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ROLE OF CEREBROSPINAL FLUID IL-8 AS A MARKER FOR DIFFERENTIATION BETWEEN ACUTE BACTERIAL AND ASEPTIC MENINGITIS

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Abstract

No doubt, the distinguishing between bacterial and aseptic meningitis in the emergency department could help to limit unnecessary antibiotic use and hospital admissions.

This study evaluated the role of cerebrospinal fluid IL-8 in differentiating acute bacterial meningitis (ABM) from aseptic meningitis (AM). A total of 80 hospitalized patients with clinical presentations of suspected acute meningitis were subjected to estimation of IL-8 CSF concentrations.

The results showed that CSF IL-8 levels were higher in acute bacterial meningitis than in aseptic ones ($p < 0.05$). The best cut-off value of CSF IL8 for early diagnosis of bacterial meningitis was 3.6ng/ml with a sensitivity of 82.5% and a specificity of 85.0%.

Key words: Egypt, Acute Bacterial, Aseptic Meningitis, Marker, Cerebrospinal Fluid Il-8

Introduction

Meningitis is inflammation of the protective membranes covering the brain and spinal cord, known collectively as the meninges. The inflammation may be caused by infection with viruses, bacteria, or other microorganisms, and less commonly by certain drugs (Ginsberg, 2004). Acute Bacterial meningitis is endemic in Egypt and sporadic cases occurring all over the year (Abdel Ghani *et al*, 2002).

The delay in the diagnosis and the consequent delay in initiation of treatment can cause death in about 10% of cases with advanced disease and severe neurological sequel as many as 80% of survivors (Van den Bos *et al*, 2005).It is important to distinguish bacterial meningitis from aseptic meningitis during the acute phase of the disease, as this could help to avoid complications and to limit unnecessary antibiotic use and hospital admissions (Tunkel *et al*, 2004). Clinical features of bacterial meningitis are nonspecific. Therefore, the discrimination of cases of bacterial meningitis from other causes by clinical feature alone is often impossible (Thwaites *et al*, 2005).

At the time being there was no clinical or laboratory method which can solely prove or disprove the diagnosis of acute bacterial or viral meningitis instantly and accurately. Interleukin 8 (IL-8) is a chemokine produced by macrophages and other cell types such as epithelial cells. It is also synthesized by endothelial cells, which store IL-8 in their storage vesicles, the Weibel-Palade bodies (Wolff *et al*, 1998).

The levels of the inflammatory cytokines, especially of IL-6 and IL-8 in the CSF of patients with bacterial meningitis (BM) were high up to 48 hours after initiation of treatment (Ruskoni *et al*, 1991) and a staging decline but not to normal limits (Tsangaropoulou-Stinga *et al*, 2003). These findings along with the observation that levels of the same cytokines in the serum are much lower, led

to the concept that the production of these cytokines is local as a result of an inflammatory process triggered by bacterial cell wall and membrane elements (Waage *et al*, 1989).

Masoud *et al*. (2013) stated that diagnostic procedures to predict the prognosis of acute meningitis are of paramount importance in order to choose the appropriate level of further surveillance. They concluded that the IgG-index was the only independent predictor for unfavourable outcome (GOS < 5) in patients especially with aseptic infection. The best cut off value of IgG index for early prediction of unfavorable outcome (GOS < 5) in bacterial meningitis group was > or = 6.75 with AUC of 0.922 and 95% CI of 0.769-1.07 and sensitivity of 75% and specificity of 93.7%. While, in aseptic meningitis infection was > or = 7.9 with AUC of 1 and 95% CI of 1.00-1.00 and sensitivity of 100% and specificity of 100%.

The aim of the present work was to evaluate of the diagnostic role of CSF IL8 in the differential diagnosis of acute meningitis (bacterial versus aseptic meningitis).

Patients, Materials and Methods

A total of 80 patients presented for the first time to Embaba Fever Hospital with clinical picture and CSF analysis suggestive of acute meningitis during the period from November 2012 to march 2013 were enrolled in this cross sectional study. They were divided into 2equal groups, based on CSF examination: acute bacterial and acute aseptic meningitis.

The patients with clinical and laboratory manifestations suggestive of tuberculous or cryptococcal meningitis, cerebro-vascular disease, brain tumours or febrile convulsion were excluded; also those patients who received antibiotic treatment more than 2 days were also excluded. Also, examination of urine by Nucleopore technique (Abo-Madyan *et al*, 2004) and stool by thick-smear technique (Katz *et al*, 1972) were carried out for endemic parasites The study aim were explained to patients who met the predesigned inclusion criteria and they were asked to sign a written informed consent form, and approvals of concerned authorities were obtained.

All patients were subjected to full medical history, thorough clinical examination and laboratory examination for CBC, ESR, CRP, random blood glucose and cerebrospinal (CSF) analysis on the first spinal tap including with assessment of: physical examination, protein and glucose and cell count including total and the differential leukocytic count, in addition to urine and stool analysis to rule out other parasitic infections. On the other hand, the Gram's stain done in all the patients.

