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Mannose Binding Lectin Serum Level and Gene Polymorphism in Patients with SLE and its Relation to the Development of Lupus Nephritis

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Abstract

Background and Aims: Systemic lupus erythematosus (SLE) is an autoimmune disease in which the complement system plays a crucial role in its pathogenesis. Mannan-binding lectin (MBL) is a recognition molecule of the lectin pathway of complement activation. The presence of several polymorphisms at the promoter and coding regions of the MBL-2 gene determines alterations in MBL serum concentration. MBL variant alleles that lead to low serum levels and/or functional deficits of MBL are postulated to contribute to the susceptibility of SLE. Moreover, the influence of MBL variation on antibody production and renal involvement in SLE patients remains controversial. Therefore, MBL serum level and genotypes were studied in our SLE Egyptian patients with evaluation of its role in auto antibodies production and lupus nephritis development.

Methods: MBL genotypes and serum level were screened in a case control study that included 30 SLE patients as well as 30 healthy controls. MBL polymorphism at exon 1 codons 54 and 57 was detected by PCR using sequence-specific priming (SSP) and serum MBL level was determined by ELISA technique.

Results: There was predominance of AA genotype (80%) in control group. Genotype frequencies of MBL variants in patients with SLE showed significant differences when compared with controls (AA 53.3% vs 80%, P=0.03, OR = 0.29 and AO+OO 46.6% vs 20%, P = 0.03, OR = 3.5, respectively). Serum MBL in our SLE patients (900 ng/ml) was significantly lower than that of the control group (2750 ng/ml, P = 0.00) with positive correlation with low MBL genotypes. SLE patients with mutant alleles were more likely to produce anti dsDNA (92.8% vs 75%, OR = 4.3) and anti-Smith (35.7% vs 18.7%, OR = 2.3). Patients carrying MBL-low genotypes have an increased risk of development of lupus nephritis than those carrying MBL-high genotype (64.7% vs 35.2%, P = 0.02, OR= 2.4).

Conclusions: MBL gene polymorphism is associated with low MBL serum levels that were found with significantly increased frequency in our SLE patients and it may be one of the genetic factors that determine the susceptibility to develop lupus nephritis.

Keywords: Mannose-Binding Lectin (MBL), Systemic Lupus Erythematosus (SLE), Lupus Nephritis (LN), Gene Polymorphism

Introduction

Systemic lupus erythematosus (SLE) is a classical autoimmune disease characterized by formation of autoantibodies and immune complexes of unclear etiology. It may affect any organ of the body and displays a broad spectrum of clinical and immunological

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manifestations. There is evidence that implicates hormonal, environmental as well as genetic factors in its pathogenesis (1).

The innate immune system plays a crucial role in the pathogenesis of SLE especially the complement system which has a dual effect. On one hand, activation of complement causes tissue injury; on the other hand, genetic deficiencies of complement components, especially the early components of the classical pathway, are strongly associated with the occurrence of SLE (2).

As a result of immunological abnormalities, renal disorders occur frequently in patients with SLE (3). Kidney involvement is a major concern in SLE, affecting ~50% of patients and accounting for significant morbidity and mortality (4). Although, even with treatment, the 5-year survival in SLE patients with renal involvement can be lower than 50%, early diagnosis and prompt treatment may dramatically modify the course of renal disease and improve the long-term survival (5).

Mannan-binding (or mannose- binding) lectin (MBL) is an acute phase protein derived from the liver and involved in innate immune defense (6). MBL recognizes mannose and N-acetyl glucosamine oligosaccharides that areexpressed by a wide range of bacteria, viruses, fungi and parasites as well as self-components including apoptotic cells, phospholipids and immune complexes (7). Upon binding, MBL interacts with mannose associated serine proteases (MASPs) and activates the complement system by initiating the lectin pathway that represents a third pathway distinct from both the classical and alternative, leading to opsonization, or promotes phagocytosis, or direct killing (8).

Structurally, MBL molecule is analogous to C1q, an innate immune effector which activates the classical complement pathway.(7) It is encoded by a single 4-exon gene located on chromosome 10 at q11.2-q21. It is a multi-chain molecule of up to 6 subunits. Each subunit consists of 3 identical 32-kDa polypeptide chains that contains a cystine-rich region, a collagenous region, an -helical coiled-coil called the neck region, and a carbohydrate binding domain encoded by exons 1, 2, 3 and 4; respectively (9).

