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Mannose Binding Lectin Gene Polymorphism and Preclinical Carotid Atherosclerosis in Patients with Systemic Lupus Erythematosus

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Patients with systemic lupus erythematosus (SLE) have increased risk of atherosclerosis and CVD that cannot be explained by traditional risk factors. Previous studies indicated that mannose binding lectin (MBL) may modify the development of atherosclerosis. This study was designed to investigate association of MBL gene polymorphism with occurrence of preclinical atherosclerosis in SLE. The study included 46 patients with SLE and 17 age and sex matched controls.MBL2 genotypes were assessed in patients and controls by the PCR-RFLP method and intima-media thickness of the common carotid artery (ccIMT) was determined by means of ultrasonography. Also, serological markers were measured and the disease activity index (SLEDAI) was estimated. SLE patients had higher frequency of MBL A/B + B/B genotypes (47.8%) than controls (29.4%). ccIMT was higher in patients having A/B, B/B, A/B+B/B genotypes when compared with wild genotype (A/A). Patients with A/B+B/B genotypes showed high serum level of LDL, TG, ESRI, CRP and SLEDAI score, but low level of HDL, C3, and C4 compared to wild genotype. ccIMT of mutant SLE subgroup correlated well with SLE risk factors for atherosclerosis. In conclusion, mutant genotypes of MBL may be atherogenic as SLE patients had a higher IMT, which correlated significantly with SLE risk factors for atherosclerosis.

Systemic lupus erythematosus (SLE) is considered the most clinically and serologically diverse autoimmune disease because it may affect any organ and display a broad spectrum of clinical manifestations. Cardiovascular events have emerged as major causes of morbidity and mortality in SLE patients (Thorburn & Ward, 2003).

SLE patients have a high prevalence of traditional risk factors for atherosclerosis, related to clinical conditions and treatment received. However, there is evidence that these factors do not fully explain the atherosclerotic process in SLE. Studies have implicated new aetiopathogenic factors such as chronic inflammation or endothelial cell injury, suggesting a multifactorial pathogenesis of cardiovascular disease in SLE (Mach *et al.,* 1998, Soubrier *et al.,* 2007).

Premature atherosclerosis is an important problem in patients with SLE and real prevalence of preclinical atheromatosis is unknown. The diagnosis of asymptomatic atherosclerotic disease in SLE may lead to early, effective treatment of potential complications (Alpert *et al.,* 2007). Various studies have attempted to evaluate the frequency of preclinical cardiovascular disease using different imaging techniques (Rumberger *et al.,* 1995, Mintz *et al.,* 1997, Maheer *et al.,* 1999, Salmon & Roman, 2008). The high-resolution duplex technique provides accurate and reliable measurements of atherosclerosis in its preclinical stage (Woford *et al.,* 1991, Doria *et al.,* 2003).

Carotid B-mode ultrasound is a reproducible method of identification of atherosclerosis in the arterial wall (Riley *et al.,* 1992). It identifies atherosclerotic plaque and has the ability to measure the artery

intima-media thickness (IMT). The frequency of atherosclerotic plaque in lupus patients identified by this method varies between 9 and 50 % (Souza *et al.,* 2005). The presence of atherosclerotic alterations in the carotids of lupus patients has been associated not only with traditional risk factors for coronary artery disease, but also with SLE associated factors (Telles *et al.,* 2008).

There is evidence that the pathogenesis of cardiovascular disease may involve components of the innate immune system, which includes specific pattern-recognition receptors such as mannose binding lectin (MBL) (Best *et al.,* 2004). Mannose-binding lectin is a liver-derived serum protein involved in innate immune defense. The ligands for mannose-binding lectin are mannose and *N*-acetyl glucosamine oligosaccharides, expressed by a wide range of microorganisms. Mannose-binding lectin may activate complement by means of the lectin pathway. When MBL binds to its target (e.g., mannose on the surface of a bacterium) and interacts with mannose-binding lectin–associated serine proteases (MASPs), results in the formation of C3-convertase, which facilitates removal of pathogens by opsonophagocytosis or complement-mediated lysis. The MBL pathway also contributes to the clearance of apoptotic cells and circulating immune complexes (Ramos-Casals *et al.,* 2009). MBL is structurally and functionally similar to C1q and shares the same phagocytic receptor on phagocytes, platelets, and endothelial cells. As with C1q, a low serum level of MBL may predispose to the development of SLE by impairing opsonization and complement-mediated clearance of immune complexes (Turner & Hamvas, 2000). Low serum levels of MBL have been associated with functional polymorphisms in the coding region of exon 1 and the promoter of the MBL gene. Accordingly, genetically defined loss-of-function variations of the MBL and MASP-2 molecules have been reported to influence susceptibility and disease expression of both infections and autoimmune diseases (Ramos-Casals *et al.,* 2009).

