Journal of Neuroimmunology 292 (2016) 102–107

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Association of ICAM-1 (K469E) and MCP-1 −2518 A>G gene polymorphism with brain abscess

Priyanka Mishra [a], Kashi Nath Prasad [a,*], Kamini Singh [a], Rabi Narayan Sahu [b], Bal Krishna Ojha [c]

[a] Departments of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow, India
[b] Department of Neurosurgery, Sanjay Gandhi Postgraduate Institute of Medical Sciences Lucknow, India
[c] Department of Neurosurgery, King George Medical University, Lucknow, India

ARTICLE INFO

Article history:
Received 10 November 2015
Received in revised form 23 January 2016
Accepted 31 January 2016

Keywords:
Brain abscess
ICAM-1
MCP-1
Polymorphisms
Serum level

ABSTRACT

Brain abscess develops in response to a parenchymal infection. Intercellular adhesion molecule-1 (ICAM-1) and monocyte chemoattractant protein-1 (MCP-1) play vital role in central nervous system (CNS) diseases. We studied ICAM-1 (K469E) and MCP-1 (−2518 A>G) polymorphisms among brain abscess patients. The genotypic distributions of ICAM-1 (K469E) and MCP-1 (−2518 A>G) were significantly different between patients and controls. Further, patient with predisposing factors, and also with culture result, we found significant association. The study revealed that the polymorphisms of these molecules lead to increased production, which appears to be a risk for the development of brain abscess.

1. Introduction

Brain abscess is as a serious infectious disease of CNS. It develops in response to a parenchymal infection by pyogenic bacteria (Kielian, 2004). Around 75% of brain abscesses originate from the contiguous site of an existing infection such as chronic otitis media, mastoiditis, sinusitis, dental caries or it can also occur directly after penetrating head injury and neurosurgical procedures (Prasad et al., 2006). Development of brain abscess leads to the activation of immune response toward rapid neutralization and elimination of infectious agent (Kielian, 2004). It was previously reported that activated immune response plays an important role in human and murine brain abscess (Bajpai et al., 2014; Kielian, 2004; Mishra et al., 2015). During CNS infection, microglia cell resident within the CNS plays an imperative role in initial cellular defense. They get activated and generate adhesion molecules, chemokines and cytokines (Davies et al., 1998). These cell surface adhesion molecules and chemokines are key mediators in inflammation and immune surveillance processes. Intercellular adhesion molecule-1 (ICAM-1) which is a member of the immunoglobulin superfamily, is constitutively expressed at a low concentration; however, under inflammatory and infectious conditions it is highly inducible in many cell types that further, lead to the activation of chemokine like monocyte chemoattractant protein-1 (MCP-1) (Combarros et al., 2004; Krakauer, 2000). MCP-1 belongs to CC chemokine family, a member of the small inducible gene, and is encoded by the CCL2 gene mapped to chromosome 17q11. After infection its production increases in the CNS by macrophages, endothelial cells, and astrocytes. Elevated levels of ICAM-1 and MCP-1 have also been reported in other inflammatory and infectious diseases such as coronary heart disease, myocardial infarction, neurocysticercosis etc. (Singh et al., 2014; Sugimoto et al., 1997). Its expression can be induced by multiple factors, including inflammatory cytokines, reactive oxygen species and shear stress (Krakauer, 2000; Miyatake et al., 1998; Sugimoto et al., 1997). Previously it has been reported that genetic variation in ICAM-1 and MCP-1 genes may result in altered expression and/or function of the resulting adhesion molecule and chemokine thus potentially contributing to a genetic predisposition to inflammatory and immune mediated events. Two polymorphisms in the ICAM-1 gene have been identified, one at position 241 of the coding region (exon 4) and the other at position 469 (exon 6) (Vora et al., 1994). The K469E polymorphism of the ICAM-1 gene plays functionally an important role in various infectious and inflammatory diseases (Mohamed et al., 2010; Singh et al., 2014). MCP-1 −2518 A>G promoter polymorphism was also associated with various infectious and inflammatory diseases (Azad et al., 2012; Chelbi et al., 2008; Flores-Villanueva et al., 2005; Gonzalez et al., 2002; Karrer et al., 2005). Previous studies from our center reported that the

Abbreviations: CNS, central nervous system; ICAM-1, intercellular adhesion molecule-1; MCP-1, monocyte chemoattractant protein-1; SNP, single nucleotide polymorphism; ELISA, enzyme linked immune-sorbent assay; CHD, cyanotic congenital heart disease; TB, tuberculosis; HIV, human immunodeficiency virus; LFA, leukocyte function associated antigen.

* Corresponding author at: Department of Microbiology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, Uttar Pradesh, India.
E-mail addresses: kashinprasad@gmail.com, knprasad@sgpgi.ac.in (K.N. Prasad).

http://dx.doi.org/10.1016/j.jneuroim.2016.01.021
0165-5728/

expression of TNF-α and IL-1β were higher in brain abscess patients and genetic variation of TNF-α and IL-1β led to the higher production of these cytokines (Bajpai et al., 2014; Mishra et al., 2015). TNF-α and IL-1β gene regulate the expression of ICAM-1 and MCP-1(Krakauer, 2000). Thus, the present study was undertaken to evaluate the level of ICAM-1 and MCP-1 molecules in brain abscess patients and role of ICAM-1 (K469E) and MCP-1 −2518 A/G gene polymorphisms as risk factors for development of brain abscess in North Indian population.

