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Prospective investigation from a North Indian tertiary care institution on the role of vascular endothelial growth factor in tuberculous meningitis

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Abstract:

There is still much uncertainty about the role of vascular endothelial-derived growth factor (VEFG) in the development of tuberculous meningitis (TBM). We prospectively assessed the function of VEFG in serum and CSF in TBM. PARTICIPANTS AND APPROACHES: From January 2018 through June 2019, researchers in this prospective study monitored patients at a hospital in northern India. Included in the research were 82 consecutive drug-naive individuals with TBM who were diagnosed using modified Ahuja's criteria. We compared the outcomes with those of 49 healthy controls (n = 49). In both the patients and controls, VEFG was measured in serum and cerebrospinal fluid. After three months after finishing antitubercular treatment, 34 individuals had their blood VEFG levels checked again. Using the human VEFG enzyme-linked immunosorbent test kit, the levels of VEFG were calculated.The average age was 29.9 ± 13.1 years. These are the results. There were 33 males and 49 females (59.8% and 40.2%, respectively) in the research group. Thirteen patients (18%) tested positive for BACTEC MGIT960, while seventy-three patients (89%) tested positive for multiplex TB polymerase chain reaction. The levels of VEFG in the cerebrospinal fluid and serum of patients with TBM were not higher than those of the control group. Decreases in serum VEFG levels during follow-up were not associated with ultimate outcome in TBM. IN SUMMARY, VEFG may not be significantly involved in the development of TBM. Further clarification of VEFG status in TBM may be possible in future investigations with bigger sample sizes.

Keywords: Treatment for tuberculosis, outcome, TB meningitis, VEFG

Introduction

In spite of extensive study over many years, tuberculous meningitis (TBM) continues to have a dismal prognosis, with 25% of patients dying and around one-third of survivors suffering from considerable residual impairment.1 through 3 Infection with tubercular bacilli may elicit either a positive or bad immunological response from the host. The articles published in this open-access journal are distributed in accordance with the Creative Commons Attribution-NonCommercial-ShareAlike 4.0 License. This license permits others to modify, adapt, and create works based on the original work without monetary compensation, provided that proper attribution is made and the new works are licensed under the same conditions.

negative or good, depending on how awful it is. The host immune system's reaction to tubercular bacilli is a major cause of mortality and other problems in tuberculosis (TBM).

The common practice of using steroids in conjunction with antitubercular therapy (ATT) in tuberculosis (TBM) is a prime example of this [4].About 40% of individuals with TBM will have cerebral infarction, making it a leading source of many problems.Among the most

The release of viscous exudates from the brain's base causes intracranial vascular strangling and occlusion, a significant cause of brain infracts. The pathophysiology of cerebral infarctions in TBM has therefore been associated with VEFG, chemokines, and cytokines.5 to 8

A 36-46 kDa homodimeric glycoprotein that controls the permeability of the vascular endothelium is known as vascular endothelial-derived growth factor (VEGF) or vascular permeability factor. It damages the blood-brain barrier (BBB), which causes inflammation and swelling in the brain and leads to the development of TBM. In bacterial meningitis, CNS neoplasms, and brain infarctions, VEFG does contribute to BBB damage.[6] Nevertheless, the function of VEFG in TBM has only been examined in a small number of investigations [9–11]. A limited sample size and the use of computed tomography scans instead of magnetic resonance imaging (MRI) to identify brain infarcts were also problems with these studies. The role of VEFG in the development of TBM remains unclear due to a lack of conclusive evidence from well-designed prospective trials. Potentially better results in TBM might be achieved with future therapy regimens centered on anti-VEFG medicines if VEFG is shown to have a significant pathogenic role in CNS TB. Therefore, the function of VEFG in TBM was assessed prospectively.

Aims and objectives

The aims and objectives of the study were to determine
the role of VEGF in the pathogenesis of TBM.

