



Oxidative and endoplasmic reticulum stress in tuberculous meningitis related seizures

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ABSTRACT

Background and purpose: High oxygen consumption and high polyunsaturated fatty acid content in the brain may render it vulnerable to oxidative stress and endoplasmic reticulum (ER) stress. We report the role of these parameters in tuberculous meningitis (TBM) patients with seizures and correlate these with clinical radiological, and laboratory findings.

Methods: Serum oxidative stress markers; Catalase, Superoxide dismutase (SOD), Glutathione (GSH), Protein-carbonyl, Malonaldehyde (MDA) were measured using spectrophotometer and ER stress markers-ATF4, CHOP, XBP1 and GRP-78 using RT-PCR in TBM patients, 29 with seizures, 20 without seizures and 20 matched controls. In 10 patients, sequential estimation of oxidative stress and ER stress markers was also measured.

Results: In comparison to controls, TBM patients had significant difference in the expression of oxidative stress and ER stress markers. Serum MDA ($P=0.02$), protein-carbonyl ($P < 0.01$) were significantly higher and SOD ($P=0.02$) and GSH ($P < 0.01$) significantly lower in the patients with seizures compared to those without seizures. The ER stress markers were insignificantly elevated in TBM patients with seizures. On sequential evaluation, oxidative stress and ER stress markers increased following seizures and returned to baseline at the time of discharge.

Conclusion: The results suggest some role of oxidative stress and ER stress in TBM, but do not predict its outcome.

1. Introduction

Tuberculous meningitis (TBM) is an important cause of seizure and epilepsy. (Misra et al., 2018). Seizures in TBM have been reported in 17–63% (Misra et al., 2018; Garcia-Monco 1999; Misra et al., 2000; Sütlaş et al., 2003; Patwari et al., 1996). A number of factors may be responsible for development of seizures in TBM, depending on various pathological changes. Some of these changes occur transiently and resolve with corrective measures, whereas others may persist requiring prolonged anti-epileptic drug treatment. Transient causes of seizures often occur during the early clinical phase and may include meningeal irritation, cerebral edema, hyponatremia, hydrocephalus and raised intracranial pressure. Multiple intracranial tuberculoma stroke, development of abnormal electric focus and other unknown causes occurring during later part of the TBM may contribute to seizures which are difficult to treat. (Misra et al., 2018).

Seizure may result in oxidative stress in the brain parenchyma. A high cerebral metabolic rate of oxygen consumption (CMRO2) and

polyunsaturated fatty acid (PUFA) content renders the brain parenchyma susceptible to lipid peroxidation and free radical injury.

Excessive production of free radicals or reduced antioxidant activity in any CNS pathology may precipitate seizures with high chances of recurrence (Hamed et al., 2004; Shin et al., 2011; Aguiar et al., 2012; Liang and Patel, 2004). On the other hand, seizures can also occur following oxidative stress. Oxidative stress may cause improper handling of misfolded proteins by the endoplasmic reticulum leading to its accumulation, resulting in cellular dysfunction and cell death due to activation of various signal transduction pathways. Impaired protein signaling alters the translocation and transcription of proteins using several pathways such as Inositol pathway, requiring 1- α protein kinase like ER kinase and Activating Transcriptional Factor 6 (ATF-6), Endoplasmic Reticulum stress (ER stress) is reduced by Unfolded Protein Response (UPR) using transcriptional factor XBP.

Various studies have established that both TBM as well as seizure may lead to a state of oxidative stress (Puttachary et al., 2015). It has also been reported that an increase in oxidative stress may induce

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seizures (Liang and Patel., 2004). It is therefore important to evaluate to what extent TBM induced oxidative stress predisposes to seizures.

The seizures in TBM may be associated with a state of increased oxidative stress and ER stress which may be related to the severity of meningitis and affect the outcome. If these changes are found to be significantly affected in TBM, it may provide therapeutic targets. The role of oxidative stress and ER stress in seizures associated with TBM has not been evaluated. In the present study, we report the oxidative stress and ER stress parameters in TBM patients with seizures and correlate these markers with clinical, laboratory and imaging findings.

2. Materials and methods

This prospective study was designed to evaluate the changes in the serum levels of various acute stress-related markers in patients of tubercular meningitis (TBM) admitted during August 2014 to August 2018 at a tertiary care teaching hospital in India. The study was approved by the Institutional Ethics Committee [2013-83-EMP-72(PGI/BE/733/2015)] and the patients or their authorized representatives gave written consent.

2.1. Inclusion criteria

Patients with TBM diagnosed according to the pre-defined criteria were included (Marais et al., 2010).

2.2. Diagnosis of TBM

Clinical diagnosis of TBM was established on the basis of clinical, MRI and CSF criteria. "Definite TBM" was defined in a patient presenting with clinical symptoms suggestive of TBM with a positive AFB smear, *M. tuberculosis* bacilli growth in the CSF culture or *M. tuberculosis* bacilli identified in the CSF by polymerase chain reaction (PCR). 'Highly-probable' TBM was defined as per the clinical scoring proposed by Torok et al after excluding alternative clinical diagnoses. (Marais et al., 2010; Kalita et al., 2014; Török et al., 2011).

