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ORIGINAL ARTICLE

Association of rs6695096 Single Nucleotide Polymorphism of Human Mannose Binding Lectin Associated Serine Protease 2 (*MASP2*) Gene Locus and MASP2 Serum Level with Systemic Lupus Erythematosus

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ABSTRACT

Background: *Systemic lupus erythematosus (SLE) represents one of the most challenging autoimmune diseases with high morbidities. MASP2 is a central enzyme in the lectin pathway of complement that has a potential role in the disease pathogenesis. This makes it a potential biomarker of interest in correlation with the disease activity.* ***Objectives:*** *The aim of the study was to assess the association between MASP2 serum level and SLE disease activity as well as the association between MASP2 rs 6695096 SNP and SLE.* ***Methodology:*** *Thirty-five patients diagnosed as SLE and Fifteen healthy control subjects were included in this study. The serum level of MASP2 was measured by ELISA and the rs 6695096 SNP of MASP2 was detected by PCR for both groups.* ***Results:*** *MASP2 serum levels were significantly lower in patients compared to control group (p=0.009). In addition, serum MASP2 level was negatively correlated with the disease activity (p=0.002). TC genotype was significantly correlated with pleural effusion (p=0.007) and hematuria (p=0.031). No significant correlation could be observed between MASP2 genotypes and both SLEDAI score and MASP2 serum level.* ***Conclusion:*** *Decreased MASP2 level could be a promising biomarker of SLE disease activity.*

INTRODUCTION

Systemic lupus erythematosus SLE is an autoimmune disease distinguished by chronic inflammation of heterotypic organs and tissues as skin, joints, serous membranes, CVS, CNS and kidney leading to severe morbidities and mortalities. The disease prevalence varies with ethnic distribution but female preponderance is consistent [1,2]. Genetic predisposition, exposome and hormonal factors are the cornerstones of SLE pathogenesis [3].

Autoantibodies against nuclear and cytoplasmic antigens are the hallmarks of the disease, these antibodies form immune complexes that have several mechanisms of the disease pathogenesis described by Craft, (2011) on of those is complement activation by both the classical pathway and the lectin pathway [5,6].

In the lectin pathway, MASP1 (mannose binding lectin associated serine protease 1) auto activates then cleaves MASP2 (mannose binding lectin associated serine protease 2). Both MASP1 and MASP2 cleave C2 but only MASP2 can cleave C4, so MASP2 is the most cardinal enzyme of the lectin pathway [7].

Complement could be a friend or enemy. Although rare, hereditary C1, C2 or C4 deficiency is strongly affiliated with SLE as the clearance of immune complexes and apoptotic cells is influenced. On the other hand, the popular vicious circle of SLE pathogenesis, excessive complement activation by the copious immune complex formation in SLE patients leads to flaring of the disease and consequently complement consumption and more immune complex deposition [8].

Few studies reported the correlation of dysregulated MASP2 level and single nucleotide polymorphisms of *MASP2* gene locus with different autoimmune diseases as rheumatoid arthritis [9], multiple sclerosis [10,11] and SLE [12–14].

The current study aimed at studying the possible association between *MASP2* serum level and SLE disease activity as well as the correlation between *MASP2* rs 6695096 SNP and SLE.

METHODOLOGY

Before collecting samples, informed consents were taken from all participants, in accordance to the “regulation of the Ethical Committee of Scientific Research (FMASU MD 217/2021) of Faculty of Medicine, Ain Shams University, Cairo, Egypt” and

according to "The Code of Ethics of the World Medical Association (Declaration of Helsinki) for experiments in humans".

Subjects

The present study is a case control study carried on over a period of 6 months from October 2021 to April 2022 and included 50 subjects. Thirty-five SLE patients diagnosed according to Systemic Lupus International Collaborating Clinics (SLICC) classification criteria for SLE [15] were recruited from Rheumatology department, Ain Shams University hospitals. Fifteen healthy age and sex matched subjects were included as control group.

For all patients, full history taking and thorough clinical examination were done and lab investigations results as CBC, ESR, CRP, ANA, anti-DNA Ab were collected from the patients' files. They were further classified into different disease activities according to SLEDAI-2K [16].

Exclusion criteria: Patients with other autoimmune disorders, immunodeficiency, acute or chronic infectious diseases were excluded.

