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ORIGINAL PAPER

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Oxidative and nitrosative stress in patients with meningitis

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ABSTRACT

Introduction and aim. Meningitis is an acute inflammation of the protective membranes covering the brain and spinal cord, known as the meninges. In this study, oxidative and nitrosative stress were evaluated in cerebrospinal fluid (CSF) and blood samples that were taken from patients with meningitis. Our goal was to identify a fast and a reliable biomarker using these parameters in order to the early diagnose of bacterial meningitis.

Material and methods. In this study, 37 bacterial meningitis, 30 tuberculous meningitis and 30 viral meningitis cases were included. Serum/CSF total oxidant status (TAS) and total antioxidant status (TOS) were measured by the Erel method. Nitrotyrosine concentrations were quantified by using ELISA in both serum and CSF

Results. Serum nitrotyrosine, CSF TAS and TOS levels were not significantly different in three groups ($p>0.05$). CSF nitrotyrosine levels were significantly higher in bacterial meningitis than tuberculous meningitis group ($p<0.05$). Viral meningitis patients had higher serum TOS and TAS concentrations than tuberculous meningitis group ($p<0.05$).

Conclusion. As a result, we can say that the oxidative and nitrosative stress markers studied are not a rapid and reliable biomarker in bacterial meningitis's diagnosis.

Keywords. bacterial meningitis, oxidative stress, nitrotyrosine, tuberculous meningitis, viral menengitis

Introduction

Meningitis is the most serious infection of the central nervous system (CNS) and affects in the meninges, the membranes that cover the brain and spinal cord. It caused by many microorganisms, including bacteria, viruses, parasites and fungi.[1,2] Meningitis is an important public health problem in developing countries, including Turkey.

The brain is particularly vulnerable to oxidative stress due to its high metabolic requirements and the presence of polyunsaturated fatty acids. Under physiological conditions, the total antioxidative activity of the cerebrospinal fluid (CSF) is only one tenth of plasma. It has been reported that free radicals can cause neuronal cell death.[3] In addition, bacterial and host-derived reactive oxygen and nitrogen species combine to form highly reactive, tissue-damaging intermediates. Therefore, it is assumed that the levels of endogenous antioxidant molecules in the CSF elevated in response to infections that can place severe stress on the human body.[4,5] There are studies that found that free radicals are produced in CNS compartment in cases with meningitis.[2,5]

Studies on children with bacterial meningitis (BM) have shown that antioxidant molecule levels and lipid peroxidation in serum/CSF increase as a result of oxidative stress.[3,5]

Nitrotyrosine (NT) is a widely used marker for the formation of reactive nitrogen species, such as peroxynitrite. It has been found that tyrosine nitration is greatly increased during meningitis. This increase has been

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dedected to be more pronounced in inflammatory cells and blood vessels in the subarachnoid space. Therefore, it has been reported that reactive nitrogen species (RNS) may contribute to oxidative brain damage during meningitis.[6,7]

Aim

Our aim was to investigate oxidative and nitrosative stress in blood and CSF samples of bacterial, viral and tuberculous meningitis (TM) cases. According to our knowledge, this is the first study.

Material and methods

Study setting and population

We conducted this study on CSF and blood samples of patients who applied to Gaziantep University Faculty of Medicine Infectious Diseases Clinic and received a pre-diagnosis of meningitis between January 2018 and June 2020. Thirty-seven BM, 30 TM and 30 viral meningitis (VM) cases were included in the study. Paired serum and CSF samples were collected on admission, before the empirical antimicrobial and supportive therapy had started.

The protocol was approved by the Clinical Research Ethics Committee of Gaziantep University with the protocol number (No: 2015 /363) and the research was conducted in compliance with the Declaration of Helsinki (version 2008).

The purpose and procedures of the study were explained and written informed consent was received from each participant or their guardians prior to participation.

Exclusion criteria: Cases who were hospitalized with a prediagnosis of meningitis, cerebrovascular event, malignant infiltration of the menix, immunocompromised patients for any reason, pregnant women, and those lacking laboratory data used for routine diagnosis were excluded from the study.