Patients and Methods

The present prospective study was done at a university standard hospital from January 2018 to June 2019. Diagnosis of TBM was made on the basis of the modified Ahuja's criteria.[1,2] We included 82 consecutive newly diagnosed patients of TBM who had not received ATT before the collection of samples. The results were compared to healthy controls (n = 49) consisting of patients who underwent cerebrospinal fluid (CSF) examination for diagnostic purposes and during surgery under spinal anesthesia. Control subjects did not have any active CNS disease or any other disease which is known to elevate VEGF levels. The study was approved by the Ethics Committee of the institute wide order number NK/4247/MD/2666-67. Patients and controls were included and excluded based on the following criteria.

Inclusion criteria

a. Age more than 14 years
b. Patients being diagnosed as TBM using modified Ahuja's criteria.

Exclusion criteria

1. Pregnancy
2. Presence of organisms other than *Mycobacterium tuberculosis*
3. Patients on antitubercular therapy or other forms of immunosuppressive therapy
4. Presence of disorders (other than TBM) which may
   elevate VEGF levels.

History was obtained and meticulous systemic and neurological examinations were performed on all patients. All the details were noted on a predesigned pro forma. All patients underwent detailed hemogram and biochemical investigations including (but not limited to) hemoglobin; total and differential leukocyte counts; erythrocyte sedimentation rate; platelet counts; blood sugars; renal, liver, and thyroid function tests; serum electrolytes; and serum calcium and phosphorus. The CSF was EXAMINED for cells, protein, glucose, acid-fast bacillus (AFB) smear, adenosine deaminase, multiplex polymerase chain reaction (PCR) for mycobacterial DNA, GeneXpert for MTB, culture and drug susceptibility testing for MTB on Lowenstein–Jensen medium, BACTEC Automated Blood Culture Systems, cultures for bacteria and fungi, and antigen testing for Cryptococcus etc . MRI brain with contrast was done in all patients at baseline. All patients received four drugs ATT (antituberculous treatment) namely rifampicin, isoniazid, pyrazinamide, streptomycin (RHZS) with steroids. Ventriculoperitoneal shunting was done wherever needed. The cases were reassessed by clinical examination every monthly and radiological examination after 3 months of treatment. They also underwent neuroimaging and clinical evaluation on an as-and-when-required basis. Eventual outcome was determined through the Glasgow Outcome Scale[12] and Schwab and England Activities of Daily Living (S and E ADL) Scale.[13] The British Medical Research Council criteria were used to determine the grade of TBM.[14] The authors obtained written informed consent from all the participants or their relatives (if the primary subject was <18 years of age or had obtunded sensorium).

Five milliliters of blood and 7–8 mL of CSF were collected with full aseptic precautions from all patients at baseline for the estimation of VEGF levels. After 3 months of treatment with ATT, blood samples were recollected for VEGF estimation. Two milliliters of CSF was taken from controls during lumbar puncture carried

out for anesthetic purposes during surgery aftertaking consent. Serum was obtained from blood and taken in an autoclaved tube. It was then centrifugedat 3000 revolutions/min for 10 min and then kept at a temperature of −80°C.

The VEGF was estimated by human VEGF DIACLONEenzyme-linked immunosorbent assay kit, a solid-phasekit designed to determine VEGF level.

Statistical analysis

All data were analyzed using the IBM Statistical Package for the Social Sciences (SPSS) for Windows, version 25.0. Armonk, NY: IBM Corp. Demographic data were analyzed using mean, median, and range. To analyze discrete variables, the Chi-square test was utilized, whereas the Mann–Whitney test was used to analyze continuous variables. Statistical significance was definedas $P \leq 0.05$. The Student's *t*-test was used to compare VEGF levels at baseline and 3 months after ATT.

Results

Demographic and clinical profile

The mean age of TBM patients (29.9 ± 13.1 years) was significantly less ($P < 0.01$) than control population (41.8 ± 9.5 years). There were 33 (40.2%) men and 49 (59.8%) women in the TBM group and 22 (44.9%)men and 27 women (55.1%) in the control group. The and clinical data are summarized in Table 1.