2.3. Clinical evaluation

A detailed clinical history and neurological examination was performed in each patient. Glasgow Coma Scale score on admission and presence of focal neurological deficits were recorded, Clinical severity grading was done as per the Medical Research Council (MRC) criteria of TBM severity (Medical Research Council, 1948). Seizures, were categorized according to the 2017 ILAE classification of epilepsy into focal-onset seizure, generalized-onset seizure, unknown onset and focal to bilateral seizure (Fisher et al., 2017).

2.4. Investigations

Baseline laboratory investigations included hemoglobin, erythrocyte sedimentation rate, liver function tests (LFT) and serum electrolytes which were repeated as required to monitor clinical course and anti-tubercular therapy.

Cerebrospinal fluid (CSF) was examined to establish the diagnosis of TBM based on features such as increased cell counts with predominant lymphocytes and abnormal biochemical findings of CSF glucose < 40 mg/dl or less than half of the value of simultaneous blood glucose levels with increase in protein levels. CSF sample was also examined for acid fast bacilli using Ziehl-Neelsen (ZN) stain, bacterial culture and polymerase chain reaction (PCR) for *M. tuberculosis*. Radiological investigations included a chest radiograph, abdominal ultrasonography cranial computerized axial tomography (CT, Siemens, West Germany) or MRI (Signa GE Medical System, Wisconsin, USA).

2.5. Assessment of serum oxidative stress markers

Serum levels of oxidative stress markers- Protein carbonyl, Malonaldehyde (MDA) and Glutathione (GSH) Catalase, Superoxide dismutase (SOD) were measured in all patients. Endoplasmic reticulum stress markers ATF-4, CHOP, XBP-1 and GRP-78 genes were evaluated using RT-PCR. All the measurements were done using standard techniques (supplementary appendix) by one of the authors (AT) who was unaware of the clinical details of the patients. Twenty healthy controls were also included for comparison of oxidative stress and ER stress markers.

2.6. Timing of blood sample collection

The blood samples of 49 patients were collected on admission and were labelled as "baseline". Of them, 10 patients with seizures underwent repeat blood sampling (within 30 min of the seizure onset (labelled as "during seizure"), and at 72 h after the subsidence of seizure (labeled as "post seizure"). In patients with recurrent seizures, the blood samples were collected after 72 h of the last seizure. All the blood samples were stored at -80°C.

2.7. Treatment

A four-drugs regimen isoniazid, rifampicin, pyrazinamide and ethambutol (HRZE) was prescribed for first six months followed by 2 drugs (HR) for the next 12 months in all patients. Standard dosages of ATT were followed - Isoniazid 5 mg/kg, Rifampicin 10 mg/kg, Pyrazinamide 25 mg/kg and Ethambutol 15 mg/kg. In addition to ATT, oral Prednisone 0.5 mg/kg and Aspirin up to 150 mg/d were given daily if there were no contraindications. The patients who developed features of raised intracranial pressure as a result of hydrocephalus underwent external ventricular drainage or ventriculo-peritoneal shunt. Patients who developed seizures were categorized according to the time of onset of seizures since the clinical diagnosis of TBM into: Early onset (< 1 month), Mid onset (1–4 months), and Late onset (> 4 months). [Table 1] These patients were treated with common first-line anti-epileptic drugs such as Phenytoin or Levetiracetam mg 12th.

2.8. Follow-up and outcome

Follow-up neurological evaluation was done on 1, 3 and 6 months following the discharge from the hospital or earlier if required. Compliance to ATT, seizure outcome, and clinical outcome using modified Rankin Scale (mRS) were evaluated and graded into; poor outcome (mRS > 2) or good outcome (mRS ≤ 2).

2.9. Lipid peroxidation

Malonaldehyde is the end product of lipid peroxidation. About 50 µL of standard/ serum sample was added in amber glass vial

Table 1

List of primers for detecting ER stress markers by real-time PCR:

S.NO.	GENE	PRIMERS
1	XBP1	Forward 5'TGCTGAGTCCGAGCAGGTG Reverse 5'GCTGGCAGGCTCTGGGGAAG
2	CHOP	Forward 5'ATGAGGACCTGCAAGAGGTCC Reverse 5'TCCTCCTCAGTCAGCCAAGC
3	ATF4	Forward 5'ATGACCGAAATGAGCTTCTCTG Reverse 5'GCTGGAGAACCCATGAGGT
4	GRP78	Forward 5'GAAAGAAGTTACCCATGCAGT Reverse 5'CAGGCCATAAGCAATAGCAGC
5	GAPDH (Housekeeping gene)	Forward 5'GAGCCACATCGCTCAGACAC Reverse 5'CATGTAGTTGAGGTCATGAAGG

Table 2
Baseline characteristics of patients with *Tuberculous Meningitis*.