Diagnosis of meningitis

The diagnosis was made according to the patient's clinics and CSF examination criteria. Lumbar puncture (LP) was performed if clinical signs and symptoms suggest meningitis. LP can be safely performed in the absence of increased intracranial pressure, focal neurological findings and/or papillary edema. The diagnosis of bacterial meningitis is based on a course of clinical history and laboratory experiments. Clinical features were such as the acute onset of headache, fever, and signs of meningeal irritation. Laboratory diagnosis of acute bacterial meningitis (ABM) was made by CSF examination. Positive CSF findings were pleocytosis (≥5/mm^3, mainly neutrophilic), elevated protein concentration (≥45 mg/dL), a reduced ratio of CSF glucose to serum glucose (≤0.60) in additionally a positive CSF culture, smear, or PCR for bacterial pathogens or a good specific response to antibacterial therapy.

Viral meningitis cases had such as clinical features, the acute onset of headache, fever, and signs of meningeal irritation. In addition, there were no signs of cortical involvement such as altered consciousness, aphasia, or seizures. Viral meningitis CSF findings were also pleocytosis (≥5/mm^3, mainly lymphocytic), a negative CSF stain, culture, or PCR for bacteria, mycobacteria, fungi and a positive PCR for viral pathogens or full recovery without any specific treatments including antibacterial or antituberculosis therapy.

Diagnosis of TM clinical symptom, CSF criteria, cerebral imaging criteria and It was placed on the basis of other evidence of tuberculosis. If mycobacterium tuberculosis was detected in CSF of cases a definite diagnosis of TM was made.

Measurements

Blood and CSF taken for analysis before starting treatment. The serum was separated with standard centrifugation procedure and immediately divided in portions which were kept tightly closed at −70°C until analysis

Measurement of TAS

TAS measurement was performed using an Aeroset 2.0 analyzer and a total antioxidant status kit (Rel Assay Diagnostic, Turkey). In this kit the reduced ABTS (2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonate)) molecule is oxidized to ABTS•+, using hydrogen peroxide alone in an acidic medium (acetate buffer 30 mmol/L; pH 3.6). The concentrated ABTS•+ molecules (deep green) remain more stable for a long time in the acetate buffer solution. The color is spontaneously and slowly bleached when it is diluted with a more concentrated acetate buffer solution at high pH (acetate buffer 0.4 mol/L; pH 5.8). In the sample's antioxidants accelerate the bleaching rate to a degree proportional to their concentrations. This reaction can be monitored spectrophotometrically and the bleaching rate is inversely related to the total antioxidant capacity (TAC) of the sample. The reaction rate is calibrated with Trolox, which is widely used as a traditional standard for TAC measurement assays, and the assay results are expressed as mmol Trolox equivalent/L.[8]

Measurement of TOS

TOS measurement was performed using an Aeroset 2.0 analyzer and a TOS kit (Rel Assay Diagnostic, Turkey). In the sample's oxidants oxidize the ferrous ion–o-dianisidine complex to ferric ions in this kit. In the reaction medium glycerol molecules enhance the oxidation reaction. The ferric ions make a colored complex with xylenol orange in an acidic medium. The color intensity, which can be measured spectrophotometrically, is

related to the total quantity of oxidant molecules in the sample. The assay is calibrated with hydrogen peroxide and the results are expressed as the micromolar hydrogen peroxide equivalent per liter (μmol H_2O_2 Equiv./L).[9]

Nitrotyrosine Analysis

The NT levels were detected in plasma samples using the Bioxytech sandwich ELISA immunoassay (OxisResearch, USA) according to the manufacturer's instructions.

Statistical Analyses

All statistical analyses were performed using PASW, version 18.0 (SPSS Inc., Chicago, IL, USA) for Windows. Continuous variables are presented as mean ± SEM and categorical variables are presented as *n* of patients (%). The Kolmogorov–Smirnov test was used to test the normality of the distribution of continuous variables. Statistical analysis of data between two groups was performed using unpaired *t*-test for parametric data and Mann–Whitney *U*-test for nonparametric data. A two-tailed *p*-value < 0.05 was considered statistically significant.

Results

Comparison of demographic and laboratory profiles of cases in CSF and serum are illustrated in Table 1.

CSF bacterial culture is known as the gold standard method for confirming acute bacterial meningitis, but most cases cannot be confirmed by culture. In this study, of 37 patients with BM, 11 had positive CSF cultures, smears, or PCR for bacterial pathogens, including 2 with Streptococcus species, 7 with Staphylococcus species, and 2 with other species.

It was determined that the oxidative/nitrosative stress parameters analyzed did not correlate with demographic data such as age and gender.