The concentration of functional multi-meric MBL is genetically determined to a major extent, although non-genetic influences are also relevant (10). MBL2 gene, the functional human gene for MBL, is highly polymorphic (9). In addition to promoter polymorphism, three independent single point mutations (SNPs) in codons 52 (Arg/Cys, allele D), codon 54 (Gly/Asp, allele B), and codon 57 (Gly/Glu, allele C) of exon 1 give rise to amino acid substitutions within the ollagenous region of MBL (11). They disrupt the collagenous structure of the protein, reduce the proportion of higher order oligomers in circulation, and dramatically reduce serum MBL concentrations. Any of these mutations (B, C, or D) is referred as O, whereas the wild-type is referred to as A (12).

In light of its structural and functional similarities to C1q and the strong association of C1q deficiency with SLE, MBL variant alleles that lead to low serum levels and/or functional deficits of MBL are postulated to contribute to the susceptibility of SLE. However, reports of such effects vary considerably (13). Moreover, the influence of MBL variation on antibody production and renal involvement in SLE patients also remains controversial (14). In the view of such inconsistent results, this study was carried out to clarify the variation of MBL serum level and its genetic polymorphism in Egyptian patients with SLE and their association with auto antibodies production and their role in development and prognosis of renal insult.

Materials and Methods

Patients and controls

A total of 30 patients with SLE who were followed up at the Out-patient Clinic of the Rheumatology & Internal Medicine Departments at Tanta University

Hospital during the period from February 2006 to July 2008 were randomly selected irrespective of their disease stage or severity.

The patients included 28 women and 2 men with mean age at diagnosis of 23±5.4 yrs. All patients fulfilled at least four of the revised American College of Rheumatology (ACR) criteria for the diagnosis of SLE (15). Patients with lupus were individually matched to 30 healthy control subjects on the basis of age and sex. Patients and controls gave written informed consent before entering the study. All patients underwent routine laboratory assessments. Blood samples were obtained for determination of the complete blood cell count, serum creatinine, serum C3, C4, ANA, anti-dsDNA and anti-Sm antibodies.

A complete urine analysis was performed and 24 hours urinary protein collection was obtained. All patients were submitted for abdominal sonography, chest X-ray, and echocardiography.

Renal involvement

Renal involvement was assessed clinically with the renal SLE Disease Activity Index (SLEDAI) (16), which consists of the 4 kidney-related parameters: hematuria, pyuria, proteinuria and urinary casts. Each item in the renal SLEDAI is assigned 4 points. Thus, scores for the renal SLEDAI ranged from 0 (inactive renal disease) to a maximum of 16. Patients were selected and classified into either the group of patients without LN at the time of their clinic visit (renal SLEDAI score of 0) or the group of patients with LN. The LN group was prospectively defined as those having a renal SLEDAI score of > 8 (i.e. at least 2 abnormal results for renal parameters).

Renal biopsies were taken from our patients. All biopsies were classified according to the modified WHO classification into six classes (17), i.e. normal, mesangial, focal segmental, diffuse proliferative, membranous and advanced sclerosis. The classification was done on the basis of the most prominent lesion. A lesion was considered active if there were cellular infiltrates, cellular crescents, fibrinoid necrosis, vasculitis and interstitial inflammatory infiltrate. Chronicity was judged by glomerular sclerosis and interstitial fibrosis

Exclusion criteria

Generally, patients with diabetes mellitus, those with malignancies and those with a diagnosis of overlap syndrome (coexistence of lupus with other connective tissue diseases such as rheumatoid arthritis or scleroderma) were excluded. Among the patients without LN, those with renal insufficiency from non-lupus-related causes were also excluded (serum creatinine level ≥1.5 mg/dl). Among the patients with LN, those undergoing hemodialysis were excluded.

