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Vascular Endothelial Growth Factor in Tuberculous Meningitis with Stroke

Cho Mangsoson Aimol, AK Nigam**, Kamna Singh***, Niteesh Singh*, Ashish Kumar**

Abstract

***Introduction:** Tuberculosis (TB) remains a global health challenge, with severe forms like central nervous system TB posing significant risks. Tuberculous meningitis (TBM) is particularly dangerous, leading to high mortality and neurological complications. This study investigates the role of Vascular Endothelial Growth Factor (VEGF) in TBM-related strokes, hypothesizing that VEGF-induced blood-brain barrier (BBB) breakdown may be a key factor.*

***Objectives:** To compare serum VEGF levels in patients of TBM with stroke and TBM without stroke, to determine the association of serum VEGF level and occurrence of stroke in TBM and its association with various clinical, biochemical and radiological features of TBM.*

***Methods:** A hospital-based case-control study was conducted among 90 TBM patients, admitted to SN Medical College and Hospital, Agra, from January 2023 to June 2024. Patients were categorised into those with clinically and radiologically confirmed TBM with stroke (cases) and TBM without stroke (controls). Data was analyzed using SPSS software version 25.0, with statistical significance set at $p < 0.05$.*

***Results:** The cerebrospinal fluid analysis showed significantly higher CSF protein and ADA levels in cases whereas CSF glucose was significantly lower in cases than controls. Serum VEGF levels were significantly higher in cases (722.9 ± 228 pg/mL) compared to controls (451.3 ± 453 pg/mL) ($p = 0.001$). Hydrocephalus was more common in cases (64.4%) than controls (33.3%). GCS <10 was considerably higher in cases (33.3%) than in controls (13.3%). The mean duration of illness was longer in cases (28.82 ± 16 days) than controls (23.0 ± 13 days).*

***Conclusion:** Elevated serum VEGF levels, hydrocephalus, poor GCS scores, and prolonged illness are associated with and may predict stroke in TBM patients. Early initiation of antiplatelet therapy could potentially reduce stroke incidence in TBM. Further prospective studies are required to confirm these findings and develop predictive markers.*

***Key words:** Tuberculous meningitis, stroke, VEGF, hydrocephalus, Glasgow Coma Scale score.*

Introduction

According to the Global Tuberculosis Report 2021[1], the most widespread infectious illnesses worldwide and a key contributor to poor health is tuberculosis (TB). According to the report, there were 188 cases of tuberculosis per one lakh people in India in 2020. Globally, one of the leading causes of mortality in 2020 was tuberculosis, accounting for up to 1.3 million fatalities among HIV-negative individuals (up from 1.2 million in 2019). Although TB typically impacts the lungs, it infects other areas of the body and result in extra-pulmonary tuberculosis (EPTB). EPTB comprises 15 - 20 per cent of all TB cases among HIV non-infected individuals and comprises around 40 - 50 per cent of new cases among HIV-infected individuals in India[22].

In impoverished nations, TBM presents a serious risk to public health because of its high fatality rate and lingering neurological effects. In India, the estimated death rate from TBM is 1.5 per 100,000 people. TBM accounts for around 5% of all EPTB cases[3]. The exact percentage of TB complicated by TBM is not exactly known. Different studies give different rates of occurrence according to the local prevalence. In high TB burden areas, large proportions (about 10%) are proposed, whereas in low TB prevalence settings, low proportions (around 1%)[4]. TBM is further complicated by the occurrence of stroke. About 25% of patients with TBM have been documented to have had a stroke in recent studies; however, earlier research has indicated that the incidence may as high as 57%[5,6]. Stroke occurs more often in TBM patients, increasing their mortality rate[7,8]. Many theories have been advocated in an effort to clarify why stroke occurs in TBM, including the role of several chemokines and cytokines, such as vascular endothelial growth factor (VEGF)[9,10]. One powerful modulator of endothelial permeability is VEGF. Acute ischaemic stroke, bacterial meningitis, brain tumours, and cerebral oedema have all been linked to VEGF-induced

**Junior Resident, **Professor, Department of Medicine, ***Associoate Professor, Department of Biochemistry, Sarojini Naidu Medical College, Agra - 282 003, Uttar Pradesh.*

***Corresponding Author:** Dr Cho Mangsoson, Junior Resident, Department of Medicine, Sarojini Naidu Medical College, Agra - 282003, Uttar Pradesh. Tel: 9910572677, E-mail: mangso2009@gmail.com*

disintegration of the BBB[11,12]. Regarding the blood level of VEGF and risk of occurring stroke in TBM patients, there is contradictory evidence.

