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# MANNOSE BINDING LECTIN GENE (MBL2) POLYMORPHISMS AND SERUM MBL IN SUDANESE PATIENTS WITH RHEUMATOID ARTHRITIS

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**Abstract**

**Objectives:** To study the association of mannose-binding lectin gene (MBL2) polymorphisms, and serum MBL levels in Sudanese patients with rheumatoid arthritis.

**Methods:** We studied 88 patients and 53 healthy controls for the variants of the MBL2 gene at codons 52 (D), 54 (B), and 57 (C) by using PCR-RFLP. MBL levels were quantitatively measured by ELISA.

**Results:** Both patients and controls showed polymorphisms in codons 54 and 57. The D allele was detected only in the patients' group; meanwhile the B allele was detected in 6.8% of the patients and 3.8% of the controls. The frequency of allele C was higher in patients (12.5%) but was not significantly different from the control group (3.8%). The prevalence of all mutations (B, C, D) in patients was significantly different from controls (p-value = 0.029, OR = 0.296, 95% CI = 0.095 – 0.926). Serum MBL in patients was significantly higher than that in controls. Individuals with mutant variant C in patients and controls showed significantly lower levels of MBL when compared with non mutant individuals (P = 0.04).

**Conclusions:** The mean MBL levels were found high in Sudanese patients compared with the controls. All of the three variant alleles (D, B, and C) of MBL2 gene were detected in our study and collectively were associated with RA in Sudanese patients.

**Keywords:** Rheumatoid Arthritis, Mannose-binding lectin, Gene polymorphisms, Sudan.

## Introduction

Rheumatoid arthritis (RA) susceptibility has been associated with defects in innate immunity (1). Mannose-binding lectin (MBL) deficiency is one of the defects incriminated. Complement system is a key component of the innate immunity that has been related to the development and clinical presentation of many autoimmune diseases (2,3). The lectin pathway is one of the three pathways of the complement system and can be triggered by pattern-recognition receptors, mainly mannose-binding lectin (MBL), ficolins, and collectin

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11 (2). The higher relative risk of developing the disease in siblings affected with MBL deficiency, suggests the importance of genetic factors that contribute to at least 60% of RA [4,5]. Mannose binding lectin (MBL) major role in innate immunity was its ability to opsonize pathogens to enhance their phagocytosis, and to activate the complement cascade via the lectin pathway [6]. Activation of the complement cascade, results from the association of the MBL with MBL-associated serine protease 2 (MASP2). Inherited insufficiency of MBL impairs the innate immune function and enhances susceptibility to infections [7]. The human MBL encoded on MBL2 gene is located in chromosome 10. Three single nucleotide polymorphisms (SNPs) in the exon1 of the human MBL2 gene at codons 52 (Arg52Cys, MBL2, D), 54 (Gly54Asp, MBL2, B), and 57 (Gly57Glu, MBL2, C), interfere with the formation of higher MBL oligomers (8,9). These polymorphisms affect serum MBL concentration, influence the stability and function of the MBL protein [10-12]. These polymorphic B, C and D alleles are collectively termed the O allele, and the wild type is designated the A allele. There is evidence that MBL2 gene mutation or MBL deficiency is a risk factor for susceptibility to autoimmune diseases, such as systemic lupus erythrimatosus and rheumatoid arthritis [13-15].

The aim of this study was to identify the MBL2 structural gene polymorphisms, its serum levels in Sudanese RA patients and their possible association with the disease.

**Materials and Methods**

This Study was carried out in Khartoum State (Sudan) in The National Ribat University Hospital. Blood samples from 88 patients with RA and 53 healthy controls were collected for DNA study and measurement of serum MBL after informed consent. Blood samples for DNA extraction and serum were stored at -70$^{o}$C until tested. All patients fulfilled the American College of Rheumatology's (ACR) selection criteria [16].

MBL levels were estimated by ELISA (R & D, USA, Minneapolis). Polymorphisms of codons (52, 54, and 57) in exon 1 of the MBL2 gene were identified by PCR-RFLP using the restriction enzymes Hha1, Mlu1, Mbo11, and Ban1, respectively. The following pairs of primers (forward (F) and reverse (R)) were amplified. The primers and the PCR-RFLP were published previously by Madsen [17].

5´AGTCGACCCAGATTGTAGGA CAGAGA-3 F

3'-AGGATCCAGGCAGTTTCCTCTG GAAGG-5R

5´CATCAACGGCTTCCCAGGCA AAGACGCG-3 F

3'-AGGATCCAGGCAGTTTCCTCTG GAAGG-5 R.