Methods

Five ml blood were collected from each patient and control subject and further divided into 3 ml in EDTA tube and 2 ml in serum separation tubes. The EDTA tube was stored at -80°C for real time PCR (qPCR) and the serum separation tube was centrifuged at 3000 rpm then stored at -80°C for enzyme-linked immunosorbent assay (ELISA).

ELISA

The serum level of MASP2 was measured by sandwich ELISA using Human Mannose Associated Serine Protease 2 ELISA Kit **Cat. No E4757Hu (Bioassay Technology Laboratory, China).** The absorbance (OD) of each well was measured under 450 nm wavelength. According to standards' concentrations and the corresponding OD values, the linear regression equation of the standard curve was calculated then according to the OD value of samples, the concentration of the corresponding sample was calculated. The standard curve range was 7-1500 ng/ml.

Genotyping:

Gene extraction:

It was done using **Gene JET Whole Blood Genomic DNA Purification Mini Kit Cat. No K0781 (Applied Biosystems,USA)** for purification of DNA from blood according to manufacturer's instructions.

Genotyping for *MASP2* rs 6695096 SNP was done by TaqMan SNP genotyping assay.

TaqMan® genotyping Master Mix Cat No 4371353 (Applied Biosystems, USA), **SNP Genotyping Assay rs6695096 Cat No 4351379 (Applied Biosystems, USA)** were used for real time PCR. The assay had two TaqMan® minor groove binder (MGB) probes:

- One probe labeled with VIC® dye which detected the Allele 1 sequence.
- One probe labeled with FAM™ dye which detected the Allele 2 sequence.

Context Sequence [VIC/FAM]
ACTCCCCAAAGCTGTGCTCTCACAG[C/T]AGTTC
CTATTCTAGTGTTTTACGAG

The real time PCR was done using **Rotor-gene Q MDx (QIAGEN Hilden, Germany)** according to the manufacturer [17].

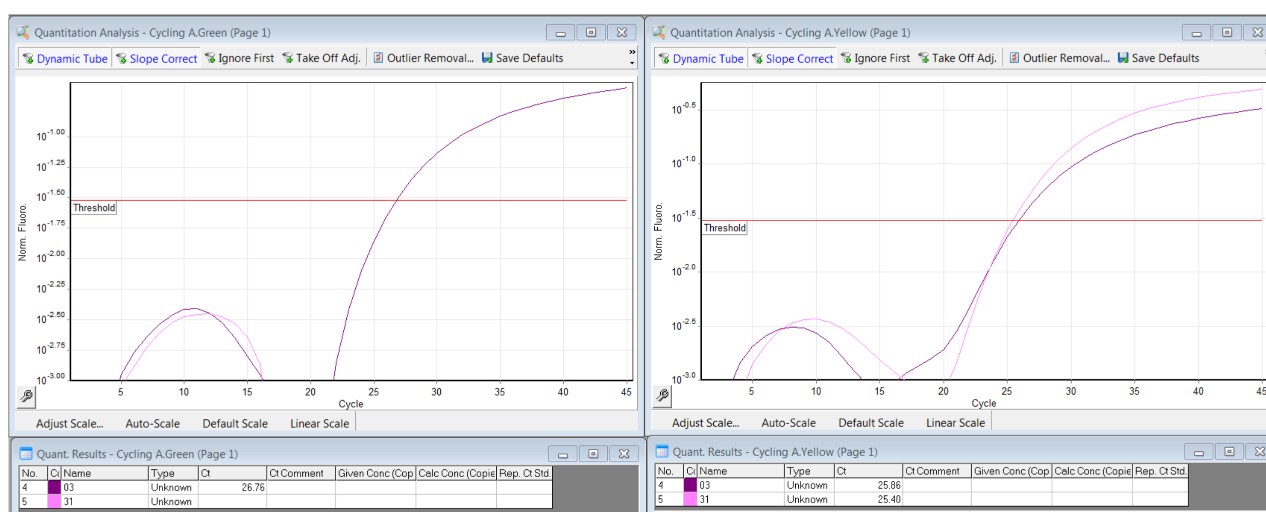

Fig. 1: The quantitation analysis of real time PCR (right panel= FAM dye, left panel= VIC dye). In heterozygous TC genotype (sample No 4= purple), the curve peaked the cycle threshold of both FAM and VIC while in homozygous CC genotype (sample No 5= pink), the curve peaked only the cycle threshold of VIC.