Parameters	Number of TBM patients (N = 49)
Median age in years (range)	26 (2 – 75)
Gender distribution	
Male	23(46.9%)
Female	26(53.1%)
Average duration of illness in days (Range)	80.7 (7-364 days)
GCS at admission	
Less than 9	5(10.2%)
9 to 12	9(18.4%)
12 to 15	35(71.4%)
Stage of TBM at admission	
Stage I	14(28.6%)
Stage II	26(53.1%)
Stage III	9(18.4%)
MRI findings – n (%)	
Tuberculoma	16 (32.7%)
Hydrocephalus	24 (49%)
Exudates	33 (67.3%)
Infarcts	25 (51%)

Table 3
Comparison of various demographic and clinico-radiological parameters of the two subsets of TBM patients.

Parameters	TBM patients with seizures (n = 29)	TBM patients without seizures (n = 20)
Median age (years)	22(2-62)	30(13-75)
Gender		
Male	14(48.3%)	9(45%)
Female	15(51.7%)	11(55%)
Average duration of illness (days)	77.3 days	85 days
GCS at admission		
Less than 9	3(10.3%)	2(10%)
9 to 12	4(13.8%)	5(25%)
13 to 15	22(75.9%)	13(65%)
Stage of TBM at admission		
Stage I	6 (20.7%)	8 (40%)
Stage II	18 (62.1%)	8 (40%)
Stage III	5 (17.2%)	4 (20%)
MRI findings –		
Tuberculoma	9 (31%)	7 (35%)
Hydrocephalus	16 (55.2%)	8 (40%)
Exudates	20 (69%)	13 (65%)
Infarcts	18 (62.1%)	7 (35%)
Seizure onset		
Early (< 1 month)	09 (31%)	Not applicable
Mid (1-4 months)	12 (41.4%)	
Late (After 4 months)	8 (27.6%)	

Table 4
Comparison of various stress markers among TBM patients versus control group.

Parameters	TBM (n = 49)	Control (n = 20)	P-Value
Oxidative stress markers			
MDA (nM/ml)	11.5 ± 3.0	2.8 ± 1.2	< 0.01
Protein carbonyl (nM/ml)	5.7 ± 0.76	4.4 ± 0.73	< 0.01
SOD (U/mg of protein)	0.08 ± 0.03	0.15 ± 0.03	< 0.01
Serum catalase (U/mg of protein)	3.7 ± 0.90	4.8 ± 1.1	< 0.01
GSH (µM/ml)	250.0 ± 35.4	581.1 ± 108.5	< 0.01
Relative gene expression of ER stress markers			
ATF-4	0.19 ± 0.28	0.09 ± 0.24	< 0.01
CHOP	0.20 ± 0.31	0.08 ± 0.22	< 0.01
XBP-1	0.31 ± 0.54	0.14 ± 0.16	< 0.01
GRP-78	0.20 ± 0.22	0.08 ± 0.09	0.01

Table 5
Comparative analysis of various stress markers in TBM patients with or without seizures.

Parameters	TBM patients with Seizures (n = 29)	TBM patients without seizures (n = 20)	P-Value
Oxidative stress markers			
MDA (nM/ml)	12.4 ± 3.2	10.2 ± 2.3	0.02
Protein carbonyl (nM/ml)	6.1 ± 0.67	5.2 ± 0.53	< 0.01
SOD (U/mg of protein)	0.07 ± 0.03	0.09 ± 0.02	0.02
Serum catalase (U/mg of protein)	3.5 ± 0.85	4.0 ± 0.83	0.11
GSH (µM/ml)	227.8 ± 24.4	281.5 ± 22.4	< 0.01
Relative gene expression of ER stress markers			
ATF-4	0.22 ± 0.34	0.15 ± 0.16	0.75
CHOP	0.22 ± 0.38	0.16 ± 0.17	0.94
XBP-1	0.36 ± 0.8	0.24 ± 0.19	0.96
GRP-78	0.20 ± 0.23	0.19 ± 0.21	0.73

containing 250 µL of 1.22 M o-phosphoric acid, 450 µL distilled water and 250 µL of TBA reagent. Tetra-methoxy propane was used as standard. The mixture was incubated at 95 °C in a water bath for 60 min. The samples were cooled on ice followed by addition of 360 µL methanol and 40 µL of 1 M NaOH to neutralize the samples. The absorbance was measured at 532 nm. Results obtained were expressed in nmol/mL for serum. (Janero, 1990; Singh et al., 2015).

2.10. Glutathione (GSH)

Concentration of SH group was measured calorimetrically in the serum. To 445 µL of PBS buffer (pH) 7.4, 25 µL of 2 mM of dithionitrobenzoic acid (DTNB) and 50 µL of standard or sample were added. The tube was centrifuged at 15,000 rpm for 10 min and absorbance was measured at 412 nm against blank with DTNB. The results were expressed in µM/ml for serum (Sedlak and Lindsay, 1968).

2.11. Protein carbonyl

Protein carbonyl concentration was determined by modified method of Levine et al. (1990). In serum 10% trichloroacetic acid was added for protein precipitation and allowed to react with 0.5 ml of 10 mM 2,4-dinitrophenylhydrazine for 1 h. The protein was washed thrice with a mixture of ethanol: ethylacetate (1:1), dissolved in 0.6 ml of a solution containing 6 M guanidine-HCl in 20 mM potassium phosphate adjusted to pH 2.3 with trifluoroacetic acid. The solution was centrifuged, and the supernatant was used for the measurement of carbonyl content following the absorbance at 362 nm. Blank was treated the same way except that DNPH was replaced with 2.5 M HCl. The carbonyl content was expressed as nM/ml.

