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Possible role of transforming growth factor β in tuberculous meningitis

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ABSTRACT

Background: Transforming growth factor β (TGF-β) is an anti-inflammatory cytokine and its role in hydrocephalus and stoke has been suggested. Tuberculous meningitis (TBM) is associated with exudates, stroke, hydrocephalus and tuberculoma, but the role of TGF-β has not been evaluated in relation to these changes.
Aim: To evaluate the cerebrospinal fluid (CSF) TGF-β level in the patients with TBM, and correlate these with clinical findings, MRI changes, paradoxical response and outcome at 6 months.
Methods: TBM patients diagnosed on the basis of clinical, CSF and MRI criteria were prospectively included. The clinical details including duration of illness, seizures, focal motor deficit, Glasgow Coma Scale (GCS) score and stage of TBM were noted. Presence of exudate, hydrocephalus, tuberculoma and infarction in MRI was also noted. MRI was repeated at 3 months and presence of paradoxical response was noted. Cerebrospinal fluid TGF-β was measured using ELISA on admission and repeated at 3 months and these were compared with 20 controls.
Results: TGF-β level was significantly higher in TBM compared to the controls (385.76 ± 249.98 Vs 177.85 ± 29.03 pg/ml, $P < 0.0001$). TGF-β correlated with motor deficit, infarction and tuberculoma on admission but did not correlate with CSF abnormalities, drug induced hepatitis, paradoxical response and outcome. TGF-β level at 3 months was significantly lower than the baseline but remained higher than the controls.
Conclusion: CSF TGF-β levels are elevated in TBM and correlate with infarction and tuberculoma.

1. Introduction

Tuberculous meningitis (TBM) is the commonest subacute/chronic meningitis especially in the developing countries. The worldwide prevalence of tuberculosis is 9.3 million in 2014 [1] and 10% of these patients develop central nervous system (CNS) tuberculosis, which is the most severe form of tuberculosis [2]. Pathologically TBM is associated with exudates, infarction, hydrocephalus and tuberculoma. The reported frequency of infarction in TBM is 20%–60% and that of hydrocephalus 42%–87%. A variable frequency of exudates and tuberculoma has also been reported in TBM [3–9]. Stroke and hydrocephalus in TBM occur at variable time points and are associated with poor outcome [8,10,11].Various pro-inflammatory and anti-inflammatory cytokines are expressed in TBM and may have important role in the pathogenesis [12]. Tumor necrosis factor α (TNFα) is expressed in brain and high level of TNFα has been reported in CSF [13,14]. Thalidomide is a TNFα blocker and was used in a randomized controlled trial in stage II and III TBM. The study was prematurely terminated because of adverse events and deaths in the thaliodomide group. The motor outcome at 6 months of antitubercular therapy was similar between the two groups, although the thalidomide group had more severe neurological deficit on admission [15]. Transforming growth factor β is a 25 kD nonglycosylated homo-dimer and is produced by various cell types. In the central nervous system (CNS), TGF-β is secreted by astrocytes, neurons and microglia. TGF-β has a neuroprotective role due to its immunosuppressive and anti-inflammatory properties and capacity to remodel extracellular matrix. TGF-β is known to protect from ischemia and has been reported to enhance fibrosis there by producing hydrocephalous in subarachnoid hemorrhage [16]. In TBM, TGF-β may also have a role in predicting stroke, hydrocephalus and exudates. In the available literature, there is no study evaluating the role of TGF-β in the patients with TBM. In the present study, we therefore report the cerebrospinal fluid (CSF) TGF-β level in the patients with TBM, and correlate these with clinical findings, MRI changes, paradoxical response and outcome at 6 months.

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2. Subjects and methods

Consecutive patients with TBM during 2012–2014 were included whose initial and 3 months clinical, MRI and CSF data were available for review. The study was approved by the Institute Ethics Committee (IEC code 2015-08-IP-82).

The diagnosis of TBM was based on clinical, CSF and MRI criteria which are as follows:

1. ***Essential criteria***
   a. Meningitis symptoms: Fever, headache and or vomiting for 2 weeks or more in whom septic and fungal meningitis, malaria, scrub typhus and leptospirosis were excluded.
2. ***Supportive criteria***
   a. CT or MRI evidence of exudate, hydrocephalus, tuberculoma or infarction in isolation or in various combinations.
   b. CSF revealing lymphocytic pleocytosis, raised protein and sterile bacterial and fungal culture.
   c. Evidence of extra CNS tuberculosis such as pulmonary, glandular, abdominal, bone or joint tuberculosis.

The patients were considered definite TBM if CSF was positive for acid fast bacilli (AFB) in smear or culture, positive polymerase chain reaction or IgM ELISA for *M. tuberculosis* [6].

2.1. Exclusion

The patients with past history of stroke, structural brain lesion other than tuberculous meningitis, pregnancy, malignancy, hepatic failure or kidney failure were excluded.