Table 1: Clinical and demographic profile of patients with tuberculous meningitis

Parameters	Number of patients (*n*=82), *n* (%)
Age (years), mean±SD	29.9±13.1
Male sex	30 (40.2)
Fever	79 (96.3)
Headache	80 (97.6)
Vomiting	64 (78)
Duration of illness (days), mean±SD	48.1±52.2 (range 7-360)
GCS score	
3 (3.7)	
Altered sensorium	

Radiological and cerebrospinal fluid data Baseline MRI (n = 77) was abnormal in 76 (98.7%)patients. The most common MRI abnormality includedthe presence of exudates seen in 85.7% of patientsfollowed by hydrocephalus in 62.2% of patients.Evidence of brain infarction was seen in 24.4%. CSF wasabnormal in 76/82 (92.7%) of patients. The remaining sixpatients had unmistakable evidence of CNS tuberculosis on neuroimaging. AFB culture (BACTEC MGIT960) waspositive in 15 (18%) patients while multiplex PCR fortubercular bacillus was positive in 73 (89%) patients. The MRI and CSF results are shown in Table 2.

Final outcome among patients with tuberculousmeningitis

The final outcome in TBM was defined either by:

1. Death (n = 24) or alive (n = 58) or
2. Using the Glasgow Outcome Scale score (good – 5,
   Lower motor neuron type of facial weakness
   ADL Scale score (100%–80% – good, 60%–70% – moderate, and <60% – poor). The outcome was taken as poor if there was death or if any scalerevealed poor outcome, moderate if any scale revealed moderate outcome, and good if both scaleshave good outcome.

Overall, death occurred in 24 (29.3%) patients. Forty (48.8%), 12 (14.6%), and 30 (36.6%) patients had good, moderate, and poor outcomes, respectively.

Table 2: Cerebrospinal fluid and radiological data of patients with tuberculous meningitis

Radiologcal data	
Parameter	**Number of patients (n=77), n (%)**
Any abnormality	76 (98.7)
Hydrocephalus	51 (62.2)
Exudates	66 (85.7)
Cerebral infarcts	20 (24.4)
Border-zone encephalitis	5 (6.1)
Meningeal enhancement	60 (73.2)
Tuberculomas	39 (26.7)
Military mottling	5 (6.1)
Cord involvement	16 (20.8)
Optochiasmatic arachnoiditis	3 (3.9)
Cerebral edema	23 (29.9)
CSF data	
Parameters	**Number of patients with TBM (n=82), n (%)**
Highly probable	9 (11)
Extraneural TB on PET	
Brain	40 (70.2)
Lung	44 (77.2)
Consolidation	23
Fibrocavitary lesions	13
Military mottling	13
Pleural involvement	5
Lymphadenopathy	44 (77.2)
Mediastinal lymphadenopathy	30
Cervical lymphadenopathy	26
Axillary	11
Mesenteric	15
Para-aortic	17
Antitubercular therapy-inducedhepatitis	
Hydrocephalus 51 (62.2)	
Ventriculoperitoneal shunt	16 (19.5)
Staging of TBM	
Stage I TBM	20 (24.4)
Stage II TBM	41 (50)
Stage III TBM	21 (25.6)
BACTEC culture positivity	15 (18)
Multiplex TB PCR positivity	73 (89)
Type of TBM	
Definitive	73 (89)

Prostate	4 (7)
Gastrointestinal	6 (10.5)
Liver	1 (1.7)
Pancreas	1 (1.7)

TB=Tuberculosis, PET=Positron emission tomography, SD=Standard deviation, GCS=Glasgow Coma Scale, TBM=Tuberculous meningitis, PCR=Polymerase chain reaction

Determinants of poor outcome

We further analyzed as to if various clinical, biochemical, CSF, or radiological parameters [Table 3] can predict the final outcome in TBM. The following associations were found as follows:

1. Positive association between low Glasgow Coma Scale and death as well as poor final outcome
2. Trends between TBM Stage III and death (P = 0.08) and poor outcome without death on any of the scales compared to lower clinical stage of TBM
3. Trend (P = 0.08) between altered sensorium and poor outcome as defined by either of the two scales
4. A trend toward death and the presence of exudates (P = 0.06) and cerebral edema (P = 0.09)
5. Positive association between poor outcome (on either of the scales) and the presence of cerebral infarcts (P = 0.02)
6. Positive association between the presence of cerebral tuberculomas and good outcome (determined by the presence or absence of mortality) (P = 0.02) and a trend between the presence of cerebral tuberculomas and good outcome as defined by either of the two scales (P = 0.06).