VEGF is a glycoprotein that regulates permeability of vascular endothelium. VEGF does play a role in damaging BBB in bacterial meningitis, central nervous system (CNS) neoplasms, and brain infarctions[10]. However, only a handful of studies[13,14] have evaluated the role of VEGF in TBM. Without definitive information from well-executed prospective research, the impact of VEGF in the pathogenesis of TBM is yet not clear. Future treatment protocols based on anti-VEGF therapies may help in improving outcomes in TBM. Our goal was to study the role of serum VEGF in TBM cases who also had stroke and compare with cases of TBM who did not have stroke.

Material and Methods

A case-control Study was conducted on 90 patients (45 cases and 45 controls) who were admitted in the Department of Medicine, Sarojini Naidu Medical College Agra, Uttar Pradesh, India from December 2022 to June 2024. This study was approved by the Institutional Ethics Committee of Sarojini Naidu Medical College. All patients fulfilling inclusion criteria were included in this study. Inclusion Criteria were age 14 - 70 years, meningitis due to tuberculosis – definitive, probable and possible Tuberculous Meningitis (TBM) cases. Patients who had meningitis clinically and fulfilled the diagnostic criteria for definite, probable and possible tuberculous meningitis with radiologically proven stroke were taken as cases. Patients aged 14 - 70 years who had meningitis clinically and fulfilled the criteria for definite, probable and possible tuberculous meningitis but without any evidence of stroke served as controls. Exclusion criteria were meningitis other than TB; patients who had chronic systemic illnesses such as diabetes mellitus, hypertension, chronic inflammatory Illnesses, autoimmune disorders, immunodeficiency disorders or any malignant disease; and suspected patients of meningitis who did not fulfill the criteria for definite, probable and possible TBM. Also those who had taken anti-tubercular treatment once or more in the past were excluded. Clinical examination was carried out for all patients. All patients were subjected to a series of tests including CSF analysis, serum VEGF, CT scan and/or MRI brain. Under all aseptic precautions blood and CSF was collected for study patients. For serum VEGF level, 2 mL of venous blood sample of each patient from both cases and control group were centrifuged at 3500 revolution per minutes for five minutes. Serum was aliquoted and stored at minus twenty degree Celsius for batch analysis by ELISA Method in the research laboratory of Biochemistry Department. The two groups (cases and controls) were compared for various parameters including duration of illness, blood haematological and biochemical parameters, clinical features, CSF parameters, serum VEGF levels and neuroimaging abnormalities.

Statistical analysis

Data was entered into Microsoft Excel and analysis was done with SPSS software v 25.0. The result was analysed using descriptive statistics and making comparisons among the two groups. Distributed variables were summarised as in proportion and percentage (%) while discrete variables were summarised as mean and Standard Deviation. Applying the chi-square/fisher-exact test for qualitative variables and the unpaired Student's t-test for quantitative variables, the two groups with normal distribution curves were compared. The results were classified as significant if $p < 0.05$.

Result

In this study, the majority participants were of age between 14 and 30 (55.5%) years in cases and 21 to 30 years in controls (51.1%) with mean age 32.8 ± 15.0 and 29.7 ± 14.2 years, respectively for cases and controls ($p > 0.05$).

Out of 90 studied patients (45 cases and 45 controls), the majority were males (64.4%) in cases whereas in controls majority were females (64.4%). We also compared hematological and biochemical variables between cases and controls and it was found that there were no significant differences in the parameters between the two groups ($p > 0.05$). The LFT variables were comparable between cases and controls with no difference between the two ($p > 0.05$). We compared the cerebrospinal fluid between cases and controls and it was found that CSF-TLC, protein and ADA levels were higher among cases than controls ($p < 0.05$). However, CSF glucose was considerably lower in cases than controls ($p < 0.05$). There was lymphocyte predominance in both cases and controls with no significant differences ($p > 0.05$) (Table I).

