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FULL PAPER

Study of oxidant-antioxidant status in cerebrospinal fluid of children with meningitis

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Meningitis, an inflammation of the meninges, is globally distributed as either sporadic or epidemic forms. Acute meningitis of infectious etiology involves viruses or bacteria making the differential diagnosis very difficult. Oxidative and reductive stress are dual dynamic phases experienced by the cells undergoing adaptation towards endogenous or exogenous noxious stimulus. Considering the issue stated, the present study was designed to evaluate the alterations in different biochemical parameters including glucose, protein, oxidative stress in the CSF samples of the meningitis patients (n=36) and compare them with control subjects (n=36). The results revealed significant increase in CSF of total protein, malondialdehyde, albumin, and NO levels in meningitis patients when compared with their respective controls. While Glucose, uric acid, glutathione, Vitamin E and C were significantly decreased in CSF samples, in meningitis patients in comparison to the control group. The findings indicated that oxidative stress due to reactive nitrogen species and altered antioxidant defenses are involved in the pathophysiology of meningitis in humans.

KEYWORDS

Cerebrospinal fluid; meningitis; oxidative stress; vitamin E and C.

Introduction

Cerebrospinal fluid (CSF) is a clear colorless liquid circulating in the intracranial and spinal compartments. At any given time, there is approximately 125 mL to 150 mL of CSF in the body, being in dynamic equilibrium and bathing neurons and glial cells. CSF analysis may support only the suspicion of a nervous system disorder and rarely provides a definitive diagnosis [1-3]. Meningitis is defined as inflammation of the membranes surrounding the brain and spinal cord, including the dura, arachnoid and pia mater [2,4]. It is usually caused by bacteria or viruses, fungal, parasites, but it can be a result of injury, cancer, or certain drugs. It is important to know the specific cause of meningitis because the treatment differs depending on the cause. It is one of the important causes of considerable morbidity and mortality in children' that associated with cerebral compromise which may be responsible for neurological squeal in nearly half of the survivors [5-7].

Oxidative and reductive stress are dual dynamic phases experienced by the cells undergoing adaptation towards endogenous or exogenous noxious stimulus. The former arises due to the imbalance between the production of reactive oxygen species (ROS) or free radicals and antioxidant defense, which may induce tissue injury. It can be assessed by measurement of reaction products of oxidative damage, like lipid peroxidation, DNA oxidation and protein oxidation [8,9]. Through pathological redox reactions, ROS can denature biomolecules

such as proteins, lipids and nucleic acids. This can initiate tissue damage via apoptosis and necrosis. It plays a significant role in the pathogenesis of meningitis and mechanisms of complications [8-12]. Accordingly, the present study aimed at evaluating the diagnostic and prognostic significance of oxidative stress and antioxidant status in cases of meningitis by serial estimation of these markers in CSF.

Material and methods

A prospective hospital-based study was done during a period of three years from January 2019 to December 2021. All patients from one month to 7 years admitted to the Salahalddin Teaching Hospital in Tikrit province, and Pediatric Hospital in Kirkuk, Iraq with a presumptive diagnosis of meningitis were included in the study.

Inclusion criteria

Inclusion criteria entailed clinical features suggestive of meningitis.

Exclusion Criteria

The exclusion criteria were patient suffering from acute or chronic liver disease, neurological disorders like Stroke, ICSOL, patient with history of muscles diseases, and patient suffering from renal diseases. Based on clinical manifestation and laboratory results of CSF which include glucose as well as protein concentration, qualitative and quantitative cytology, Gram stain, ZN stain, 36 number of patients were finally selected for the study diagnosed as either Pyogenic or Tubercular Meningitis.

Biochemical assays

The fluid was withdrawn by lumbar puncture, using a spinal needle No.20). The patients lay on a hard bench, taking the lateral reclined position and the needle was gently placed above or beneath the fourth lumber vertebra. The amount of withdrawn C.S.F was not fixed, but usually in the range of (1-3) ml. The CSF was examined grossly for appearance and color. Freshly collected specimens were stored at 4 C°. Turbid specimens were centrifuged at 3000 rpm for 10 minutes before storage.

The CSF glucose, protein, GSH, MDA, G-Px, and TAS levels were measured by spectrophotometric kit via Bio labo kit CHOD/PAP-France. Nitric oxide was estimated by the method of Smarason et al., (1997) [13]. Measurement of Vitamin E and C were performed according to the method described by Tietz [14].

Statistical methods

Statistical analysis was performed using standard statistical software (SPSS version 16.0). All data were expressed as mean ± S.D. The data were also tested using student's t-tests; P-value of less than 0.05 was taken as significant.