Human MBL is encoded by the MBL2 gene on chromosome 10 (MBL1 is a pseudogene) and contains 4 exons (Sastry *et al.,* 1989). Three single nucleotide polymorphisms at exon 1 of MBL2 at codons 52, 54 and 57 (named D, B and C variants, respectively, and collectively referred as O variants) are major determinants of serum MBL levels. The wild-type variant is referred to as A. Polymorphisms in exon 1 at 54 (aspartic acid for glycine, allele "B"), result in disruption of assembly of MBL peptides into functional polymers and profound reduction in serum levels of functional MBL. An individual heterozygous for MBL2 54 coding region containing the B mutations is referred to as "A/B" and a homozygote "B/B" (Turner & Hamvas, 2000).

The normal genotype is associated with the highest serum concentrations of MBL. Heterozygosity for MBL2 structural variant alleles causes an 85–90% drop in the serum concentration of functional MBL and homozygosity for structural variant alleles is devoid of functional MBL (Dorthe *et al.,* 2007, Huh *et al.,* 2009). A previous report showed an association between the deficient homozygous O/O MBL-genotype and the development of arterial thrombosis in patients with SLE (Øhlenschlaeger *et al.,* 2004). The possible association between low serum MBL levels and accelerated atherosclerosis has been reported. It has been hypothesized that patients with low serum MBL levels may mount a suboptimal defense against infections with Chlamydia pneumoniae, thus allowing its colonization of the vascular wall. MBL promotes the clearance of this and other potentially atherogenic agents from the vascular endothelium; this is more efficient in

subjects with high MBL levels (Saevarsdottir *et al.,* 2005).

This study aimed to investigate association of MBL gene polymorphism with occurrence of preclinical atherosclerosis in systemic lupus erythematosus patients.

Subjects and Methods

Subjects

Forty-six Egyptian patients with SLE (44 female, 2 male, with mean age of 35.23 ± 4.67 yr.), were recruited from Internal Medicine, and Rheumatology and Rehabilitation Departments, Assiut University Hospitals from January 2009- January 2010. All patients fulfilled at least four of the revised American College of Rheumatology (ACR) criteria for the diagnosis of SLE (Hochberg, 1997), and were either in an active or an inactive stage of the disease defined as SLE disease activity index score (SLEDAI) ≥ 8 or ≤ 8 respectively (Jacobi *et al.,* 2003). Forty-five patients received steroid therapy. Seventeen age and sex matched healthy volunteers (15 female, 2 male, with mean age 34.80 ± 2.11 yr.) were also included as a control group. None of them had clinical evidence of cardiovascular or cerebrovascular disease. Full history and clinical examination were performed for all the participants. The study was approved by the Ethics Committee of Faculty of Medicine, Assiut University and informed consent was obtained from all the participants.

Exclusion Criteria

Subjects with diabetes mellitus, hypertension, obesity, or smoking habit as well as those in the postmenopausal state were excluded from the study. Patient who had pre-existing clinical cardiovascular or cerebrovascular events (angina, myocardial infarction, peripheral vascular disease, transient ischemic attack or stroke) were also excluded.