2.12. Catalase activity

Serum catalase activity was determined by the method described by Aebi (1984). Catalase activity was measured spectrophotometrically at 240 nm. The reaction mixture containing 2.9 mL of phosphate buffer pH 7.4 with 30 mM H₂O₂ and 10 µL of serum sample was added in the cuvettes. The reaction was measured for three minutes and the specific activity was expressed as units/mg of protein.

2.13. Superoxide dismutase activity

Superoxide dismutase activity was measured by the method described by Marklund and Marklund (1974). The reaction mixture containing 2 mL of Tris-cacodylate buffer pH 8.5 and 250 µL of the sample was added and the reaction was started by addition of pyrogallol. The

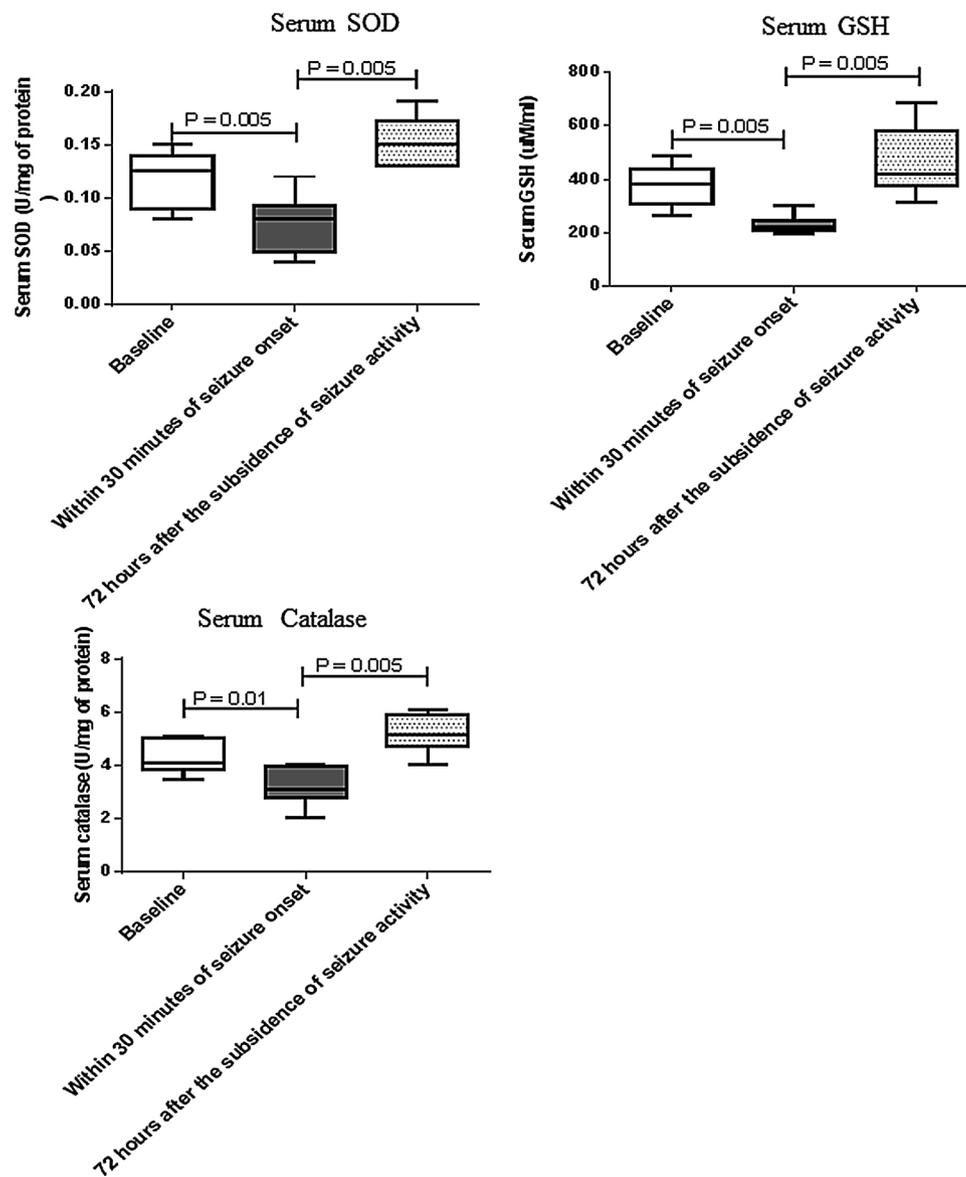


Fig. 1. Box plots showing the levels of SOD, Catalase and GSH in patients with TBM at different time points. The serum levels of these markers were lower at the time of seizures. The panel shows the levels at baseline (white bars), during seizures (grey bars) and 72 h post seizure (dotted bars) respectively. The horizontal line within each box indicates the median, the top and bottom of the box indicate the inter-quartile range, and the I bars indicate the minimum and maximum values.

solutions were mixed well with a plastic paddle. The rate of auto-oxidation was measured as the incremental difference in A420 ($\Delta O/D$) for 3 min on UV spectrophotometer. The specific activity was expressed as units/mg of protein.

