Int J Clin Exp Pathol 2015;8(11):15123-15127
www.ijcep.com /ISSN:1936-2625/IJCEP0015206

Original Article

The role of *MBL2* gene polymorphism in sepsis incidence

Lei Liu, Bo Ning

Intensive Care Unit, Air Force General Hospital of PLA, 30 Fucheng Road, Beijing 100142, China

Received August 27, 2015; Accepted September 28, 2015; Epub November 1, 2015; Published November 15, 2015

Abstract: Aim: This case-control study was aimed to explore the role of mannose-binding lectin 2 (*MBL2*) gene rs1800450 polymorphism (codon 54 A/B, G230A) in the development of sepsis in Han Chinese. Methods: *MBL2* rs1800450 polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). MBL serum level was detected by enzyme-linked immunosorbent assay (ELISA). Associations between rs1800450 and sepsis susceptibility was detected by Chi-square test and represented by odds ratios (ORs) and 95% confidence intervals (CIs). Correlation of rs1800450 genotypes and MBL serum level was assessed using t test. Result: Variant A allele frequency was significantly observed in cases than that in controls, indicating a significant association with the susceptibility of sepsis (OR = 1.979, 95% CI = 1.200-3.262). GA genotype also relate to the onset of sepsis (OR = 2.090, 95% CI = 1.163-3.753). MBL serum concentrations were significantly different between case and control groups (P<0.001). Meanwhile, variant allele carriers had lower serum level compared with wild homozygous (P<0.001). Conclusion: Variant A allele in *MBL2* gene rs1800450 polymorphism might increase the risk of sepsis via decrease the MBL serum level.

Keywords: Sepsis, *MBL2*, polymorphism, PCR-RFLP, ELISA

Introduction

Sepsis is defined as the host response to infection including the infection and the systemic manifestations of infection [1]. Sepsis, which will lead to severe sepsis and septic shock, is a kind of systemic inflammatory response syndrome (SIRS). It is a category of infection complication for some diseases, disorder and severe trauma. Symptoms of sepsis mainly include abnormal body temperature, heart rate, breathing rate and the abnormal peripheral blood leukocyte. Sepsis has a high mortality [2-4], and is one of the most prevailing cause of death in intensive care unit (ICU) [5]. Although many therapy methods have made great progress in recent years, the sepsis mortality is still very high [6]. Multiple researches were carried out to detect the pathogenesis of sepsis. It was found that this disease is influenced by various genetic and environmental factors [7-9]. However, the sepsis etiology is still unclear.

As we all know, mannose-binding lectin (MBL) belong to the C-type collectin family, and is an important element in innate immunity [10]. MBL is compounded by liver and stored in plasma. It was verified that MBL may be contribute to activate complement system, facilitate opsono-phagocytosis, mediate inflammation and promote apoptosis [11]. MBL could mediate phagocytosis via directly combined with the oligose group receptors which is in the surface of pathogenic microorganisms; and activate the complement system via MBL-MASP pathway [12].

In human, MBL protein is encoded by *MBL2* gene. Recent researches indicate that *MBL2* gene relate to some diseases which is caused by infection including sepsis [13-17]. Recently, genetic polymorphisms play a potential role in the investigations of disease pathogenesis. These polymorphisms could alter the structure and function of the corresponding protein. *MBL2* exon 1 encode the signal peptide of MBL. Many studies indicated that polymorphisms in the promoter region and exon1 of *MBL2* gene contribute to the MBL serum concentration and MBL activation [18-20].

Table 1. Genotype distributions of rs1800450 polymorphism in cases and controls

SNP	Case n = 107 (%)	Control n = 134 (%)	P	OR (95% CI)
GG	67 (62.62)	105 (78.36)	-	-
GA	36 (33.64)	27 (20.15)	0.013	2.090 (1.163-3.753)
AA	4 (3.74)	2 (1.49)	0.173	3.134 (0.559-17.588)
G	170 (79.44)	237 (88.43)	-	-
A	44 (20.56)	31 (11.57)	0.007	1.979 (1.200-3.262)

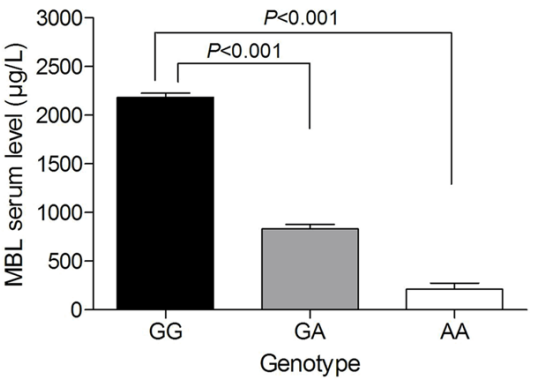

Figure 1. MBL serum level in different genotypes of rs1800450 polymorphism in sepsis patients.

