between VCAM-1 and disease activity. Our findings also agree with Robak and his colleagues who stated that it does not find any statistically significant differences in numbers of circulating EMPs and their particular subpopulations between patients with active and non-active type of SLE [30]. But our results disagree with Kassem who stated that regarding their relations to disease activity VCAM-1 significantly increased with disease activity and correlate positively with circulating endothelial cells microparticles count and such variation in results may reflect small sample sizes and heterogeneity of populations studied [31].

In the present study, the mean ± SD of CD105 in SLE patients group was 59,495 ± 21,511.5 while that in the control group was 31,267 ± 1,871.66. Comparative study between both groups revealed high significant elevation in SLE group in comparison with the control group (P<0.001). Similar results were reported by Duval who measured the EMP in the plasma of SLE patients and healthy individuals and found significant elevation of EMP in SLE patients in comparison with the control [32,33].

In the present work CD105 showed no significant difference in their levels in SLE patients with ACL antibodies or with anti-dsDNA. Also, there was no significant difference between patients with normal IMT and patients with increased IMT as regard CD105 level.

In our study, a comparative study between SLE patients with no or mild activity (SLEDAI ≤ 5) and SLE patients with high activity (SLEDAI>5) revealed no significant difference as regard CD105 (P>0.05). Our results agree with Bassouyi who stated that they failed to detect any association between the disease activity as assessed by SLEDAI and s-Endoglin concentrations [34].

Conclusion
Our results show that SLE patients had a significant increase in cIMT, VCAM-1 and CD105 compared with the controls. This significant increase in these atherosclerotic biomarkers was not correlated with indices of disease activity or presence of anti-dsDNA nor ACL antibodies but correlated with duration of steroids, duration of the disease, steroid cumulative dose, age and LDL-C level. We can conclude that the increase in these atherosclerotic biomarkers could be attributed to immune complex-mediated autoimmune inflammation and its effect upon endothelial system.

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References
1. Aravanis C, Toutouzas P, Yatzidis I (1964) Fatal myocardial infarction in lupus erythematosus: Report of a case of a young female patient. Vasc Dis 1: 258-260.
2. Urowitz MB, Bookman AAM, Koehler BE, Gordon DA, Smythe HA, et al. (1976) The bimodal mortality pattern of SLE. Am J Med 60: 221-225.
3. Abu-Shakra M, Codish S, Zeller L, Wolak T, Sukenik S (2008) Atherosclerotic cardiovascular disease in systemic lupus erythematosus: The Beer Sheva experience. Isr Med Assoc J 10: 43-44.
4. Mahajan K (2015) Microparticles in atherosclerosis: Biomarkers of disease. J Clin Exp Cardiolog 6: 356.
5. França CN, de Oliveira Izar MC, Amaral JB, Tegani DM, Francisco FA (2015) Microparticles as potential biomarkers of cardiovascular disease. Arq Bras Cardiol 104: 169-174.
6. Pathe S, Celermann D (2006) Assessment of vascular disease using arterial flow mediated dilation. Pharmacol Rep 58: 3-7.
7. Tushuizen ME, Diamant M, Sturk A, Nieuwland R (2011) Cell-derived microparticles in the pathogenesis of cardiovascular disease: friend or foe? Arterioscler Thromb Vasc Biol 31: 4-9.
8. Cook-Mills JM, Marchese ME, Hiam Abdala-Valencia (2011) Vascular cell adhesion molecule-1 expression and signaling during disease: Regulation by reactive oxygen species and antioxidants. Antioxid Redox Signal 15: 1607-1638.
9. Nachtgall P, Zemankova VL, Rathouska J, Strasky Z (2012) The role of endoglin in atherosclerosis. Atherosclerosis 224: 4-11.
10. Hochberg MC (1997) Updating the American college of rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum 40:1725.
11. Frerix M, Stegbauer J, Kreuter A, Weiner SM (2014) Atherosclerotic plaques occur in absence of intima-media thickening in both systemic sclerosis and systemic lupus erythematosus: a duplex sonography study of carotid and femoral arteries and follow-up for cardiovascular events. Arthritis Res Ther 16: R54.
12. Lam GK, Petri M (2005) Assessment of systemic lupus erythematosus. Clin Exp Rheumatol 23: 120-132.
13. Touboul PJ, Labreuche J, Vicaud E, Amarenco P, GENIC Investigators (2005) Carotid intima-media thickness, plaques, and Framingham risk score as independent determinants of stroke risk. Stroke 36: 1741-1745.
14. Libby P (2001) Current concepts of pathogenesis of the acute coronary syndromes. Circulation 104: 365-372.
15. Colombo BM, Murdaca G, Cattir M, Rodriguez G, Grassia L, et al. (2007) Intima-Media thickness: a marker of accelerated atherosclerosis in women with systemic lupus erythematosus. Ann N Y Acad Sci 1108: 121-126.
16. Caccipaglia F, Zardi EM, Coppolino G, Buzzulini F, Margiotta D, et al. (2009) Stiffness parameters, intima-media thickness and early atherosclerosis in systemic lupus erythematosus patients. Lupus 18: 249-256.
17. Au K, Singh MK, Bodukam V, Bae S, Maranian P, et al. (2011) Atherosclerosis in systemic sclerosis: A systematic review and meta-analysis. Arthritis Rheum 63: 2078-2090.
18. Tyrell PN, Beyeve J, Feldman BM, McCrindle BW, Silverman ED, et al. (2010) Rheumatic disease and carotid intima-media thickness: A systematic review and meta-analysis. Arterioscler Thromb Vasc Biol 30: 1014-1026.
19. Gallelli B, Burdick L, Quaglini S, Bani G, Novembrino C, et al. (2010) Carotid plaques in patients with long-term lupus nephritis. Clin Exp Rheumatol 28: 386-392.
20. Doria A, Shoenfeld Y, Wu R, Gambari PF, Puato M, et al. (2003) Risk factors for subclinical atherosclerosis in a prospective cohort of patients with systemic lupus erythematosus. Ann Rheum Dis 62: 1071-1077.

