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Mannose Binding Lectin Levels Was Not Associated with Resistance to Tuberculosis Infection in the Population of Uyo Metropolis in Nigeria

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Abstract
Mannose-binding lectin (MBL2) is an important pattern recognition molecule that identifies and binds to specific sugar molecules on the surface of pathogens thereby activating its destruction by the immune system. Samples for study were recruited from Uyo metropolis of Akwa Ibom state in Nigeria. In this study, levels of MBL2 was measured by enzyme-linked immunosorbent assay in tuberculosis patients and healthy individuals to determine if the immune protein protects against tuberculosis infection. MBL2 levels in tuberculosis patients and healthy controls were 14.0ng/ml ± 13.9 and 19.9ng/ml ± 18.5 respectively. The results from the study showed that there was no association in MBL2 levels between tuberculosis and controls (p=0.107) as well as between the different sub-groups. Therefore, MBL2 is not a contributory factor in resistance against tuberculosis in the population under study.
Keywords: Mannose binding lectin, tuberculosis, pattern recognition molecule, immune system
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1. Introduction

Mannose-binding lectin (MBL), also known as mannose-binding protein, mannan-binding protein and core-specific lectin (Turner, 1996) is classified among the collectin protein family characterized by the possession of a collagenous region and a lectin domain (Auriti *et al*., 2017). It is a pattern recognition molecule which recognizes and binds to exposed specific sugar surfaces of microorganisms thereby triggering immune response (Takahashi *et al.*, 2006).

The C-type liver serum lectin plays a key role in innate immune response (Eisen & Minchinton, 2003). As an acute phase protein (Ezekowitz *et al.*, 1991), the Human mannan-binding protein immunologically responds as an acute phase reactant shown by an increase in its serum concentration during acute phase response - a systemic reaction to an inflammatory response (Thiel *et al.*, 1992). MBL acts against a wide variety of micro-organisms including bacteria (aerobic and anaerobic), fungi and viruses as well as parasites (Neth *et al*., 2000; Townsend *et al.*, 2001; Ezekowitz *et al.*, 1989; Ying *et al*., 2004). This underlines the role of MBL in first-line immune defense against pathogens. The binding of the MBL to microbial surface carbohydrates activates the lectin complement pathway and enhances opsonophagocytosis (Neth *et al.*, 2002).

Structural mutations in exon 1 and promoter polymorphisms in the MBL-2 gene can result in differential MBL levels in humans (Eisen & Minchinton, 2003). Three point mutations at codon 54, 57 and 52 of the MBL-2 gene, associated with low level serum MBL (Sumiya *et al*., 1991) has been found to be of high frequency among the world populations (Lipscombe *et al*., 1993).Several studies have reported that polymorphisms that produce low level MBL provides protection against tuberculosis (Mombo *et al*., 2003; Cosar *et al*., 2008; Capparelli *et al*., 2009; Singla *et al*., 2012) while others conclude that these polymorphisms confer susceptibility to TB (Alagarasu *et al*., 2007; Shen *et al.*, 2020). MBL deficient individuals are more susceptible to infections. However, the susceptibility is more pronounced among individuals homozygous for the mutant alleles (Mombo *et al*., 2003).

MBL level deficiency has been shown to be associated with increased susceptibility to various infectious diseases such as sepsis, meningococcal disease, aspergillosis as well as invasive pneumococcal infections (Peterslund *et al.*, 2001; Eisen & Minchinton, 2003; Lambourne *et al*., 2009). It has been suggested that the best way to unravel the association between MBL levels and predisposition to tuberculosis could be through direct measurement of MBL levels in blood (Denholm *et al*., 2010). An earlier study that deployed this method suggested that high level MBL may play a considerable disadvantageous role in tuberculosis susceptibility, due to their findings that there was a significantly higher MBL level in tuberculosis infected individuals than non-infected individuals (Bonar *et al.,* 2004).

No study has been conducted or reported on the association of mannose binding lectin with tuberculosis in this population. Therefore, this study set out to investigate the association of MBL levels with predisposition to tuberculosis infection among the population of Uyo Metropolis, Akwa Ibom State in Nigeria by directly

measuring the MBL levels in the serum of tuberculosis patient samples and healthy individuals as controls.

2. Materials And Methods

The study recruited 60 tuberculosis patients as cases from St. Luke's Hospital, Anua, Uyo and 60 healthy individuals were recruited from the University of Uyo Teaching Hospital as controls. Healthy individuals were defined as those who did not have any disease condition. Ethical approval was obtained from the two hospitals and blood samples were collected from cases and control in line with ethical guidelines.

Blood samples were centrifuged to obtain serum/plasma for subsequent application in Enzyme-linked Immunosorbent assay (ELISA). The ELISA reagents were purchased from OriGene Technologies. The MBL concentrations in serum/plasma was measured by ELISA technique in line with manufacturer's protocols. Samples with outlier values were excluded from further analysis.