The comparison of serum VEGF levels in both groups was done. It was observed that the mean serum VEGF was significantly higher in cases (722.9 ± 228 pg/mL) than in controls (451.3 ± 453 pg/mL) ($p < 0.001$) (Table III). The distribution of patients based on hydrocephalus in both groups was studied and found that hydrocephalus was significantly higher numbers among cases (64.4%) than controls (33.3%) with p value < 0.006 (Table IV). The presence of tuberculomas was comparable between cases (33.3%) and controls (28.9%) ($p > 0.05$) (Table V). Regarding the distribution of GCS score in both groups, it was found that among cases, GCS <10 was in a much higher proportion (33.3%) than controls (13.3%) but it was not significant ($p > 0.05$). Based on duration of their illness both groups were studied. The mean duration of illness among cases was

28.82 ± 16 days which was higher than controls (23.0 ± 13 days) but the difference was insignificant (p >0.05) (Fig. 2). Different locations of infarctions in the stroke groups were also studied and it was found that the majority of the cases had basal ganglia infarct (44.44%) followed by cortical infarct (37.77%), multifocal infarcts (31.11%), thalamic infarct (8.88%), and least had brain stem infarct (4.44%) and cerebellar infarct (4.44%) (Fig. 3).

Table I: Cerebro-Spinal Fluid (CSF) in both groups.

CSF	Cases (n = 45) (Mean ± SD)	Controls (n = 45) (Mean ± SD)	p-value
TLC (per cumm)	134.0 ± 25.8	121.5 ± 22.6	**0.016**
DLC- Polymorphs (%)	9.62 ± 8.21	8.8 ± 6.92	0.609
DLC- Lymphocytes (%)	90.48 ± 8.30	89.53 ± 13.89	0.693
Protein (mg/dL)	414.9 ± 121.5	233.4 ± 116.6	**0.004**
Glucose (mg/dL)	39.3 ± 11.8	44.98 ± 12.3	**0.030**
ADA (IU/L)	26.7 ± 21.0	19.5 ± 14.8	**0.005**

Table II: Clinical features in both groups.

Clinical features		Cases (n = 45)	Controls (n = 45)	p-value
Fever		40 (88.9)	41 (91.1)	1.00
Headache		37 (82.2)	33 (73.3)	0.447
Vomiting		30 (66.7)	30 (66.7)	1.00
Seizure		8 (17.8)	7 (15.6)	1.00
Weight Loss		24 (53.3)	24 (53.3)	1.00
Neck Rigidity/Kernig Sign		39 (86.7)	37 (82.2)	0.772
Altered Sensorium		39 (86.7)	40 (88.9)	1.00
Hemiparesis		35 (77.7)	0 (0.0)	0.001
Cranial Nerve Palsy	6th Cranial Nerve	3 (6.7)	2 (4.4)	0.510
	7th cranial nerve	2 (4.4)	0 (0.0)	
	3rd cranial nerve	1 (2.2)	1 (2.2)	
Ataxia		2 (4.4)	0 (0.0)	0.152
Hemianesthesia		4 (8.8)	0 (0.0)	0.040

Table III: Comparison of serum VEGF levels in both groups.

VEGF	Cases (n = 45)	Controls (n = 45)	p-value
Mean ± SD (pg/mL)	722.9 ± 228	451.3 ± 453	**0.001**

Table IV: Hydrocephalus in both groups.

Hydrocephalus	Cases (n = 45) (%)	Control (n = 45) (%)	p-value
Yes	29 (64.4)	15 (33.3)	**0.006**
No	16 (35.6)	30 (66.7)	

Table V: Tuberculoma in both groups.

Tuberculoma	Cases (n = 45) (%)	Control (n = 45) (%)	p-value
Yes	15 (33.3)	13 (28.9)	**0.820**
No	30 (66.7)	32 (71.1)	