2.2. Clinical evaluation

The demographic details, duration of illness and presence of seizure, focal weakness, cranial nerve palsy and altered sensorium were noted. The depth of coma was assessed by Glasgow Coma Scale (GCS). The severity of meningitis was graded as follows:

Stage I- meningitis only.
Stage II- meningitis with focal weakness or GCS score 11–14.
Stage III- meningitis with GCS score <11 [17].

2.3. Investigations

Hemoglobin, blood counts, ESR, blood sugar, serum creatinine, bilirubin, transaminase, sodium, potassium and alkaline phosphate, and HIV serology were done. MRI was carried out using 3T MRI scanner, Signa GE Medical System, USA. T1, T2, FLAIR, DW1 and T1 contrast images were obtained. Presence of exudate, hydrocephalus, infarction and tuberculoma were noted.

Lumbar puncture was done in aseptic condition and CSF was analyzed for cells, protein and sugar. Microbiological study includes smear and culture for AFB, pyogenic bacterial and fungal culture and cryptococcal antigen. CSF PCR and IgM ELISA were also done for *M. tuberculosis*. DNA was isolated from CSF by cetyl-trimethyl-ammonium bromide (CTAB)-phenol chloroform extraction method [18]. Specific pair of primers was used to amplify an insertion sequence IS6110 (~123-bp). The primer sequence of these FP1 and RP2 primers were: 5′-CCT GCG AGC GTA GGC GTC GG3′ and 5′ CTC GTC CAG CGC CGC TTC GG 3′, respectively [19]. Amplification was carried out in PCR Machine (PTC-100 Thermal Cycler, GMI, Inc, USA) which involved 40 cycles of denaturation at 94 °C for 2 min, annealing at 68 °C for 2 min, and primer extension at 72 °C for 1 min. The presence of 123 bp fragment indicated a positive test for *M. tuberculosis*.

2.4. Transforming growth factor β

CSF TGF-β was measured on admission and at 3 months follow up. 2 ml CSF was collected in a cryotube and centrifuged at 3000 rpm for 10 min at 4 °C. The supernatant was stored at −80 °C till analyzed. TGF-β level in CSF was measured using ELISA (Invitrogen Camarillo, CA, USA). Absorbance was read at 450 nm using spectrophotometer (Lab life ER2007). TGF-β concentration of samples was extracted from the standard curve plot and the OD values were multiplied by a factor of 2.2. The value of TGF-β was expressed in pg/ml. CSF TGF-β was also done in 20 age (36.9 ± 15.3 Vs 36.2 ± 13.2 years; P = 0.85) and gender (males 30 Vs 16; P = 0.12) matched controls. CSF of controls was obtained from urology operation theatre from the patients undergoing spinal anesthesia for urology surgery.

2.5. Treatment

The patients were treated with 4 drug antitubercular treatment. Rifampicin 10 mg/kg (maximum 450 mg/day), isoniazid 5 mg/kg (maximum 300 mg/day), pyrazinamide 25 mg/kg (maximum 1500 mg/day) and ethambutol 15 mg/kg (maximum 800 mg/day) were prescribed for 6 months and thereafter 2 drugs (rifampicin and isoniazid) were continued for a total of 18 months. Prednisolone 0.5 mg/kg/day (maximum 40 mg/day) was prescribed for 1 month and tapered in the next one month. Aspirin 150 mg/-day was also administered [20]. The patients were prescribed anticonvulsant (levetiracetam, lorazepam) for seizure, paracetamol and cold sponging for high fever, and ventriculo-peritoneal shunt for hydrocephalus with raised intracranial pressure. General care, fluid, electrolytes and calories were also provided.

2.6. Follow up

The patients were followed up at 3 months and their clinical details were recorded, and cranial MRI was also repeated. Disappearance of existing lesions, worsening of existing lesion and appearance of new lesions were noted. The appearance of new lesion or enlargement of existing lesion was considered as paradoxical response. Outcome at 3 and 6 months was defined as survived or death. The functional outcome of surviving patients was categorized as complete (independent for activities of daily living), partial (partially dependent for activity of daily living) and poor (wheel chair bound or bedridden) [6].

2.7. Statistical analysis

The TGF-β level of the patients and the controls was compared by Mann-Whitney *U* test. The baseline and follow up TGF-β levels of the TBM patients were also compared by paired *t*-test or Wilcoxon rank test. The TGF-β level in the TBM patients was also correlated with baseline clinical parameters, baseline MRI findings and outcome at 3 and 6 months using various parametric and non-parametric tests. The variable having a two tailed P value of <0.05 was considered significant. The statistical analysis was done using SPSS 16 version software and graphs were prepared using GraphPad prism 5.

3. Results

There were 51 patients with TBM whose age ranged between 5 and 70 (median 35) years and 21 were females. The median duration of illness was 45 (range 16–240) days. At the time of admission, 38 patients had altered sensorium and their median GCS score was 11 (3–14). Focal weakness was present in 34