Measurement of MBL serum concentrations

The MBL oligomer ELISA (KIT 029) assay was performed according to the instructions provided by the manufacturer, BioPorto Diagnostics, Gentofte, Denmark with a detection range of 0 - 7000 ng/ml. Briefly, diluted serum samples were added to microtiter wells pre-coated with a mouse monoclonal antibody specific for the carbohydrate-recognition domain (CRD domain) of MBL (MAb anti-CRD-MBL) in duplicates of 100 ml per well. Following incubation for 1 h at room temperature with shaking at 200/minute, the wells were washed twice with 300 ml wash solution. Biotin conjugated MAb anti-CRD-MBL (100 ml) was added and, incubated for 1 h, and the bounded immune complexes were detected with HRP-conjugated streptavidin followed by 100 ml TMB as substrate. The optical density was read at 450 nm and values were detected from the standard curve. This method has intra-assay and inter-assay coefficients of variation of 3.7% and 6.7%, respectively.

Genotyping of MBL

Genomic DNA was extracted from EDTA-treated blood cells by using the QIAamp DNA blood mini kit, following the manufacturer's instructions (QIAGEN, USA) and stored at -20°C until the time of use. MBL codon 54 and 57 wild and mutant alleles were detected using polymerase chain reactions-sequence-specific primer (SSP-PCR) technique as previously described by Garred et al, 2003.(18) Oligonucleotide primers encoding sequences Beta globulin gene were used to amplify a 110 bp internal control fragment.

PCRs were performed in 20 µl volumes that contained 100 ng of genomic DNA, 1.0 µM of A-non-B/A-non-C forward primers and B/C reverse primers, 0.8 µM of A-non-B/A-non-C reverse primers and B/C forward primers and in the presence of 1.5 mM MgCl2, 0.2 mM of dNTPs, 50 mM KCl, 10 mM Tris-HCl (pH 8.3), and 0.025 U/µl of Taq DNA polymerase (Life Technologies, USA). AllPCR were initiated by 3 min of denaturation at 94°C and com pleted by 10 min of extension at 72°C. PCR cycles were 30 cycles as follows: 30 s at 94°C, 30 s at 56°C, and 45 s at 72°C. PCR amplicons were resolved on 2% agarose gels containing ethidium bromide and visualized by UV transillumination. The primers sequences are listed in Table 1. Wild allele is A, mutant alleles are B&C.

Statistics

The collected data were organized, tabulated and statistically analyzed using SPSS software statistical computer package version 12. Data were presented as mean ± SD for continuous variables and as proportions for categorical variables. The difference between median level was statistically analyzed using Mann Whitney test or Kruskal Wallis tests with a minimum level of significance of <0.05. The frequency of studied allelic polymorphisms among cases was compared to that of controls describing number and percent of each and tested for positive association using Chi- square and Odds ratio.

Table 1. The primers sequences

Allele		Sequence	Product size (bp)
AnonB	Forward	5'-AGTCGACCCAGATTGTAGGACAGAG-3'	227
	Reverse	5'- CCTTTTCTCCCTTGGTGC-3'	
B	Forward	5'- GCAAAGATGGGCGTGATGA-3'	224
	Reverse	5'- GGGCTGGCAAGACAACTATTA-3'	
AnonC	Forward	5'-AGTCGACCCAGATTGTAGGACAGAG-3'	287
	Reverse	5'- CCTGGTTCCCCCTTTTCTC-3'	
C	Forward	5'-CAAAGATGGGCGTGATGGCACCAAGGA-3'	222
	Reverse	5'-GGGCTGGCAAGACAACTATTA-3'	
Beta Globulin	Forward	5'-ACACAACTGTGTTCACTAGC-3'	110
	Reverse	5'-CAACTTCATCCACGTTCACC-3'	

Results

Thirty patients with SLE (female/male = 28/2) as well as 30 healthy controls (female/male = 26/4) with a mean age at SLE diagnosis of 23+ 5.4 years were included in this case control study. The ACR criteria includes: articular affection in 90%, renal involvement in 56.7%, cardiovascular disease was detected in 33.3% and vasculitis were present in 26.7%.