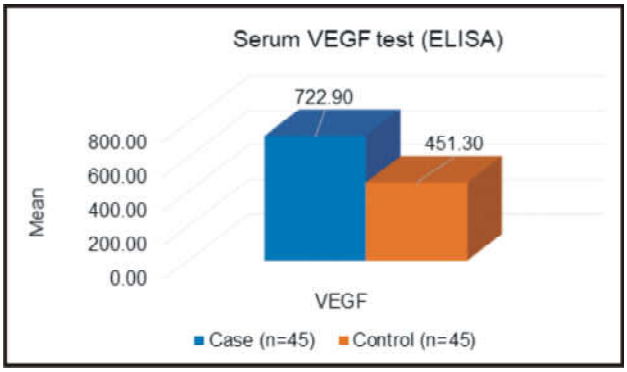

Fig. 1: *Comparison of serum VEGF levels in both studied groups.*

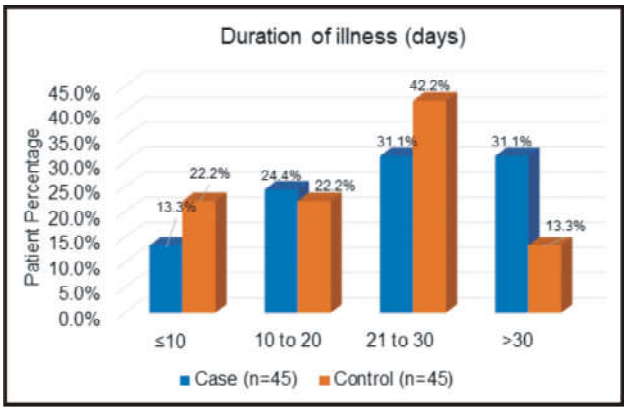

Fig. 2: *Duration of illness (days).*

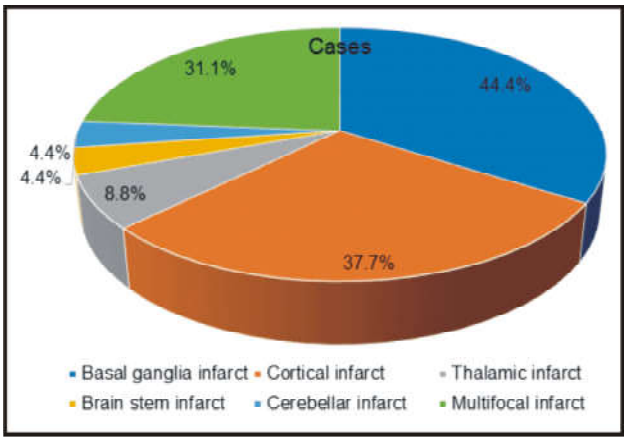

Fig. 3: *Locations of infarction in the stroke group.*

Discussion

Our results were similar to those of Jhamb *et al*[15] who

examined blood VEGF values in TBM patients with and without stroke. We compared 40 patients with suspected or confirmed tuberculous meningitis who have stroke with those who did not have stroke. The 2 groups were comparable regarding age and gender (p value greater than 0.05). Likewise, Tang *et al*[16] reported that the mean age of cases with TBM with stroke was 59.50 ± 18 years, and TBM without stroke was 54.00 ± 13. years with male predominance. Similarly, Kumar *et al*[17] reported that the mean age of TBM patients (29.9 ± 13.1 years) was significantly less (p <0.05) than the population of controls (41.82 ± 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%) among control group.

The current research revealed that the mean VEGF was significantly higher in cases (722.9 ± 228 pg/mL) than in controls (451.3 ± 453 pg/mL) (p <0.05). There were significantly greater numbers of hydrocephalus in the cases group (64.4%) than control group (33.3%). Tuberculoma was comparable between cases (33.3%) and controls (28.9%) (p >0.05). Our findings were supported by Jhamb *et al*[15] who reported that the mean serum levels of VEGF in TBM without stroke was 499.33 ± 230.32 pg/mL, but those with TBM plus stroke, the mean levels were 1218.37 ± 570.11 pg/mL. MRI results revealed that cases of hydrocephalus were notably more common than controls (p = 0.018). Tuberculomas on MRI have shown negligible difference between the patients and controls (p = 0.644). Comparable blood level of VEGF has been documented by Husain *et al*[1,18] whereby they measured the levels of VEGF in serum as well as CSF in twenty cases of intraparenchymal tuberculoma and twenty-two cases of TBM using an enzyme-linked immunoassay (ELISA). The mean blood levels of VEGF were 694.9 ± 820.6 pg/mL for active TBM, 499.62 ± 238.34 pg/mL for inactive TBM, and 541.02 ± 389.06 pg/mL for tuberculoma. In CSF, active TBM cases had significantly higher VEGF levels (106.02 ± 50.04 pg/mL) than inactive TBM (14.72 ± 10.08 pg/mL, p <0.05). VEGF expression was shown to be highly expressed in excised tuberculoma when stained with immuno-histochemistry. An increasing length of therapy for tuberculoma was associated with a serial decline in serum VEGF values. They concluded that elevated CSF and serum VEGF levels reflect disease activity in neuro-tuberculosis, and that a slow decline in these levels over time likely indicates a favourable therapy outcome.