Because the polymorphism distributions are different in ethnicity and region. In this study, we explore the association between *MBL2* rs1800450 polymorphism (codon 54 of exon1, A/B) and sepsis incidence, then analyzed the association of rs1800450 genotypes with serum MBL level in sepsis patients in unrelated Han Chinese.

Materials and methods

Study subjects

This case-control study was approved by the ethic committee of Air Force General Hospital of PLA. All of the participants were unrelated China Han population, understand this study, and signed the informed consent form. Demographic and clinical characteristic were collected via questionnaire and interview.

107 sepsis patients (69 males, 38 females, mean age 52.47 ± 14.69) who were diagnosed by two physicians in Air Force General Hospital of PLA during 2011 to 2015 were recruited as cases. Sepsis diagnosis accorded with previous standards [21]. Inclusive criteria of sepsis patients were as follows: (1) adult patients (≥18 years); (2) had no congenital and acquired immune deficiencies, as well as diabetes mellitus; (3) did not received any immunosuppressive therapy in recent 12 months; (4) had no history of sepsis and malignancy; (5) non-pregnancy.

134 healthy individuals (86 males, 48 females, mean age 49.65 ± 14.98) who received health examination in the healthy check-up center of Air Force General Hospital of PLA during the same time were enrolled as controls. Controls matched with the cases in age, gender, ethnicity and living area.

Collection and treatment of specimens

5 mL peripheral blood was collected from every participant who had a 12 hrs fasting. These samples were anti-coagulated with EDTA, and 2000 r/min centrifuged for 10 min. Plasma and leukocytes were respectively stored in -80°C until to use.

MBL2 genotyping

Genomic DNA was extracted from leukocytes using a TIANamp Blood DNA Kit (Tiangen Biotech, China). *MBL2* rs1800450 polymorphism was genotyped by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP), referring to an early study [22].

Serum MBL measurement

Plasma was utilized to detect the functional MBL via enzyme-linked immunosorbent assay (ELISA). Serum MBL was measured by a MBL human kit (Hycult Biotech, Netherlands) according to the manufacturer's specification.

Statistical analysis

Hardy-Weinberg equilibrium (HWE) was used to examine the representativeness of the cases and controls. Genotype and allele frequencies were calculated by direct count. Associations of *MBL2* rs1800450 polymorphism with sepsis incidence was evaluated by Chi-square test. MBL serum level presented by mean ± SD. Difference of MBL serum level between cases

and controls was assessed by t test. The associations were represented by odds ratios (ORs) with 95% confidence intervals (CIs). All of the calculations were performed by PASW 18.0. Statistical significance was presented when P<0.05.

Results

Association of rs1800450 polymorphism with sepsis

In order to explore the association of *MBL2* gene rs18004550 polymorphism with sepsis incidence, we genotyped this polymorphism and compared them between cases and controls. We found that genotype and allele distributions in cases and controls were in accordance with the HWE test. Variant allele carriers were frequently discovered in case group than that in control group (**Table 1**). A allele might obviously increase the risk of sepsis (OR = 1.979, 95% CI = 1.200-3.262). Meanwhile, GA genotype significantly associated with the onset of sepsis (OR = 2.090, 95% CI = 1.163-3.753). But AA genotype had no significant association with the occurrence of sepsis.

MBL serum level

We detected the MBL serum level both in cases and controls, so as to evaluate the association of *MBL2* gene and sepsis. ELISA results demonstrated that the MBL serum levels in case and control groups were respectively as 1533.44 ± 698.17 and 3017.56 ± 573.90 μg/L, these concentrations had a significantly difference (P<0.001). In case group, the MBL concentrations among rs1800450 genotypes were respectively as GG = 1990.07 ± 396.50, GA = 830.58 ± 266.71, AA = 210.50 ± 125.62 μg/L, the differences had statistically significance (**Figure 1**, P<0.001).