Statistical analysis

Data were evaluated statistically using IBM SPSS statistics (Statistical Package for Social Sciences) software version 28.0, IBM (SPSS Inc., Chicago, IL, USA). Descriptive statistics: Mean, Standard deviation (± SD) and range were used for parametric numerical data, while Median and Interquartile range (IQR) were used for non-parametric numerical data. Frequency and percentage were used to describe non-numerical data. Suitable statistical tests were used according to the type of data. Chi-square test was utilized to compare the association between the qualitative while Mann-Whitney test was utilized to compare differences between medians of two independent groups. Kruskal-Wallis test was applied for comparing differences between medians of more than two independent groups. Independent t-test was used to assess the statistical difference between means of unrelated groups.

RESULTS

This study included 50 participants; the SLE patient's groups were 30 females and 5 males with a mean age of 32.89±9.96 years. The control group included 13 females and 2 males with a mean age of 34.00±9.32 years. No statistically significant difference regarding the age and gender of SLE patients and healthy controls was detected (*p*=0.714 and 0.929, respectively).

The patients were classified according to SLEDAI-2K[16,18] as demonstrated in figure 2. Arthritis, proteinuria and pyuria were the most frequent clinical parameters represented as 31.4, 28.6 and 22.9% respectively.

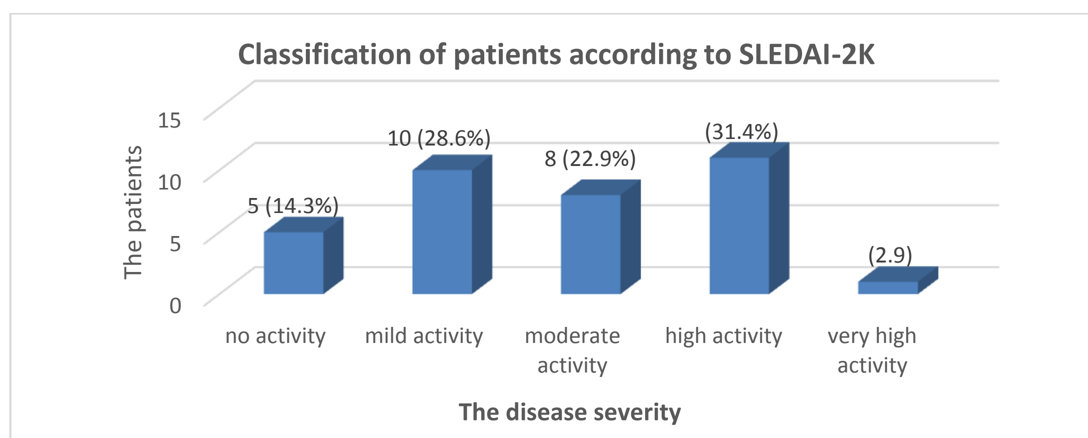

Fig. 2: Classification of patients group according to SLEDAI score

The serum level of *MASP2* in patients group ranged from 25 to 750 ng/ml with a median of 140 ng/ml, while it was 25-800 ng/ml with a median of 450 ng/ml in control group. The difference between patient and control group was statistically highly significant as presented in figure (3).

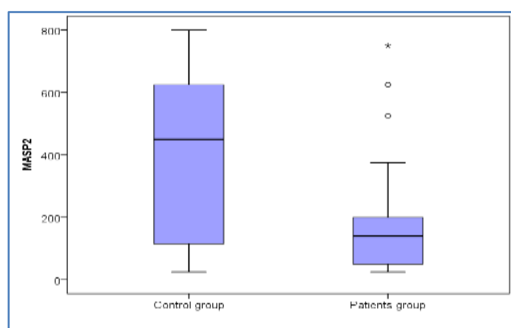

Fig.3: Comparison between control and patients group regarding MASP2 level (ng/ml).

ROC curve analysis showed that the cut off level of MASP2 ≤255 ng/ml and it could be used to discriminate cases from controls with 88.57% sensitivity, 66.67% specificity, 86.1% positive predictive value and 71.4% negative predictive value. Area under the curve was 0.734 and *P*-value was 0.009 as demonstrated in figure (4).