2.14. RNA extraction and cDNA synthesis

Total RNA was isolated from EDTA blood using Trizol reagent (Invitrogen, Rockville, MD, USA). The concentration and integrity of the RNA were determined by measuring the absorbance at 260 and 280 nm by Spectro-photometer. Total RNA was reversely transcribed to c-DNA using a cDNA reverse transcription kit (Applied Biosystem, USA) and amplified by RT-PCR and the melting temperature (T_m) of all the primers ATF-4, GRP-78, CHOP, and XBP-1 was standardized by RT-PCR. Real-time PCR was performed using SYBR Green Supermix and Single-Color Real-Time PCR Detection System. The amount of template used in the PCR reactions was cDNA corresponding to 200 ng reverse-transcribed total RNA. DNA polymerase was first activated at 95 °C for 3 min, denatured at 95 °C for 30 s, and annealed/extended at 61 °C for

30 s, for the recommended number of 40 cycles according to the manufacturer's protocol. Expression of the housekeeping gene GAPDH served as an internal positive control in each assay performed.

2.15. Endoplasmic reticulum stress (ER stress)

The ER stress/ unfolded protein response (UPR) genes consist of molecular chaperone BiP/Grp78, indicator of the onset of the UPR, as well as key regulators of the UPR pathway including transcription factors ATF4, and XBP1. Expression of the housekeeping gene GAPDH served as an internal positive control in each assay performed. The Primer sequences for genes are given in Table 1.

2.16. Statistical analysis

Patients' demographic details, clinical and radiological information was recorded at time of admission. Follow-up data regarding outcome was obtained at outpatient visits. All data was entered prospectively into patient proforma and subsequently in SPSS software version 24

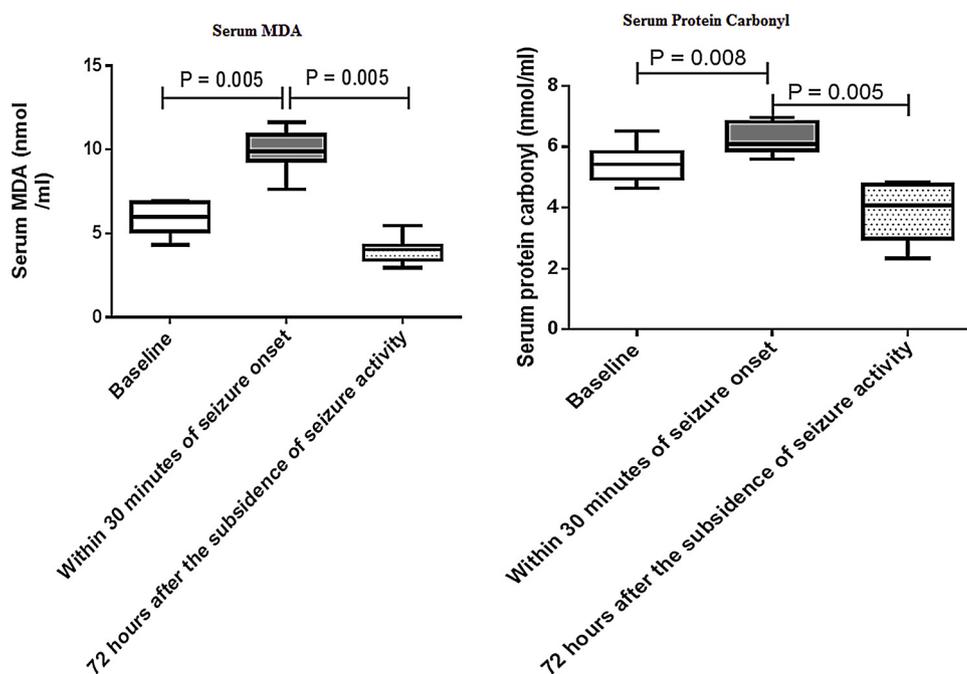


Fig. 2. Box plots showing the levels of MDA and protein carbonyl in patients with TBM at different time points. The serum levels of these markers were higher at the time of seizures. The panel shows levels at baseline (white bars), during seizures (grey bars) and 72 h post seizure (dotted bars). The horizontal line within each box indicates the median, the top and bottom of the box indicate the inter-quartile range, and the I bars indicate the minimum and maximum values.

(IBM Corp, Armonk, NY, USA). The categorical data were presented as median and range and groups were compared using Chi square or Fisher exact test. The continuous variables were recorded as mean and standard deviation and the groups were compared using Student *t*-test. A two tailed *p*-value < 0.05 was considered significant. Statistical analysis was performed using SPSS version 24 (IBM Corp, Armonk, NY, USA).