Laboratory Tests

- Laboratory investigations were performed for every subject. For each participant, 10 ml of venous blood were aseptically collected; 3 ml in tubes containing EDTA as anticoagulant for PCR, 1 ml in tubes containing EDTA for complete blood picture, and 1.6 ml in tubes containing 3.8% sodium citrate for ESR. Remaining sera were aliquot and stored at -20°C for biochemical tests.
- Complete blood count (CBC) was performed using Beckman Coulter HMX, ESR was done according to Westergren.
- Glucose, kidney function tests, total cholesterol, high density lipoprotein cholesterol (HDL), low density lipoprotein cholesterol (LDL), very low density lipoprotein cholesterol (VLDL) and triglycerides (TG) were performed using a chemical analyzer Hitachi 911 (Boehringer Mannheim, Germany).
- C3 and C4: Serum levels of C3 and C4 was assessed using the single radial immunodiffusion technique (The Binding site Ltd, Birmingham, UK).
- C-reactive protein (CRP) was determined by latex test (Omega Diagnostics, United Kingdom).
- Anti-dsDNA: An indirect solid phase ELISA for quantitative measurement of IgG class autoantibodies against double-stranded DNA in human serum or plasma was performed according to the manufacturer instructions (Cat. No. ORG 604; ORGENTEC Diagnostika, GmbH, Mainz, Germany).
- Mannose binding lectin gene polymorphisms: Codon 54 polymorphism of the MBL gene was performed by polymerase chain reaction and restriction fragment length polymorphism (PCR-RFLP) method as mentioned by Wang *et al*., 2001. Briefly, DNA was isolated from blood samples using the QIA amp DNA mini kit (QIAGEN GmbH, D-40724 Hilden), and amplified by PCR using the primer sequence as follow; forward (5'GTAGGACAGAGGGCATGCTC -3') and Reverse (3'- CAGGCAGTTTCCTCTGGA AGG-5'). For DNA amplification we used Ready-To –Go PCR beads Kit (GE Health Care, UK Limited, Code: 27-9557-01). The PCR mixture contained 10 µl of the extracted DNA as template, 1µl of each primer and 13 µl water. The amplification profile was as follows: initial denaturation at 94°C for four minutes, 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for one minute, and extension at 72°C for one minute; and a final elongation at 72°C for five minutes. The amplified product (329 bp) was digested by restriction enzyme *BanI*. The digestion mixture contained 10µl of amplified DNA, 2µl 10x buffer, 1µl *BanI* enzyme and 5 µl of free water; and incubated at 37°C for 6 hours. The digested products were subjected to 2% agarose gel electrophoresis, stained with ethidium bromide, visualized on a UV box and fragment patterns were determined. A fragment with the wild type allele (A) is cleaved into two bands (245 bp and 84 bp), while that with the mutant allele (B) shows one band (329 bp). Three patterns were determined: A/A, B/B, and A/B.

Ultrasonographic Studies (using LOGIQ 7; General Electric Company, UK):

Patients and controls were examined in the supine position with the head hyper extended and the neck either straight or oblique in the contra lateral direction. A high frequency imaging probe was used to visualize vessel anatomy and vascular pathology optimally in both transverse and sagittal planes. When the imaging analysis was complete, Doppler was used to assess blood flow in the common, internal, and external carotid arteries on both sides. In areas of carotid plaque, Doppler measurements were taken proximal to, at, and distal to the point of maximum stenosis. Precise placement of the Doppler sample volume relative to the vessel on pathology was facilitated by the simultaneous duplex B-mode image.

Atherosclerotic plaque (defined as a focal protrusion into the vessel lumen with at least 50% greater thickness than that found in the surrounding area) was investigated bilaterally in the aforementioned arteries in multiple projections. Intima-media thickness was measured three times on each side of the posterior wall of the right and left distal common carotid arteries immediately below the bulb. The average of the right and left arteries was calculated and the highest one was considered in statistical analysis (Telles *et al*., 2008).

Statistical Analysis

The statistical analysis was performed using SPSS for Windows (version 17.0). Data were expressed as mean, standard deviation (SD), and percentage. The Mann-Whitney test was used to assess differences in quantitative variables. The Chi-square (χ^2 test) was used to compare proportions. The Person´s correlation was performed to determine the relationship between variables. *P* values ≤0.05 were considered significant.

Results

Levels of serum cholesterol, triglyceride, LDL, CRP, anti-ds-DNA, ESR, and intima-media thickness were significantly elevated; while HDL,C3,and C4 were reduced in SLE group compared to control group (Table, 1).

Table (2) shows the distribution of mannose binding lectin genotypes in which the percentage of A/B + B/B genotypes was significantly increased in lupus patients (47.8%) when compared to control group (29.4%), ($P < 0.01$).

Table1. Serum Levels of Studied Parameters and intima-media thickness in SLE Patients and Controls

Parameters (mean±SD)	SLE(46)	Controls(17)	*P value
Cholesterol (mg/dl)	227.28±86.41	150.38±30.29	<0.01
Triglyceride (mg/dl)	218.28±145.45	117.29±37.28	<0.01
LDL (mg/dl)	131.59±64.46	115.72±38.41	<0.05
HDL (mg/dl)	29.46±07.39	47.82±10.81	<0.05
ESR (mm/h)	86.00±23.44	12.34 ±2.3	<0.001
CRP (mg/L)	19.04±4.87	3.10±1.1	<0.01
C3 (mg/L)	758.61±216.42	1401.12±271.93	<0.01
C4 (mg/L)	191.55±92.41	293.82±145.24	<0.01
Anti-ds-DNA (IU/ml)	616.63±100.25	16.62±3.86	<0.001
Intima-media thickness (mm)	0.80 ± 0.2	0.57 ± 0.13	<0.05
SLEDAI score	11.45± 3.89	--------	--------

**P* values ≤0.05 were considered significant

Table 2. Frequency of mannose binding lectin genotype in SLE patients and controls

MBL	SLE(46)	Controls(17)	*P value
A/A	24 (52.2 %)	12 (70.6 %)	NS
A/B	10 (21.7 %)	3 (17.6 %)	NS
B/B	12 (26.1 %)	2 (11.8 %)	NS
A/B + B/B	22 (47.8 %)	5 (29.4 %)	<0.01

MBL, mannose-binding lectin. Allele A corresponds to wild-type MBL gene. Allele B corresponds to MBL gene with codon 54 mutation. *P values ≤0.05 were considered significant. NS= not significant.