Among the analyzed markers, a weak positive correlation was found between only serum TAS and laboratory profile parameters CSF glucose (r: 0.267; p: 0.036).

Cerebrospinal fluid and serum NT, TAS and TOS concentrations of the subjects are given in Table 2.

Discussion

Activated phagocytic cells (neutrophil eosinophil and macrophages of all types) produce $O_2\bullet^-$ by NADPH oxidase. This event, called oxidative burst (burst), is important in clearing phagocytosed bacteria. Meanwhile, O_2 consumption in phagocytic cells increases 4 to 100 times. Reactive oxygen (ROS) and nitrogen species are produced by the human immune system in response to infection such as meningitis. According to in the results of cases with bacterial meningitis different animal, and several human studies it has been determined that ROS/RNS are produced in activated PMNs during the inflammatory response of the host when bacteria reach the subarachnoid space.[1,10-13] The mechanisms of central nervous system damage during meningitis are not

Table 1. Comparison of demographic and laboratory profiles of cases with bacterial, tuberculosis and viral meningitis

Parameters	Bacterial meningitis (n=37)	Tuberculosis meningitis (n=30)	Viral meningitis (n=30)	p
Demographic Profile				
Men, n (%)	18 (48.6)	17 (56.7)	19 (63.3)	>0.05
Age*, years	40.19±2.73	42.60±3.31	45.40±2.56	>0.05
Blood Profile WBC†x10^6/L	9.42 (2.35-26.21)	8.80 (3.61-17.82)	7.91 (4.60-20.80)	>0.05
Neutrophil† (%)	75.50 (46.0-91.0)	76.0 (46.0 -90.0)	75.0 (51.0-92.0)	>0.05
ESH† (mm/hour)	35.0 (2-103)	56.0 (2-120)	29.0 (2-96)	>0.05b <0.05a,c
CRP† (mg/L)	55.0 (0.5-328.0)	55.0 (2-250)	24.0 (0.78-145.0)	>0.05a <0.05b, c
CSF Profile				
WBC†/mm^3	450 (70-1100)	250 (40-850)	90 (20-450)	>0.05a <0.05b, c
Lymphocyte percentage†	40 (10-75)	87.5 (20-90)	90 (60-100)	<0.05a,b >0.05c
Protein† (mg/dl)	153 (26-1361)	647 (34-1640)	90 (34-215)	<0.05
Glucose ratio *(CSF/Blood)	0.41±0.06	0.30± 0.04	0.59±0.03	<0.05a,c >0.05b
Clinical Profile				
Systolic Blood Pressure† (mmHg)	120 (90-171)	120 (100-206)	119 (90-168)	>0.05
Diastolic Blood Pressure† (mmHg)	70 (50-96)	70 (47-99)	70 (60-86)	>0.05
Death, n (%)	10 (27)	4 (10)	1 (3.3)	<0.05
Sequelae, n (%)	5 (13.5)	2 (6.7)	1 (3.3)	<0.05

† Median (interquartile range)*Mean±SEM a: BM vs. TM; b: BM vs. VM; c: TM vs. VM

CRP: C-reactive protein, CSF: cerebrospinal fluid, WBC: white blood cell, ESH: erythrocyte sedimentation rate; CRP: C-reactive protein; BM: Bacterial meningitis; TM: Tuberculous meningitis; VM: Viral meningitis

fully elucidated, but a wealth of evidence suggests that reactive oxygen and nitrogen species may contribute to brain damage.

Some studies have shown that ROS may have an important role in various pathological processes such as vascular damage, cerebral edema formation and cerebrospinal fluid pleocytosis in BM.[14,15] It has been suggested that oxidative stress has a role in the pathophysiology of TM-associated seizures.[16]

It has been determined that the nitration of tyrosine is increased at the cellular level, especially in cerebral vessels and inflammatory cells in BM. In these cells, 4-hydroxynonenal compound, a lipid peroxidation marker, was detected suggesting a role for RNS in oxidative brain injury. In addition, high concentrations of NT in CSF were associated with worsening of the disease.[6]

Previous studies found significant increases in CSF nitrite and NT levels in children with BM. In this study in cases of BM serum and CSF NT levels were found higher than TM and VM groups.[12,17,18] However, only NT levels in CSF samples of cases with BM were statistically significant compared to the levels of TM cases ($p<0.05$).