Prevalence of MBL genotypes

Genotypic study of single nucleotide substitution at codon 54 (B) and codon 57 (C) of MBL2 exon 1gene in the same two groups was examined. The distribution of wild (AA) and mutant (AO/OO) genotypes was 24 (80%) and 6 (20%) in control group with slightly higher frequency of B allele (8.3%) than C (5%) allele. On the other hand, significantly few patients (53.3%, P = 0.03, OR = 0.3) showed wild homozygote genotype (AA) and significantly more patients (46.6%, P = 0.03, OR = 3.5) showed mutant genotypes with the predominance of C than B allele (23.3% vs 10%, P = 0.05) in diseased group as well as than the C allele frequency in control group (23.3% vs 5%, P = 0.004, OR = 5.8). Although it did not reach statistical significance, patients with homozygous MBL mutation are more frequent in SLE patients (20%) than in controls (6.7%, OR = 3.5) (Table 2).

MBL serum level and its relation to MBL genotype

Overall, the median serum MBL concentration in SLE patients was 900 ng/ml which was significantly lower than that of the control (2750 ng/ml) with a p value of <0.000. Analyzing MBL serum level within different genotypes reveals positive correlation between low MBL serum level and MBL genetic mutation in both controls and patients with P value of 0.04 and <0.0001, respectively. AA genotype was associated with higher level of circulating MBL in

Table 2. Genotypic frequencies of MBL-2 variants in patients with SLE and healthy control

MBL-2	Patients		Control		P-value^	OR (95%CI)
	n =30	%	n =30	%		
Alleles						
A	40	66.7	52	86.7	0.01*	0.3 (0.1-0.8)
B	6	10	5	8.3	0.7	1.2 (0.4-4.2)
C	14	23.3	3	5	0.004*	5.8 (1.6-21.3)
Genotypes						
Wild (A/A)	16	53.3	24	80	0.03*	0.3 (0.1-0.9)
Mutant (A/O +O/O)	14	46.6	6	20	0.03*	3.5 (1.1-11)
A/O	8	26.7	4	13.3	0.2	2.4 (0.6-8.9)
O/O	6	20	2	6.7	0.1	3.5 (0.7-19)

^Chi-square test

* Statistical significance (P ≤ 0.05)

controls (2950 ng/ml) compared with SLE patients (1360 ng/ml, p<0.000). Mutant alleles had a suppressive effect on MBL serum levels, both in SLE and healthy controls. In heterozygous (A/O) individuals, MBL levels were reduced 1.9 times in SLE and 1.3 times in the controls, whereas in O/O homozygotes, the reduction in circulating protein was 9.3 and 2.8 times for both groups, respectively. Moreover, there was significant reduction in serum MBL level in mutant genotypes in patients compared to the same genotype in control group (P<0.05) (Table 3).

Based on reference values in adults, MBL level of 1000 ng/ml fairly divided the cohort into those with sufficiently high MBL concentrations from those with insufficient concentrations (19). Twenty-one (70%) SLE patients compared to 7 (23%) of controls had MBL concentration under 1000 ng/ml (P = 0.0) (data not shown).

Immunological features

To examine the relationship between MBL geneand autoantibody production, the prevalence of some autoantibodies in SLE patients was analyzed. Although the results showed that SLE patients with mutant alleles were more likely to produce anti dsDNA (92.8% vs 75%, OR = 4.3) and anti-Smith (35.7% vs 18.7%, OR = 2.3), the differences did not reach statistical significance. Regarding the complement level, SLE patients with mutant alleles were significantly less likely to produce C3 (78.5 vs 43.7) and C4 (71.4 vs 31.3) with p values of 0.05 and 0.03 and OR 0f 4.7 and 5.5, respectively (Table 4).

SLE-related features

Among SLE patients, lupus nephritis and vasculitis were more frequently observed in those patients with mutant MBL2 genotypes[(78.6% vs 37.5%, P = 0.02, OR = 6.1) and (50% vs 6.2%, P = 0.006, OR = 15)]. Regarding the other clinical features of SLE patients, no significant associations were found with MBL2 genotypes (Table 4).

