Mannose-binding lectin in pre-menopausal women with recurrent urinary tract infections

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Abstract

Mannose-binding lectin (MBL) comprises an oligomeric serum protein that is a member of the collectin class of the C-type lectin superfamily. Its deficiency is genetically determined and confers predisposition to recurrent infections as well as increased infection severity. This correlation has been demonstrated in recurrent furunculosis caused by Staphylococcus aureus, and in pneumococcal and Candida infections. The present study aimed to determine whether there is a correlation between MBL serum levels and recurrent urinary tract infections (UTI) in pre-menopausal women. The present aged-matched double-blind controlled study was conducted in 100 pre-menopausal adult women: 50 who suffered from recurrent UTI and 50 without UTI. The MBL concentration was measured in a single serum sample from each patient using an enzyme-linked immunosorbent assay. MBL serum levels [median (range)] were 2500 (4–12 000) ng/mL and 2105 (4–22 800) ng/mL for the research and control groups, respectively. The results from the two groups were compared and were not statistically different (p 0.4). According to these results, MBL serum levels are not associated with an increased risk for recurrent UTI in pre-menopausal women.

Keywords: Lectin, mannose-binding, pre-menopausal, recurrent, UTI

Introduction

Mannose-binding lectin (MBL), also named mannose or mannan-binding protein, comprising an oligomeric serum protein, is a member of the collectin class in the C-type lectin superfamily that plays a central role in the acute phase of infections. MBL has the capacity to recognize carbohydrate patterns found on the surface of a large number of pathogenic microorganisms, including bacteria, viruses, protozoa and fungi. Binding of MBL to a micro-organism results in activation of the classical complement pathway, long before specific antibodies are formed, eventually leading to the death of the pathogen [1–4]. Reduced plasma levels of MBL may impair the normal innate immune response and increase susceptibility to infections [5]. MBL deficiency occurs in 20–25% of the population, which is a relatively high incidence compared to other deficiencies of the complement system [6,7].

Variability in plasma MBL levels and function among the population correlates with multiple polymorphisms of the MBL2 genes. Low plasma MBL levels and impaired function are seen in structural gene homozygosity and the LX promoter haplotype.

A relationship between low MBL serum levels and recurrent furunculosis caused by Staphylococcus aureus [8] and severe pneumococcal infections [9,10] has been reported. On the basis of a laboratory model, Ip and Lau [11] concluded that MBL has also a significant influence on the host innate immunity against Candida albicans.

Urinary tract infection (UTI) is one of the most frequent bacterial infections worldwide [12]. Pathogenesis and risk factors for recurrent UTI in pre- and post-menopausal women have been extensively described [13–16]. The major pathogen causing more than 90% of UTI in young women is Escherichia coli and Shang et al. [17] demonstrated that this bacterium demonstrates a high binding capacity to MBL.

In addition, purified recombinant human MBL was found to enhance phagocytosis of E. coli directly by acting as a
bridge between macrophages and the target E. coli [18]. Another study found that patients with MBL-2 genotypes associated with serum MBL deficiency undergoing acute pyelonephritis caused by E. coli have a higher risk of developing septic shock [19].

However, Li et al. [20] have postulated a molecular theory of complement activation during colonization of urinary epithelium by E. coli that could support a lack of association between low serum levels of MBL and UTI. To the best of our knowledge, these issues have not been investigated previously in a clinical setting.

In the present age-matched case–control study, we aimed to determine whether there is any association between MBL serum levels and recurrent UTI in pre-menopausal women.

Materials and Methods

Pre-menopausal adult (age >18 years) women suffering from recurrent UTI were enrolled in this case–control study. Recurrent UTI was defined as two documented episodes of UTI in the past 6 months, or a history of three or more episodes in 1 year. A control group was also enrolled, including an age-matched (±3 year) woman without recurrent UTI for every woman in the research group. Controls were randomly selected among nursing students, hospital personnel and patients at two outpatient clinics.

From every woman in the study, a single peripheral blood sample was taken at enrollment and serum was separated within a maximum of 4 h and frozen at −70°C. An additional citrated blood sample was taken and kept frozen for further possible genetic investigation. After providing their written informed consent, the women filled in a questionnaire including demographic data and clinical information that were relevant to UTI, such as the number of documented UTI episodes and prophylactic antimicrobial or replacement hormone therapy.

