

## ASSESSMENT OF C-REACTIVE PROTEIN AND OXIDATIVE/ANTIOXIDATIVE STATUS IN CHILDREN WITH ACUTE BACTERIAL MENINGITIS

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*The objectives of this study were to compare the serum levels of C-reactive protein (CRP), serum malondialdehyde (MDA), antioxidant parameters [represented by serum ferritin, uric acid and total antioxidant status (TAS)] in children with bacterial meningitis with a control group. Twenty-seven children with bacterial meningitis were included in this study. Thirty apparently healthy children were also included as the control group. Assays of serum CRP, uric acid, ferritin and TAS were performed on samples from controls and from patients prior to antibiotic therapy. After two weeks of antibiotic therapy, assays of the same parameters were repeated in the patients. A significant rise in the serum levels of MDA and CRP, and a significant reduction in serum uric acid levels and TAS were noted in children with acute bacterial meningitis (before therapy) compared to controls. Serum ferritin levels showed no significant differences. When measured parameters of children with bacterial meningitis after therapy were compared with those of the controls, highly significant differences in the mean serum levels of uric acid, CRP and TAS were noted. There were no significant differences in the mean serum ferritin levels. Serum oxidative/antioxidative balance shifted to the oxidative side in meningitis patients before therapy, and improved after therapy. Elevation of CRP in cases with bacterial meningitis may reflect its importance as an aid in the diagnosis of such cases.*

**Keywords:** Acute bacterial meningitis, C-reactive protein, Oxidant/antioxidant status

### INTRODUCTION

Bacterial meningitis continues to result in significant brain injury in many affected patients, despite the use of highly active antibiotics (Lieb and Tauber 1999). The pathological mechanisms of central nervous system injury in human meningitis are not yet completely understood, although recent studies indicate that the host's inflammatory responses are as important in brain damage as they are during infection by organisms and toxins (Jafari and McCracken Jr 1994). Under normal conditions, host cells are protected from the toxic effects of reactive oxygen species by

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enzymatic and non-enzymatic antioxidants (Tsukahara 2002). The marked increase in reactive oxygen species production during pathologic conditions, such as during acute and chronic inflammation, can overwhelm the body's defense mechanisms and lead to oxidative cell and tissue injury (Roedel and Pfister 1999). In the early phase of experimental bacterial meningitis, reactive oxygen species and related compounds are important mediators of meningitis-associated pathophysiologic alterations (Roedel and Pfister 1999). There are several lines of evidence indicating that reactive oxygen species may contribute to brain injury (Lieb and Tauber 1999; Roedel and Pfister 1999; Schaper *et al.* 2002).

The aim of this study was to assess the levels of C-reactive protein (CRP), malondialdehyde (MDA) an indicator of lipid peroxidation, some individual antioxidant parameters (uric acid and serum ferritin) and total antioxidant status (TAS) in children with acute bacterial meningitis before and after therapy.

## METHODS

The study was conducted from January 2005 to April 2007. Approval to conduct this study was obtained from the ethical committees of the Main Health Centre in Mosul City, Iraq, and the College of Medicine, University of Mosul. Children with positive signs of meningitis were admitted to Ibn Al-Atheer Pediatric Hospital in Mosul City, Iraq. Blood and CSF samples were obtained from all study subjects, and patients positive for bacterial meningitis, as determined by CSF cultures, were selected for the study. Thirty apparently healthy children served as a control group.

Initially, 7 mL of venous blood were taken from both the meningitis patients and the control group. Assays for serum CRP, serum MDA, uric acid, TAS and serum ferritin were carried out. After a two-week course of ceftriaxone 2 g IV 12 hourly, another 7 mL of venous blood were taken from the patient group and the same assays were repeated.

Serum CRP concentrations were measured using a nephelometric immunoassay (Wener, Daum and McQuillan 2000). TAS was estimated using a commercial assay kit obtained from Randox Company, according to the methods of Miller *et al.* (1993). Serum ferritin concentration was estimated using the immunoturbidimetric method, as described by Bellod and Seco (1999). Serum uric acid concentration was estimated using the

uricase enzymatic method, as described by Newman and Price (1999) and serum MDA levels were measured manually using a method described by Ohkawa, Ohisi and Yagi (1979).

Data from patients with acute bacterial meningitis before and after therapy were compared using paired *t*-test. Comparison of results from children with acute bacterial meningitis with those from controls was performed using unpaired *t*-test. All values are expressed as mean  $\pm$  SD. A *p* value of  $< 0.05$  was considered to be statistically significant.

## RESULTS AND DISCUSSION

Out of 37 cases admitted to the hospital, 29 proved to have bacterial meningitis (all due to *H. influenzae*). Two patients died during follow up, leaving 27 cases to be included in the study. They were eight females and 19 males with a mean age of  $4.96 \pm 2.19$  years (range: 2 to 10 years). In the control group, there were nine females and 21 males with a mean age of  $4.76 \pm 2.24$  years (range: 2 to 10 years).