Data obtained was subjected to statistical tests to determine the level of association of MBL concentration with tuberculosis susceptibility. T-test and ANOVA were used to assess the level of association between the different groups tested. Significant difference was based on a cut off mark of $p \leq 0.05$

3. Results

The assay result showed that the mean levels of MBL2 in healthy controls was 19.9ng/ml ± 18.52 while that of tuberculosis cases was 14.0ng/ml ± 13.92 (Table 1). The t-test analysis of MBL2 levels in tuberculosis patients and healthy controls showed no significant difference between the two groups (p= 0.107).

The sample population was also assessed based on gender. The population size of males was 14 while that of the females was 26 for healthy controls. Additionally, the males constituted 18 cases whereas females made up 22 cases for tuberculosis. The mean MBL2 levels for male healthy controls was 15.5 ± 15.9 while that of male tuberculosis patients was 12.3 ± 13.93. The female population of our study subjects had mean MBL2 levels of 15.5 ± 14. 12 and 20.9 ± 18.80 for TB patients and healthy controls respectively (Table 1). There was no significant difference in mean MBL2 levels between male and female healthy controls (p= 0.404When the male and female tuberculosis cases were compared statistically, there was no significant difference between them (p=0.534). Figure 1 shows the mean distribution of MBL2 levels across the study populations.

The MBL2 levels in sample population was further stratified according to different age groups (Table 2 and Figure 2) showing the mean levels MBL2 of healthy controls and TB patients based on age groups. Age-based analysis of variance (ANOVA) showed no differences between the age groups in both healthy controls (p= 0.533) and TB cases (p=0.538).

Table 1: MBL2 levels of study subjects by gender

Population	TB Cases	Healthy Controls
Total population	14.0 ± 13.92	19.9 ± 18.52
Male	12.3 ± 13.93	15.5 ± 15.90
Female	15.5 ± 14. 12	20.9 ± 18.80

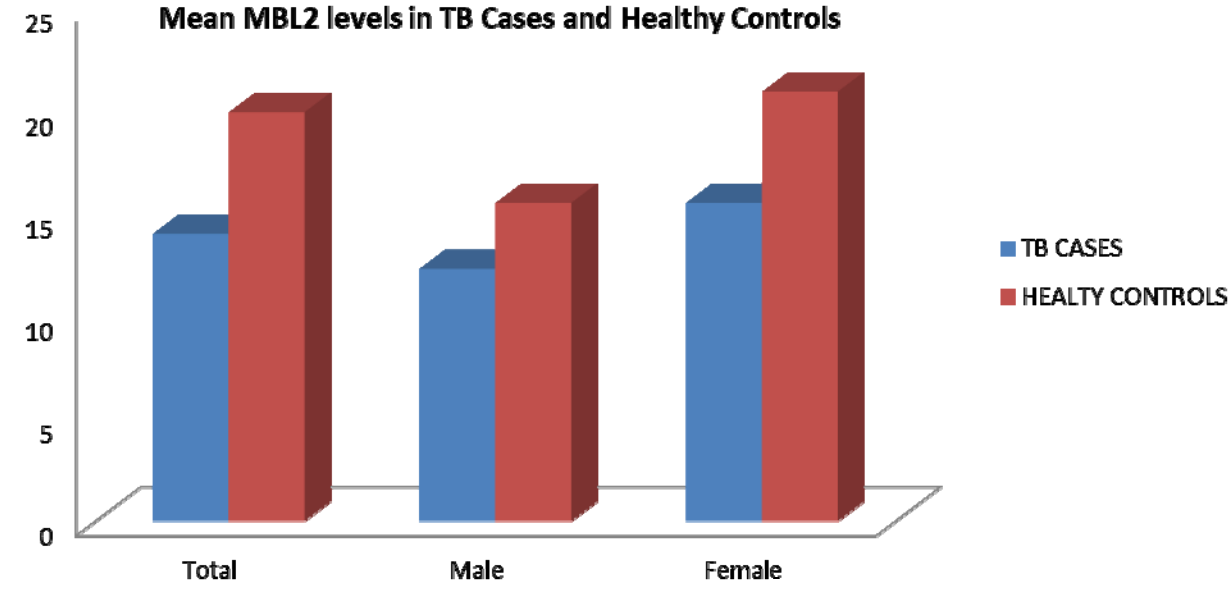

Fig 1: Mean MBL2 levels in TB Cases and Healthy Controls across study populations

Table 2: table showing MBL2 levels (ng/ml) of study subjects according to age group