21. Rizk A, Gheita TA, Nassef S, Abdallah A (2012) The impact of obesity in systemic lupus erythematosus on disease parameters, quality of life, functional capacity and the risk of atherosclerosis. Int J Rheum Dis 15: 261-267.

22. Souza AW, Hatta FS, Miranda Jr F, Sato El (2005) Atherosclerotic plaque in carotid arteries in systemic lupus erythematosus: frequency and associated risk factors. Sao Paulo Med J 123: 137-142.

23. Skeoch S, Haque S, Pemberton P, Bruce IN (2014) Cell adhesion molecules as potential biomarkers of nephritis, damage and accelerated atherosclerosis in patients with SLE. Lupus 23: 819-824.

24. Roman MJ, Shanker BA, Davis A, Lockshin MD, Sammaritano L, et al. (2003) Prevalence and correlates of accelerated atherosclerosis in systemic lupus erythematosus. N Engl J Med 349: 2399-2406.

25. Petri M, Hampler U (1997) Frequency of atherosclerosis detected by carotid duplex in SLE. Arthritis Rheumatol 40: 4219.

26. Sazliyana S, Mohd Shahrir MS, Kong NT, Tan HJ, Hamidon BB, et al. (2011) Thickened carotid intima media thickness and carotid atherosclerosis among lupus nephritis patients: the role of traditional and lupus-specific factors. Int J Rheum Dis International 14: 267-275.

27. Kisiel B, Kruzewski R, Juszkiewicz A, Raczkiewicz A, Bachta A, et al. (2015) Systemic lupus erythematosus: the influence of disease-related and classical risk factors on intima media thickness and prevalence of atherosclerotic plaques–a preliminary report. Beneficial effect of immunosuppressive treatment on carotid intima media thickness. Acta Cardiol 70: 169-175.

28. Nassef S, El Guindey H, Fawzy M, Nasser A, Reffai R, et al. (2016): Microparticles (CD146) and arterial stiffness versus carotid intima media thickness as early predictors of vascular affection in systemic lupus patients. Arch Rheumatol 31: 31-40.

29. Rho YH, Chung CP, Oeser A, Solus J, Raggi P, et al. (2008) Novel cardiovascular risk factors in premature coronary atherosclerosis associated with systemic lupus erythematosus. J Rheumatol 35: 1789-1794.

30. Robak E, Kierstan M, Cebula B, Krawczynska A, Sysa-Jedrzejowska A, et al. (2009) Circulating endothelial cells and angiogenic proteins in patients with systemic lupus erythematosus. Lupus 18: 332-341.

31. Kassem E, El-Sergany M, El-Saadany H, Shahba A, Salah W (2010) Circulating endothelial cells and cardiovascular risk in systemic lupus erythematosus. J Am Sci 11: 700-707.

32. Rubio-Guerra AF, Vargas-Robles H, Serrano AM, Lozano-Nuevo JJ, Escalante-Acosta BA (2009) Correlation between the levels of circulating adhesion molecules and atherosclerosis in type-2 diabetic normotensive patients: Circulating adhesion molecules and atherosclerosis. Cell Adh Migr 3: 369-372.

33. Duval A, Helley D, Capron L, Youinou P, Renaudineau Y, et al. (2010) Endothelial dysfunction in systemic lupus patients with low disease activity: evaluation by quantification and characterization of circulating endothelial microparticles, role of anti-endothelial cell antibodies. Rheumatology (Oxford) 49: 1049-1055.

34. Bassouyuni IH, El-Shazly R, Azkalany GS, Zakaria A, Bassouyuni RH (2012) Clinical significance of soluble-endoglin levels in systemic lupus erythematosus: possible association with anti-phospholipid syndrome. Lupus 21: 1565-1570.