2. Material and method

2.1. Study population

The study was conducted at Sanjay Gandhi Postgraduate Institute of Medical Sciences (SGPGIMS) Lucknow. A total of 100 MRI confirmed brain abscess patients (74 male and 26 female) admitted to the neurosurgery ward of SGPGIMS and King George's Medical University, Lucknow were included. A total of 100 healthy controls (70 male and 30 female) without any history of apparent infectious illness within the last 4 weeks were also included. The study was approved by the institutional ethics committee and written informed consent was obtained from all the study subjects.

2.2. Genomic DNA isolation

Genomic DNA was extracted from ethylene diamine tetra acetic acid (EDTA) anticoagulated peripheral blood by commercially available kit (Invitrogen, Carisbad, USA) according to the manufacturer's instructions and stored at −20 °C. DNA samples of 100 ng/μl concentrations were used for single-nucleotide polymorphism detection.

2.3. Genotyping

ICAM-1 (K469E) (rs5498) polymorphism was detected by PCR-RFLP method, using sense 5′-GGAACCCATTGCCCGAGC-3′ and antisense 5′-GGTGAGGATTGCATTAGGTC-3′ primers with a PCR product of 223 bp (Mohamed et al., 2010). After restriction analysis with BstU I restriction enzyme (Fermentas, New England Biolabs, Beverly, MA) at 37 °C for 8 h, the polymorphism was analyzed on 3% polyacrylamide gel electrophoresis and categorized as indigestible K/K homozygous (223 bp), digestible E/E homozygous (136 and 87 bp) and digestible E/K heterozygous (223, 136 and 87) genotypes (Nonomura et al., 2006). MCP-1 −2518 A>G (rs1024611) polymorphism was also detected by PCR-RFLP method, using sense 5′- CTTTCCCTTGTGTGTCCCC -3′ and antisense 5′- TTAC TCCTTTTCTCCCCAACC -3′ primers respectively with a PCR product of 940 bp. After restriction analysis with PvuII (Fermentas, New England Biolabs, Beverly, MA) at 37 °C for 8 h, three genotypes could be distinguished: G/G (650 and 290 bp), G/A (940, 650 and 290 bp) and A/A (940 bp) (Piotrowski et al., 2010).

2.4. Measurement of ICAM-1 and MCP-1 in serum and brain abscess

Serum samples were separated from blood of patients and healthy controls and stored in aliquots at −80 °C until use. ICAM-1 and MCP-1 levels in sera of patients and healthy controls, and also in brain abscess samples were determined by enzyme linked immune-sorbent assay (ELISA) using commercially available immunoassay kits (R&D System, Canada and USA) and expressed as picograms per milliliter (pg/ml) based on the standard provided with the kits. The assay was performed in triplicates independently for each sample according to the manufacturer's instructions.

2.5. Statistical analysis

The SPSS 15 statistical package (SPSS Inc., Chicago, IL) was used for data management and analysis. The sample size was calculated using Quanto software version 1.0 (http://hydra.usc.edu/gxe) at 80% power and 5% level of significance for OR = 2. Hardy–Weinberg equilibrium (HWE) was checked in controls by goodness of fit χ^2 test. For comparisons between the groups of study populations χ^2 test was used. ELISA data were expressed as mean ± SD of triplicate experiments performed independently for each sample. The independent t test was performed to determine the expression levels of cytokines. Logistic regression analysis was applied to estimate predictors of the brain abscess susceptibility and considered significant if the p values were ≤0.05.

Table 1
Demographic characteristics of study subjects.

	Patients ($n = 100$)	Controls ($n = 100$)
Characteristic		
Age, y (mean ± SD)	24 ± 14.8	27 ± 14.47
Gender		
Male	74(74%)	70(70%)
Female	26(26%)	30(30%)
Culture result		
Culture positive	66 (66%)	
Culture negative	34 (34%)	
Predisposing factors		–
Present	70 (70%)	
Otitis media	27 (48.2%)	
Head trauma	06 (10.7%)	
CHD	05 (8.9%)	
Dental infection	04 (7.1%)	
Sinusitis	04 (7.1%)	
TB	03 (5.3%)	
Meningitis	03 (5.3%)	
HIV infection	01 (1.8%)	
Diabetic ketoacidosis	01 (1.8%)	
Liver transplant	01 (1.8%)	
Glioma	01 (1.8%)	
Absent	30 (30%)	
Symptoms		–
Headache	74 (82.2%)	
Fever	60 (66.6%)	
Vomiting	67 (74.4%)	
Seizure	11 (12.2%)	
Motor weakness	10 (11.1%)	
Blurring of vision	10 (11.1%)	
Unconsciousness	09 (10%)	
Paralysis	05 (5.5%)	
Hemiparesis	06 (6.7%)	

CHD, cyanotic congenital heart disease; TB, tuberculosis; HIV, human immunodeficiency virus.

Table 2
Genotype and allele frequencies of ICAM-1and MCP-1 genes in brain abscess patients and healthy controls.

ICAM-1 (K469E)	Brain abscess patients (n = 100)	Control (n = 100)	p value	OR (95% CI)
Genotype				
K/K	34 (34%)	46 (46%)	–	reference
E/K	42 (42%)	49 (49%)	0.631	1.16 (0.63–2.12)
E/E	24 (24%)	05 (5%)	0.001	6.49 (2.24–18.75)
Allele				
K	110 (55%)	141 (75%)	–	reference
E	90 (45%)	59 (25%)	0.001	1.95(1.29–2.95)
MCP-1 −2518 A>G				
A/A	49 (21%)	43 (43%)	–	reference
A/G	30 (30%)	50 (50%)	0.039	0.52 (0.28–0.96)
G/G	21 (49%)	07 (7%)	0.045	2.63 (1.02–6.79)
Allele				
A	128 (60%)	136 (68%)	–	reference
G	72 (40%)	64 (32%)	0.399	1.19 (0.79–1.80)