When bacterial infections occur, neutrophils and macrophages gather at the site of infection to combat the microorganisms. During this process, these cells consume molecular oxygen, which is converted into toxic oxygen metabolites (Ray *et al.* 2000). Despite the experimental and clinical evidence that reactive oxygen species are important mediators of meningitis-associated pathophysiologic changes, there has been little research on changes in oxidant status in meningitis (Tsukahara *et al.* 2002). Recent studies have suggested that reactive oxygen species play an important role in several pathologic processes in septic meningitis (Tsukahara *et al.* 1998).

MDA assay is still widely used in clinical chemistry laboratories to monitor oxidative stress, and is also used as an index of lipid peroxidation (Draper and Hadley 1990). Our findings showed a significant elevation in serum MDA levels in children with acute bacterial meningitis ( $1.91 \pm 0.36$   $\mu\text{mol/L}$ ) compared to controls ( $1.00 \pm 0.2$   $\mu\text{mol/L}$ ) (Table 1). However, these levels decreased significantly after therapy (Table 2). The mean serum MDA level in meningitis patients who had received the full course of antibiotics was found to be  $1.26 \pm 0.18$   $\mu\text{mol/L}$  compared to pre-treatment levels (mean  $1.91 \pm 0.36$   $\mu\text{mol/L}$ ). Even after receiving

a two-week course of antibiotic therapy, the mean MDA level was significantly higher than that in patients in the control group (Table 3). This result is in agreement with the findings of many previous researchers (Lieb and Tauber 1999; Roedel and Pfister 1999; Ray *et al.* 2000).

**Table 1:** Comparison of measured parameters between children with meningitis before therapy and the control group.

Parameters	Mean $\pm$ SD		p-value
	Control group (n = 30)	Patient group before therapy (n = 27)	
Uric acid (mg/dL)	4.17 $\pm$ 0.71	3.47 $\pm$ 0.54	0.018
Serum ferritin (ng/mL)	99.50 $\pm$ 32.04	95.93 $\pm$ 33.31	0.681 (NS)
MDA ( $\mu$ mol/L)	1.00 $\pm$ 0.21	1.91 $\pm$ 0.36	<0.001
TAS (mmol/L)	2.03 $\pm$ 0.25	1.13 $\pm$ 0.14	<0.001
CRP (mg/L)	9.93 $\pm$ 2.60	88.33 $\pm$ 38.46	<0.001

Note: NS = Not significant (unpaired *t*-test)

Similarly, our results also showed a highly significant elevation in serum CRP levels in children with acute bacterial meningitis (88.33  $\pm$  38.46 mg/L) compared to controls (9.93  $\pm$  2.60 mg/L) (Table 1). The role of the CRP level in acute bacterial meningitis has been well-established. Sormunen *et al.* (1999) concluded that a combination of careful physical examination, CSF analysis and measurements of CRP levels provide a substantial aid in the diagnosis or exclusion of acute bacterial meningitis. In addition, Dias Leite, Ribeiro and Farhat (1999) concluded that in complicated cases of acute bacterial meningitis, CRP levels showed either secondary elevation after an initial drop with treatment, or remained consistently high. Sutinen *et al.* (1998–1999) reported that CRP levels were elevated in all cases of bacterial meningitis, and that in the presence of a normal CRP concentration (below 10 mg/dL), a diagnosis of acute bacterial meningitis can be ruled out.

There was a significant reduction in serum CRP in the patients after receiving a course of antibiotics (17.59  $\pm$  3.60 mg/L) compared to pre-treatment levels (88.33  $\pm$  38.46 mg/L) (Table 2). However, the levels of CRP in patients with acute bacterial meningitis remained elevated compared to patients in the control group even after receiving the two week course of antibiotic therapy (Table 3). A reduction in CRP levels

**Table 2:** Comparison of measured parameters in children with meningitis before and after antibiotic therapy.

Parameters	Mean $\pm$ SD, (n=27)		P-value
	Before therapy	After Therapy	
Uric acid (mg/dL)	3.47 $\pm$ 0.54	3.50 $\pm$ 0.54	0.018
Serum ferritin (ng/mL)	95.93 $\pm$ 33.31	95.00 $\pm$ 32.43	0.096 (NS)
MDA ( $\mu$ mol/L)	1.91 $\pm$ 0.36	1.26 $\pm$ 0.18	<0.001
TAS (mmol/L)	1.13 $\pm$ 0.14	1.22 $\pm$ 0.11	<0.001
CRP (mg/L)	88.33 $\pm$ 38.46	17.59 $\pm$ 3.60	<0.001

Note: NS = Not significant using paired *t*-test

**Table 3:** Comparison of measured parameters between children with meningitis after therapy versus the control group.

Parameters	Mean $\pm$ SD		P-value
	Control (n = 30)	Patient group after therapy (n = 27)	
Uric acid (mg/dL)	4.17 $\pm$ 0.71	3.50 $\pm$ 0.54	0.018
Serum ferritin (ng/mL)	99.50 $\pm$ 32.04	95.00 $\pm$ 32.43	0.601 (NS)
MDA ( $\mu$ mol/L)	1.00 $\pm$ 0.21	1.26 $\pm$ 0.18	<0.001
TAS (mmol/L)	2.03 $\pm$ 0.25	1.22 $\pm$ 0.11	<0.001
CRP (mg/L)	9.93 $\pm$ 2.60	17.59 $\pm$ 3.60	<0.001

Note: NS = Not significant using unpaired *t*-test

would be expected with uneventful recovery, and CRP should eventually reach the levels seen in the control (i.e. 9.93  $\pm$  2.60 mg/L). In another study of oxidant/antioxidant activities in the CSF, Tsukahara *et al.* (2002) reported a several-fold increment in acrolein-lysine adducts (a marker of lipid peroxidation) in children during the early phase of bacterial meningitis, as compared with the CSF of children without meningitis and patients with aseptic meningitis.