Lupus nephritis related features

According to the previous findings, SLE patients with LN were compared to those without LN. Noticeably, patient characteristics did not differ between the two subgroups (data not shown). A higher prevalence of LN was observed in patients carrying MBL-low genotypes compared with those carrying MBL-high genotypes (64.7% vs 35.2%, P = 0.02, OR= 2.4). Mutant genotypes were significantly higher in patients

Table 3. Serum MBL concentration in SLE patients and control groups according to genotype

Genotype	MBL (ng/ml)		Mann Whitney Test	
	Control Median (range)	SLE Median (range)	z	p-value
Wild (A/A)	2950 (982-4954)	1360 (650-2170)	-4.129	0.000*
Mutant (A/O)	2200 (968-3520)	685 (395-1260)	-2.378	0.017*
Mutant (O/O)	1009 (730-1288)	155 (43-272)	-2.000	0.046*

* Statistical significance (P ≤ 0.05)

Table 4. Clinic data and autoantibodies in SLE patients breakdown by mutation

	Mutant		Wild		P-value^	OR (95% CI)
	n =14	%	n =16	%		
Arthritis	13	92.8	14	87.5	0.6	1.9 (0.2-23)
Vasculitis	7	50	1	6.2	0.007*	15 (1.5-146.6)
Cardiovascular	6	42	4	25	0.3	2.3 (0.5-10.6)
Lupus nephritis	11	78.6	6	37.5	0.02*	6.1 (2-31.2)
ANA	13	92.9	15	93.7	0.9	0.9 (0.05-15.1)
Anti ds DNA	13	92.8	12	75	0.2	4.3 (0.4-44.4)
Anti-smth	5	35.7	3	18.7	0.3	2.4 (0.46-12.7)
Low C3	11	78.5	7	43.7	0.05*	4.7 (0.9-23.7)
Low C4	10	71.4	5	31.3	0.03*	5.5 (1.1-26.4)

Wild, A/A genotype; Mutant, A/0 + 0/0

^ Chi-square test

* Statistical significance (P ≤ 0.05)

with than in patients without LN (64.7vs 23.1, P = 0.02, OR = 6.1) and the prevalence of A allele was significantly less frequent (52.9 vs 84.6, P = 0.01, OR 0.2) and C allele was more frequent (35.3 vs 7.7, P = 0.01, OR = 6.5) in LN than in non- LN patients (Table 5).

Serum MBL level in control group, patients with LN and those without LN are shown in (table 6). Significant difference was observed between those groups with a p value of <0.0001. Levels of mutant and wild-type MBL were compared within and between controls, lupus nephritis and non-lupus nephritis groups (Table 7). In each group, the levels of mutant MBL were lower than those of wild-type MBL with a p value of <0.05 for all 3 groups. Of notice, the maximum reduction in MBL serum level was detected in nephritic patients with mutant MBL genotype with a median ± SD of 272 ng/ml.

MBL and degree of renal involvement

The results of renal biopsy samples were obtained for LN patients. Among these patients, 3 had grade I, 4 had grade II, 4had grade III, 4 had grade IV, and 1 had grade V. Only 1 patient had pure grade VI.

Table 5. Genotypic frequencies in Lupus nephritis (LN) compared to non lupus nephritis patients (non LN)

MBL-2	LN		Non LN		p- value	OR (95%CI)
	n=17	%	n=13	%		
Alleles						
A	18	52.9	22	84.6	0.01*	0.2 (0.06-0.7)
B	4	11.8	2	7.7	0.6	1.6 (0.27-9.5)
C	12	35.3	2	7.7	0.01*	6.5 (1.3-32.6)
Genotypes						
A/A	6	35.2	10	76.9	0.02*	0.2 (0.03-0.8)
A/O+O/O	11	64.7	3	23.1	0.02*	6.1 (1.2-31.2)
A/O	6	35.2	2	15.3	0.2	3 (0.5-18.2)
O/O	5	29.4	1	7.6	0.1	5 (0.5-49.4)

^ Chi-square test

*Statistical significance ($p \leq 0.05$)

Table 6. Serum MBL concentration in lupus nephritis (LN) compared to non lupus nephritis (non LN) patients and control group

MBL (ng/ml)	Control (n=30)	Non LN (n=13)	LN (n=17)	z	p-value
Range (median)	730-4954 (2750)	465- 2170 (1600)	43-1420 (650)	32.9	0.000*

Kruskal Wallis Test

*Statistical significance ($p \leq 0.05$)