Discussion

As an important member of complement system, MBL play a potential role in innate immunity. MBL could recognize the antigen via bind to oligosaccharide group which displayed on the surface of pathogenic microorganisms, and directly serve as an opsonin [23]. Many studies indicated that the low or the lack of serum MBL content closely relate to many diseases [11, 24, 25]. Immune defensive function of the MBL is bound up with the serum level and oligomeric type of MBL [24]. Peptide chain of MBL is comprised by N-terminal segment, collagen-like region (CLR), helical coiled hinge region, and carbohydrate-recognition domain (CRD). In human plasma, the common type of MBL is hexamer, MBL molecules which less than tetramer cannot bring into play the complete physiological functions [12]. Human MBL protein is encoded by *MBL2* gene.

MBL2 gene is located in 10q11.2, and included 5 exons. Variations in *MBL2* gene will alter the peptide chain structure of it, influencing the formation and stability of oligomeric MBL [26-28]. Five widely studied *MBL2* gene polymorphisms were reasonably related to reduced MBL serum level. These polymorphism include three structure variants, codon 52 (rs5030737C/T, A/D), 54 (rs1800450G/A, termed A/B), 57 (rs1800451G/A, termed A/C) of exon1 (wild allele termed A and the three variant alleles collectively termed O) and two promoter variants -550 (rs11003125G/C, termed H/L), -221 (rs7096206G/C, termed Y/X) [12, 29, 30]. B, C alleles could block the formation of helix structure in CLR; D allele could destroy the disulfide bond in peptide chain; and promoter polymorphisms will affect the MBL concentration in transcription level.

Sepsis, which is caused by infection, significantly relate to the immune system. It is proved by many studies that MBL could control the development of sepsis, and low MBL level maybe increase the risk of sepsis [16, 29, 30]. However, the association of *MBL2* gene polymorphisms with sepsis risk had inconsistent results. Klostergaard et al. [31] found that A/O polymorphism had no significant association with sepsis risk. Huh et al. [32] indicated that A/B variant had no significant association with sepsis, but could decrease the risk of septic shock in Korean. Another study performed by Davis et al. [33] demonstrated that B allele of A/B polymorphism was a risk factor for sepsis in Turks. Therefore, the association of *MBL2* gene polymorphisms with the occurrence of sepsis is still unclear. In additionally, gene polymorphism was different among regions and ethnicity. So, in this study, we explored the association between *MBL2* gene rs1800450 polymorphism and sepsis occurrence, as well as the serum concentration in the different genotypes of rs1800450 in China Han population.

Current study found that GA genotype and A allele frequencies of *MBL2* rs1800450 polymorphism was obviously discovered in sepsis patients. Further ELISA test demonstrated a significantly decreasing trend of MBL serum level in cases compared with controls, moreover, the same trend was observed in rs1800450 genotypes. That was according to early studies [16, 33]. We speculated that rs1800450 A allele increase the sepsis risk via decrease the MBL serum level.

In conclusion, we suggested that rs1800450 might significantly increase the risk of sepsis in Han Chinese. This finding will contribute to the diagnosis and therapy of sepsis. However, this finding was insufficient to certify the pathogenesis of MBL2 gene in sepsis because there were some limitations in our study. Firstly, the sample size was small. Secondly, sepsis is a complex disease which will be affected by various genetic and environmental factors, in this study other polymorphisms in *MBL2* gene and other risk factors were not included. Thirdly, this result was not adjusted by confounding factors. Finally, the sepsis grade and the infections which lead to sepsis were had no detailed classification. So, in order to understand the pathogenesis of sepsis, well designed further cohort studies were imperative.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Bo Ning, Intensive Care Unit, Air Force General Hospital of PLA, 30 Fucheng Road, Beijing 100142, China. E-mail: nibogn142@126.com

References

[1] Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, Sevransky JE, Sprung CL, Douglas IS, Jaeschke R, Osborn TM, Nunnally ME, Townsend SR, Reinhart K, Kleinpell RM, Angus DC, Deutschman CS, Machado FR, Rubenfeld GD, Webb SA, Beale RJ, Vincent JL and Moreno R. Surviving sepsis campaign: international guidelines for management of severe sepsis and septic shock: 2012. Crit Care Med 2013; 41: 580-637.

