Acta Scientific MICROBIOLOGY (ISSN: 2581-3226)

Volume 5 Issue 12 December 2022

Research Article

Expression of Leukotriene A4 Hydroxylase Gene and their Association with Tuberculous Meningitis in the North India Population: A Pilot Study

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Received: November 08, 2022

Published: November 18, 2022

Abstract

Background: Tuberculosis meningitis (TBM) is the lethal and disabling form of tuberculosis, resulting in increased death and disability rates. The expression of the Leukotriene A4 Hydrolase (LTA4H) gene in TBM helps in the inflammatory pathogenesis of the disease.

Aim: This study aimed to investigate the expression of the LTA4H gene and its association with TBM in the North Indian population.

Methods: This Case-control study was conducted in the Department of Respiratory Medicine in collaboration with the Department of Biochemistry at KGMU, Lucknow, which includes 50 TBM cases and 50 control. LTA4H gene expression was analyzed by using quantitative(q) RT-PCR.

Result: No significant difference in age and gender between case and control group showed adequate ($p = 0.61$ and $p = 0.41$). The expression of LTA4H was twofold higher in TBM patients as compared to the control ($p < 0.002$)**.** White blood cells were elevated in TBM patients as compared to control (19.2 ± 15.7 vs. 8.7 ± 4.2, $p < 0.0001$). LTA4H expression was positively correlated with white blood cells ($r = 0.389$, $p = 0.022$) and negatively correlated with aspartate transaminase ($r = -0.300$, $p = 0.038$).

Conclusion: The elevated expression of LTA4H mRNA expression is associated with TBM via controlling the inflammation.

Keywords: LTA4H Gene; q-PCR; TBM.

Introduction

Tuberculosis (TB) is a major public problem worldwide, including in India. Mycobacterium tuberculosis (Mtb) affects any part of the body, but its effects on the brain are devastating. Approximately one hundred thousand new cases of tuberculosis meningitis (TBM) are identified yearly [1-3]. The epidemiological study demonstrated that tuberculosis increases morbidity, mortality and infections recurrences. According to WHO's Global TB report [4,5], 10 million newly diagnosed tuberculosis cases were reported in 2019, and 1.4 million people died from the disease (WHO 2019). The incidence of tuberculosis was highest in Southeast Asia at 44%, followed by Africa at 25% and the Western Pacific at 18% [6,7]. Two-thirds of all TB cases were reported in India, China, Indonesia, the Philippines, Pakistan, Nigeria, Bangladesh, and South Africa in 2017. Although TBM is difficult to diagnose, it is frequently not reported. Neonatal vaccination with Bacillus Calmette-Guerin (BCG) has a 73% chance of avoiding TBM [2,3,9].

During primary tuberculosis, Mtb bacilli move from a damaged lung granuloma, causing bacteremia and hematogenous dissemination to the brain. The pathogen discharge moves into the meninges via cerebrospinal fluid resulting in inflammation of the meninges and forming a thick exudate [5,8]. The present study hypothesized that Mtb patients have a high degree of inflammatory and expression of proinflammatory markers Leukotriene A4 Hydrolase (LTA4H) gene is associated with TBM. Leukotriene A4 Hydrolase gene encodes an enzyme that catalyzes the formation of leukotriene B4 (LTB4) from its unstable precursor LTA4 and modulates the ratio of LTB4 to Lipoxin A4 (LXA4). LTA4H enzyme is responsible for LTB4 production and is a crucial TNF level regulator. LTB4 is a proinflammatory eicosanoid that is perhaps associated with Mycobacterial infection. The human LTA4H gene is a single-copy gene located on chromosome 12q22 with a length larger than 35 kb. The LTA4H gene encodes a protein having 610 amino acid residues and a molecular weight of 69,140 Da [10,11]. In this context purpose of the present study is to identify the mRNA expression of the LTA4H by the highly sensitive technique qRT-PCR.

Material and Methods

This case-control pilot study was conducted in the department of Respiratory Medicine and the experimental work was carried out in the department of biochemistry at KGMU in Lucknow. The study involved 100 participants, including 50 patients with TBM and 50 healthy controls.

Diagnostic criteria for TBM: Guidelines

TBM is diagnosed by confirmed meningitis having symptoms like evidence of meningeal irritation for at least two weeks, fever, headache, irritability, and by MRI and clinical examination of cerebrospinal fluid (CSF). Based on the Criteria by Ahuja., *et al*. Patients were categorized into 4 groups: 1. Definitive TBM, 2. Highly Probable TBM, 3. Probable TBM, 4. Possible TBM and was included in the study. Severe or chronic diseases, other sites of tuberculosis and any other inflammatory disease alignment were excluded from the study.

