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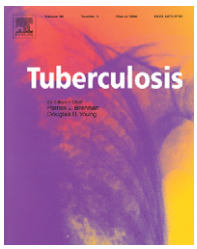

Mannose binding lectin gene variants and susceptibility to tuberculosis in HIV-1 infected patients of South India

Kalichamy Alagarasu, Paramasivam Selvaraj*, Soumya Swaminathan, Sampathkumar Raghavan, Gopalan Narendran, Paranji R. Narayanan

Tuberculosis Research Centre, Indian Council of Medical Research, Mayor V.R. Ramanathan Road, Chetput, Chennai 600031, India

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Summary
Mannose binding lectin (MBL) plays an important role in innate immunity. Plasma MBL levels and MBL2 gene polymorphisms were studied in HIV-1 infected patients without tuberculosis (HIV+TB−) ($n = 151$) and with tuberculosis (HIV+TB+) ($n = 109$), HIV negative tuberculosis patients (HIV−TB+) ($n = 148$) and healthy controls ($n = 146$) by ELISA and genotyping by polymerase chain reaction based methods. MBL levels were significantly increased among HIV−TB+ and HIV+TB+ patients than controls and HIV+TB− patients ($P < 0.05$). A significantly increased frequency of OO genotype of structural polymorphism and YY genotype of −221Y/X was observed among HIV−TB+ patients than controls. In HIV+TB+ patients, a significantly increased frequency of YA/YA diplotype (associated with very high MBL levels) was observed compared to controls ($P = 0.03$). In HIV+TB+ patients, a significantly decreased frequency of medium MBL expression diplotypes (XA/XA and YA/YO) were noticed compared to HIV+TB− and healthy controls. The results suggest that YA/YA diplotype associated with very high MBL levels may predispose HIV-infected patients to tuberculosis while O/O genotype associated with very low MBL levels may be associated with susceptibility to tuberculosis in HIV uninfected individuals. Medium MBL expression diplotypes might protect against development of TB in HIV-infected patients.

Introduction

Human immunodeficiency virus-1 (HIV-1) infection is the largest health problem being faced by developing nations today. There are an estimated number of 5.206 million people infected with HIV living in India according to National

*Corresponding author. Tel.: +91 44 2836 9761; fax: +91 44 2836 2528.
E-mail address: psraj21@hotmail.com (P. Selvaraj).

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AIDS Control Organization (NACO) report for the year 2005.[1] The incidence of tuberculosis (TB) caused by *Mycobacterium tuberculosis*, is high in HIV-infected patients.[2] The dual epidemic of HIV and TB is a cause for concern in India, where these two infections are prevalent in epidemic proportions. The seroprevalence of HIV among TB patients in various parts of India has been increasing steadily.[3]

Not all people exposed to HIV-1 become infected, and those who do, progress to AIDS defining illness at different intervals. Infected individuals have heterogeneity in the strength of their innate, humoral and cell mediated immune responses. This heterogeneity in the epidemic is at least partially determined by host genetic factors that control viral replication and immunity. Variants in the two major families of host genes, chemokine receptors and human leukocyte antigen (HLA) were shown to influence susceptibility to HIV-1 infection/disease progression.[4] Apart from chemokine receptor and HLA genes, variants of mannose binding lectin (MBL) gene MBL2 due to their role in innate immunity, are also thought to influence disease free survival and susceptibility to HIV-1 infection. MBL is a pattern recognition receptor protein found in plasma, produced by hepatocytes and plays an important role in complement activation, a vital component of innate immunity. MBL can recognize carbohydrate surface structures of several clinically important bacteria, yeast and viruses including HIV-1.[5,6] Interaction between microbes and MBL can initiate complement activation and phagocytosis as well as induction of inflammatory cytokine responses.[7] MBL deficiency is common and predisposes to serious infections.[8] Low serum MBL levels may also confer protection against some intracellular parasites.[9]

Inter-individual variations in the serum MBL levels are mainly due to the presence of three common point mutations in the exon 1 of MBL2 gene at the codons 52, 54 and 57, which encodes for the variant alleles namely D, B, and C, respectively; wild type allele is referred to as A and mutant alleles are collectively termed as O. Variant alleles are known to cause amino acid changes that disrupt the collagenous backbone of the MBL molecule leading to dysfunctional protein. Further, the MBL concentrations are independently influenced by a number of nucleotide substitutions in the promoter region of MBL2 gene. Particularly, a base-pair substitution (G→C) at position −221 leading to the presence of X allele instead of Y allele has a down regulating effect on serum MBL levels.[10] Data from previous studies about the role of MBL variant alleles and genotype on susceptibility to HIV-1 and TB infection and disease are inconsistent.[11–19] Studies on the association of promoter variants of MBL2 gene with HIV and TB are scanty. In the present study, we have investigated the MBL levels as well as association of structural and promoter alleles, genotypes, haplotypes and diplotypes of MBL2 gene with HIV-1 and TB separately as well as with TB in HIV-infected individuals of south India.