Results

TABLE 1 Demographic characteristic of study population

Diagnosis	No. of patients		
	Total	Male	Female
Normal (control)	36	15	21
Tuberculous meningitis	4	1	3
Bacterial meningitis	5	4	1
Viral meningitis	16	10	6
Partial treated meningitis	1	-	1

The study included 36 patients with meningitis and 36 healthy subjects as controls. It is evident from Table 2 that there is decrease in levels of MDA (1.29 ± 0.18 vs. 1.01 ± 0.21µmol/L), and NO (23,6 ± 2,8 vs20,3 ± 2,2 µmol). The activities in the patient group were compared with those of control. However, the levels of TAC ,uric acid, Vitamin-C , and GSH, GPx, and Vitamin E were significantly lower in the experimental group than those of the controls (0.391 ± 0.15 vs 1.67 ± 0.25 mmol/L), (1.8 ± 0.09 vs 3.48 ± 1.75 mg/dl), (0.39 ± 0.24 vs. 0.88 ± 0.12 mg/dl), and (36± 4.15 vs 42.64±4.76 mg/dl), (40±3.81 vs. 57.53±6.53 U/g Hb) ,and (0.740± 0.204 vs 1.34 ± 0.221 mg/L), respectively.

TABLE 2 The (mean ±SD) CSF concentrations of measured biochemical parameters of the patients and the control group

Parameters	Patients group	Control group	P
Glucose (mg/dl)	120 ± 300	33.1 ± 2.8	0.05
Total protein (g/l)	25.80 ±4.30	63.05 ± 8.08	0.00001
MDA (µmol/L)	1.29 ± 0.18	1.01 ± 0.21	<0.001
Uric acid(mg/dl)	1.8 ± 0.09	3.48 ± 1.75	<0.001
NO (µmol)	23.6 ± 2.8	20.3 ± 2.2	<0. 01
Albumin (g/dl)	1.19 ± 0.079	2.13 ± 0.39	p < 0.0001
Total antioxidant capacity (mmol/L)	0.391 ± 0.15	1.67 ± 0.25	<0.001
Glutathione peroxidase (U/g Hb)	40±3.81	57.53±6.53	<0.0084
Vitamin-C (mg/dl)	0.39 ± 0.24	0.88 ± 0.12	<0.001
GSH (mg/dl)	36± 4.15	42.64±4.76	<0. 01
Vitamin E (mg/L)	1.34 ± 0.221	0.740± 0.204	P < 0.05

Discussion

CSF proteins originate from serum and from local intracranial production. A breakdown in the integrity of the blood-brain barrier allows increased levels of serum proteins to access the CSF. Probably the increased membrane permeability may lead to increase CSF enzymes proportionately, helping the differential diagnosis of meningitis [15]. This contributes to the development of increased intracranial pressure, hydrocephalus, brain edema, and cerebral ischemia, all of which can cause neuronal cell death [16, 17]. CSF levels of glucose were significantly increased in the patients compared with the control group. These results were in agreement with a study done by Entedhar *et al* [17], due to the fact that microorganisms in meningitis uses CSF glucose as a source of energy for their metabolism which leads to increase in its level in CSF.

The brain is naturally protected from the body's immune system by the barrier that the meninges create between the bloodstream and the brain [18]. The blood-brain barrier is formed by microvascular endothelial cells, astrocytes, and pericytes. This barrier acts by controlling the exchange of substances into and out of the brain and thereby protects the brain from toxins and pathogens [19]. The BBB breakdown occurred at 12 hours after pneumococcal meningitis induction, subsequent to the cytokine production. ROS and RNS have been implicated as mediators of the BBB breakdown, suggesting that the increase of the BBB permeability appears to be related to the presence of $NO/O_2^{\bullet -}$ [20].

Malondialdehyde is a reactive aldehyde and is one of the many reactive electrophile species that cause toxic stress in cells [21]. CSF malondialdehyde considered one of the final products of lipid peroxidation was found to be significantly increased in present study indicating the destruction in the tight junctions of the endothelial monolayer of BBB and increasing its permeability. This result is in agreement with those of Miric (2010) and Ramakrishnan (2009) [22,23]. Increased levels of MDA in this study are an indication of increased oxidative stress. Antioxidants like superoxide dismutase and glutathione peroxidase are jointly accessed and they are both known to counteract free radicals (reactive oxygen species) [24].

Glutathione has a role in scavenging ROS and reactive nitrogen species. In accordance with the findings of Hamed *et al. (2009.*), GSH levels were significantly lower in patients with meningitis compared with those in the control group [25]. **S**uperoxide dismutase as metalloenzymes catalyzes superoxide anion into oxygen and hydrogen peroxide. It is located in mitochondria and plays a critical role as an antioxidant [26].