In addition to the measurement of TAS, serum uric acid and ferritin (as an individual antioxidant parameter) (Jacob 1995) were also measured to identify any changes during acute bacterial meningitis. Our study revealed that mean serum uric acid levels were significantly lower in patients with acute bacterial meningitis (3.47  $\pm$  0.54 mg/dL) compared to controls (4.17  $\pm$  0.71 mg/dL) (Table 1). Serum uric acid levels showed a significant increase in patients after receiving antibiotic therapy (3.50  $\pm$  0.54 mg/dL) compared to pre-treatment values (3.47  $\pm$  0.54 mg/dL) (Table 2). There were also significant differences in the levels of serum uric acid in patients with acute bacterial meningitis after two-week

antibiotic treatment ( $3.50 \pm 0.54$  mg/dL) compared to controls ( $4.17 \pm 0.71$  mg/dL). Similarly, Nakazato *et al.* (1998) reported that serum urate levels were lower in patients with bacterial meningitis compared to controls, and the levels rose gradually upon improvement of meningitis with therapy. They suggested that such effects were due to the development of syndrome of inappropriate antidiuretic hormone secretion (SIADH).

The only study addressing ferritin levels in meningitis patients was that of Katnik (1995). He reported that CSF ferritin levels were higher in patients with proven meningitis, and these levels did not change after an average treatment of 15 days. We did not find any significant difference between mean serum ferritin levels of patients and controls. In children with meningitis, there was no significant difference in mean serum ferritin levels before and after therapy (Table 2). Even after two weeks of antibiotic therapy, there was no significant difference between mean serum ferritin levels in children with acute bacterial meningitis and those in controls. Variations in the levels of other antioxidants associated with meningitis and its treatment have been reported, and it appears that different antioxidants might be affected to different degrees. For example, serum vitamin E, beta carotene and retinol levels did not change (Caksen *et al.* 2004), while both uric acid and vitamin C were significantly reduced (Aycicek *et al.* 2006). In this study, we have tried to address this variability in antioxidant levels by measuring total antioxidant status.

Serum ferritin levels were not affected by meningitis and drug therapy. Although serum ferritin levels may be of no value in meningitis, the measurement of CSF ferritin levels is a potentially useful diagnostic test in such cases (Gray *et al.* 1986). On the other hand, serum ferritin levels were significantly elevated in cases of encephalitis for unknown reasons. A study conducted by Cunha, Sachdev and Cnario (2004) of West Nile Encephalitis reported a highly significant elevation of serum ferritin levels ( $\geq 500$  mg/mL) in such patients compared to patients with viral meningitis.

TAS was significantly increased in patients after receiving antibiotic therapy ( $1.22 \pm 0.11$  mmol/L) compared to pre-treatment values ( $1.13 \pm 0.14$  mmol/L) (Table 2). However, serum TAS levels were significantly lower in children with acute bacterial meningitis after therapy ( $1.22 \pm 0.11$  mmol/L) than in the control group ( $2.03 \pm 0.25$  mmol/L) (Table 3). This is in agreement with the observation of Aycicek *et al.* (2006), who reported that TAS levels were significantly lower in patients with meningitis and meningism compared to controls, indicating

that a considerable amount of serum TAS undergoes oxidation under these conditions. Only two studies have dealt with the assessment of TAS in patients with acute bacterial meningitis before and after therapy. The first study, which produced results that are in agreement with our findings, was conducted by Ray *et al.* (2000). They reported an increase in antioxidant enzymes and a decrease in MDA in patients with bacterial meningitis after two days of therapy. The second study was conducted by Aycicek *et al.* (2007), who reported that TAS levels were lower in patients with acute bacterial meningitis. These findings were similar to ours. However, unlike our study and that of Ray *et al.* (2000), Aicycek (2007) also reported that TAS levels in their patients did not change with therapy. This lack of change in TAS levels reported by Aicycek (2007) could be attributed to the type of drug treatment given to their patients. Their patients were given either ampicillin or ceftriaxone for 10 days with dexamethasone at 0.6 mg/kg/day during the first four days. In contrast, our patients were given ceftriaxone 2 g IV every 12 h for two weeks. This difference in drug therapy could have affected patient responses.

## CONCLUSION

These findings showed a dramatic rise in CRP levels in children with acute bacterial meningitis, which may reflect its importance as an aid in the diagnosis beside the clinical examination and CSF analysis. This study also showed that the serum oxidative/antioxidative balance shifted to the oxidative side in patients with meningitis before therapy and improved gradually after therapy.

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