All serum samples were thawed at the same time and quantitatively tested for MBL level using MBL Oligomer ELISA kit (BioPORTO® Diagnostics, Gentofte, Denmark). This CE marked kit allows the testing of MBL in range 2–3000 ng/mL. Test results were read in a manual ELISA reader and plotted. All internal controls fitted the expected results according to the manufacturer’s instructions. Sera showing levels higher than 3000 ng/mL were diluted 1:100 and retested to reach endpoint.

Statistical analysis was performed using chi-squared for dicotomic parameters and Student’s t-test for serial numbers. This study was approved by the local ethics committee.

Results

In total 100 women (50 in the research group and 50 in the control group) were included. Age-matching between groups was successfully performed with a median (range) of 32 (18–51) years for the research group and 34 (20–53) years for the control group (p 0.44).

The mean (range) number of documented episodes of UTI in the past year in the research group was 3 (0–6) and none for the control group. Among the 50 patients in the research group, seven had not suffered any episode of UTI in the previous year and two had experienced only one episode. However, these nine patients were enrolled in the research group as having a documented history of recurrent UTI in the past. Of these nine patients, four were receiving prophylactic nitrofurantoin macrocrystals and five had been consuming cranberry juice on a daily basis during the last year.

The demographic characteristics of the two groups are compared in Table 1. Mean (range) and median levels of MBL were 2820 (4–12 000) ng/mL and 2105 ng/mL in the research group vs. 3466 (4–22 800) ng/mL and 2105 ng/mL in the control group (p not significant). The distribution of

### Table 1. Clinical and demographic characteristics of patients in research and control groups

<table>
<thead>
<tr>
<th></th>
<th>Research group</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Recurrent UTI within family</td>
<td>21 (42.0)</td>
<td>4 (8.0)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Recurrent non-UTI infections</td>
<td>5 (10.0)</td>
<td>2 (4.0)</td>
<td>0.23</td>
</tr>
<tr>
<td>UTI in childhood</td>
<td>11 (22.0)</td>
<td>1 (2.0)</td>
<td>0.002</td>
</tr>
<tr>
<td>UTI in pregnancy</td>
<td>10 (20.0)</td>
<td>5 (10.0)</td>
<td>0.16</td>
</tr>
<tr>
<td>Sex activity related UTI</td>
<td>22 (44.0)</td>
<td>0</td>
<td>0.0000</td>
</tr>
<tr>
<td>Upper UTI in the past</td>
<td>23 (46.0)</td>
<td>2 (4.0)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Hospitalization due to UTI</td>
<td>13 (26.0)</td>
<td>1 (2.0)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Previous ABTx for UTI</td>
<td>18 (36.0)</td>
<td>0</td>
<td>0.0000</td>
</tr>
<tr>
<td>Prophylactic ABTx for UTI</td>
<td>18 (36.0)</td>
<td>0</td>
<td>0.0000</td>
</tr>
<tr>
<td>Birth control use</td>
<td>19 (38.0)</td>
<td>3 (6.0)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Cranberry juice prophylactic use</td>
<td>27 (54.0)</td>
<td>0</td>
<td>0.0000</td>
</tr>
</tbody>
</table>

ABTx, antibiotic treatment; UTI, urinary tract infection.

### Table 2. Mannose-binding lectin (MBL) serum levels by category in both groups

<table>
<thead>
<tr>
<th>MBL (ng/mL)</th>
<th>Research group</th>
<th>Control group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>0–10</td>
<td>2 (4.0)</td>
<td>5 (10.0)</td>
<td>0.23</td>
</tr>
<tr>
<td>11–100</td>
<td>3 (6.0)</td>
<td>4 (8.0)</td>
<td>0.70</td>
</tr>
<tr>
<td>101–1000</td>
<td>11 (22.0)</td>
<td>8 (16.0)</td>
<td>0.44</td>
</tr>
<tr>
<td>1001–2000</td>
<td>7 (14.0)</td>
<td>7 (14.0)</td>
<td>1.0</td>
</tr>
<tr>
<td>2001–3000</td>
<td>3 (6.0)</td>
<td>6 (12.0)</td>
<td>0.29</td>
</tr>
<tr>
<td>&gt;3000</td>
<td>24 (48.0)</td>
<td>20 (40.0)</td>
<td>0.42</td>
</tr>
<tr>
<td>Total</td>
<td>50 (100)</td>
<td>50 (100)</td>
<td></td>
</tr>
<tr>
<td>Mean value</td>
<td>2500</td>
<td>2105</td>
<td>0.40</td>
</tr>
</tbody>
</table>

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MBL serum levels in the two groups is presented in six incremental categories in Table 2 and graphically presented in Fig. 1 indicating that no cut-off value could be established between the groups.