Table 7. comparison of wild- type and mutant MBL concentration among lupus nephritis (LN), non lupus nephritis (non LN) patients and control group

Group	wild	mutant	Mann Whitney	
	median (Renge)	median (Renge)	z	p-value
Control	2950 (982-4954)	1554 (730-3526)	-2.076	0.038*
non LN (n=13)	1845 (650-2170)	1100 (465-1260)	-1.690	0.091
LN (n=17)	1025 (850-1420)	272 (43-820)	-3.317	0.001*

*statistical significance ($p \leq 0.05$)

Table 8. Comparison of the hazards ratio of the degree of renal involvement in nephritis patients

OR (95% CI)	p-value	wild		mutant		Grades
		%	n=6	%	n=11	
0.04 (0.003-0.6)	0.009*	83.3	5	18.2	2	1-3 (7)
22.5 (1.6-314.6)		16.7	1	81.8	9	3-4 (10)

*Statistical significance ($p \leq 0.05$)

The majority of wild MBL2 genotype (83.3%) was detected in early stage of renal involvement (Grades I and II) while the majority of mutant genotype was detected in late stage of nephritis (81.8%). Of notice, significant increase in Hazard ratio in patient with homo- and hetero-zygous mutant alleles than wild homozygous allele was observed for late stage nephritis (OR = 22) (Table 8).

Discussion

When the components of the classical pathway ofcomplement are deficient, it has been suggested that abnormal clearance of not only immune complexes but also apoptotic cells contributes to the occurrence of SLE (2). Recently, it was reported that MBL can bind to apoptotic cells and initiate their uptake by macrophages though lectin pathway, and thus, abnormal clearance of apoptotic cells due to MBL deficiency may provide a source of auto antigens in SLE (20). So, we have investigated MBL serum level and the possible association of MBL polymorphisms with the broader spectrum of clinical manifestations that may be presented by SLE patients and with the development of renal disease among lupus patients.

Initially, we investigated MBL mutations in exon 1 region at codon 54 and 57 in our SLE patients. We also investigated those 2 variant alleles rather than D allele because B and C alleles lead to profound reduction of serum MBL levels, whereas the D allele has minimal impact, predominance of structural variant C allele in sub-Saharan African populations and difficulty of confronting allele D primer design (21). This work revealed high A allele frequency with nearly similar low frequency of B and C alleles in control group. Although these results are against the predominance of structural variant C allele in sub-Saharan African populations (22) and B allele in many ethnic groups (23), they are in agreement with some studies in other populations (21, 24). As in other studies (25), mutant alleles in this study were associated with reduction of MBL serum level.

On comparing our SLE patients with controls, significantly more patients showed mutant genotypes than healthy control with predominance of C allele. The genotyping data of different populations ranges from supportive results (21), impressively similar results that can be calculated that the presence of B variant allele of MBL approximately doubles the relative risk of developing SLE (26), or lack of association (27, 28). Such diverse results may originate from difference in the sample size, homogenicity of samples, selection of controls, or different ethnic background of patient populations (29).

Among previous reports, Lau et al,1996 (30) pointed out that the serum MBL concentration is lower in SLE patients than in controls, a result which was supported by others (13, 31-33).

Interestingly, Seelen et al, 2005 (13) explained this-difference by differences in MBL genotype distributions, difference of disease activities of SLE in the individuals studied, or other unknown factors.

Although Garred et al, in 1992 failed to show such association in their black African population (34), now, there is increasing data showing that MBL serum concentrations are dependent on MBL genotype (25, 35-39). Individuals with variant alleles have decreased MBL serum concentrations compared with serum concentrations of individuals with wild type MBL genotype and those with homozygous mutations have even lower or almost undetectable concentration of this protein (37, 39). Indeed this association may be intensified by SLE (1, 26, 40, 41). The mutation occurs in MBL coding regions may results in decrease synthesis of such protein or production of dysfunctional variants incapable of oligomerization (18, 21).

In line with the above data, our results showed that MBL mutations reduced MBL serum level in controls. Serum MBL of the patients group was significantly lower than that of the healthy controls. Moreover, serum MBL level of SLE patients with MBL mutation was significantly lower than that of SLE patients without MBL mutation with marked reduction in those with homozygous alleles.