Age Group	TB Cases	Healthy Controls
0-17	0.3 ± 0.00	21.4 ± 19.59
18-35	13.7 ± 13.64	14.0 ± 18.34
36-50	13.8 ± 13. 06	12.8 ± 15.36
51-65	21.0 ± 17.60	18.9 ± 9.61
66-80	N/A	41.8 ±0.00

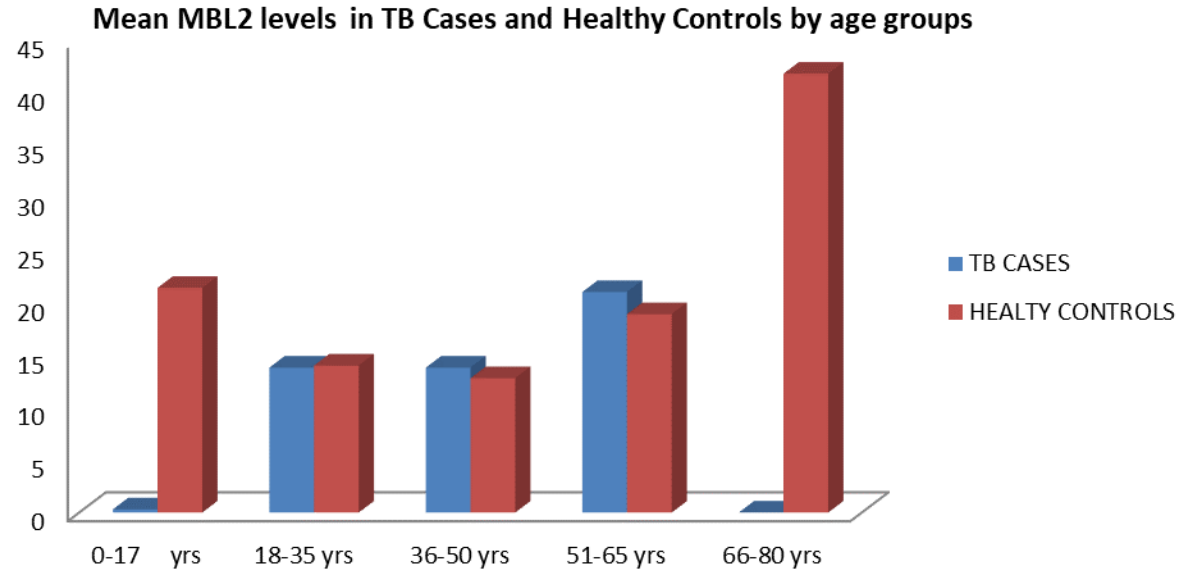

Fig 2: MBL2 levels in TB Cases and Healthy Controls based on age groups

4. Discussion

MBL is one of the major pathogen associated molecular patterns (PAMPs) molecule that binds to array of carbohydrates on the surfaces of micro-organisms (Turner, 2004; Neth *et al*., 2000; Jack & Turner, 2003; Presanis *et al*., 2003). As a serum complement factor, it plays a dominant role in first line defence through the lectin complement pathway (Kuipers *et al*., 2003; Jack & Turner, 2003).

MBL is reported to interact with 3- and 4-hydroxyl groups of many sugars such as N-acetyl-D-glucosamine, mannose, N-acetyl-mannosine, fucose, mannoheptulose, sedoheptulose and glucose on micro-organisms (Turner, 2004; Kawasaki *et al*., 1989) as well as lipoarabinomannan of mycobacterial cell wall (Chatterjee *et. al*., 1992). These observations were what informed the basis for this study.

Adult individuals that are homozygous for all wild-type alleles have MBL levels of about 1400µg/l and those that are heterozygous for codon 54 variant have reduced levels of the protein between 26 and 396µg/l depending on the promoter polymorphisms present (Madsen *et al*.,1995).

In a study in UK Caucasoid by Crosdale and Co-workers (2000), they found that in the low producing sub-population, heterozygosity for codon 52 variants have lower serum MBL of 29.24µg/l than heterozygotes for codon 54 (65.51µg/l). Also in the population, they found that heterozygotes for codon 52 variant had a mean level of 601µg/l with the mean level for codon 54 heterozygotes being 297µg/l.

From this study, healthy individuals had mean MBL levels of 19.9ng/ml which is far below the levels observed in Europeans conducted by Madsen and Colleagues (1995) and Crodale and Co-Workers (2000). Eventhough MBL levels in healthy individuals were higher than tuberculosis patients (14.0ng/ml), there was no significant difference between healthy controls and tuberculosis cases. There was no association of mannose binding lectin levels with tuberculosis. The results from this study imply that mannose binding lectin is not an important factor in conferring resistance to tuberculosis infection in the population of Uyo Metropolis in Nigeria. A major limitation of this study was the lack of genetic screens to discover the occurrence of MBL genetic mutations in the population study. As a consequence, the design of therapeutic agents based on MBL levels would not be effective against the disease condition in this population.

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