Matsuyama *et al*[19] found a significant increase in VEGF (in blood and CSF) in TBM (n = 28) than other CNS infections (n = 31). Follow-up VEGF levels decreased in patients who showed clinical improvement (n = 12). They stressed the importance of VEGF in TBM. In another study conducted on the paediatric population, CSF VEGF was significantly higher in TBM (n = 26) than healthy children (n = 20). VEGF levels and the number of CSF mononuclear cells correlated positively. Thus, inflammatory cells in CSF secrete VEGF which, in turn, damages BBB. The authors suggested that steroids may exert their benefit in TBM by antagonizing the effects of VEGF.

Jhamb *et al*[15] reported that blood VEGF levels and stroke was associated with a low GCS score in patients suffering from TBM, according to a multivariate logistic regression study comparing TBM patients with and without stroke. Studies comparing the blood VEGF levels of TBM patients who have experienced a stroke to those who have not are extremely rare in the literature. In research done by Van Der flier *et al*[13,20], twenty children with fever whose lumbar puncture ruled-out meningitis were compared to a sample of blood as well as CSF samples of twenty-six children with TBM. There was elevated VEGF in the CSF of fifteen out of twenty-six (58.10%) patients with TBM (98 ± 32 pg/mL), while not a single control patient (all less than 25 pg/mL; p = 0.0393) had any detectable levels. Additionally, in contrast to controls (69 ± 13 pg/mL), the plasma VEGF concentrations in TBM patients (182 ± 52 pg/mL) were considerably greater (p = 0.045). The computed VEGF index was 486 ± 976, indicating intrathecal VEGF production. In their study, periventricular oedema, tuberculoma, and cerebral infarction were all seen on cranial CT scans in 66% of patients, 17%, and 38% patients, respectively. However, there was no correlation between the presence of these pathologic abnormalities with CSF VEGF concentrations[1,24].

Also, among cases lower GCS <10 (33.3%) were much higher in number than that of the controls (13.3%) but in those patients who have higher GCS score between 10 - 14 and GCS score ≥15 the p value was insignificant (p = 0.058) between the two studied groups. Our results aligned with those of Jhamb *et al*[15] that there were no significant variations between the two groups of participants with respect to the presenting signs and symptoms (cranial nerve palsy, fever, headache, vomiting, weight loss, and seizures) or duration of illness at presentation, except hemiparesis. Patients with stroke who had advanced TBM (stage 3 BMRC) were more prone to have low GCS, hemiparesis, and other symptoms at presentation (p = 0.05). This was corroborated by a similar observation made in another study by Kalita *et al*[21], where the incidence of stroke in TBM was associated with the presence of a localised neurological impairment at presentation.

In this study, the average length of illness for the cases was 28.82 ± 16 days which was higher than controls (23.0 ± 13 days) but the difference was insignificant (p >0.05). The majority of cases had basal ganglia infarct (44.4%) followed by cortical infarct (37.7%), multifocal infarct (31.1%), thalamic infarct (8.8%), least had brain stem infarct (4.4%)

and had cerebellar infarct (4.4%). Jhamb *et al*[15] reported that, at the time of presentation, the mean illness duration in cases was considerably longer (33.1 ± 9.34 days) than in controls (24.6 ± 6.92 days) ($p < 0.01$). Anuradha *et al*[22] state that among the locations where an infarct was looked for were the thalamus, internal capsule, cerebellum and brainstem. They also concluded that the regions of the main cerebral arteries namely the posterior cerebral arteries, anterior cerebral arteries, and middle cerebral arteries can also be affected.

The results of this study suggest a rise in VEGF levels during hyper-acute as well as acute phases of haemorrhagic or ischaemic stroke implying a shift of tissue and circulation abnormalities that are common in early stroke. Early subacute phases of ischaemic and haemorrhagic strokes are marked by elevated VEGF levels, which point to continued regeneration, including neo-angiogenesis.

Conclusion

We can conclude that characteristics like high serum VEGF levels, hydrocephalus, hemiparesis, poor GCS at presentation, and prolonged illness duration at presentation are associated with stroke in TBM and could be predictive markers for stroke in TBM. Early TBM diagnosis and treatment with anti-platelet medications (dipyridamole and aspirin) may reduce and even prevent stroke in TBM patients. If any metric or disease marker can be created that can preduct stroke in the initial phase of TBM, more prospective studies are essential to clarify the significance of the aforementioned factors in stroke prediction.

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