The clinical, laboratory, and radiological investigations as well as SLEDAI score in patients with lupus were delineated in table (3) according to A/A, A/B and B/B mannose binding lectin genotypes.

Table (4) shows the results of laboratory and radiological investigations in A/A genotype subgroup and A/B + B/B genotypes subgroup. The intima-media thickness showed increase in A/B + B/B subgroup when compared to group A/A subgroup ($P < 0.05$). Serum LDL, TG, ESR1,CRP, and SELDAI showed increase in A/B + B/B subgroup when compared to group A/A subgroup ($P < 0.05$ for each), while serum HDL, C4, and C3 showed decrease in A/B + B/B subgroup when compared to group A/A subgroup ($P < 0.05$ for each).

Table 3. Clinical, Laboratory and radiological investigations in various mannose binding lectin genotypes in patients with systemic lupus erythematosus.

Parameters (mean±SD)	A/A gene (24)	A/B gene (10)	B/B gene (12)	AA vs A/B	AA vs B/B	A/B vs B/B
Age/Year	35.00±5.42	33.94±5.62	35.56±2.65	NS	NS	NS
Disease duration/ year	3.38±2.62	4.03±3.13	2.16±1.56	NS	<0.05	<0.05
Cholesterol (mg/dl)	210.67±59.16	258.50±102.83	235.55±92.84	<0.05	<0.05	<0.05
Triglyceride (mg/dl)	199.36±115.13	248.18±196.86	190.11±123.88	<0.05	N.S.	<0.05
LDL (mg/dl)	122.00±49.84	115.37±54.50	174.22±95.24	NS	<0.05	<0.05
HDL (mg/dl)	35.50±14.23	32.43±7.36	26.44±8.67	NS	<0.05	NS
ESR 1 (mm/h)	89.17±49.47	86.18±44.15	87.78±38.25	NS	NS	NS
CRP (mg/L)	12.67±13.56	27.88±34.22	16.18±19.96	<0.05	<0.05	<0.01
C3 (mg/L)	789.04±571.47	589.50±385.14	804.78±599.10	<0.05	NS	<0.05
C4 (mg/L)	150.92±79.04	158.25±102.06	138.80±103.33	NS	NS	<0.05
Anti-ds-DNA (IU/ml)	547.44±1271.68	643.97±631.87	698.33±578.30	<0.05	<0.05	NS
Intima-media thickness (mm)	0.62 ± 0.19	0.86 ± 0.22	0.95 ± 0.25	<0.05	<0.05	NS
SLEDAI score	10.45± 3.17	10.56± 3.99	11.44 ± 3.84	NS	NS	NS

*P values ≤0.05 were considered significant. NS= not significant.

Table 4. Comparative analysis of clinical, Laboratory and radiological investigations in mannose binding lectin A/A genotype and A/B + B/B genotypes in patients with systemic lupus erythematosus.

Parameters (mean±SD)	A/A gene (24)	A/B+B/B gene (22)	*P-value
Age/Year	35.00±5.42	35.50±3.81	NS
Disease duration/ year	3.38±2.62	3.66±2.94	NS
Cholesterol (mg/dl)	210.67±59.16	242.67±79.16	NS
Triglyceride (mg/dl)	199.36±115.13	235.63±169.21	<0.05
LDL (mg/dl)	122.00±49.84	142.05±77.22	<0.05
HDL (mg/dl)	35.50±14.23	30.95±16.21	<0.05
ESR 1 (mm/h)	89.17±49.47	96.27±35.68	<0.05
CRP (mg/L)	12.67±13.56	21.67±25.39	<0.05
C3 (mg/L)	789.04±571.47	705.41±259.08	<0.05
C4 (mg/L)	150.92±79.04	143.15±99.86	<0.05
Anti-ds-DNA (IU/ml)	547.44±1271.68	692.11±637.88	NS
Intima-media thickness (mm)	0.62 ± 0.19	0.88 ± 0.25	<0.05
SLEDAI score	10.45± 3.17	11.92± 4.51	<0.05

*P values ≤0.05 were considered significant. NS= not significant.