Patient assessment

Self-questionnaire containing the patient's history with demographic details like age, gender, duration of infection, and Stage of TBM was recorded.

Status of LTA4H gene expression

Real-time PCR (RT-PCR) was utilized to examine the expression of LTA4H mRNA. Blood RNA was extracted using the Trizol technique (Invitrogen, Rockville, MD, USA). RNA was quantified two hours after elution. The extracted RNA's purity was determined by measuring optical density (OD) on A260/280 with a NanoDrop (DS-11-spectrophotometer, Bio-rad, USA).

Complementary DNA (cDNA) synthesis

The GeneSure First Strand cDNA Synthesis Kit (Catalog Number: PGK 162 B) was used to convert total RNA to complementary DNA (cDNA). (Puregene, genetix). 1µg RNA (260/280 = 2.0) was added to a 0.5 µL PCR tube. Add 1 µL of oligo(dt)18 primer and enough nuclease-free water to bring the volume to 12 µL. After mixing, quickly centrifuge and incubate at 65°C for five minutes. Chill on ice, spin down, and then place vials back on ice. Each chill vial contains 4 µL of reaction buffer, 20U of RNAse Inhibitor, 2 µL of 10Mm dNTP mix, and 200U of reverse transcriptase (M-MuLV RT). Then, 1 hour of incubation at 42°C. The process is terminated by heating at 70°C for 5 min.

cDNA (100 ng) was used to amplify the housekeeping genes glyceraldehyde 3-phosphate dehydrogenase GAPDH(forward sequence; CATCACTGCCACCCAGAAGACTG and reverse sequence ATGCCAGTGAGCTTCCCGTTCAG) and LTA4H (forward sequence; ACTCTGGTGTGGTCCGAGAAAG) and (reverse sequence; GGCAGAACCAAGAGGTCGTACT) using qPCR in a 20 µL incubation mixture containing Sybergreen Dye and master mix. The δCt value of the gene was used to estimate the LTA4H fold expression.

Statistical analysis

The student's t-test was evaluated to compare continuous data, while categorical data were analyzed using the χ 2 test to assess the difference in routine parameters in both groups. The correlation was determined by using the Pearson correlation coefficient. All data were analyzed by using SPSS 16.0 version (Chicago, Inc. USA).

Result

General characteristics of TBM patients and control

No statistically significant difference was found in age and gender, indicating good matching with $p = 0.61$ and $p = 0.44$, respectively. Total protein and Hb were considerably decreased, whereas TLC, Total Bilirubin, SGOT, and SGPT were elevated

considerably in TBM patients relative to controls ($p < 0.05$) (Table 1). Table 1 displays the clinical signs of TBM patients, of whom 47 (94%) had fever and headaches and 44 (88%) had vomiting.

Variables	TBM Case (n = 50)	Control (n = 50)	p-value
Age (Years) Mean ± SD	30.9 ± 15.9	32.6 ± 18.6	0.61
Gender: Male Female	 27 (54%) 23 (46%)	 31 (62%) 19 (38%)	0.41
TBM Stage I II III	 17 (34%) 22 (44%) 11 (22%)	-	
Routine Parameters	**Mean ± SD**	**Mean ± SD**	**p-value**
Hb (g/dL) Non -Anemic Anaemic	11.8 ± 1.68 27(54%) 23(46%)	13.2 ± 2.9	0.003*
WBC (/mm^3)	19.2 ± 15.7	8.7 ± 4.2	<0.0001*
Total Bilirubin (mg/dL)	0.74 ± 0.48	0.42 ± 0.23	<0.0001*
AST (U/L)	130.1 ± 86.1	34.4 ± 19.8	<0.0001*
ALT (U/L)	98.6 ± 84.3	32.8 ± 20.1	<0.0001*
Total protein (g%)	6.5 ± 0.8	7.1 ± 0.3	<0.0001*
Diagnosis Definitive TBM Highly probable TBM Probable TBM	 12 (24%) 30 (60%) 8 (16%)	-	

Table 1: Demographical characteristics of Study Population.

P-value was calculated by comparing the TBM case and control.

*p-value < 0.05 considered as statistically significant.