These results indicate that ROS/RNS production is more increased in CSF and serum of cases with BM and that oxidative damage may contributes to the pathophysiology of such cases. CSF and blood TOS, TAS, oxidative stress index and S-100B levels were found to vary in pediatric cases with BM. This change was found to be parallel to inflammation.[19]

In our study TAS and TOS levels in CSF samples were not statistically different from each other in all 3 meningitis groups ($p>0.05$). In serum samples, TAS and TOS values showed statistically significant difference only between TM and VM groups ($p<0.05$)

In BM, ROS and RNS are considered to mediate the disruption of the blood brain barrier. Moreover, it has been found that treatment with antioxidants prevents the deterioration of the blood brain barrier.[1,20]

This may be because the inflammation that occurs is due to the proliferation of bacteria, the release of excessive amounts of oxidants produced in phagocytes to destroy bacteria, and the use of antioxidants to neutralize them.

The CSF/plasma glucose ratio in healthy adults is about 0.6 and therefore an abnormal level is less than that, usually 0.5 or less.[21] In our study, this ratio was determined as BM (Mean±SEM: 0.41±0.06) TM (0.3±0.04) and VM (0.59±0.03). The CSF/plasma glucose ratio of the TM group is statistically significantly lower than the other two groups ($p<0.05$).

In our study, the percentage of sequelae (13.5%) in cases with BM was found to be higher than in cases with other meningitis (TM: 6.7% and VM: 3.3% ; $p<0.05$). ROS and RNS have been associated with cognitive sequelae, especially since they cause cellular damage.[22]

In addition, the mortality rate was found to be statistically significantly higher in cases with BM than in TM and VM cases (BM: 27%, TM: 10%, VM: 3.3% ; $p<0.05$).

Conclusion

As a result, we can say that the oxidative and nitrosative stress markers studied are not a rapid and reliable biomarker in BM's diagnosis, but we might conclude that oxidative stress contributes at least in part to the severe neurological dysfunction found in meningitis especially bacterial meningitis. However, in parallel with the inflammation in bacterial meningitis, changes had been occured in CSF and blood TOS, TAS and NT levels, and these changes should be examined in larger case groups in future studies.

Table 2. Cerebrospinal fluid and serum NT, TAS and TOS concentrations of the subjects

	Bacterial Meningitis (n:37)	Tuberculous Meningitis (n:30)	Viral Meningitis (n:30)	p value
Serum				
NT (ng/ml)	393.48±73.4	268.74±31.1	237.14±44.0	>0.05[a,b,c]
TAS (mmol/L)	1.38±0.1	1.22±0.1	1.43±0.1	>0.05[a,b] <0.05c
TOS (µmol/L)	12.16±0.9	9.41±1.1	13.4±1.1	>0.05[a,b] <0.05c
CSF				
NT (ng/ml)	183.39±13.4	129.29±10.8	156.08±14.7	<0.05[a] >0.05b,c
TAS (mmol/L)	0.95±0.1	0.91±0.1	1.09±0.1	>0.05[a,b,c]
TOS (µmol/L)	8.07±0.9	6.97±0.9	9.06±0.9	>0.05[a,b,c]

BM: Bacterial meningitis; TM: Tuberculous meningitis; VM: Viral meningitis CSF: cerebrospinal fluid, Data are given as mean ± standard error, a: BM vs TM; b: BM vs VM; c:TM vs VM

Declarations

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Author contributions

Conceptualization, E.S.N. and M.N.; Methodology, E.S.N; M.N. and I.K.; Software, E.S.N. and M.N; Validation, E.S.N., M.N. and K.K.; Formal Analysis, E.S.N.; Investigation, M.N. and I.K; Resources, E.S.N and M.N; Data Curation, M.N. and K.K; Writing – Original Draft Preparation, E.S.N.; Writing – Review & Editing, E.S.N. and M.N; Visualization, E.S.N.; Supervision, E.S.N., M.N. and I.K; Project Administration, E.S.N.; Funding Acquisition, E.S.N.

Conflicts of interest

The authors declare no conflicts of interest.

Data availability

All data generated or analyzed during this study are included in this article [and/or] its supplementary material files. Further enquiries can be directed to the corresponding author.

Ethics approval

The protocol was approved by the Clinical Research Ethics Committee of Gaziantep University with the protocol number (No: 2015 /363)

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