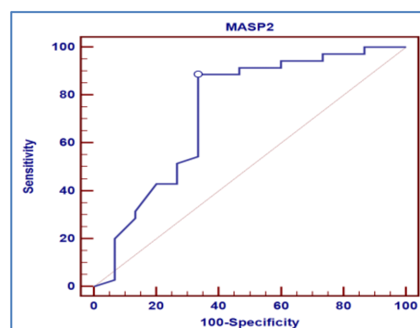

Fig. 4: Receiver operating characteristic (ROC) curve for the MASP2 level discriminating SLE patients from healthy control.

MASP2 levels were negatively correlated with SLEDAI score with high statistical difference as delineated in table (1) and figure (5).

Moreover, decreased MASP2 level was correlated with arthritis and mucosal ulcers with statistical significance. It was found that patients with arthritis and ulcers had lower serum levels of MASP2 (median=50 ng/ml and 30 ng/ml respectively) than in patients without these manifestations (median =172.5 ng/ml and 160 ng/ml respectively) as displayed in table (1).

Table 1: Correlation between *MASP2* serum level and the disease activity represented by SLEDAI score and its parameters in the patient group.

SLEDAI		MASP2		Test value	P-value	Sig.
		Median (IQR)	Range			
SLEDAI score	No activity Mild activity Moderate activity High or very high activity	525 (255 – 625) 180 (75 – 199) 85 (62.5 – 132.5) 60 (47 – 170)	255 – 750 30 – 375 25 – 200 30 – 205	14.722≠	0.002	S
Arthritis	No Yes	172.5 (75 – 250) 50 (30 – 170)	30 – 750 25 – 199	-2.189•	0.029	S
Proteinuria	No Yes	175 (50 – 245) 75 (50 – 95)	25 – 750 49 – 205	-1.426•	0.154	NS
Pyuria	No Yes	140 (49 – 205) 142.5 (72.5 – 180)	25 – 750 50 – 375	-0.216•	0.829	NS
Vasculitis	No Yes	150 (70 – 199) 62 (45 – 200)	25 – 750 30 – 205	-1.074•	0.283	NS
Fever	No Yes	145 (50 – 205) 75 (50 – 185)	30 – 750 25 – 200	-0.732•	0.464	NS
Alopecia	No Yes	115 (50 – 199) 190 (111.5 – 225)	25 – 750 48 – 245	-0.701•	0.483	NS
Hematuria	No Yes	115 (50 – 200) 172.5 (170 – 175)	25 – 750 170 – 175	-0.498•	0.618	NS
Increased DNA binding	No Yes	150 (50 – 200) 85 (75 – 95)	25 – 750 75 – 95	-0.463•	0.644	NS
Hem granular cast	No Yes	140 (50 – 200) 120 (70 – 170)	25 – 750 70 – 170	-0.320•	0.749	NS
Pericardial effusion	No Yes	150 (50 – 205) 85 (62.5 – 135)	25 – 750 50 – 175	-0.571•	0.568	NS
Pleural effusion	No Yes	127.5 (50 – 202.5) 170 (75 – 175)	25 – 750 75 – 175	-0.148•	0.883	NS
Serositis	No Yes	145 (50 – 205) 95 (75 – 170)	25.00 – 750.00 50.00 – 175.00	-0.401•	0.688	NS
Ulcers	No Yes	160 (60 – 202.5) 30 (25 – 75)	30 – 750 25 – 75	-2.154•	0.031	S
Rash	No Yes	170 (70 – 205) 50 (40 – 100)	25 – 750 30 – 150	-1.662•	0.097	NS
Granular cast	No Yes	150 (50 – 200) 82.5 (50 – 115)	25 – 750 50 – 115	-0.712•	0.477	NS
Low complement	No Yes	160 (60 – 202.5) 50 (45 – 75)	25 – 750 45 – 75	-1.564•	0.118	NS
Visual disturbance	No Yes	140 (50 – 200) 100 (30 – 170)	25 – 750 30 – 170	-0.890•	0.374	NS
Others	No Yes	150 (70 – 205) 50 (40 – 112.5)	25 – 750 30 – 175	-1.480•	0.139	NS

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.001: Highly significant
•: Mann-Whitney test; ≠: Kruskal-Wallis test