In lupus patients with A/A genotype, there was a positive correlation between intima-media thickness and serum cholesterol, and SLEDAI score (r = 0.725, $P< 0.05$, r= 0.592, $P < 0.01$ respectively), while there was a negative correlation between serum HDL and intima-media thickness (r = -0.970, $P< 0.05$). (Fig. 1)

In lupus patients with A/B + B/B genotypes there was a significant positive correlation between intima-media thickness and duration of disease, serum cholesterol, serum LDL, serum triglycerides (TG), ESR1, C reactive protein, Anti-ds DNA and SLEDAI score (r = 0.725, $P< 0.05$, r = 0.802, $P< 0.05$, r= 0.652, $P < 0.05$, r = 0.835, P< 0.01, r = 0.521, $P< 0.05$ r = 0.395, $P< 0.05$ r = 0.925, $P< 0.05$ r = 0.824, $P< 0.05$ respectively).

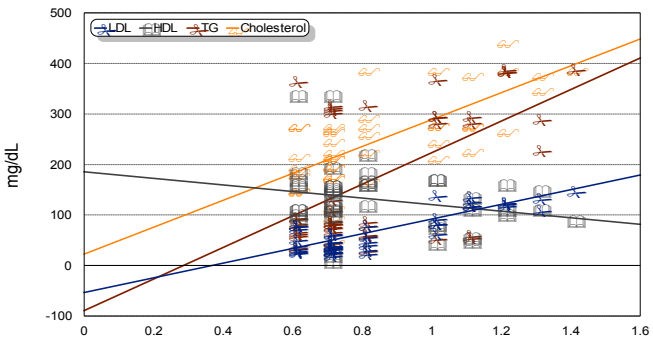

Figure 1. Correlation between IMT and serum cholesterol, LDL, HDL, and TG in SLE patients with A/B+ B/B genotypes.

However, there was a significant negative correlation between steroid therapy, serum HDL, C4 and C3 and intima-media thickness (r = -.625, $P< 0.05$, r = -.725, $P< 0.05$, r= -0.608, $P < 0.05$ r= -0.743, $P < 0.05$ respectively). Study data revealed that none of the patients had carotid atherosclerotic plaque.

Discussion

Cardiovascular disease is a major cause of illness and death in patients with systemic lupus erythematosus. The increased risk of cardiovascular disease in young patients is not fully accounted for by traditional atherosclerotic risk factors. In previous studies MBL was associated with myocardial infarction and ischemic heart disease in patients with SLE (Øhlenschlæger *et al.,* 2004, Font *et al.,* 2007, Troelsen *et al.,* 2010). Accordingly we tried to demonstrate if polymorphism of MBL in SLE patients were associated with increased subclinical atherosclerosis measured by ultrasonography.

In the present study, the frequency of individuals, with homozygous MBL gene codon 54 mutation allele B (B/B genotype), was higher in SLE patients compared to healthy controls, but the difference did not reach statistical significance. On the other hand, the frequency of MBL (A/B + B/B) genotypes was significantly increased in SLE patients when compared to healthy controls. These findings are in agreement with Lau *et al.,* 1996 and Øhlenschlæger *et al.,* 2004, they reported that codon 54 substitution is slightly more frequent in SLE patients than in normal Chinese population, although statistical significance was not reached. Davies *et al.,* (1995), reported that, the frequency of mutated codon 54 allele is increased in UK and Spanish population, although the frequency of homozygous codon 54 mutation was not significantly increased. Our result are inconsistent with Tsutsumi *et al.,* (2001), who found that the frequency of individuals with homozygous codon 54 mutation (allele B) of the MBL gene was significantly increased in Japanese patients with autoimmune disorders, in particular, SLE or Sjogren's syndrome and most individuals with heterozygous MBL gene mutations have significantly low but easily detectable serum concentration of MBL. On the other hand, in individuals with homozygous MBL gene mutations, serum MBL will be almost undetectable.

The present study revealed that the mean intima-media thickness was higher in A/B+ B/B genotypes than that of both wild genotype (A/A) and healthy control. This was due to loss of the protective antiatherogenic effect or role of MBL in A/B+ B/B genotypes as a result of its deficiency. This finding is in agreement with Robert *et al.,* (2009), who, stated that MBL binds to sugars on cells undergoing apoptosis or necrosis as a results of injury, and mediates their disposal through phagocytosis. This process serves to minimize local inflammation. So with reduced or absent MBL, endothelial damage caused by infection may undergo elevated and prolonged inflammation, which in turn may accelerate the process of atherogenesis. Over an extended period and exposure to multiple other pro-atherogenic factors throughout life, the absence of MBL may ultimately contribute to increased risk of severe atherosclerosis. Conversely in the presence of MBL, damage may be managed and repaired with reduced inflammation reducing the likelihood of fatty streak development.

