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Estimation of Some Interleukins in Cerebrospinal Fluid in Children with Meningitis

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Meningitis is often associated with cerebral compromise which may be responsible for neurological squeal in nearly half of the survivors. Little is known about the mechanisms of CNS involvement in bacterial meningitis. the current study was to analyze the clinical significance of cerebrospinal fluid (CSF) concentrations of interleukin (IL)- 6, IL-1, IL-8, IL-10, tumor necrosis factor-alpha (TNF-a), and C-reactive protein in children with meningitis (n=35) and compared with control subjects (n=28). Serum total protein, interleukine-6, IL-1 , IL-8, IL-10 , and, CRP, and TNF-a levels were found to be significantly high in study group, whereas serum glucose IL-10 levels were significantly low comparing with control group. Positive correlation was observed between IL-6with glucose , CRP , and IL-8 , also between IL-10 with protein , TNF with IL-8 ,and IL-1 with CRP . whereas there was negative correlation between IL-6 with protein , TNF , IL-1 , IL-10 , also between IL-1 with IL-8 ,between IL-10 with, and TNF , IL-10 with glucose . More studies performed in suitable models of meningitis are required in order to establish the routine use of inflammatory markers in the diagnosis of infectious diseases of the central nervous system

Keywords: Interleukin; cerebrospinal fluids; meningitis.

Central nervous system(CNS) cytokine production may lead to protection or damage of neural cells, depending on the specific cytokine, timing of its production, and the amount produced. Production of pro-inflammatory cytokines by microglia can induce neuronal damage or death, but the effects of pro-inflammatory cytokines may be modulated by production of anti-inflammatory cytokines, or by other proinflammatory cytokines. Disruption of BBB in meningitis cytokines and chemokines could cross the BBB in an area of breakdown and affect brain tissue directly, and stimulate cytokine and chemokine production by microglial cells and astrocytes[1]. Cytokines are small polypeptides with a wide range of inflammatory ,metabolic and immunomodulatory properties[2,3]. There is different types of cytokines including interleukin. Studies have indicated the pathological role of interleukins in both peripheral and CNS diseases such as shock, meningitis and head injury[4].

The present study aimed at analyzing cytokine profiles(IL-6 , IL-1 , IL-10, IL-8),TNF-a, and CRP among patients with meningitis in comparison with healthy controls.

MATERIAL AND METHODS

A prospective hospital based study was done during a period of one year (from 1st.March / 2017 to 1st.April 2018). All patients from one month to 7.5 years admitted to the Salahalddin Teaching Hospital in Tikrit, and Pediatric Hospital in Kirkuk, Iraq with a presumptive diagnosis of meningitis were included in the study.

The fluid was withdrawn by lumbar puncture, using a spinal needle No.20).The patient lies on a hard bench, taking the lateral reclined position and the needle is gently placed above or beneath the forth lumber vertebra. The amount of withdrawn C.S.F is 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 4C°.Turbid specimens were centrifuged at 3000 rpm for 10 minutes before storage. The biochemical parameters such as IL-6 , IL-1 , IL-10, IL-8 , CRP, and TNF were measured by using the commercial enzyme-linked immunosorbent assay (ELISA) kits.

The C.S.F samples were divided into two groups. Normal control group , this group comprises 26 healthy individuals, and meningitis group which they were classified into four groups (N= 26 patients)

Statistical analysis

The statistical package for social science (SPSS version 21 for Windows® Microsoft, USA) was used for data analysis. Comparison between patients and controls was made using the t-test for quantitative data and ?2 -test for qualitative data. $P < 0.05$ was considered statistically significant.

RESULTS

The results of table(2)indicate the mean serum of Total protein, interleukine-6, IL-1 , IL-8, and, CRP, and TNF-a , IL-10 levels was higher in patients with (63.05 ± 8.08 g/l), (0.487 ± 0.184 ng/mL),(11.25 ± 1.10 pg/ml), (1.12 ±2.80 ng/mL), (5.532 ± 0.566 mg/dl) and (57±142 pg/ml), (70 ± 176) respectively than in controls (25.80 ±4.30 g/l), (0.1635±0.0340 ng/mL),(6.450 ± 0.887 pg/ml), (0.135 ± 0.339 ng/mL) (1.940 ± 0.227 mg/dl), and (5.8± 14.5 pg/ml) , (0.1635±0.0340), and (24.3 ± 60.7) respectively. Also the results show that there is a significant decrease in serum Glucose level in patients(33.1± 82.8 mg/dl)) comparing with control group (120 ± 300 mg/dl) .

Table 1. Demographic characteristic of study population

No. of patients	Control	Diagnosis Tuberculous meningitis	Bacterial meningitis	Viral meningitis	Partial treated meningitis
Male	15	4	6	8	1
Female	13	3	3	6	4
Total	28	7	9	14	5

Table 2. Biochemical parameters of patients and the controls.