Amplification For codon 57 was carried out using 10x PCR buffer (10mM tris-HCl, pH 8.3, 1.5 mM $MgCl_2$, 200µM of dNTPs (Sigma, UK), 100nM of each of oligonucleotides, DNA template, and 1unit of Tag DNA polymerase (Roche, Germany) in a final volume of 25 µl. Reactions were performed in thermocycler (BIO-RAD, UK) with the following thermal profile: primary denaturation at 94 $^{O}$C for 2 min, denaturing at 94 $^{O}$C for 30 seconds,, annealing at 55 $^{O}$C for 2 mins, extension at 72 $^{O}$C for 2 mins, and final extension at 72 $^{O}$C for 5 mins. The thermal profile for codon 52, 54 was: primary denaturation at 95 $^{O}$C for 5 mins, denaturation at 95 $^{O}$C for 20 seconds, annealing temperature at 62 $^{O}$C for 20 seconds, extension at 72 $^{O}$C for 30 seconds for 34 cycles, and final extension at 72 $^{O}$C for 2 mins. The master mix digestive enzyme 10 µl volume was prepared according to the instructions of manufacturers, as fallows: pure sterile distilled water 7.3 µl, buffer 2 µl, BSA buffer 0.2 ul, and 0.5 µl of (Hha 1, Ban 1) enzyme, whereas BSA buffer was excluded for (Mlu 1, Mbo 11) enzymes. 10 µl of the mixture was placed into 0.5 ml PCR tube with patients DNA amplified with specific primer of the specific codons in a total volume of 20 µl, incubated overnight for 16 hours.

Statistical analysis: SPSS 18 was used for all statistical analysis. Differences between continuous variables were analysed by unpaired t-tests. Comparison of allele prevalence in patients and controls, and genotypes AA/AB or AC and CC. was calculated by Chi-square analysis. Confidence interval CI and odds ratios (OR) also were performed by Chi-square test. P-values <0.05 were considered to be statistically significant.

**Results**

MBL levels were significantly higher in patients when compared with controls, (p-value <0.03) (Table 1). MBL was undetectable in 3 patients (3.4%) and one of the controls (1.9%). Also there were 10/19(52.6%) of the total mutant patients, revealed decreased MBL levels (<600 ng/ml). Seven out of the ten patients with reduced MBL were mutant codon 57 and the remaining 3 were mutant codon 54. Mean MBL levels of patients and controls with mutant variants (C, B and D) of MBL2 gene, and the group of patients and controls without mutations are shown in Table1. The mean levels of MBL of patients and controls with the mutant variant C when compared with not mutant patients and controls group showed significantly lower values (p-value = 0.04) (Table 1). The distribution of MBL2 gene alleles amongst RA patients and controls is shown in Table 2. MBL2 gene codon 57 variant C was found in 11 of 88 patients, heterozygous (n= 8) or homozygous (n= 3) and 2 heterozygous in controls. There was no significant difference in the prevalence of this allele between patients

and controls (p = 0.08, OR = 0.275, 95% CI = 0.058-1.29). B variant, (codon 54) was found in 6 of the patients and 2 of the controls (p-value = 0.70, OR = 0.536, 95% CI = 0.104-2.76). Variant D (codon 52) was detected in 2 of the patients and not seen in the control group. Mutations in the three codons of the gene collectively (52, 54, 57) in patients was 19 of 88, when compared with the controls 4 of 53, revealed a significant difference (p-value = 0.029, OR = 0.296, 95% CI = 0.095 – 0.926). Table 3 shows the distribution of mutations in the three codons 52, 54 and 57 of the MBL2 gene among patients.