[2] Tulloch LG, Chan JD, Carlbom DJ, Kelly MJ, Dellit TH and Lynch JB. Epidemiology and Microbiology of Sepsis Syndromes in a University-Affiliated Urban Teaching Hospital and Level-1 Trauma and Burn Center. J Intensive Care Med 2015; [Epub ahead of print].

[3] van der Wekken LC, Alam N, Holleman F, van Exter P, Kramer MH and Nanayakkara PW. Epidemiology of Sepsis and Its Recognition by Emergency Medical Services Personnel in the Netherlands. Prehosp Emerg Care 2015; [Epub ahead of print].

[4] Harrison DA, Welch CA and Eddleston JM. The epidemiology of severe sepsis in England, Wales and Northern Ireland, 1996 to 2004: secondary analysis of a high quality clinical database, the ICNARC Case Mix Programme Database. Crit Care 2006; 10: R42.

[5] Rochwerg B, Alhazzani W, Sindi A, Heels-Ansdell D, Thabane L, Fox-Robichaud A, Mbuagbaw L, Szczeklik W, Alshamsi F, Altayyar S, Ip WC, Li G, Wang M, Wludarczyk A, Zhou Q, Guyatt GH, Cook DJ, Jaeschke R and Annane D. Fluid resuscitation in sepsis: a systematic review and network meta-analysis. Ann Intern Med 2014; 161: 347-355.

[6] Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, Moreno R, Carlet J, Le Gall JR and Payen D. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 2006; 34: 344-353.

[7] Darlow BA, Voss L, Lennon DR and Grimwood K. Early-onset neonatal group B streptococcus sepsis following national risk-based prevention guidelines. Aust N Z J Obstet Gynaecol 2015; [Epub ahead of print].

[8] Qiao L and Liu Z. [Analysis of the risk factors of acute respiratory distress syndrome of Berlin new definition in patients with sepsis in emergency department]. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 2015; 27: 558-562.

[9] Krajcinovic SS, Doronjski A, Barisic N and Stojanovic V. Risk Factors for Neonatal Sepsis and Method for Reduction of Blood Culture Contamination. Malawi Med J 2015; 27: 20-24.

[10] Worthley DL, Bardy PG and Mullighan CG. Mannose-binding lectin: biology and clinical implications. Intern Med J 2005; 35: 548-555.

[11] Turner MW. The role of mannose-binding lectin in health and disease. Mol Immunol 2003; 40: 423-429.

[12] Turner MW and Hamvas RM. Mannose-binding lectin: structure, function, genetics and disease associations. Rev Immunogenet 2000; 2: 305-322.

[13] Chong YP, Park KH, Kim ES, Kim MN, Kim SH, Lee SO, Choi SH, Jeong JY, Woo JH and Kim YS. Association of mannose-binding lectin 2 gene polymorphisms with persistent Staphylococcus aureus bacteremia. PLoS One 2014; 9: e89139.

[14] Zhang H, Zhou G, Zhi L, Yang H, Zhai Y, Dong X, Zhang X, Gao X, Zhu Y and He F. Association between mannose-binding lectin gene polymorphisms and susceptibility to severe acute respiratory syndrome coronavirus infection. J Infect Dis 2005; 192: 1355-1361.

[15] Sheng AJ, Liu LX, Wu H, Wang YX, Peng XX and Wang W. [Correlation of mannose-binding lectin gene promoter polymorphism and plasma MBL concentration with HIV susceptibility in northern Chinese Han population]. Nan Fang Yi Ke Da Xue Xue Bao 2008; 28: 567-571.

[16] Luo J, Xu F, Lu GJ, Lin HC and Feng ZC. Low mannose-binding lectin (MBL) levels and MBL genetic polymorphisms associated with the risk of neonatal sepsis: An updated meta-analysis. Early Hum Dev 2014; 90: 557-564.