Thanks to its ability in activating the complement system and inducing phagocytosis, MBL deficiency may predispose patients to the accumulation of apoptotic cells that serve as a major source of autoantigens and hence resulting in an imbalance on the mechanisms of immunological tolerance, immune system activation, and autoantibody production in SLE patients (42). Most of these autoantibodies are directed against membranous, intracellular or nuclear target antigens (13).

To clarify such effect, prevalence of different autoantibodies was studied in this work. Both anti-dsDNA and anti-Smith antibodies were more frequently found in patients with MBL variant alleles compared with patients with the MBL wild type genotype. The presence of a wide variety of autoantibodies is a characteristic finding in SLE (13). Tan and his colleagues concluded that autoantibodies against nuclear proteins, such as anti-ANA and anti-dsDNA antibodies, are used as diagnostic markers in SLE. They added that due to their specificity for SLE, anti-dsDNA antibodies constitute one of the diagnostic criteria for the disease (43). Piao and his co-workers showed that in their SLE patients, heterozygous genotypes of MBL were found to be associated with increased probability of generation of anti-Smith antibody whereas those with homozygous mutant genotype were more likely to produce anti-dsDNA (21). These results, together, not only support the hypothetical link between MBL deficiency and SLE, but also imply the complex interplay between genetic factors and autoantibody phenotype in different individuals.

On comparing our wild and mutant SLE subgroups, mutant subgroup showed significant low C3 and C4 serum level than wild subgroup. As MBL protein activates the complement system, 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 result in immune complex deposition in tissues causing organ damage (13).

Renal involvement

The MBL serum level was significantly reduced in lupus nephritis than non lupus nephritis patients. In correlation, lupus nephritis was more frequently found in patients with MBL variant alleles compared to the patients with the MBL wild type genotype. Alternatively, a significantly increased number of patients with mutant alleles were observed when comparing SLE patients with nephritis to those without. The mutant alleles especially C allele therefore, appears to be acting as a susceptibility factor for the development of renal disease in our SLE patients. This would account for a reduction in immune complex clearance and complement activation in development of renal disease in our SLE patients.

This would account for a reduction in immune complex clearance and complement activation in these individuals, resulting in immune complex deposition in renal tissues which could ultimately lead to increased susceptibility to renal disease in SLE patients (7).

The factors that influence the involvement of different target organs in individual patients with SLE are currently unknown. Indeed, little is known about the role of MBL serum level and polymorphism in SLE lupus nephritis patients. More than 90% of people with C1q deficiency develop SLE, but only one-third of them develop glomerulonephritis (3). The existence has been suggested of nephropathy susceptibility genes independent of SLE susceptibility genes by Freedman et al, 1997 (44). Garred et al 2001 pointed out that homozygous MBL mutation may possibly be associated with increased risk of renal involvement (45). In contrary, Bertoli et al, have reported that renal involvement was more likely to occur in patients with the wild-type than patients with MBL variant alleles (46). These inconsistent data were explained by that the MBL deficiency may be a weak risk factor for renal disorders in SLE patients of certain ethnicities and or the small number of patients enrolled in the study by Garred, et al, 2001 (45).

Finally, our work pointed to the possible association between MBL low serum level and mutant alleles and the severity of kidney affection in lupus nephritis patients. Interestingly, Monticielo et al, 2008 expected that the balance of MBL production and consumption determines serum MBL levels (41). As the presence of MBL deposits in tissues of autoimmune patients has been demonstrated, so that MBL would be consumed during active disease, and that serum MBL concentration and subsequently polymorphism might reflect disease activity and pathological features of SLE in individual patients (6, 41, 47).

Conclusions

In conclusion, MBL polymorphism and deficiency seems to play a role in the development of SLE in Egyptian patients. Because the causes of SLE are heterogeneous, the contribution of the MBL gene should be considered in combination with other genetic and environmental factors. However, further studies using larger patient population are needed before definitive conclusions can be reached. The present study supports a role for MBL as a disease modifier in patients with SLE. It demonstrates that renal involvement in SLE patients is strongly associated with MBL. MBL substitution therapy has now become available. SLE patients might be candidates for adjunctive MBL therapy.

Conflict of Interest

None declared.

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