CSF pleocytosis	76 (92.7)
Lymphocytic predominance	42
Polymorphonuclear predominance	34
Low glucose	72 (87.8)
CSF glucose≤30 mg/dL	46 (56.1)
Raised CSF protein (>50 mg/dL)	71 (86.6)
AFB culture	15/82
AFB staining	Nil
CSF high ADA (>10)	54
CSF TB PCR	73

CSF=Cerebrospinal fluid, AFB=Acid-fast bacillus, ADA=Adenosine deaminase, PCR=Polymerase chain reaction, TB=Tuberculosis, TBM=Tuberculous meningitis

VEGF in serum and cerebrospinal fluid samples of tuberculous meningitis and controls

Comparison of serum and cerebrospinal fluid VEGF in cases versus controls

In the present study, we evaluated the role of VEGF in the pathogenesis of TBM. The levels of serum and CSF VEGF were measured in TBM and compared with controls. On analysis, serum and CSF VEGF were greater (though statistically insignificant; P = 0.01) in TBM than controls [Table 4].

Correlation between VEGF and various clinical parameters

We also compared serum and CSF VEGF with the stage of TBM and with the final outcome among patients with TBM. On analysis, there was no correlation of the severity of TBM (as suggested by clinical staging) or final outcome of TBM with either serum or CSF VEGF.

Table 3: Correlation of clinical and radiological parameters with final outcome in tuberculous meningitis

Parameters	"Final outcome as defined by death or alive dead"	*P*	"Final outcome as defined by Schwab and England score of GOS score		*P*
			Good	Poor	
GCS					
15	6	0.01	25	10	0.05
8-14	15		14	17	
<7	3		1	3	
TBM stage					
1 or 2	14	0.06	6	34	0.09
3	10		11	19	
Altered sensorium					

Present	17	0.5	21	19	0.08
Absent	7		21	9	
Radiological parameters					
Cerebral infarct					
Infarct absent		NS	43	14	0.02
Infarct present			6	12	
Exudates					
Present	12	0.06	12	15	0.07
Absent	8		28	11	
Cerebral edema					
Present	9	0.09	-	-	-
Absent	11		-	-	
Cerebral tuberculomas					
Present	6	0.02	25	9	0.06
Absent	14		15	17	

NS=Not significant, TBM=Tuberculous meningitis, GCS=Glasgow Coma Scale

Table 4: Serum and cerebrospinal fluid vascular endothelial-derived growth factor in patients with tuberculous meningitis versus controls

Parameter	**Mean±SD (pg/mL)**	***P***

Correlation between VEGF and various radiological parameters

We determined the role of VEGF in radiological compilations of TBM such as hydrocephalus, exudates, infarcts, tuberculomas, border zone encephalitis, and cerebral edema. Analysis revealed a positive association ($P = 0.01$) between serum VEGF and exudates on MRI. There was no correlation between serum and CSF VEGF with any other radiological parameter.

Follow-up serum VEGF levels

In this present study, we also determined levels of serum VEGF at follow-up. Follow-up VEGF testing was done in 34 patients. At follow-up serum VEGF decreased only in 15 (44.1%) patients. When compared, change in serum levels of VEGF did not have any bearing on the final outcome. These results are shown in Table 5.