3. Results

The results are based on 49 patients with TBM [Definite 26 (53%) and, Highly probable 23 (46.9%)]. The median age of TBM patients was 26 (Range 2–75) years with a Female : Male of 1.13:1. The mean duration of illness was 80.7 ± 78.9 days. The stage of TBM at the time of admission was stage I in 14, stage II in 26 and stage III in 9 patients. (Table 2)

Extra CNS tuberculosis was present in 15 patients and included pulmonary ($n = 3$, 6.1%), abdominal ($n = 3$, 6.1%), disseminated ($n = 3$, 6.1%), spinal ($n = 5$, 10.2%) and lymph node involvement ($n = 1$, 2%). Twenty-nine (59.1%) patients developed seizure early onset ($n = 9$, 31%), mid onset ($n = 12$, 41.4%) and late onset ($n = 8$, 27.6%). About 80% of TBM patients who developed seizures presented in stage II or stage III while 40% of TBM patients who never developed seizures presented in stage I TBM. Comparison of TBM patients with and without seizures is presented in Table 3.

3.1. Oxidative stress markers

The serum levels of various oxidative stress markers were measured in all the patients as well as in the controls. As compared to the controls, TBM patients had significantly higher levels of MDA ($P < 0.01$), Protein-carbonyl ($P < 0.01$) and significantly lower levels of SOD ($P < 0.01$) Catalase ($P < 0.01$) and GSH ($P < 0.01$) [Table 4]. < 0.01) and significantly lower levels of SOD ($P < 0.001$) Catalase ($P < 0.001$) and GSH ($P < 0.001$).

Comparison of TBM patients with and without seizures serum levels of MDA ($P < 0.02$), and Protein-carbonyl ($P < 0.01$) significantly higher. The serum levels of SOD ($P < 0.02$) and GSH ($P < 0.01$) were significantly lower in patients with seizures compared to those without seizures. The serum level of catalase was insignificantly lower in

patients with seizures compared to those without seizures, ($P = 0.11$) [Table 5].

3.2. ER stress markers

The expression of ATF-4 gene (0.19 vs 0.09), GRP-78 gene (0.20 vs 0.08), CHOP gene (0.20 vs 0.08), and XBP-1 gene (0.31 vs 0.14) were significantly elevated in TBM patients compared to controls. [Table 4] The subset analysis of TBM patients showed insignificantly higher levels of these markers in TBM patients with seizure compared to those without seizures, Results have been presented in Table 5.

3.3. Serial levels of oxidative stress and ER stress markers

Sequential evaluation of serum levels of the oxidative stress and ER stress markers were possible in 10 out of 29 TBM patients with seizures. The ER stress markers, MDA and Protein-carbonyl were elevated immediately following the seizure compared to their respective baseline levels and subsequently returned to the baseline at the time of discharge. The TBM patients who developed seizures had a higher baseline mean serum levels of MDA, Protein-carbonyl and lower mean levels of SOD, catalase and GSH as compared to the patients who did not develop seizures. The results have been presented in the boxplots. [Figs. 1–3].

The oxidative stress markers; MDA ($P = 0.19$), Protein carbonyl ($P = 0.7$), GSH ($P = 0.25$) SOD ($P = 0.18$), Catalase ($P = 0.39$); and ER stress markers: ATF 4 gene ($P = 0.07$) GRP78 gene ($P = 0.97$), CHOP gene ($P = 0.74$) and XBP-1 gene ($P = 0.18$) were not significantly related to stage of TBM. Admission TBM stage, however, significantly correlated with the outcome at time of hospital discharge. While all patients who presented in stage I TBM survived, 4 out of 26 patients in stage II and 2 out of 9 patients who presented in stage III TBM died ($P = 0.01$). In TBM, patients the serum oxidative and ER stress markers in early seizure were similar, except for Protein carbonyl levels, which was elevated in patients with early seizures compared to late seizure (6.5 ± 0.54 nM/ml vs 5.8 ± 0.74 nM/ml, $P = 0.04$).

Paradoxical worsening was present in 14 (28.6%) patients in our study. Nine (31%) patients in the seizure group had paradoxical worsening as compared to 5 (25%) in the no-seizure group. The level of various stress markers did not predict development of paradoxical worsening [Table 6]. Moreover, Oxidative stress markers and ER stress