Parameters	ControlMean ± SD	PatientsMean ± SD
Glucose (mg/dl)	120 ± 300	33.1 ± 82.8 *
Total protein (g/l)	25.80 ±4.30	63.05 ± 8.08 **
Interleukine-6(ng/mL)	0.1635±0.0340	0.487 ± 0.184**
Interleukine-1(pg/ml)	6.450 ± 0.887	11.25 ± 1.10**
CRP (mg/dl)	1.940 ± 0.227	5.532 ± 0.566 **
TNF (pg/ml)	5.8 ± 14.5	57 ± 142*
Interleukine-8 (ng/mL)	0.135 ± 0.339	1.12 ± 2.80 *
Interleukine-10 (pg/ml)	24.3 ± 60.7	70 ± 176 (NS)

Table 3. Correlation within Parameters

CHF Group (r)	Parameters
0.178	IL-6 with glucose
0.155	IL-6 with IL-8
-0.036	IL-10 with IL-6
0.131	IL-6 with CRP
-0.221	TNFwithIL-6
-0.209	IL-1 with IL-6
-0.402	IL-10 with glucose
0.265	IL-10 with protein
0.106	IL-1 with CRP
-0.176	IL-1 with IL-8
-0.404	TNFwith CRP
-0.110	IL-1 with IL-10
0.272	TNFwithIL-8
-0.123	TNFwithIL-1
-0.105	IL-8 with glucose

Correlation within Parameters

The results revealed that there was positive correlation between IL-6with glucose (0.178), CRP (0.131), and IL-8 (0.155), also between IL-10 with protein (0.265), TNF with IL-8 (0.272),and IL-1 with CRP (0.106). whereas there was negative correlation between IL-6 with protein (-0.049), TNF (-0.221), IL-1 (-0.209), IL-10 (-0.036), also between IL-1 with IL-8 (-0.176),between IL-10 with(-0.110), and TNF (-0.123).between IL-10 with glucose (-0.402), CRP (-0.065).

DISCUSSION

Interleukin-6 (IL-6) is a multifunctional inflammatory cytokine that plays an important role in the response to environmental stress and has been implicated in the pathogenesis of many chronic diseases. Interleukin-6 is a proinflammatory cytokine involved in the inflammatory response in the CNS. It is produced within CNS, i.a. by astrocytes and glial cells as a response to initiated or ongoing infection. Its production is also affected by other cytokines, i.a. TNF and IL-1. IL-6 has mainly proinflammatory properties. It persuades the synthesis of acute-phase proteins and contributes to BBB. IL-6 may act as an anti-inflammatory cytokine. It is validated by leukocyte transmission from blood to CSF[5-7]. The level of IL-6 is higher in patients with meningitis than control group. Our findings are in the line with[8,9] whereas disagreement with the results obtained by Pinto *et al*[10].

Interleukin-10 is an acid-labile anti-inflammatory cytokine produced by monocytes, macrophages, B and T lymphocytes, brain cells such as neurons and microglia that capable of inhibiting proinflammatory responses[11,12]. The level of IL-10 is higher in patients with meningitis ,These findings are in harmony with preceding reports[13,14]. who stated that initially high IL-10 concentration gradient across the blood-brain barrier, comparably low amounts of IL-10 were detected in serum in the acute phase of meningitis, suggesting an initial compartmentalized release of IL-10 in the subarachnoid space in patients presenting with meningitis without persistent circulatory impairment.

Interleukin-8 is a pro-inflammatory cytokine which acts as a chemoattractant for neutrophils to the site of inflammation[14]. Serum level of IL-8 was found to be significantly elevated in patients with meningitis, Because IL-8 contributes to leucocyte extravasation during infectious meningitis of viral and bacterial origin. The role of this molecule has been implicated in the blood–brain barrier disruption and meningeal infiltration by immune cells[15]. Similar observation was supported by[13,16].

Tumor necrosis factor alpha (TNF-a) and interleukin-1 (IL-1) a proinflammatory cytokine are present in the CSF which are produced in the subarachnoid space by different cells, e.g., leukocytes, astrocytes, and microglia., Therefore, TNF-a contributes to the accumulation of leukocytes in the CSF, brain edema , blood-brain barrier damage ,and damage of cells within the CNS[17]. TNF may leak from CSF to serum during meningitis. However, in spite of the marked leakage of protein, and the high concentration gradient of TNF across the blood-brain barrier, the negligible amounts of bioactive TNF leak from CSF to serum during meningitis. By leakage from the subarachnoid space to the systemic circulation there is a dilution factor of 10-20.

Interleukin-1 (IL-1) produced within tissues and has multifunctional cytokine contributes to local inflammatory reactions, primarily complicated in the regulation of inflammatory processes, it mediates stimulation of fibroblast growth, and differentiation of B and T cells[18,20].

Interleukin -1 contributes to disruption of the blood-brain barrier and CSF leukocyte recruitment and stimulates the production of other cytokines, such as IL-6 and TNF-a. The recruitment of polymorphonuclear leukocytes may induce damage not only to the bacteria but also to the brain[21]. Our analysis revealed statistically significant increase in the level of TNF-a and IL-1 in patients compared to the control group. This result is consistent with the other studies[6,21,22], whereas disagree with[23].

C-reactive protein is produced by the monocytes of the tissue factor which initiates the coagulation process. C-reactive protein, together with fibrinogen, acts as a chemotactic factor. Fibrinogen is responsible for the adhesion of macrophages to the endothelial surface for their migration into the intima[24]. Direct hepatic release of CRP in to plasma which then undergoes ultra-filtration to form CSF meningeal irritation stimulate CRP production. Once CRP enters the CSF it binds to the damaged tissues[25]. So that the increased CRP concentrations in patients with meningitis may stimulate production by the monocytes of the tissue factor which initiates the coagulation process(26). The findings of the present study concurs with earlier studies[27,28]. Corral *et al*. found positive CSF CRP in 24/32 patients with culture-proved bacterial meningitis, while only 2/32 children with non-bacterial meningitis had CSF which was positive for CRP. According to Corral *et al*., this was a more sensitive test for differentiating bacterial from non-bacterial[29].

CONCLUSION

More studies performed in suitable models of meningitis are needed in order to establish the routine use of inflammatory markers in the diagnosis of infectious diseases of the central nervous system.

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