Study subjects and methods

Study subjects

The study population consisted of 151 HIV-1 seropositive patients without tuberculosis (HIV+TB−) (64 males, mean age ± standard deviation (SD):32.1 ± 6.9; 87 females, mean age ± SD:28.6 ± 6.7), 109 HIV-1 seropositive patients with tuberculosis(HIV+TB+) (80 males, mean age ± SD:35.1 ± 5.8; 29 females, mean age ± standard deviation (SD):31.6 ± 7.0), 148 HIV-1 seronegative patients with pulmonary tuberculosis (HIV−PTB+) (101 males, mean age ± SD:35.3 ± 10.8; 47 females, mean age ± SD:24.7 ± 6.9) recruited from HIV/TB clinics of Tuberculosis Research Centre, Chennai, India and 146 healthy controls (88 males, mean age ± SD:32.8 ± 8.76; 58 females, mean age ± SD:33.16 ± 8.5). Among the HIV+ TB+ patients, 78 patients had pulmonary TB (HIV+PTB+) and 31 patients had extrapulmonary forms of tuberculosis (HIV+ETB+). The clinical features of HIV+TB+ patients are given in Table 1. The diagnosis of TB was confirmed by clinical, radiographic and/or bacteriological findings (pulmonary TB) or by the presence of acid fast bacilli in the aspirated fluid/tissue (extra pulmonary TB). HIV-1 was diagnosed using two rapid tests (HIV tridot, J. Mitra, India; CombiAids, Span Diagnostics, India), and was confirmed by a third test (Western blot, J. Mitra, India). The present study was approved by the institutional ethical committee and a written informed consent was obtained from all participants of the study. Both patients and controls represented the same ethnic group of south Indian population of Dravidian descent from the state of Tamil Nadu.

MBL plasma levels

Plasma samples obtained from the study subjects were stored at −80 °C. MBL levels were estimated using an MBL oligomer ELISA kit (Antibody Shop, Denmark).

Genotyping of MBL2 gene polymorphisms

Genomic DNA was isolated from peripheral blood mononuclear cells and granulocytes obtained from the blood of

Table 1 Stratification of HIV+TB+ patients based on the clinical features of tuberculosis.

Clinical features of tuberculosis among HIV patients	Number of patients
HIV patients with PTB only	
Smear positive PTB	71
Smear negative PTB	7
HIV patients with extrapulmonary TB	
PTB and pleural effusion	2
PTB and lymph node TB	1
PTB and mediastinal lymph node TB	1
PTB and tuberculoma	1
PTB and pneumothorax	1
PTB and TB abdomen	1
Miliary TB	5
Lymph node TB	10
Pleural effusion	5
Pericardial effusion	2
Mediastinal lymph node TB	1
Pleural effusion and mediastinal lymph node TB	1

patients and healthy controls using salting out procedure.[20] Structural genotypes (52, 54 and 57) of MBL2 gene were identified using polymerase chain reaction (PCR) with sequence specific primers followed by hybridization with biotinylated sequence specific oligonucleotide probes as described earlier.[14] Promoter genotypes (−221 X/Y) were identified using PCR with allele specific primers as described earlier.[21] Sequences of primers and probes are given in Table 2.

Statistical analysis

Genotype and allele frequencies of individual polymorphisms were determined by direct counting. Confirmation to Hardy–Weinberg equilibrium was tested using χ^2 test with one degree of freedom. Haplotypes and diplotypes of MBL2 gene were identified using HAP version 3.[22] Haplotypes with mutant alleles (YB, YC and YD) were combined and collectively represented as YO. The other haplotypes are YA and XA. The diplotypes based on these haplotypes are YA/YA, YA/XA, XA/XA, YA/YO, XA/YO and YO/YO. Frequencies of genotypes, haplotypes and diplotypes of patients and healthy controls were compared by χ^2 test, *P* values with Yates correction and odds ratio (OR) with 95% confidence limits were calculated using Statcalc program (Epi info version 6.0.4, CDC, Atlanta, GA, July 1996). HIV+TB+ patients with different clinical features were grouped as HIV+PTB+ and HIV+ETB+ for further comparisons since the number of patients in each extrapulmonary form of TB (ETB) group was very low. Comparison of MBL plasma levels between different groups of study subjects and between different genotypes/diplotypes of each study group was performed by Mann–Whitney *U* test for two independent samples or by Kruskal–Wallis test for more than two categories using Graphpad prism (version 4). *P* value less than 0.05 was considered significant.