[17] Wu L, Deng H, Zheng Y, Mansjo M, Zheng X, Hu Y and Xu B. An association study of NRAMP1, VDR, MBL and their interaction with the susceptibility to tuberculosis in a Chinese population. Int J Infect Dis 2015; 38:129-35.

[18] Garred P, Larsen F, Madsen HO and Koch C. Mannose-binding lectin deficiency--revisited. Mol Immunol 2003; 40: 73-84.

[19] Liu W, Zhang F, Xin ZT, Zhao QM, Wu XM, Zhang PH, de Vlas S, Richardus JH, Habbema JD, Yang H and Cao WC. Sequence variations in the MBL gene and their relationship to pulmonary tuberculosis in the Chinese Han population. Int J Tuberc Lung Dis 2006; 10: 1098-1103.

[20] Zhang DF, Huang XQ, Wang D, Li YY and Yao YG. Genetic variants of complement genes ficolin-2, mannose-binding lectin and complement factor H are associated with leprosy in Han Chinese from Southwest China. Hum Genet 2013; 132: 629-640.

[21] Bone RC, Balk RA, Cerra FB, Dellinger RP, Fein AM, Knaus WA, Schein RM and Sibbald WJ. Definitions for sepsis and organ failure and guidelines for the use of innovative therapies in sepsis. The ACCP/SCCM Consensus Conference Committee. American College of Chest Physicians/Society of Critical Care Medicine. 1992. Chest 2009; 136: e28.

[22] Kang M, Wang HW, Cheng PX, Yin ZD, Li XO, Shi H and Hu XF. Lack of association between mannose-binding lectin gene polymorphisms and juvenile idiopathic arthritis in a Han population from the Hubei province of China. Arthritis Res Ther 2006; 8: R85.

[23] Ogden CA, deCathelineau A, Hoffmann PR, Bratton D, Ghebrehiwet B, Fadok VA and Henson PM. C1q and mannose binding lectin engagement of cell surface calreticulin and CD91 initiates macropinocytosis and uptake of apoptotic cells. J Exp Med 2001; 194: 781-795.

[24] Scorza M, Liguori R, Elce A, Salvatore F and Castaldo G. Biological role of mannose binding lectin: From newborns to centenarians. Clin Chim Acta 2015; 451: 78-81.

[25] Geng P, Ding Y, Qiu L and Lu Y. Serum mannose-binding lectin is a strong biomarker of diabetic retinopathy in chinese patients with diabetes. Diabetes Care 2015; 38: 868-875.

[26] Seyfarth J, Garred P and Madsen HO. The 'involution' of mannose-binding lectin. Hum Mol Genet 2005; 14: 2859-2869.

[27] Garred P, Larsen F, Seyfarth J, Fujita R and Madsen HO. Mannose-binding lectin and its genetic variants. Genes Immun 2006; 7: 85-94.

[28] Gordon AC, Waheed U, Hansen TK, Hitman GA, Garrard CS, Turner MW, Klein NJ, Brett SJ and Hinds CJ. Mannose-binding lectin polymorphisms in severe sepsis: relationship to levels, incidence, and outcome. Shock 2006; 25: 88-93.

[29] Presanis JS, Kojima M and Sim RB. Biochemistry and genetics of mannan-binding lectin (MBL). Biochem Soc Trans 2003; 31: 748-752.

[30] Eisen DP and Minchinton RM. Impact of mannose-binding lectin on susceptibility to infectious diseases. Clin Infect Dis 2003; 37: 1496-1505.

[31] Klostergaard A, Steffensen R, Moller JK, Peterslund N, Juhl-Christensen C and Molle I. Sepsis in acute myeloid leukaemia patients receiving high-dose chemotherapy: no impact of chitotriosidase and mannose-binding lectin polymorphisms. Eur J Haematol 2010; 85: 58-64.

[32] Huh JW, Song K, Yum JS, Hong SB, Lim CM and Koh Y. Association of mannose-binding lectin-2 genotype and serum levels with prognosis of sepsis. Crit Care 2009; 13: R176.

[33] Ozkan H, Koksal N, Cetinkaya M, Kilic S, Celebi S, Oral B and Budak F. Serum mannose-binding lectin (MBL) gene polymorphism and low MBL levels are associated with neonatal sepsis and pneumonia. J Perinatol 2012; 32: 210-217.