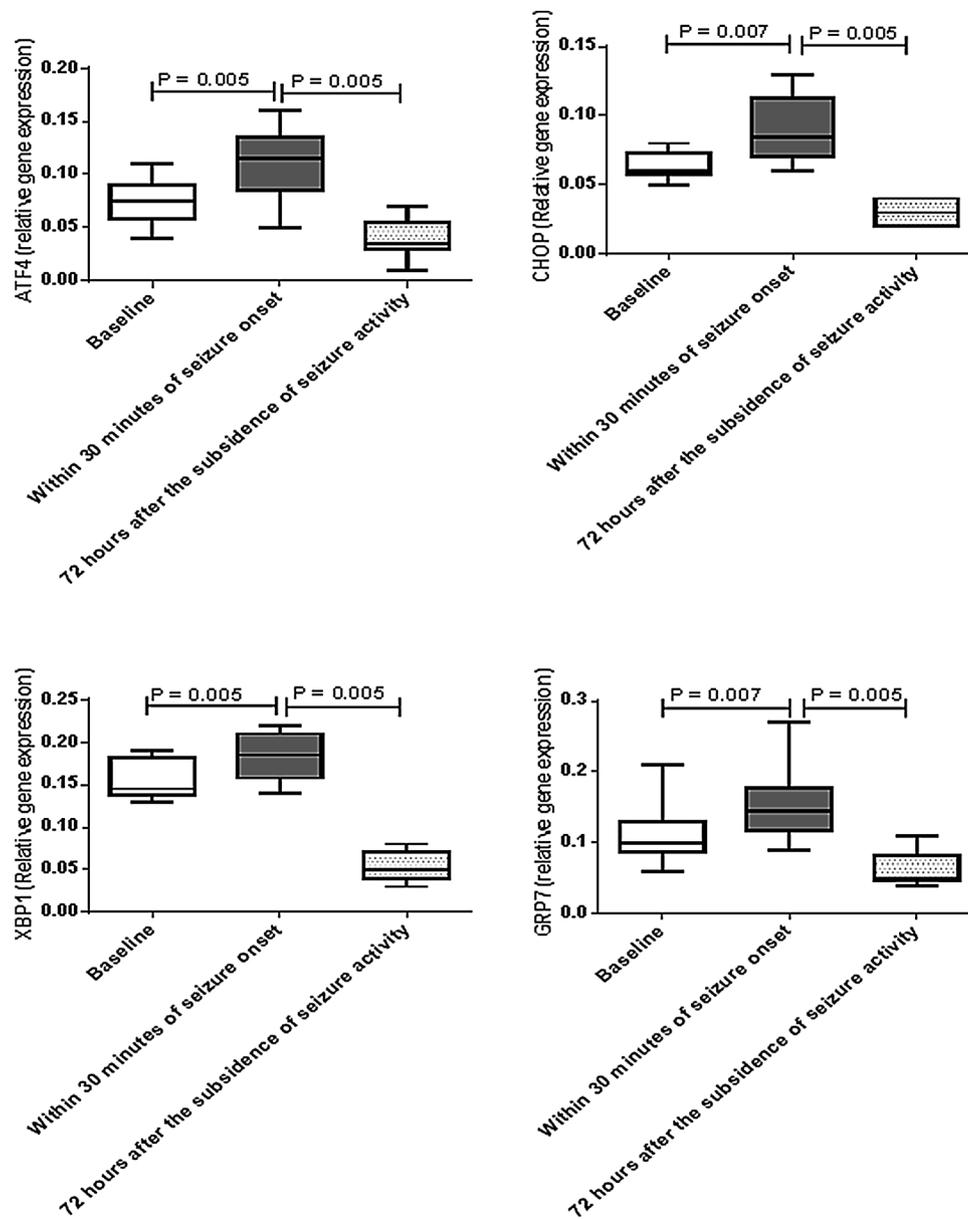


Fig. 3. Box plots showing the levels of ER stress markers in patients with TBM at different time points. The serum levels of ER stress markers were higher at the time of seizures. The panel shows ER stress markers levels at baseline (white bars), during seizures (grey bars) and 72 h post seizure (dotted bars) The horizontal line within each box indicates the median, the top and bottom of the box indicate the inter-quartile range, and the I bars indicate the minimum and maximum values.

Table 6
Comparison of stress markers in TBM patients with and without paradoxical worsening.

Parameters	Paradoxical worsening(N = 14)	No paradoxical worsening(N = 35)	P-Value
Oxidative stress markers			
MDA (nM/ml)	12.3 ± 3.5	11.5 ± 3.0	0.277
Protein carbonyl (nM/ml)	5.7 ± 0.62	5.6 ± .80	0.745
SOD (U/mg of protein)	0.08 ± 0.03	0.08 ± 0.02	0.727
Serum catalase (U/mg of protein)	3.8 ± 0.90	3.6 ± 0.87	0.685
GSH (µM/ml)	247.7 ± 42.5	249.1 ± 32	0.902
Relative gene expression of ER stress markers			
ATF-4	0.14 ± 0.13	0.20 ± 0.32	0.411
CHOP	0.27 ± 0.46	0.16 ± 0.23	0.464
XBP-1	0.19 ± 0.13	0.36 ± 0.63	0.297
GRP-78	0.17 ± 0.18	0.19 ± 0.23	0.735

markers were not different in the patients with focal vs secondary generalized seizures, those with and without status epilepticus and those with abnormal versus normal MRI.

4. Discussion

In this study, the role of oxidative stress and ER stress in TBM was evidenced by the elevation of MDA and protein carbonyl and reduction of SOD, catalase and GSH in comparison to healthy controls. However, the oxidative stress markers except catalase in the patients with seizures revealed significant difference compared to those without seizures. Moreover, the oxidative and ER stress markers did not correlate with the severity of TBM. The seizures in TBM patients was associated with significant elevation of oxidative stress and ER stress markers and returned to the baseline values 72 h post-seizure. However, the oxidative stress and ER stress markers did not correlate with the outcome of TBM.