to VEGF A, a 45 kDa glycoprotein produced by many different cell types. Primarily released from vascular endothelial cells in response to hypoxia, VEGF potently stimulates angiogenesis. In cerebral ischemia, VEGF can have both pathogenic and protective roles depending on the stage of disease process. While, in early stages, VEGF contributes to brain damage through increase in vascular permeability with consequent cerebral inflammation and edema; in the late stages, it protects against ischemia through the formation of new blood vessels. As disruption of BBB (with increased vascular permeability and influx of inflammatory cells) remains a major factor for cerebral damage in TBM, it is likely that VEGF contributes to the pathology of TBM at least in the early stages of disease process. VEGF also stimulates the genesis of nitric oxide resulting in vasodilatation with increased blood flow and consequent cerebral vasodilatation. Accordingly, it is imperative to study the role of VEGF in TBM where vascular complications are important determinants of death and other disabling sequelae.[9-11]

Matsuyama *et al*.[9] found a significant increase in VEGF (in blood and CSF) in TBM ($n = 28$) than other CNS

Table 5: Follow-up serum vascular endothelial-derived growth factor levels and their influence on the outcome as defined by dead or alive

Parameter	**Final outcome as defined by dead or alive (*n*=38) Alive**		***P***	**Dead**
Decrease in serum VEGF	15	0	0.6	
Increase in serum VEGF	18	1		
Parameter	**"Final outcome as defined by Schwab and England ADLs or GOS (*n*=38)**			***P***
	Good	**Moderate**	**Poor**	
Decrease in serum VEGF	11	3	1	0.4

VEGF=Vascular endothelial-derived growth factor, ADLs=Activities of dailyliving, GOS=Glasgow Outcome Scale

infections (n = 31). Follow-up VEGF levels decreasedin patients who showed clinical improvement (n = 12). They stressed on the importance of VEGF in TBM. In another study conducted on pediatric population,[10] CSF VEGF was significantly higher in TBM (n = 26) than healthy children (n = 20). There was a positive correlation between CSF mononuclear cell count and VEGF levels. Thus, inflammatory cells in CSF secrete VEGF which, in turn, damages BBB. The authors suggested that steroidsmay exert their benefit in TBM by antagonizing the effectsof VEGF. Husain *et al*.[11] reported significantly higher blood and CSF VEGF in ongoing (n = 20) compared to inactive TBM (n = 20).

The demographic, clinical, laboratory, positron emission tomography, and MRI data of the present study were consistent with that reported previously from our center.[1,2,15] Regarding serum and CSF VEGF, our results contrasted with previous studies as we did not find a roleof VEGF in the pathogenesis of TBM. Although blood and CSF VEGF were more in TBM than healthy subjects, the difference did not reach statistical significance. Our results were similar to those of Misra *et al*.[6] who reported insignificantly higher serum VEGF in TBM (n = 40) patients compared to controls. Similar to Misra *et al*.,[6] the present study did not find any correlation between VEGF levels and disease severity or time of presentation.

Among various radiological parameters, we did finda significant correlation between basal exudates and serum VEGF levels but not with CSF VEGF levels. The association between increased serum VEGF and basal exudates can be explained due to VEGF-induced vasodilatation and increased vascular permeability. However, the above inference is putative as there was noassociation between CSF VEGF levels and the presence ofbasal exudates. Similar to the study by Misra *et al*.,[6] wedid not find any effect of correlation between the presenceor absence of cerebral infarction and VEGF levels in TBM.

In our study, we compared change in the value of VEGF after treatment with ATT for 3 months. Unlike a previous study,[9] we did not find any correlation between decreasein levels of VEGF with the final outcome.

Our findings are significant. Our results could not reiterate the previously suggested role played by elevated VEGF in the pathogenesis of TBM. The likely reason could be the unique genetic structure of our population wherein a different set of cytokines rather than VEGF may be more operative in the pathogenesis of TBM. The point in favor of the above hypothesis is the fact that a previous study from Indian subcontinent also did not find a significant role of VEGF in TBM.

Conclusion

In conclusion, our investigation failed to identify a significant involvement of VEFG in the development of TBM. Two of our study's strongest features were the large sample size and the use of magnetic resonance imaging (MRI) of the brain in all patients at both the baseline and follow-up assessments. Our study's strength lies in the fact that 89% of patients were classified as having confirmed TBM. The exact function of VEFG in TBM may be further understood in future trials with bigger samples.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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