Results

MBL levels in different patient groups and healthy controls

Plasma MBL levels were significantly higher in HIV−PTB+ patients (median 1160 ng/ml, $P<0.05$) than healthy controls (median 695.9). HIV+TB+ patients had significantly higher MBL levels (median 1205 ng/ml, $P<0.05$) compared to HIV+TB− patients (median 825.9 ng/ml). Among HIV+TB+ group, MBL levels did not differ significantly between HIV+PTB+ (median 1065 ng/ml) and HIV+ETB+ (median 1980 ng/ml) patients. However, MBL levels in HIV+PTB+ ($P = 0.04$) and HIV+ETB+ ($P = 0.02$) were significantly higher than HIV+TB− patients. Although serum MBL levels were higher in HIV+TB+ patients than healthy controls, it was not statistically significant (Figure 1).

MBL2 genotypes in healthy controls and patient groups

Allele and genotype frequencies for all polymorphisms studied are given in Tables 3 and 4. All the polymorphisms examined in different study groups were in Hardy–Weinberg equilibrium with the sole exception of codon 54 polymorphism in HIV−PTB+ group, in which reduced frequency of heterozygotes was observed ($P<0.01$). A significantly increased frequency of OO genotype (collective representation

Table 2 Primers and probe sequences used for detecting structural and promoter polymorphisms of MBL2 gene.

Primers/probes specificity	Sequence of the primers/probes	Product size
Primers for exon variants	5′-GCA CCC AGA TTG TAG GAC AGA G-3′	339 bp
	5′-CAG GCA GTT TCC TCT GGA AGG -3′	
Biotinylated probes		
Codon 52 W	5′-AAG ATG GGC GTG ATG G-3′	
52 M	5′-AAG ATG GGT GTG ATG G-3′	
Codon 54 W	5′-CGT GAT GGC ACC AAG GA-3′	
54 M	5′-CGT GAT GAC ACC AAG GA-3′	
Codon 57 W	5′-CAC CAA GGG AGA AAA GGG-3′	
57 M	5′-CAC CAA GGA AGA AAA GGG-3′	
Primers for promoter variants		
−221 Y allele	5′-CTG GAA GAC TAT AAA CAT GCT TTC C-3′	443 bp
X allele	5′-GGA AGA CTA TAA ACA TGC TTT CG-3′	440 bp
Common primer	5′-CCT GCC AGA AAG TAG AGA GG-3′	
Control primers*		
C3	5′-GCA TCT TGC TCT GTG CAG AT-3′	
C5	5′-TGC CAA GTG GAG CAC CCA-3′	796 bp

W = wild allele.
M = mutant allele.
*Control primers amplify a fragment of human growth hormone gene.