Discussion

The role of MBL in the innate immune system has been extensively described. Once MBL recognizes a microbial surface, the complement pathway is activated, enhancing opsonophagocytosis [21]. A lack of MBL may predispose the host to recurrent infections, Super et al. [1] described a correlation between an MBL-dependent opsonic defect in human serum and a phenotype of recurrent infection. Analysis of the MBL gene has revealed at least seven distinct MBL haplotypes in humans, four of which express low MBL serum levels [22]. There is a high rate of haplotype variation in different human populations, with heterozygosity rates ranging from 15% in Caucasians to 30% in African populations [6,7].

Low serum levels of MBL have been associated with increased risk of recurrent furunculosis [8], severe infections caused by Streptococcus pneumoniae [9,10], and tuberculosis [23], and a possible, although not definitely proven, association with cryptosporidiosis [24]. In addition, the role of MBL in the innate defence against C. albicans has been postulated by Ip and Lau [11] and a higher frequency of the MBL2 codon 54 morphotype has been found in Belgian women with recurrent vulvovaginal candidosis [25]. Moreover, MBL deficiency may play an important role in specific populations at risk; for example, a significant association between low concentrations of MBL and serious infections in patients receiving chemotherapy has been reported [26].

On the other hand, other studies have found a lack of association between low serum levels of MBL and Helicobacter pylori seropositivity, HPV infection and infections in patients with systemic lupus erythematosus [27–29].

Equally, in a population-based study performed in 9245 individuals, no evidence for differences in infectious disease or mortality between MBL-deficient subjects and controls was found [30].

UTI is one of the most common bacterial infections in humans. Women are more likely than men to develop recurrences, and do so especially in the post-menopausal era. However, recurrent UTI in pre-menopausal women is not an infrequent phenomenon: many young fertile women suffer from this very unpleasant and sometimes dangerous infection.

Given the fact that both UTI and MBL-defective halotypes are widely spread in the human population, we decided to investigate the relationship between serum MBL levels and recurrent UTI, as a first step for a further genetic investigation to determine whether any association exists.

We decided to focus first on pre-menopausal women, representing a more homogeneous group of patients, in that post-menopausal women often present co-morbidities such as diabetes or urogynaecological problems and have lower oestrogen secretion. All these factors were identified as potential confounders. For these reasons, post-menopausal women were not included in the present study.

With this goal in mind, we performed an age-matched case–control study in a total of 100 women volunteers, with half of them suffering from recurrent UTI.

The categorical (Table 2) and graphical (Fig. 1) distribution of MBL serum levels in the two groups revealed no significant differences between the groups. Moreover, the median value among patients in the control group was slightly lower (but not statistically significantly) compared to those in the research group.

The lack of association between low MBL levels and recurrent UTI found by us in the present study could be explained by the fact that MBL does not appear to be the principal pathway for complement activation during urinary infections by E. coli. As postulated by Li et al. [20], the initiation of complement activation during colonization of urinary epithelium by E. coli depends mainly on the classical or the alternative pathway, as related to complement protein

![FIG. 1. Mannose-binding lectin serum levels of patients in research and control groups.](image-url)
concentration. According to Li et al. [20], the MBL pathway has little role in the opsonization of uropathogenic Escherichia coli. To the best of our knowledge, the present study is the first to provide clinical results supporting this molecular hypothesis.

One of the limitations of the present study concerns the potential use of the samples in a further genetic investigation: the ethical stipulations prevented any identification of individual samples that could allow matching of epidemiological data to the MBL serum levels obtained. Thus, the results of the present study could only be compared between groups and no regression analysis could be performed to test whether a low MBL serum level is an independent risk factor for recurrent UTI. However, given the results detailed in Table 2, this possibility appears to be very unlikely.

Other limitations of the present study were the relatively small number of cases that were enrolled and the fact that participants were not asked about their sexual activity, which is a well known risk factor for UTI in young women. However, the data obtained convinced us not to perform the genetic investigation of our patients that was planned originally, given the fact that we did not find phenotypic differences in MBL between the research and control groups.

The results obtained reveal no association between low MBL serum levels and recurrent UTI in pre-menopausal women, suggesting that MBL deficiency does not play a role in recurrent UTI in pre-menopausal women. This lack of association should be confirmed in studies including a larger number of cases.

Transparency Declaration

All authors have no commercial relationship or conflict of interest of any nature related to the present study.

References


