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ASSESSMENT OF THE DIAGNOSTIC AND PROGNOSTIC ROLE OF CEREBEROSPINAL FLUID INTERLEUKIN-8 LEVEL IN ADULT PATIENTS WITH MENINGITIS

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Abstract

Meningitis necessitates immediate diagnosis and therapy. It is important to distingu- ish bacterial from aseptic meningitis, as this help to avoid complications and unnece- ssary antibiotic use. This work assessed the diagnostic and prognostic role of cerebro-spinal fluid interleukin-8 (IL-8) level in adult patients with meningitis.

Ninety adult patients with meningitis were studied. They were divided into 3 groups: bacterial, tuberculous and aseptic meningitis. Full clinical examination and laboratory workup of meningitis were done. Cerebrospinal fluid (CSF) IL-8 levels were assessed. Patients were followed up till discharge or death.

CSF IL-8 level was significantly higher in bacterial and tuberculous meningitis in comparison to aseptic meningitis. At cut off value 121.77 pg/ml, the area under ROC curve was 0.774 with efficacy 69% for differentiating viral from non-viral meningitis. The test efficacy is low in differentiating tuberculous from bacterial meningitis. There is no correlation of CSF IL-8 levels and disease severity or prognosis.

Keywords: Bacterial meningitis, Tuberculous meningitis, Aseptic meningitis, IL-8.

Introduction

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). Microbiological culture of the cerebrospinal fluid (CSF) is the most sensitive method for diagnosis but results can take up to 48 hours to become available (Graeff-Teixeira *et al,* 2009). In tuberculous meningitis acid fast bacilli seen in only 10-87% of patients and results of culture are only available after 2-6 weeks of incubation (Puccioni-Sohler and Brandão, 2007).

In practice, before definitive CSF bacterial cultures were available, most patients with acute meningitis are treated with broad spectrum antibiotics targeting bacterial meningitis. In general, this does not seriously harm the aseptic meningitis patient; however, it may enhance the local frequency of antibiotic resistance, and cause antibiotic adverse effects, nosocomial infections, and high medical costs (Parasura-man *et al,* 2001). Thus, it was not only important to recognize bacterial meningitis patients who promptly need antimicrobial therapy but also aseptic meningitis patients who do not need antibiotics and/or hospital stays (Huy *et al,* 2010). Extensive research has shown that the choroid plexus plays multiple roles during infectious diseases of the central nervous system (CNS). These roles include the function as an entry site of the pathogens into the CNS as well as active participation by producing cytokines and chemokines. These molecules serve both to attract other inflammatory effector cells and to activate those (Schwerk *et al,* 2015).

This study aimed to evaluate the role of IL-8 in CSF in diagnosis of bacterial, tuberculous and aseptic meningitis compared to the standard techniques and to evaluate the role of IL-8 level in CSF as a predictor of the prognosis in those patients.

Patients, Materials and Methods

The present study was carried out on ninety adult patients diagnosed with meningitis at Abbasia fever hospital, from January 2014 to December 2014. Informed consent was obtained from all the patients included in the study. The collected data and samples were kept in confidentiality and patient's privacy was considered.

The inclusion criteria were picture suggestive of meningitis, in bacterial meningitis were positive culture or positive gram staining of cerebrospinal fluid or dominance of polymorphonuclear cells, low sugar and high protein in cerebrospinal fluid. TB meningitis was diagnosed with positive Ziehl-Neelsen stain and/or culture. Aseptic meningitis was diagnosed by the presence of more than five leukocytes in cubic millimeter of cerebrospinal fluid with the dominance of mononuclear cells, almost normal sugar and protein in cerebrospinal fluid and negative gram staining and culture of cerebrospinal fluid (David, 2005).

According to the final diagnosis patients were divided into 3 groups G1: patients with bacterial meningitis. G2: patients with tuberculous meningitis. G3: patients with aseptic meningitis. Patients with clinical picture suggestive of cerebro-vascular disease, brain tumors or other neurological insults, other causes of coma (drug-induced meningeal irritation as intravenous immuno-globulins, azathioprine, and methotrexate) were excluded from the study.