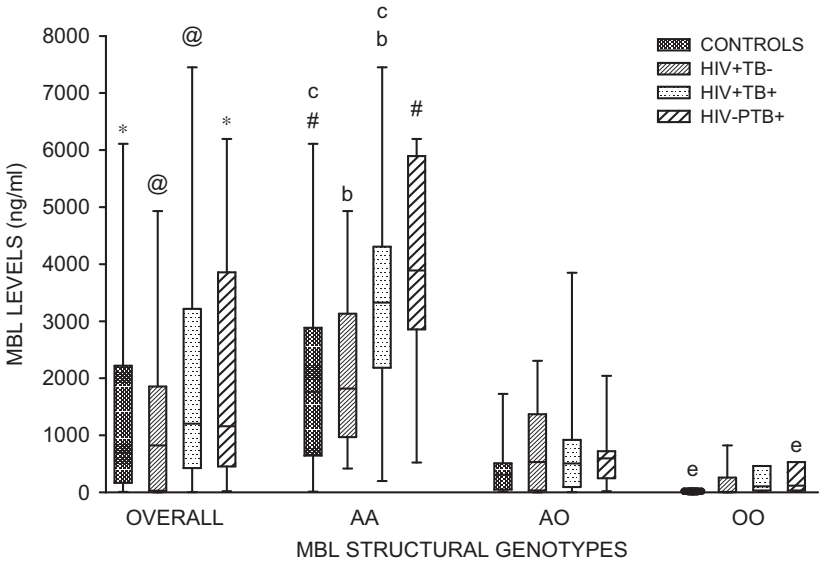

Figure 1 Influence of MBL2 structural genotypes on serum MBL levels in HIV-infected patients with and without tuberculosis (HIV+TB− and HIV+TB+), HIV negative patients with tuberculosis (HIV−PTB+) and healthy controls. Results are represented by Box–Whisker plots. Central horizontal line in the box represents median while the borders of boxes represent 25th and 75th percentiles. Whiskers indicate the minimal and maximal values. Total number of subjects studied, $n = 49$ for controls, 47 for HIV+TB−, 67 for HIV+TB+ and 55 for HIV−PTB+. Number of subjects studied for each genotype, for AA, $n = 29$ for controls, 18 for HIV+TB−, 31 for HIV+TB+ and 25 for HIV−PTB+; for AO $n = 17$ for controls, 19 for HIV+TB−, 31 for HIV+TB+ and 24 for HIV−PTB+; for OO, $n = 3$ for controls, 10 for HIV+TB−, 5 for HIV+TB+ and 6 for HIV−PTB+ MBL levels in overall genotypes: *controls vs HIV−PTB+ $P<0.05$; [@]HIV+TB− vs HIV+TB+ $P<0.05$; MBL levels in AA genotypes: [#]controls vs HIV−PTB+ $P = 0.0002$; [b]HIV+TB− vs HIV+TB+ $P = 0.008$; [c]Controls vs HIV+TB+$P = 0.002$; MBL level in OO genoptypes: [e] controls vs HIV−PTB+ $P<0.05$.

Table 3 Allele frequencies of MBL2 gene polymorphisms in healthy controls, HIV patients with and without tuberculosis and HIV negative patients with tuberculosis.

Alleles	Healthy controls ($n = 146$)	HIV+TB− ($n = 151$)	HIV+TB+ ($n = 109$)	HIV−PTB+ ($n = 148$)*
Structural alleles				
A	0.771	0.735	0.780	0.699
D	0.038	0.053	0.046	0.088
B	0.147	0.179	0.142	0.172
C	0.048	0.031	0.032	0.047
Promoter alleles				
Y	0.702	0.778	0.771	0.793
X	0.298	0.222	0.229	0.203

n = number of individuals studied.
*$n = 147$, one failed PCR for promoter polymorphism.
A = Common allele for structural polymorphisms.
D, B and C = less frequent alleles for 52, 54 and 57 codon polymorphisms, respectively.
Y = common allele of −221 polymorphism.
X = less frequent allele of −221 polymorphism.

of homozygous mutant genotypes and double heterozygotes of structural polymorphisms) ($P = 0.04$) and YY genotype (−221 Y/X polymorphism of promoter region) ($P = 0.016$) was observed among HIV−PTB+ patients than healthy controls. A significantly increased frequency of AA genotype was seen among HIV+ETB+ patients compared to HIV+TB− patients ($P = 0.035$). A trend towards an increased frequency of YY genotype of −221 Y/X polymorphism was found in HIV patients without tuberculosis when compared with healthy controls. In contrast, a trend towards a decreased frequency of XX genotype was observed in HIV patients with TB as compared to healthy controls (Table 4).

Table 4 Percent genotype frequencies of MBL2 gene polymorphisms in healthy controls, HIV patients with and without tuberculosis and HIV negative patients with tuberculosis.