In addition, there was a positive correlation between IMT and duration of SLE and negative correlation between IMT and steroid therapy. These findings are consistent with Telles *et al.,* (2008), who reported that in SLE there was a correlation between IMT and disease duration. On the other hand De Leeuw *et al.,* 2009, reported that steroid treatment is often believed to be atherogenic, because its adverse effects, including hypertension, diabetes mellitus (which were not observed in our patients), and dyslipidemia, are all risk factors for cardiovascular disease. However, steroid treatment could actually prevent atherosclerosis as well, because it suppresses inflammation, which is implicated in atherosclerosis.

Among SLE related modifiers of atherosclerosis in patients with A/B + B/B genotypes, there was a positive significant correlation between IMT and serum cholesterol, LDL, and triglycerides in addition to a negative significant correlation with HDL. This was consistent with De Carvalho *et al.,* 2008, who stated that definitive evidence for primary abnormalities of lipoprotein metabolism due to SLE persi could be recognized at its onset. The so-called "lupus pattern" of dyslipoproteinemia was defined by elevated levels of very low-density lipoprotein cholesterol (VLDL) and triglycerides (TG), and lower high density lipoprotein cholesterol (HDL) levels. Interestingly, activity aggravates these alterations since a striking increase of VLDL and TG levels and decrease of HDL levels were directly correlated with SLEDAI scores. The most common abnormality due to SLE itself is low HDL levels which are detected in almost 80% of patients with active disease but also in almost one-third of inactive patients. HDL levels are known to be inversely related to coronary artery disease (CAD) risk and therefore are additional risk factors in SLE. Moreover, increased triglyceride levels are also related to low HDL levels, and both promote a proatherogenic lipid profile in SLE. These abnormalities observed in untreated active and inactive SLE.

Furthermore, SLE patients with MBL A/B+ B/B genotypes showed a significant positive correlation between IMT and CRP and ESR. Both CRP and ESR are markers of systemic inflammation which is an essential element or component for both systemic lupus and atherosclerosis. This is in agreement with Libby *et al.,* (2002) who mentioned that systemic chronic inflammation, manifested by increased levels of C- reactive protein has been associated with atherogenesis. In addition, William *et al.,* (2005), reported that as elevated levels of CRP have been demonstrated in SLE, systemic inflammation might enhance atherosclerosis in this disease.

The present work revealed that the serum levels of C3 and C4 were significantly lower in SLE MBL (A/B+ B/B) genotypes when compared to those with (A/A) genotype. These finding are supported by Seelen *et al.,* (2005), who reported that as MBL protein activates the complement system and quantitative or functional deficiency of MBL may lead to insufficient activation of the complement system with reduction of its level, inadequate clearance of immune complex, which in turn results in immune complex deposition in tissues causing organ damage. Ramos-Casals *et al.,* (2009) also reported that the MBL pathway also contributes to the clearance of apoptotic cells and circulating immune complexes, suggesting a possible implication in the aetiopathogenesis of systemic autoimmune diseases. These findings also confirmed in our study by the significant negative correlation between IMT and C3and C4 in the SLE patients with A/B and B/B genotypes.

Our study indicated a positive correlation between IMT and anti-ds DAN in SLE patients with MBL (A/B + B/B) genotypes. These results are consistent with Yazici *et al.,* (2001) who stated that systemic inflammation, autoantibodies directed to double stranded DNA (dsDNA), ribonucleoproteins (nRNP), endothelial cells, phospholipids, circulating immune complexes, lupus nephropathy, dyslipidemia represent some factors related to SLE which contribute to appearance of endothelial dysfunction. Also, Mastej & Adamiec, (2003) found that anti-dsDNA may play a role in atherosclerosis by promoting accumulation of cholesterol in macrophages and smooth muscle cells, influence on dyslipoproteinemias, cytotoxicity and releasing cytokines. Moreover, Seelen *et al.,* (2005) reported that both anti-ds DNA and anti-Smith antibodies were more frequently

found in patients with MBL variant alleles compared with patients with the MBL wild type.

Also, there was a significant positive correlation between IMT and SLEDAI score in patients with A/B+ B/B genotypes, and this finding is in agreement with Caraba *et al.,* (2008) who mentioned that in patients with systemic lupus erythematosus, atherosclerosis has a long period of subclinical evolution. The first reversible step in the atherogenesis process is represented by the endothelial dysfunction.

In conclusion, the SLE patients with mutant MPL alleles had a higher IMT which correlated significantly with SLE related risk factors for atherosclerosis. Further studies are needed to investigate mechanisms by which MBL and MBL genotypes affect atherosclerosis development.