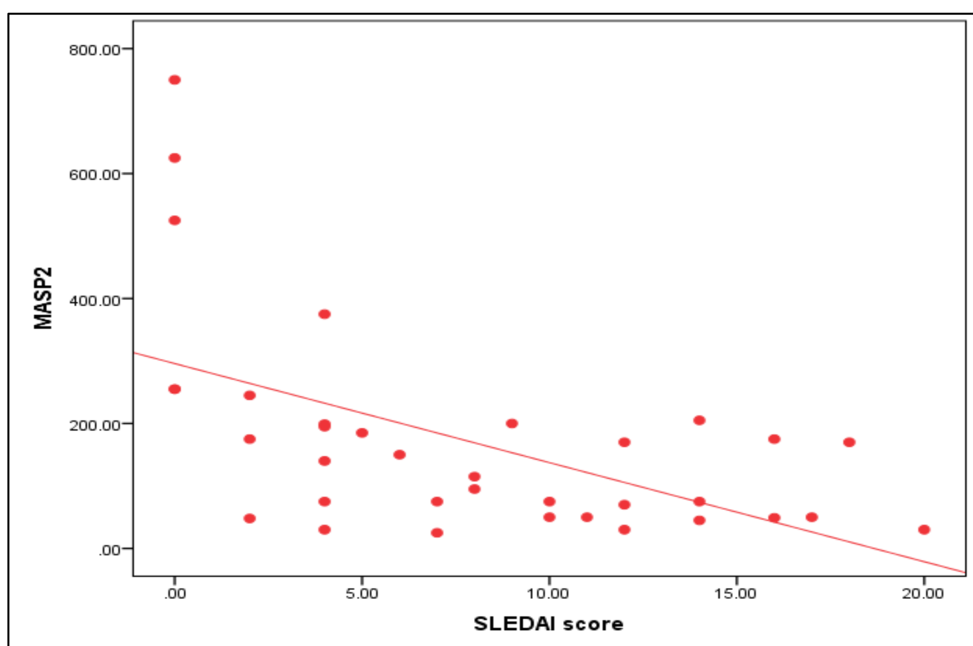

Fig. 5: Correlation between MASP2 and SLEDAI score.

As regards genotyping, in the patient's group, 24 patients (68.6%) were homozygous TT genotype, 11 patients (31.4%) were heterozygous TC genotype while no patient showed homozygous CC. while in the control group, 11(73.3%) subjects were homozygous TT, 3 (20.0%) subjects were heterozygous TC and one (6.7%) subject was homozygous CC. The diversities between the two groups for the genotypes and alleles were statistically non-significant as presented in table (2). Furthermore, the association between MASP2 genotypes and disease activity was statistically non-significant. Statistically significant association between MASP2 genotype TC and some SLEDAI parameters i.e., hematuria and pleural effusion was detected. No other parameters were found to be statistically significant as illustrated in table (3). The correlation between MASP2 level and MASP2 genotypes was statistically non-significant as exhibited in table (4).

Table 2: The distribution of MASP2 genotypes among the patients and control groups.

		Control group II	Patients group I	Test value	P-value	Sig.
		No. = 15	No. = 35			
Genotype	TT	11 (73.3%)	24 (68.6%)	2.857*	0.240	NS
	TC	3 (20.0%)	11 (31.4%)			
	CC	1 (6.7%)	0 (0.0%)			
Gene allele	T	25 (83.3%)	59 (84.3%)	0.014*	0.906	NS
	C	5 (16.7%)	11 (15.7%)			

*: chi-square test NS: non-significant

Table 3: Correlation between MASP2 genotype and the disease activity represented by SLEDAI score and its parameters among patients' group.

	Genotype		Test value	P-value	Sig.
	TT	TC			
	No. = 24	No. = 11			
SLEDAI score Median (IQR) Range	8 (4 – 12) 0 – 17	4 (0 – 16) 0 – 20	-0.679≠	0.497	NS
No activity Mild activity Moderate activity High or very high activity	2 (8.3%) 6 (25.0%) 8 (33.3%) 8 (33.3%)	3 (27.3%) 4 (36.4%) 0 (0.0%) 4 (36.4%)	5.922*	0.115	NS
Arthritis	10 (41.7%)	1 (9.1%)	3.714*	0.054	NS
Proteinuria	8 (33.3%)	2 (18.2%)	0.848*	0.357	NS
Pyuria	4 (16.7%)	4 (36.4%)	1.660*	0.198	NS
Vasculitis	5 (20.8%)	1 (9.1%)	0.732*	0.392	NS
Fever	4 (16.7%)	1 (9.1%)	0.354*	0.552	NS
Pericardial effusion	2 (8.3%)	2 (18.2%)	0.723*	0.395	NS
Alopecia	2 (8.3%)	2 (18.2%)	0.723*	0.395	NS
Hematuria	0 (0.0%)	2 (18.2%)	4.628*	0.031	S
Increased DNA binding	2 (8.3%)	0 (0.0%)	0.972*	0.324	NS
Hemegranular cast	1 (4.2%)	1 (9.1%)	0.339*	0.560	NS
Pleural effusion	0 (0.0%)	3 (27.3%)	7.159*	0.007	S
Ulcers	2 (8.3%)	1 (9.1%)	0.006*	0.941	NS
Rash	3 (12.5%)	1 (9.1%)	0.087*	0.769	NS
Granular cast	2 (8.3%)	0 (0.0%)	0.972*	0.324	NS
Low complement	2 (8.3%)	1 (9.1%)	0.006*	0.941	NS
Visual disturbance	2 (8.3%)	0 (0.0%)	0.972*	0.324	NS
Others	2 (8.3%)	2 (18.2%)	0.723*	0.395	NS