Genotypes	Healthy controls ($n = 146$)	HIV+TB− ($n = 151$)	HIV+TB+ ($n = 109$)	HIV+ PTB+ ($n = 78$)	HIV+ ETB+ ($n = 31$)	HIV−PTB+ ($n = 148$)[§]
Structural genotypes						
AA*	58.9 (86)	55.0 (83)[¶]	62.4 (68)	55.1 (43)	77.4 (24)[¶]	52.0 (77)
AO[†]	36.3 (53)	37.1 (56)	31.2 (34)	37.2 (29)	16.1 (5)	35.8 (53)
A/D	5.8 (8)	7.9 (12)	6.4 (7)	9.0 (7)	0	12.8 (19)
A/B	22.6 (33)	23.2 (35)	19.3 (21)	24.3 (19)	6.4 (2)	17.6 (23)
A/C	8.2 (12)	6.0 (9)	5.5 (6)	3.8 (3)	9.7 (3)	5.4 (8)
OO[‡]	4.8 (7)[‖]	7.9 (12)	6.4 (7)	7.7 (6)	3.2 (1)	12.2 (18)[‖]
D/B	0.68 (1)	2.0 (3)	0.92 (1)	1.2 (1)	0	2.0 (3)
D/DB	0.68 (1)	0	0	0	0	0.68 (1)
D/D	0	0	0.92 (1)	1.2 (1)	0	0.68 (1)
B/DB	0	0.66 (1)	0	0	0	0
B/B	2.7 (4)	4.0 (6)	3.7 (4)	5.1 (4)	0	5.4 (8)
B/BC	0	0.66 (1)	0	0	0	0.68 (1)
B/C	0	0	0.92 (1)	0	3.2 (1)	2.0 (3)
C/C	0.68 (1)	0.66 (1)	0	0	0	0.68 (1)
Promoter (−221) genotypes						
YY	49.3 (72)**	60.3 (91)	56.9 (62)	53.8 (42)	64.5 (20)	64.0 (94)**
YX	41.8 (61)	35.1 (53)	40.4 (44)	43.6 (34)	32.3 (10)	30.6 (45)
XX	8.9 (13)	4.6 (7)	2.7 (3)	2.6 (2)	3.2 (1)	5.4 (8)

n = number of individuals studied.
Genotypes were represented as percent genotype frequencies and numbers within the parenthesis represent individuals positive for a particular genotype.
*AA genotype represents homozygous wild genotypes for structural polymorphisms.
†AO genotype represents heterozygous genotypes of structural polymorphisms.
‡OO genotype represents homozygous mutant genotypes of structural polymorphisms as well as double heterozygous genotypes (D/B, B/C) of structural polymorphisms.
§$n = 147$ for −221 polymorphism.
¶$P = 0.035$, Odds ratio (OR) 2.81; 95% Confidence limit (Cl): 1.08–8.16.
‖$P = 0.04$, OR 2.75; 95% Cl: 1.05–8.03.
**$P = 0.016$, OR 1.82; 95% Cl: 1.11–2.99.

Distribution of MBL2 gene haplotypes and diplotypes among different patient groups and healthy controls

A significantly decreased frequency of XA haplotype was observed among HIV−PTB+ patients than healthy controls ($P = 0.016$). A trend towards a decreased frequency of XA haplotype was seen among HIV patients with TB as compared to healthy controls and further analysis revealed a significantly decreased frequency of XA haplotype in HIV+ETB+ than HIV+TB− patients ($P = 0.008$).

A significantly increased frequency of YA/YA diplotype was observed among HIV+TB+ compared to healthy controls ($P = 0.0108$) and an increased trend was noticed as compared to HIV+TB− patients. Further stratification of HIV+TB+ patients revealed a significantly increased frequency of YA/YA diplotype among HIV+ETB+ patients compared to HIV+TB− ($P = 0.027$) and healthy controls ($P = 0.0004$). A trend towards a decreased frequency of YA/YO diplotype was observed in HIV+TB+ than in HIV+TB− patients. Comparison of HIV patients with different forms of TB with HIV+TB− patients revealed a significantly decreased frequency of YA/YO among HIV+ETB+ patients ($P = 0.049$). A trend towards an increased frequency of YO/YO diplotype was observed among HIV−PTB+ patients than in healthy controls.

MBL levels, MBL2 genotypes and diplotypes in different study groups

When patients and controls were stratified based on structural genotypes (AA, AO, OO) and compared, plasma MBL levels in HIV−PTB+ patients of AA group were significantly higher than AA group of healthy controls ($P = 0.0002$). MBL levels in OO group of HIV−PTB+ patients were also higher compared to OO group of healthy controls ($P = 0.047$). MBL levels of AA group HIV+TB+ patients were significantly higher than in AA group HIV+TB− patients ($P = 0.0087$) and AA group healthy controls ($P = 0.0021$). Plasma MBL levels were significantly higher in AA genotypes