References

1. Alpert D, Davis A, Erkan D, Roman MJ, Salmon JE (2007). Subclinical carotid atherosclerosis in a patient with systemic lupus erythematosus Nat Clin Pract Rheumatol. 3(8):473-78.
2. Best LG, Davidson M, North KE, MacCluer JW, Zhang Y, Lee ET, Howard BV, DeCroo S, Ferrell RE (2004). Prospective analysis of mannose-binding lectin genotypes and coronary artery disease in American Indians—the strong heart study. Circulation; 109: 471–75.
3. Caraba A, Germaine S, Viorica C, Corina G, Iuliana Z, Romosan I (2008). Endothelial dysfunction in systemic lupus erythematosus. Romanian J. Biophys; 18(3): 237–44.
4. Davies EJ, Snowden N, Hillarby MC (1995). Mannose-binding protein gene polymorphism in systemic lupus erythematosus. Arthritis Rheum; 38:110–114.
5. De Carvalho JF, Bonfá E, Borba EF. (2008). Systemic lupus erythematosus and "lupus dyslipoproteinemia".Autoimmun Rev. 7(3):246-50.
6. De Leeuw K, Smit AJ, de Groot E, van Roon AM, Kallenberg CG, Bijl M (2009). Longitudinal study on premature atherosclerosis in patients with systemic lupus erythematosus. Atherosclerosis; 206(2): 546-50.
7. Doria A, Shoenfeld Y, Wu R (2003). Risk factors for subclinical atherosclerosis in a prospective cohort of patients with systemic lupus erythematosus. Ann Rheum Dis; 62: 1071–77.
8. Dorthe H, Anders L, Hans OM, Jan B, Jens OJ, Jørgen W,Torsten F, Jørn W, Peter G (2007). Heterozygosity of mannose-binding lectin (MBL2) genotypes predicts advantage (heterosis) in relation to fatal outcome in intensive care patients. Hum. Mol. Genet. 16 (24): 3071-80.
9. Font J, Ramos-Casals M, Brito-Zeron P, Nardi N, Ibanez A, Jimenez S,Tassies D, Garcıa-Criado A, Ros, J. Sentıs E, Reverter JC,Suarez B, Lozano F (2007). Association of mannose-binding lectin gene polymorphisms with antiphospholipid syndrome, cardiovascular disease and chronic damage in patients with systemic lupus erythematosus. Rheumatology; 46: 76–80.
10. Hochberg MC (1997). Updating the American college of Rheumatology revised criteria for the classification of systemic lupus erythematosus. Arthritis Rheum; 40: 1725.
11. Jacobi AM, Odendahi M, Reiter K, Bruns A, Burmester GR, Radruch A, Vale G, Lipsky PE, Dorner T (2003). Correlation between circulating CD27 high plasma cells and disease activity in patients with systemic lupus erythematosus. Arthritis Rheum. 48(5):1332-42.
12. Huh JW, Song K, Yum JS, Hong SB, Lim CM, Koh Y (2009). Association of mannose-binding lectin-2 genotype and serum levels with prognosis of sepsis. Critical Care; 13(6):176.
13. Lau YL, Lau CS, Chan SY, Karlberg J, Turner MW. (1996). Mannose-binding protein in Chinese patients with systemic lupus erythematosus. Arthritis Rheum. 39: 706–708.
14. Libby P, Ridker PM, Maseri A (2002). Inflammation and atherosclerosis. Circulation; 105: 1135-43.
15. Mach F, Shonbeck U, Sukhova GK, Atkinson E, Libby P (1998). Reduction of atherosclerosis in mice by inhibition of CD40 signaling. Nature; 394:200–03.
16. Maheer JE, Bielak LF, Raz JA, Sheedy PF, Schwartz RS, Peyser PA (1999). Progression of coronary artery calcification: a pilot study. Mayo Clin Proc. 74: 347–55.

17. Mastej K, Adamiec R (2003). The role of anti-dsDNA autoantibodies in the pathogenesis of atherosclerosis. Przegl lek. 60 (11):748-50.

18. Mintz GS, Pichard AD, Pompa JJ (1997). Determinants and correlates of target lesion calcium in coronary artery disease: A clinical, angiographic and intravascular ultrasound study. J Am Coll Cardiol. 29:.268–74.

19. Øhlenschlaeger T, Garred P, Madsen HO, Jacobsen S (2004). Mannose-binding lectin variant alleles and the risk of arterial thrombosis in systemic lupus erythematosus. N Engl J Med. 351:260–67.