Uric acid $C_5H_4N_4O_3$ (7,9-dihydro-1H-purine-2,6,8(3H)-trione) is an end product of purine nitrogen base metabolism and powerful antioxidant [27], as effective as ascorbate, and a potent scavenger scavenger of radicals formed by the reactive oxygen and nitrogen species. Uric acid can act as a neuro-protective agent not only by eliminating PN's oxidative toxicity but also by effectively scavenging downstream radicals of peroxynitrite (PN) – that is, $CO_3^{\bullet -}$ and $NO_2^{\bullet}$, produced following the rapid reaction of PN with CO_2 [28]. UA might suppress increased BBB permeability by protecting against PN-induced damage and directly scavenging. SUA protects the BBB's integrity and reduces its permeability. It also reduces inflammatory cell infiltration and thereby relieves brain inflammation [29]. Our results showed that patients with meningitis have lower CSF UA due to overconsumption of UA in scavenging excessive oxidative stress [30], being in line with previous reports [31].

Albumin, accounting for about 70% of the plasma colloid osmotic pressure, plays a vital role in maintaining the normal fluid distribution and constitutes the main circulating antioxidant system in the body. As potent scavengers of ROS derived from oxidative stress, albumin is the major source of extracellular reduced sulfhydryl groups (-SH) [32]. Recent studies have shown that evaluation of albumin in CSF and serum specimen obtained at the same time can predict disruption of BBB and meningitis [33]. Increased amount of specific plasma protein in CSF is neither synthesized, nor metabolized intra thecally so in CSF which is free of contaminating blood so albumin in CSF must come from plasma through BBB [34]. Munoz-Sanchez [35] stated that the serum albumin, specifically block echovirus by inhibiting the un coating step in the virus replication cycle. That is to say that in man, echovirus infection may be modulated by serum albumin.

Nitric oxide (NO) is produced by the action of endothelial nitric oxide synthase [36]. The mechanism by which NO may contribute to the pathophysiology of meningitis is not understood. NO produced by phagocytes has been shown to be either cytocidal or cytostatic for a variety of cells. Within the target cells, NO disrupts various enzyme systems associated with mitochondrial respiration, DNA replication, and the citric acid cycle. The enzyme inactivation is accomplished by NO chelation of iron cofactors necessary for the function of these enzymes. A plausible scenario in which NO inactivates these iron-containing enzyme systems in the microvascular endothelial cells that constitute a major site of the BBB, thereby causing cellular destruction or alteration and loss of integrity of the BBB may be postulated [37]. The NO activity in CSF in M significantly is higher in patients than in

that of the control group. This was similar to the previous studies [38, 39]. Increased nitric oxide production in cerebrospinal fluid during the acute phase of bacterial meningitis may result from the inflammatory process and tissue injury.

Vitamin C is a water-soluble antioxidant compound. It reduces compounds such as free radicals through its electron donating capacity, being oxidized to dehydroascorbate, and acts as a co-antioxidant by regenerating alpha tocopherol from alpha-tocopheroxyl radical produced during scavenging of oxygen free radicals [40,41]. A significant decrease in CSF vitamin C is seen in patients with meningitis compared with the control group in this study. A similar observation was made by Mirice*t al* [23], showed low vitamin C levels in CSF of tuberculous meningitis patients. Low CSF vitamin C level as observed here was probably due to increased utilization of the vitamin to mitigate the toxicity of free radicals.

In this study we found that the CSF vitamin E level in the patients was significantly lowered than that of the healthy group due to their free radical scavenging action and to preserve the body antioxidant reserve and in normalization of vascular superoxide formation [42].

Total Antioxidant Capacity (TAC), defined as the moles of oxidants neutralized by one liter of solution, is a biomarker measuring the antioxidant potential of body fluids [43]. In the present study, serum TAC was significantly lower in patients with meningitis when compared with the control group, which was in accordance with that of Aycicek *et al*. [44]., and Imad A.J. Thanoon *et al* [45], showing that there is significant decrease in TCA in meningitis, indicating that a considerable amount of serum TAS undergoes oxidation under these conditions. In the conditions of excessive ROS production, the decrease can also induce the damage of BBB endothelial cells, contributing to further CNS infiltration by macrophages and T-lymphocytes [46].

In sum, changes in oxidants and antioxidants were observed and their possible role in pathogenesis of meningitis. This study may play an important role in the diagnosis and more accurate treatment for the patients suffering from viral meningitis.

Conclusion

Changes in oxidants and antioxidants observed suggest production of reactive oxygen species and their possible role in pathogenesis of meningitis.

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