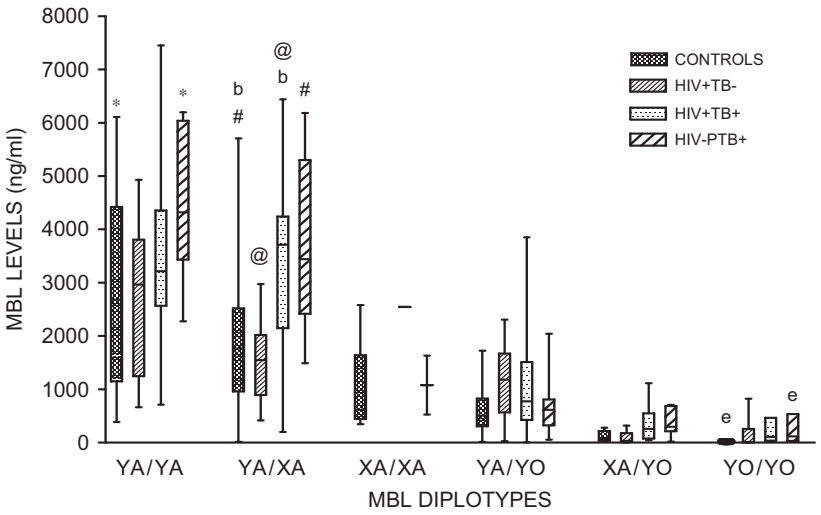

Figure 2 Influence of MBL2 diplotypes on serum MBL levels in HIV-infected patients with and without tuberculosis (HIV+TB− and HIV+TB+), HIV negative patients with tuberculosis (HIV−PTB+) and healthy controls. Results are represented by Box–Whisker plots. Number of subjects studied for each diplotype, for YA/YA, $n = 8$ for controls, 10 for HIV+TB−, 18 for HIV+TB+ and 15 for HIV−PTB+; for YA/XA, $n = 17$ for controls, 8 for HIV+TB−, 12 for HIV+TB+ and 8 for HIV−PTB+; for XA/XA, $n = 4$ for controls, 0 for HIV+TB−, 1 for HIV+TB+ and 2 for HIV−PTB+; for YA/YO, $n = 11$ for controls, 12 for HIV+TB−, 17 for HIV+TB+ and 17 for HIV−PTB+; for XA/YO, $n = 6$ for controls, 7 for HIV+TB−, 14 for HIV+TB+ and 7 for HIV−PTB+; for YO/YO, $n = 3$ for controls, 10 for HIV+TB−, 5 for HIV+TB+ and 6 for HIV−PTB+; MBL level in YA/YA diplotype: * controls vs HIV−PTB+ $P < 0.05$; MBL levels in YA/XA diplotypes: $^{\#}$controls vs HIV−PTB+ $P = 0.018$; $^{@}$HIV+TB− vs HIV+TB+ $P = 0.028$; ^{b}Controls vs HIV+TB+ $P = 0.04$; MBL level in YO/YO diplotypes: ^{e} controls vs HIV−PTB+ $P < 0.05$.

of all study groups followed by AO and OO genotypes ($P < 0.0001$ for all groups) (Figure 1).

When the diplotypes were compared for MBL levels within themselves in each study group, MBL levels in YA/YA diplotype were higher followed by YA/XA, XA/XA, YA/YO, XA/YO and YO/YO ($P < 0.0001$ for HIV−PTB+, HIV+TB− and HIV+TB+ and $P = 0.0002$ for healthy controls). When the MBL levels of each diplotype were compared between study groups, MBL levels were significantly higher in YA/YA diplotype of HIV−PTB+ patients compared to the YA/YA group of healthy controls ($P = 0.036$). Among YA/XA diplotype, higher MBL levels were noticed in HIV−PTB+ ($P = 0.018$) and HIV+TB+ ($P = 0.04$) patients compared to healthy controls. When YA/XA diplotype group of HIV+TB− and HIV+TB+ patients were compared MBL levels were higher among HIV+TB+ patients ($P = 0.028$) (Figure 2)

When the diplotypes were grouped based on MBL levels of the present study as well as other studies[10,23] as high (YA/YA and YA/XA), medium (XA/XA and YA/YO) and low MBL (XA/YO and YO/YO) expression diplotypes and compared, a significantly decreased frequency of medium MBL expression diplotypes was observed among HIV+TB+ patients than HIV+TB− ($P = 0.047$) and healthy controls group ($P = 0.031$). However, the decrease was significantly evident only among HIV+ETB+ compared to HIV+TB− ($P = 0.042$) and healthy controls ($P = 0.033$) (Table 5).