The meningitis was defined as acute bacterial according to CSF laboratory findings increased protein >100mg/dl, decreased glucose <40mg/dl, and leukocyte count 100-5000/mm3 with polymorph nuclear leukocyte domination > 80%), identification of the bacterial agents in Gram staining, and/or positive bacterial culture (Razonable, 2011). Also, CSF/serum glucose ratio ≤0.4 is indicative of bacterial meningitis (Straus *et al,* 2006). On the other hand viral meningitis was defined so, if the viral culture, serological testing, pleocytosis, or reverse transcriptase polymerase chain reactions were positive and the bacterial culture was negative (Dubos *et al,* 2008).

The CSF IL8 was measured for all patients using the AviBion Human IL8 ELISA (Enzyme-Linked Immunosorbent Assay) Kits, which employs an antibody specific for human IL8.

The collected data were coded, tabulated, and statistically analysed using IBM Statistical package for Social Science (SPSS) statistics (V. 20.0, IBM Corp., USA, 2011). Data was presented and suitable analysis was done according to the type of data obtained for each parameter. Receiver operating characteristic curve (ROC) was computed, and the area under the ROC curve (AUROC) was used to evaluate the ability of IL-8 to discriminate bacterial meningitis from asep-

tic meningitis. Optimum cut-off was defined as the value that maximized the AUROC.

Results

The demographical characteristics, the results for routine CSF analysis and IL-8 assay results are shown in Table 1. CSF IL-8 concentrations were higher in the bacterial meningitis group with a significant difference when compared to the aseptic meningitis group ($p < 0.05$).

The IL-8 appeared as an excellent marker for differentiating ABM from aseptic meningitis with cut off value of CSF IL8 for early diagnosis of bacterial meningitis equal to 3.6ng/ml with a sensitivity of 82.5%, a specificity of 85.0%, a negative predictive value of 84.6%, a positive predictive value of 82.9% and a diagnostic efficacy of 83.8% (Fig. 1).

In both groups with patient characteristics, there was significant correlation between CSF IL8 and each of ESR & CSF cells in ABM group. In the aseptic group SCF IL8 was correlated significantly with CSF glucose, otherwise there was no statistical significant correlation found regarding the other parameters (Tab. 2).

Table 1: demographical characteristic, CSF findings and IL 8 concentration in both groups

Parameter	Acute bacterial meningitis (n=40)	Aseptic meningitis (n=40)	p-value	Sig.
Age (years)	35.18±17.62	35.18±17.62	0.769	NS
Male Sex (%)	42.5%	32.5%	0.356	NS
WBCs (cell/cmm)	1766.25±1918.36	215±181.20	0	HS
Polymorph %	86.83±10.73	22.68±20.64	0	HS
Lymphocytes %	13.18±10.73	76.58±21.20	0	HS
Protein	312.48±174.38	96.95±63.65	0	HS
CSF/ serum glucose	0.163±0.171	0.539±0.166	0	HS
CRP (N):0-6 mg/l	24±9.11	2.2±3.2	0	HS
ESR	44.53±34.28	20.15±20.38	0	HS
CSF IL8 (ng/ml)	6.156±2.9539	2.2595±1.73259	0	HS

Figure 1: ROC curve analysis showing diagnostic performance of CSF-IL8 for discriminating ABM from AM