This is the first study estimating the role of oxidative stress and ER stress in TBM related seizures. Our results are in agreement with a study in 19 TBM patients suggesting the role of free radicals in the pathogenesis of meningitis; higher levels of lipid peroxidation and percentage hemolyzed plasma ceruloplasmin in patients compared to controls. Moreover, antioxidant levels (glutathione reductase) and antioxidant vitamins A, C and E were significantly lower compared to the controls. After the anti-tubercular therapy, serum levels of anti-oxidants - glutathione reductase and catalase were significantly higher compared to pretreatment levels (Sudha et al., 2002). In another study on HIV and TB co-infection, lipid peroxidation (MDA) was high in naïve TBM patients compared to the HIV co-infected patients whereas reduction in GSH was noted in both naïve and TBM co-infected patients (Awodele et al., 2011). Increase in lipid peroxidation products have also been reported in pneumococcal and meningococcal meningitis and correlated with its severity (Roslyi et al., 1990). In meningitis, superoxide anions, hydro-peroxide and hydroxyl radicals have a bactericidal role after the bacteria is engulfed by leucocyte, macrophage and microglia (Brückner et al., 1981). Excessive production of free radicals in pathological conditions often overwhelms the cell defense mechanism leading to lipid peroxidation and cell death (Southorn and Powis 1996). At cellular level, bacterial meningitis leads to increased nitration of tyrosine, particularly involving the cerebral vessels and inflammatory cells. These cells have been found to exhibit 4-hydroxynonenal (a marker of lipid peroxidation), suggesting the role of reactive nitrogen species induced oxidative brain damage. Moreover, a high concentration of nitro-tyrosine in the CSF was associated with poor outcome. There was also reduction of ascorbic acid and oxidation of uric acid to allantoin (Kastenbauer et al., 2002). Tuberculous meningitis is associated with multiple pathological changes including meningeal irritation, basal exudate, hydrocephalus, infarction and tuberculoma which could contribute to seizures independently or in combination. There was no significant difference in oxidative stress and ER stress markers on comparing secondary generalized seizures vs focal seizures, paradoxical worsening vs no paradoxical worsening, abnormal MRI vs normal MRI and patients with status epilepticus (SE) vs no SE in our study. The lack of significance in the subgroup analysis in our study may be attributed to a relatively small sample size and multiplicity of variables influencing these endpoints. The sequential evaluation of oxidative stress and ER stress markers in 10 patients with seizures, however, did reveal significant change compared to the baseline (Figs. 1–3).

Increased plasma levels of MDA have been reported in epilepsy patients as a biomarker of oxidative stress. Additionally, accumulation of ROS byproducts occurs as a result of oxidized genomic DNA in patients with epilepsy (Ercegovac et al., 2010a, 2010b). In a study of 60 patients with first unprovoked seizures, enhanced protein (RCDPSH), lipid and DNA oxidation with upregulated antioxidant enzyme (SOD and GPX), urinary 8-epi-prostaglandin-2 α and 8-hydroxy-2-oxyguanosine levels were consistent with increased oxidative stress in epilepsy

(Seimon et al., 2010a, 2010b). Oxidative stress, NO and ROS produces ER stress which ultimately may induce apoptosis and neuronal damage after seizure or TBM or both. Upregulation of about 73% genes involved in UPR has been reported in tubercular granuloma which leads to apoptosis of ER-stressed macrophages. Apoptosis was more frequent in granulomatous compared to non-granulomatous tissue isolated from the patients with pulmonary tuberculosis (Seimon et al., 2010a, 2010b). Apoptosis is known to be associated with UPR effector CHOP expression in the areas surrounding caseation (Schroder and Kaufman 2005). Early Secreted mycobacterial Antigen ESAT6 is reported to increase intracellular calcium concentration which results in ROS accumulation which causes ER stress in the cell (Brown, 1996).

The oxidative and ER stress parameters did not predict the outcome of TBM. It is possible that these are markers of stress may correlate with the conditions at a particular time and may not predict the long-term outcome because of protracted and fluctuating course of TBM. Superoxide dismutase, catalase, GPX glutamate reductase, peroxy-reductase and non-enzymatic vitamin C, E and reduced glutathione are able to handle normal physiological levels of ROS (Shin, et al., 2011). However, when the defense system is overwhelmed by the infection or other stress, it affects calcium homeostasis, and results in protein unfolding response which leads to apoptosis and cell death (Malhotra and Kaufman 2007). Specific and non-specific therapy may alter or modulate the stressful condition and determine the outcome.

We depended upon serum levels of oxidative stress and ER stress markers as a surrogate marker of the changes in the CNS. The sequential studies were possible in ten patients only and the biomarkers could not be studied at different time points to provide a more robust clinical correlation. Our results are based on selected hospitalized patients. Hence a larger study is needed before these results can be applied to TBM in general. This study however does provide some evidence of oxidative stress and ER stress in TBM patients with seizures.

5. Conclusion

To conclude, there seems to be some role of oxidative stress and ER stress in pathophysiology of TBM related seizures. However, these cellular stress biomarkers neither co-relate with the severity of TBM nor does it predicts the outcome.

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Ethical approval

This study was approved by Institutional Ethics Committee, SGPGIMS, Lucknow, INDIA.

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