NS: non-significant

Table 4: Correlation between MASP2 serum level and MASP2 genotypes.

		Genotype		Test value	P-value	Sig.
		TT	TC			
		No. = 24	No. = 11			
MASP2	Median (IQR) Range	85 (50 – 199.5) 25 – 625	175 (75 – 255) 30 – 750	-1.334≠	0.182	NS

P-value > 0.05: Non significant; P-value < 0.05: Significant; P-value < 0.001: Highly significant
*: Chi-square test; ≠: Mann-Whitney test

DISCUSSION

SLE is one of the most common autoimmune diseases worldwide. Dysregulation of the complement system has been demonstrated to participate in the pathogenesis of SLE. The disease represents a challenge that should be faced to decrease its disabling morbidities and mortalities [19].

MASP2 is the key effector enzyme of lectin pathway of the complement system. MASP2 binds mannose binding lectin (MBL), M ficolin, L ficolin or H ficolin then it's activated to cleave both C2 and C4 leading to C3 convertase formation [20].

There are few studies that examined the role of MASP2 in autoimmune diseases especially its association with SLE and the disease activity, although it's recently targeted in the treatment of different disorders as COVID-19 SIRS, myocardial and gastric ischemia [21,22]. Herein comes the importance of this study.

To the best of our knowledge, this study is the 1st in Egypt and about the 4th worldwide done on MASP2 level and genotypes and its association with SLE.

The current study showed that there were decreased levels of MASP2 in the patients group compared to the control group and the difference between both groups was statistically significant. The median and range of serum level of MASP2 were 140 (50 – 200) ng/ml in patients' group vs 450 (80 − 650) ng/ml in the control group at a cutoff point ≤ 255 ng/ml and AUC=0.734. A significant negative correlation was detected between serum MASP-2 levels and the disease activity (SLEDAI score). This highlights the possible role of MASP2 levels in the prediction of the disease activity.

There is a discrepancy between the previous findings and a study that measured the plasma protein levels encoded by *MASP2* gene (MASP2 and Map19, the alternative splice of *MASP2* gene[23]) in SLE done by Troldborg et al.[14]. They included 58 female SLE patients and 65 healthy controls and demonstrated that the plasma levels of *MASP2* were not significantly different from that in healthy controls. A negative correlation between Map19 and SLE was statistically highly significant. Also, Troldborg et al.[13] concluded 372 SLE patients and 170 healthy controls, MASP2 median and range levels were found as follows 545 (65–3612) ng/ml and 391 (51–1592) ng/ml respectively. It was significantly correlated with SLEDAI score. Moreover, Xu et al.[12] included 61 SLE patients and 98 healthy control. This study revealed that MASP2 serum levels were significantly higher in lupus patients as compared to that in controls (12.230 ± 0.779 vs 7.174± 0.999 ng/mL). In addition, MASP2 serum level is elevated in patients with active disease in contrast to patients with less active disease.

Other investigators revealed deficiency of other components of the lectin pathway in SLE patients. ***Perazzio et al.*** [24] reported that there is association between MBL deficiency and SLE especially in those with lupus nephritis in the Brazilian population. In addition, Hein et al.[25] reported that decreased ficolin-1 levels were associated with increased SLEDAI score in patients with SLE.