**Discussion**

This cross-sectional case-control study revealed statistically significant difference (Table 1) between mean MBL levels of patients and controls. As an acute phase protein, expression of MBL is expected to rise in many inflammatory conditions including RA (18). In this study MBL have been undetected in 3 patients (3.4%) and only 1 of controls (1.9%). These findings were in agreement with those of previous reports [14, 19] who found (4.3%, 11%) of RA patients, and (1%, 3%) of controls with undetectable MBL. In our study there were 10 (52.6%) patients from the total 19 mutants revealed decreased MBL levels (<600 ng/ml). These findings again confirm previous findings (16) that, heterozygous people of D, B or C mostly have MBL levels between 0.5 and 1µg/ml. In other population studies the serum MBL level was reported to be low in RA patients as compared with controls [17]. Another study (18) found similar levels of mean serum MBL in both the patients and controls (1280, 1230 ng/ml), respectively. This study have shown that there was a significant differences in the means of the MBL levels of the 13 variants C and the 120 patients and controls without mutant variant C, (p-value = 0.04)(Table 1). The 8 variant allele B mean MBL levels was compared with the patients and controls without mutation of the same allele, (p-value = 0.066) (Table 1).Our results also found an allelic association with the MBL levels in codon 57 variant C. However, there was no association with the other variants (B and D). These findings were not similar to previous findings [18], Meanwhile S.J Stanworth [15] also found no association between MBL2 polymorphisms and RA, whereas T. Horiuchi (23) reported that MBL2 gene polymorphisms were not risk factors for SLE or RA in Japanese individuals. Our results findings apparently clarified that, variant C frequency was the highest mutation among Sudanese RA patients, but not significantly different from the control group, then variant B, and lastly variant D (table 2) contrary to these, mutations in codon 57 were not detected by Sasaki K and Cheong JY [24, 25]. However, mutations in this codon were found in 50 to 60% of African populations. Our study has shown that 22 mutations were found in 19

patients (21.6%) and 4 mutations in the controls which reveal a significant difference (p =0.029) (Table 3). There was no significant difference between patients and controls in the presence of the different alleles. Contrary to our findings in codon 54 Ip WK [14] found a significantly more RA patients had heterozygous or homozygous alleles than the controls (p = 0.027, OR = 1.96, 95% CI = 1.084-2.640). Other investigators [26] found slight increase of the homozygous allele B in RA patients. The findings of allelic mutants in this study were also different from previous reports in Japanese and Korean populations [14, 22].

In conclusion the mean MBL level was found high in Sudanese patients compared with the controls, and was found low in patients with mutant variant allele C. All of the three variant alleles (D, B, and C) of MBL2 gene were detected in our study among the Sudanese patients, and the frequency of the C allele was the highest. Our data provides evidence that homozygous and heterozygous D, B and C variant MBL2 alleles in exon 1 of the gene are associated with rheumatoid arthritis in Sudanese patients.

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**TABLE 1:** Mean serum MBL levels in patients and controls, mutant (C, B) alleles and non mutant individuals (patients and controls).

| Patients and controls | Mean MBL (ng/ml) | P-value |
|---|---|---|
| Not mutants (n = 120) | 1624 ± 196 | |
| Mutant variant C (n= 13) | 1028 ± 147 | 0.04 |
| Mutant variant B (n= 8) | 1418 ± 181 | 0.8 |
| Total patients (n= 88) | 1871 ± 154 | 0.003 |
| Total controls (n= 53) | 1202 ± 129 | |

**TABLE 2:** Distributions of MBL2 structural genotypes and alleles amongst the RA patients and control groups.

| MBL2 (A/C) | Patients (n=88) | Controls(n=53) | P-values |
|---|---|---|---|
| A/A | 77(87.5) | 51(96.2) | |
| A/C | 8(9.1) | 2(3.8) | = 0.15 |
| C/C | 3(3.4) | zero | |
| Allele | | | |
| A | 162(92.1) | 104(98.0) | |
| C | 14(7.9) | 2(2.0) | |
| MBL2(A/B) | | | |
| A/A | 82(93.2) | 51(96.2) | |
| A/B | 6(6.8) | 2(3.8) | = 0.45 |
| Allele | | | |
| A | 170(96.6) | 104(98.0) | |
| B | 6(3.4) | 2(2.0) | |
| MBL2(A/D) | | | |
| A/A | 86(97.7) | | |
| A/D | 2(2.3) | | = 0.7 |
| Allele | | | |
| A | 174(98.9) | | |
| D | 2(1.1) | | |

**TABLE 3:** Wild-type A, heterozygous and homozygous alleles of the MBL2 gene in the three codons (D, B, C) among RA patients.

| The alleles among patients (A,D,B,C) | Alleles (n= 176) | P-value |
|---|---|---|
| Wild type Allele A | 154(87.5%) | |
| Heterozygote Alleles (D,B,C) | 16(9.1%) | 0.08 |
| Heterozygotes plus homozygotes Alleles in the three codons (D,B,C) | 22(12.5%) | 0.029 |