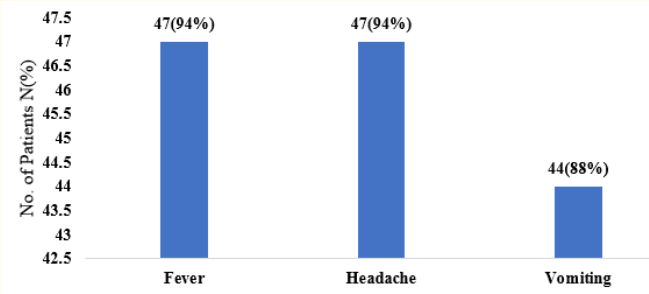

Figure 1: Details of Symptoms of TBM patients.

LTA4H gene expression in TBM and control

As shown in Figure 2, the expression of LTA4H was significantly 2-fold higher in TBM patients (relative fold;1.24 ± 0.85) than in controls (relative fold;0.62 ± 0.41) ($p = 0.002$).

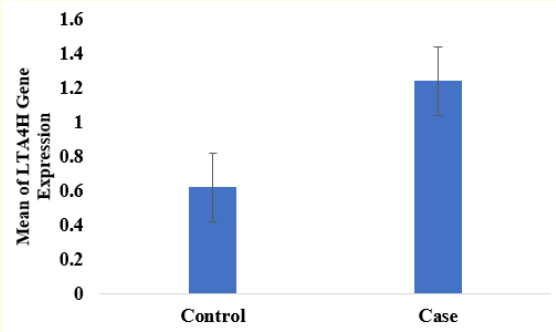

Figure 2: Bar Graph shows the expression of LTA4H in TBM patients compared to controls.

Correlation of LTA4H gene expression with biomarkers:

LTA4H expression was positively correlated with WBC count ($r = 0.389$, $p = 0.022$) and negatively correlated with AST levels ($r = -0.300$, $p = 0.038$) (Table 2).

LTA4H	WBC (mm^3)	Protein (g/dL)	ALT (U/L)	AST (U/L)
r-value	0.389	-0.262	-0.196	-0.300
p-value	0.022*	0.41	0.176	0.038*

Table 2: Correlation between Biomarkers with LTA4H gene expression.

r- Pearson Correlation. *p-value <0.05 considered as statistically significant.

Discussion

In the present study, a caseload with tuberculous meningitis was investigated for the gene expression of LTA4H with a set of biochemical and clinical parameters. The TBM patients were middle-aged and predominantly male with common symptoms of fever, headache, and vomiting. Previous studies have demonstrated that the mean patient's age was TBM and males were more prone than females [13]. Various studies have demonstrated that hematological and biochemical abnormalities are common

in tuberculosis and anemia is one of the common manifestations [14,15]. Anemia occurred in 46% of TBM patients. In addition, patients with tuberculosis have significant lymphopenia associated with anemia, neutrophilia, and monocytosis [16]. Parallel to overhead lines, the increase of white blood cells and decrease in the total protein in TBM patients was 2.2 fold more that than in normal controls, indicating a chronic infection [16] (Morris., *et al*.), usually a prolonged infection with many days to weeks and induces inflammatory cascading [9]. This study revealed that the expression of the LTA4H gene was considerably 2 fold higher in tuberculous meningitis. Since LTA4H is a proinflammatory biomarker and induces TNF-α during inflammation. In the infection of TBM, LTA4H was elevated. Nair., *et al*. [17] identified the link between LTA4H genes and LTB4 overproduction in myocardial infarction [18]. An *in vitro* study by David., *et al*. [19] demonstrated a link between LTA4H and LTB4DH RNA levels; elevated LTA4H transcript levels expressed more LTB4DH ($r^2 = 0.2$, $p = 0.0003$).

However, some limitations of these findings should be considered. First, the number of participants in this study was relatively small, and dietary conditions, environmental and occupational exposure, and the effects were not recorded. These findings are probably influenced by other causative and genetic factors. Finally, as this study was conducted at a single centre and the study cohort was confined to one ethnic group, the data may not represent the whole population. However, in future investigations, more inflammatory biomarkers at the gene and protein levels must be employed with impenetrable factors and anti-inflammatory drugs are strongly recommended.

Conclusion

TBM is associated with inflammation and with increasing infection load, the levels of proinflammatory markers increased. Our study highlights that the levels of LTA4H inflammatory markers could be employed as predictors of TBM severity in patients with respect to infection and Mtb load. The higher expression of LTA4H in TBM patients suggests that these patients will likely benefit from targeted anti-inflammatory remedies based on the transcription levels of this biomarker.

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