In addition Goeldner et al.[27] found that *MASP2* levels were significantly lower in rheumatoid arthritis patients (median 181 ng/ml, range 21–1200 ng/ml, IQR=199 ng/ml) than controls (median 340 ng/ml, range 42–1200 ng/ml, IQR=398 ng/ml). They explained their findings by increased MASP2 consumption and/or lower gene expression due to the expression of other proteins such as Map19, an alternative splice product of the *MASP2* gene.

The discrepancies between the present study findings and others could be explained by the difference in the sample size, medical treatments given to the patients that may affect *MASP2* synthesis or decreased *MASP2* gene expression in those patients according to ethnic differences. In addition, the possible contributions of multiple polymorphisms in MASP2 gene that may be associated with low MASP2 levels and functional deficiency were not examined.

Many antibodies against different complement components were detected in SLE patients as C1q, MBL, C3, C4, factor I, factor H and factor B [28] and this leads to acquired complement deficiency which may explain the low serum level of *MASP2* in the present study. It could be also explained by the possible MASP2 consumption in the complement pathway known to be hyper activated in SLE patients as the well-known C2 and C4 consumption in high disease activity. Also, the reported differences between the serum and plasma levels of the same proteins could justify the variability between the results [29].

As regards the genotype results, the present study showed that the SNP rs6695096 genotypes of *MASP2* gene were distributed as follows: 24/35 (68.6%) patients were homozygous TT genotype, 11/35 (31.4%) patients were heterozygous TC genotype while no patient showed homozygous CC. As regards the control group, 11/15 (73.3%) subjects were homozygous TT, 3/15 (20.0%) subjects were heterozygous TC and 1/15 (6.7%) subject was homozygous CC. The differences between the two groups for genotypes and alleles were statistically not significant. Also, there was no significant correlation between *MASP2* level and *MASP2* genotypes. Higher frequencies of TC genotypes were detected in patients with pleural effusion and haematuria as compared to others.

Only one study analyzed the correlation between rs6695096 SNP and SLE. ***Xu et al.(2020)*** included 61 SLE patients of the Chinese Hans and revealed that it was significantly correlated with the genetic predisposition to SLE. Furthermore, increased frequency of genotype TT and TC and allele T were detected in SLE patients with oral ulcers and hypocomplementemia compared to patients without these manifestations. Also, TT and TC genotype revealed higher *MASP2* expression in the patients group.

Another study by Ytting et al.[30] analyzed the D120G within the coding regions of *MASP2* and found no association between this *MASP2* genotype and postoperative infectious complications or recurrence of the disease in patients with colorectal carcinoma. However other studies found an association between *MASP2* SNP and different disorders as ischemic stroke[31], HIV prognosis [32], HCV [33] and pulmonary tuberculosis [34]. Other studies performed by Chen et al.[36,37] found that SNP rs6695096 genotype TC was associated with increased susceptibility to TB.

The discrepancy of the results could be elucidated by different study population or in better words the ethnic differences between Egyptian, Chinese and Brazilian populations. Different sample size between the studies could also affect the results. Also, there are many polymorphisms of the *MASP2* gene were reported

in different populations. Nine SNPs were detected [38] and we did not analyze the impact of other polymorphisms and haplotypes of *MASP2* gene on SLE susceptibility.

Consequently, further larger scale studies evaluating the association between MASP2 serum level and SLE disease activity as well as the association between MASP2 SNPs and SLE are needed.

CONCLUSION

This study concluded that serum level of MASP2 is significantly decreased in SLE patients' group compared to control's group. Furthermore, decreased MASP2 serum level was correlated with increased disease activity as expressed by SLEDAI-2K score. So, serum *MASP2* could be a promising biomarker to help monitoring the disease activity. On the other hand, no significant correlation was detected between *MASP2* genotypes of rs 6695096 SNP and the disease activity as well as *MASP2* serum level.

Limitations

First, multiple gene polymorphisms were not examined in patients with SLE and in other group of patients. Second, a larger sample size applied separately in different ethnic groups to evaluate association of *MASP2* gene polymorphisms with SLE risk will help better understanding of the genetic susceptibility of SLE.

Ethical considerations:

This manuscript has not been previously published and is not under consideration in the same or substantially similar form in any other reviewed media. I have contributed sufficiently to the project to be included as author. To the best of my knowledge, no conflict of interest, financial or others exist. All authors have participated in the concept and design, analysis, and interpretation of data, drafting and revising of the manuscript, and that they have approved the manuscript as submitted

Conflict of interest:

All authors declare no conflict of interest in this work.

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