Discussion

In the present study, significantly increased plasma MBL levels were observed in HIV−TB+ and HIV+TB+ patients (HIV+PTB+ and HIV+ETB+) than those without TB. An earlier study has shown increased MBL levels in HIV-infected patients with advanced clinical disease.[24] Our earlier report has shown that MBL levels in active pulmonary TB (PTB) patients are significantly higher than in healthy controls.[25] Hence the increased MBL levels in HIV+TB+ patients may be due to active tuberculosis rather than HIV infection. MBL is known to play a modulatory role on TNF-α responses after stimulation with HIV proteins and mannans.[26] Enhanced proinflammatory cytokine production may act to increase MBL synthesis[27] elevating MBL levels in HIV patients with advanced disease and may be further augmented by tuberculosis. Since the cell wall of *M. tuberculosis* contains lipoarabinomannan,[28] stimulation of MBL is possible in HIV patients with TB infection. If high MBL levels can modulate TNF-α levels in vivo, potentially harmful inflammatory responses may occur since TNF-α responses has been suggested to promote HIV replication.[29]

In the present study, a significantly increased frequency of OO genotype of structural gene polymorphisms and YY genotype of −221 (Y/X) promoter polymorphism was observed among HIV−PTB+ patients suggesting their possible association with susceptibility to pulmonary tuberculosis confirming our earlier finding that functional mutant homozygotes of MBL2 gene were associated with susceptibility to PTB.[14] OO genotype is shown to be associated with low or deficient MBL levels while YY genotype is associated with high MBL levels.[10,23] However, the amount of functional MBL available depends on the combination of promoter and structural alleles present. Since the mutant alleles of structural polymorphisms are in strong linkage with Y allele of −221 promoter polymorphism, the observed increase in YY genotype may be due to the increased frequency of OO

Table 5 Percent frequencies of haplotypes and diplotypes of MBL2 gene polymorphisms in healthy controls, HIV patients with and without tuberculosis and HIV negative patients with tuberculosis.

Haplotypes and diplotypes	Healthy controls ($n = 146$)	HIV+TB− ($n = 151$)	HIV+TB+ ($n = 109$)	HIV+ PTB+ ($n = 78$)	HIV+ ETB+ ($n = 31$)	HIV−PTB+ ($n = 147$)
Haplotypes						
YA	75.3 (110)	78.6 (118)	78.0 (85)	74.4 (58)	83.9 (26)	76.2 (112)
XA	50.6 (74)*,†	39.7 (60)‡	43.1 (47)	46.1 (36)	12.9 (4)†,‡	36.1 (53)*
YO	42 (60)	45.6 (69)§	37.6 (41)	44.9 (35)	22.6 (7)§	47.6 (70)
Diplotypes						
YA/YA	19.2 (28)¶,‖	23.2 (35)**	32.1 (35)¶	24.4 (19)	51.6(16)‖,**	23.1 (34)
YA/XA	30.8 (45)	26.4 (40)	27.6 (30)	28.2 (22)	22.6 (7)	23.8 (35)
XA/XA	8.9 (13)	4.6 (7)	2.8 (3)	2.6 (2)	3.2 (1)	5.4 (8)
YA/YO	25.3 (37)	28.5 (43)□	18.3 (20)	21.8 (17)	9.7 (3)□	29.3 (43)
XA/YO	11 (16)	8.6 (13)	12.8 (14)	15.4 (12)	9.7 (3)	6.8 (10)
YO/YO	4.8 (7)	8.6 (13)	6.4 (7)	7.7 (6)	3.2 (1)	11.6 (17)
Diplotypes based on MBL expression						
High MBL (YA/YA and YA/XA)	50.0 (73)[a]	49.7(75)[b]	59.6 (65)	52.6 (41)	74.2 (23)[a,b]	46.9 (69)
Medium MBL (XA/XA and YA/YO)	34.2(50)[c,e]	33.1(50)[d,f]	21.1(23)[c,d]	24.3 (19)	12.9 (4)[e,f]	34.7 (51)
Low MBL (XA/YO and YO/YO)	15.8 (23)	17.2 (26)	19.3 (21)	23.1 (18)	12.9 (4)	18.4 (27)

n = number of individuals studied. Haplotypes and diplotypes were represented as percent frequencies and numbers within the parenthesis represent individuals positive for a particular haplotype/diplotype.