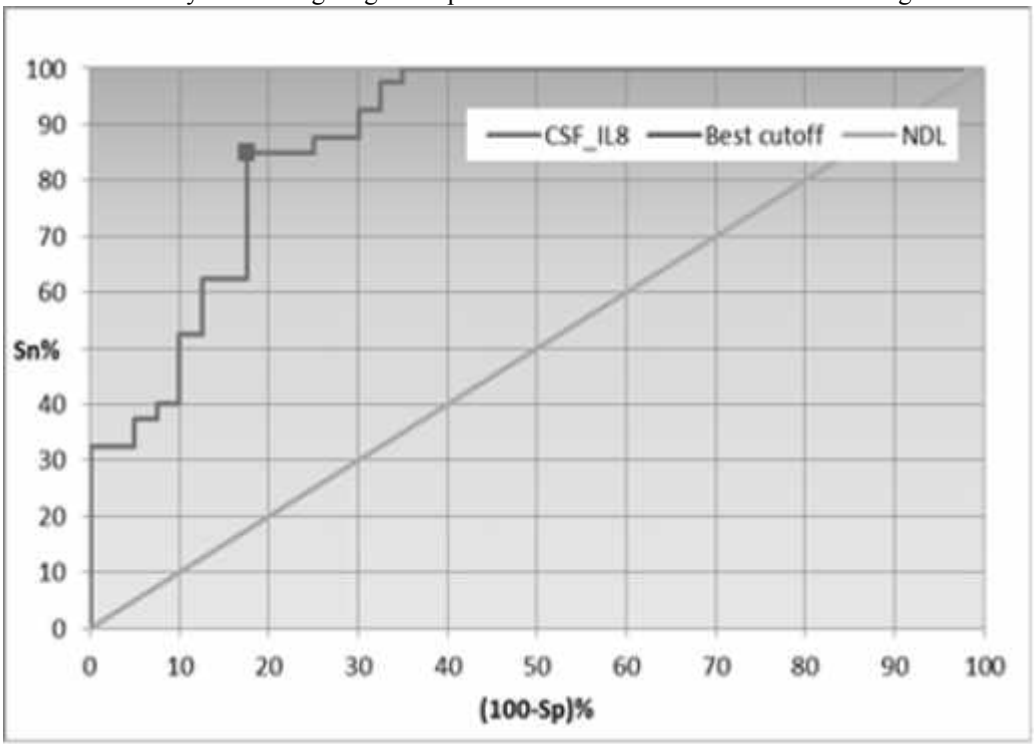

Table 2: Correlation between patients' characteristics and CSF IL8 among patients of both groups

Variable	CSF IL8 in ABM			CSF IL8 in AM		
	R value	P value	Sig	R value	p value	Sig.
CBC TLC (cell/ml)	-0.01	0.949	NS	0.308	0.53	NS
CBC neutrophil %	0.303	0.057	NS	0.308	.053	NS
CRP	-0.005	0.974	NS	-0.119	0.465	NS
ESR	0.459	0.003	HS	0.081	0.681	NS
CSF TLC	0.364	0.015	S	0.039	0.813	NS
CSF glucose	0.255	0.112	NS	-0.341	0,021	S
CSF protein	-0.203	0.208	NS	0.019	0.91	NS
serum/CSF glucose	-0.203	0.208	NS	-0.14	0.311	NS

Discussion

At the time being there was no clinical or laboratory method which can solely prove or disprove the diagnosis of acute bacterial or viral meningitis instantly and accurately. In this work we noted that the patients of the acute bacterial meningitis group had a higher value of CSF IL8 than those of the acute aseptic meningitis group with statistically significant difference between them. This result is in accordance with the similar studies (Ostergarrd *et al*, 1996; Yilmaz *et al*, 2012).

In the present study, the best cut-off value of CSF IL8 for early acute bacterial meningitis was 3.6ng/ml with a sensitivity of 82.5 %, specificity of 85 %, negative predictive value of 84.6%, a positive predictive value of 82.9 % and a diagnostic efficacy of 83.9 %.Comparably, Ostergarrd *et al.* (1996) reported sensitivity value of 81%, while, Printo-Geniour *et al.* (2011) reported high sensitivity (100%) for CSF IL8 in differentiation between bacterial and aseptic meningitis. It is important to note that cut-off values for IL-8 may vary depending on variations of the methodology or reagents used, and before standardized tests are available one must determine the specific cut-off value under the conditions employed to ensure the best accuracy (PrintoGeniour *et al*, 2011).

Also, this may be due to false low levels obtained due to estimation of IL8 in early course of bacterial infection or using variable cut off points of CSF IL8 (Somasundaram *et al*, 2013).

In this study, the patients with acute bacterial meningitis had higher values of C reactive protein and serum leukocytes and polymorphs than those with aseptic ones with significant difference between them. but no significant difference in haemoglobin concentration, This result agreed with those of El-Kapany (2011) and Ibrahim *et al.* (2011), Arun and Sunit (2006) who stated that there was no significant difference in haemoglobin concentration and Viallon et *al.* (2011) and Alkholi *et al.* (2011) who revealed a highly statistical significant difference in CRP between patients with bacterial meningitis and those with aseptic meningitis This may be explained as serum C reactive protein and ESR are reactive proteins activated by acute inflammatory process or acute bacterial infection. Makoo *et al.* (2010) reported that there was no significant difference in CRP between patients with bacterial and aseptic meningitis.

This might be explained as the initial CRP level can occasionally be low in bacterial disease, especially in the early stages. CRP concentrations begin to rise between 6 and 12 hr. and reach a peak level only at 24 to 48 hr. (Mary *et al*, 2003).

In the present study, in septic cases there was significant correlation between CSF IL8 and leukocytes, and a highly significant correlation between CSF IL8 and ESR). While, there was no correlation between CSF IL8 and other parameters as C reactive protein, CSF glucose, CSF protein, CSF/serum glucose ratio, age, duration and GCS. Regarding aseptic cases,

there was a significant correlation between CSF IL8 and CSF glucose ($P<0.05$), without correlation between CSF IL8 and other parameters.

These results agreed with Ostergarrd *et al.* (1996), but disagreed with Ostergarrd *et al.* (1996) reported correlation between CSF IL8 level and each of CSF protein and CSF/serum glucose ratio.

The differential diagnosis between aseptic and bacterial meningitis in some instances can be difficult. A trust worthy laboratory marker would facilitate the clinical decision of interrupting antimicrobial therapy and avoiding unnecessary hospitalization. CSF IL-8 concentrations above 3.6ng/mL would indicate bacterial meningitis confirming other clinical and laboratory findings. 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.

Conclusion

The outcome results showed that the CSF concentration of IL-8 increased in the presence of meningeal inflammation. Consequently, the CSF IL8 could be an important tool to differentiate the acute bacterial meningitis from the aseptic meningitis.

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