20. Ramos-Casals M, Brito-Zero´ P, Soria N, Nardi N, Vargas A, Munoz S, Bove´ A, Sua´ rez B. Lozano F (2009). Mannose-binding lectin-low genotypes are associated with milder systemic and immunological disease expression in primary Sjougren's syndrome.Rheumatology; 48:65–69.

21. Riley WA, Barnes RW, Applegate WB (1992). Reproducibility of noninvasive ultrasonic measurement of carotid atherosclerosis - the asymptomatic carotid artery plaque study. Stroke; 23:1962–68.

22. Robert AM, Menno PJ de Winther, Dian K, Ingeborg van der Made, Christian W, Veronica MH, Marion JJ G, Wim AB (2009). Macrophage-Specific Expression of Mannose-Binding Lectin Controls Atherosclerosis in Low-Density Lipoprotein Receptor–Deficient Mice. Circulation; 119: 2188-95.

23. Saevarsdottir S, Oskarsson OO, Aspelund T, Eiriksdottir G, Vikingsdottir T, Gudnason V (2005). Mannan binding lectin as an adjunct to risk assessment for myocardial infarction in individuals with enhanced risk. J Exp Med; 201:117–25.

24. Salmon JE, Roman MJ (2008). Subclinical atherosclerosis in rheumatoid arthritis and systemic lupus erythematosus. Am J Med. 121 (10 Suppl 1): S3-8.

25. Sastry K, Herman GA, Day L, Deignan E, Bruns G, Morton CC, Ezekowitz RA.(1989). The human mannose-binding protein gene. Exon structure reveals its evolutionary relationship to a human pulmonary surfactant gene and localization to chromosome 10. J Exp Med. 170:1175- 89.

26. Seelen MA, van der Bijl EA, Trouw LA, Zuiverloon TCM, Munoz LR,Fallaux-van den Houten FC, Schlagwein N, Daha MR, Huizinga TWJ, Roos A (2005). A role for mannose-binding lectin dysfunction in generation of autoantibodies in systemic lupus erythematosus. Rheumatology; 44: 111–119.

27. Soubrier M, Mathieu S, Dubost JJ. (2007). Atheroma and systemic lupus erythematosus. Joint Bone Spine; 74(6): 566-570.

28. Souza AW, Hatta FS, Miranda F-Jr, Sato EI (2005). Atherosclerotic plaque in carotid arteries in systemic lupus erythematosus: frequency and associated risk factors. São Paulo Med J; 123: 137–42.

29. Telles RW, Lanna CCD, Ferreira GA, Souza AJ, Navarro TP, Ribeiro AL (2008). Carotid atherosclerotic alterations in systemic lupus erythematosus patients treated at a Brazilian university setting. Lupus; 17: 105–113.

30. Thorburn CM, Ward MM (2003). Hospitalizations for coronary artery disease among patients with systemic lupus erythematosus. Arthritis Rheum; 48:2519–23.

31. Troelsen L N, Garred P, Christiansen B, Torp-Pedersen C, Jacobsen S (2010). Genetically Determined Serum Levels of Mannose-Binding Lectin Correlate Negatively with Common Carotid Intima-Media Thickness in Systemic Lupus Erythematosus. J Rheumatol. 37(9):1815-21

32. Tsutsumi A, Sasaki K, Wakamiya N, Ichikawa K, Atsumi T, Ohtani K, Suzuki Y, Koike T, Sumida T (2001). Mannose-binding lectin gene polymorphisms in Japanese patients with systemic lupus erythematosus, rheumatoid arthritis and SjÖgren's syndrome. Genes and Immunity; 2: 99–104.

33. Turner MW, Hamvas RM (2000). Mannose-binding lectin, structure, function, genetics and disease associations. Rev Immunogenet; 2: 305–322.

34. Wang, Z-Y, Morinobu, A, Kanagawa, S, Kumagai, S (2001). Polymorphisms of the mannose binding lectin gene in patients with Sjögren's syndrome. Ann Rheum Dis. 60:483–486.

35. William Jr RC, Harmon ME, Burlingame R, Du Clos TW (2005). Studies of serum C-reactive protein in systemic lupus erythematosus. J. Rheumatol. 32: 454-461.

36. Woford JL, Kahl FR, Howard GR, McKinney WM, Toole JF, Crouse JR (1991). Relation of extent of extra cranial carotid artery atherosclerosis as measured by B-mode ultrasound to the extent of

coronary atherosclerosis. Arterioscler Thromb. 1: 1786–94.

37. Yazici ZA, Raschi E, Patel A (2001). Human monoclonal anti-endothelial cell IgG-derived from a systemic lupus erythematosus patient binds and activates human endothelium in vitro. Int. Immunol. 13:349–57.