*$P = 0.016$, OR 0.55; 95% Confidence limit (Cl): 0.33–0.99.
†$P = 0.0002$, OR 0.14 95% Cl: 0.04–0.45.
‡$P = 0.008$, OR 0.22; 95% Cl: 0.05–0.70.
§$P = 0.03$, OR 0.35; 95% Cl: 0.12–0.90.
¶$P = 0.026$, OR 1.99; 95% Cl: 1.08–3.70.
‖$P = 0.0004$, OR 4.42; 95% Cl: 1.79–10.81.
**$P = 0.027$, OR 3.54; 95% Cl: 1.46–8.49.
□$P = 0.049$, OR 0.27; 95% Cl: 0.05–0.95.
[a]$P = 0.023$, OR 2.88; 95% Cl: 1.14–7.89.
[b]$P = 0.021$, OR 2.91; 95% Cl: 1.16–7.98.
[c]$P = 0.031$, OR 0.51; 95% Cl: 0.28–0.94.
[d]$P = 0.047$, OR 0.54; 95% Cl: 0.29–0.99.
[e]$P = 0.033$, OR 0.28; 95% Cl: 0.07–0.89.
[f]$P = 0.042$, OR 0.30; 95% Cl: 0.07–0.93.

genotype. Significantly increased frequency of AA genotype was observed in HIV+ETB+ patients suggesting that AA genotype may be associated with susceptibility to ETB in HIV patients. No significant differences in genotype frequencies were observed between HIV+TB− patient and healthy controls. A study from a Colombian population also failed to detect an association between MBL polymorphisms and HIV-1 infection.[16] However, other studies have shown that homozygous carriers of variant alleles of structural gene polymorphisms are at increased risk of HIV infection and progress rapidly towards AIDS defining illness.[11] In contrary to the above studies, an Amsterdam cohort study on HIV-1 infection has indicated weak protective effect of variant MBL alleles.[13] Differences between the present study results and other studies may be due to ethnicity, environmental and social conditions of the populations involved.

In the present study, a significantly increased frequency of diplotype YA/YA were observed in HIV+ETB+ patients compared to healthy controls and HIV+TB− patients suggesting that at least in HIV patients, diplotype YA/YA may exert a predisposing effect towards the development of ETB. This is further supported by the observation of decreased frequency of medium MBL expression diplotypes among HIV+ETB+ patients suggesting that medium MBL expression diplotypes may protect against development of ETB in HIV-infected individuals. This is a cross-sectional study and further follow-up studies in HIV-infected patients are needed.

Associations between MBL alleles and MBL levels have been reported earlier.[10,23] In the present study also, significantly increased MBL levels were observed among AA genotype and YA/YA diplotype individuals. Very low level of MBL was noticed among individuals with YO/YO diplotype similar to earlier studies. Increased MBL levels observed in individuals with YA/YA diplotype might have been activated by interaction with HIV and mannans of opportunistic pathogens leading to increased TNF-α levels, in addition to those produced due to TB infection. This may enhance HIV replication contributing to immunodeficiency paving way to the dissemination of *M. tuberculosis* resulting in the development of extrapulmonary TB

In the present study, an increased frequency of YO/YO diplotype was observed among HIV−PTB+ patients suggesting that YO/YO diplotype may be associated with susceptibility to PTB in HIV negative individuals. Low level of MBL may lead to direct uptake of *M. tuberculosis* by mannose receptors.[30] Our earlier study has reported a negative correlation between phagocytosis of *M.tuberculosis* and MBL levels in HIV negative individuals.[25] The influence of MBL gene polymorphisms on the susceptibility to ETB in HIV negative individuals could not be assessed in the present study due to the lack of HIV negative ETB samples. Contradicting results observed in HIV-infected patients with different forms of TB and HIV negative PTB patients suggests that MBL may exert opposing effects in the same disease entity under different clinical situations given the involvement of two different pathogens. Genetic susceptibility for TB may be influenced by coinfections.[18] Excessive increases in MBL levels due to TB, which might have a physiological role in antituberculosis immunity, could be unfavorable in HIV infection due to its activation.

We conclude that diplotypes associated with very high MBL levels (YA/YA) might have a predisposing effect in the development of ETB in HIV-infected individuals and diplotypes associated with very low MBL levels (YO/YO or O/O) may be a risk factor for development of PTB in HIV negative individuals. Diplotypes associated with medium MBL levels might have a protective effect against the development of ETB in HIV-infected individuals.

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