All patients were subjected to full history taking and clinical examination with stress on presence of headache, fever, neck stiffness and altered mental status, seizures on admission and full neurological examination. Duration of hospital stay, ICU days, mortality and complications were recorded. Complete blood picture, ESR, random blood sugar, liver function tests, renal function tests and electrolytes were done for all the included patients. CT and MRI brain were done whenever possible.

CSF is obtained by a sterile lumbar puncture needle under complete aseptic conditions. CSF analysis including: Cell counts, differential count, cytology, glucose and protein levels, Ziehl-Neelsen stain and Gram stain. CSF specimens were cultured on blood agar and chocolate agar media, suspected cases of *Mycobacterium tuberculosis* were cultured on Lowenstein Jensen medium. The isolated organisms were worked up microbiologically and identified by conventional methods (Cheesebrough, 2007).

Measurement of the CSF IL-8 by ELISA technique was done for all cases: The AviBion Human IL-8 ELISA (Enzyme-Linked Immunosorbent Assay) kit is an in vitro enzyme-linked immunosorbent assay for the quantitative measurement of human IL-8 in serum, plasma, cell culture supernatants and urine. This assay employs an antibody specific for human IL-8 coated on a 96-well plate. Standards, samples and biotinylated anti-human IL-8 antibody are pipetted into the wells and IL-8 present in a sample is captured by the immobilized antibody and by biotinylated IL-8 specific detection antibody. After washing away unbound biotinylated antibody, Horse radish peroxidase (HRP)-conjugated streptavidin is pipetted to the wells. The wells are again washed, a Tetramethylbenzidine (TMB) substrate solution is added to the wells and color develops in proportion to the amount of IL-8 bound. The Stop solution changes the color from blue to yellow, and the intensity of the color is measured at 450 nm (AviBion Human IL-8 ELISA Kit Protocol).

Ethical approval: All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Results

The results are shown in tables (1, 2, 3, 4, 5 & 6) and figure (1).

The demographic data of the patients showed no difference as regard to age and sex distribution in groups (Tab.1).

Table 1: Demographic data of studied patients

Variables	Bacterial meningitis (49 patients)		Viral meningitis (30 patients)		Tuberculous meningitis (11 patients)		*t*	*p*
Age (years)	46.8±9.5		42.7±10.06		45.6±6.4		1.43	0.052
	No.	%	No.	%	No.	%	x^2	p
Male	27	55.1	18	60	7	63.63	0.36	0.84
Female	22	44.9	12	40	4	36.4		
Smoking	20	40.82	12	40	4	36.36	0.45	0.75
IV drug addiction	9	18.37	7	23.33	3	27.27		
Other forms of drug addiction	4	8.16	3	10	2	18.18		
DM	3	6.12	1	3.33	1	9.09	0.92	0.63
HTN	9	18.37	1	3.33	1	9.09	5.82	0.054

In bacterial meningitis group, *Streptococcus pneumoniae* was detected in 35 patients (71.43%), *Neisseria meningitidis* in 10 patients (20.41%) & *Haemophilus influenzae* in 4 patients (8.16%).

Clinical pictures of patients showed fever, skin rash, convulsions and photophobia are significantly higher in patients with bacterial and tuberculous meningitis in comparison to aseptic meningitis ($p<0.05$). Signs of meningeal irritation are significantly higher in bacterial and tuberculous meningitis than in aseptic meningitis. There was longer duration of hospital stay in bacterial and tuberculous meningitis patients in comparison to viral meningitis patients. The patients with aseptic meningitis had significantly better outcome in comparison to patients with bacterial and tuberculous meningitis (Tab. 2)

Table 2: Clinical data of patients

		Bacterial meningitis (49 patients)		Viral meningitis (30 patients)		TB meningitis (11 patients)		x^2	p
		No.	%	No.	%	No.	%		
Fever		46	91.8%	29	90%	11	100%	0.92	0.63
Headache		30	61.2%	20	66.6%	8	72.7%	0.96	0.79
DCL (GCS<15)		45	91.8%	27	90%	11	100%	0.93	0.69
Vomiting		47	95.9%	27	90%	6	54.5%	0.95	0.75
Convulsions		40	81.6%	4	13.3%	5	45.5%	35.41	*0.000
Neck rigidity		47	95.9%	24	80%	8	72.7%	7.041	*0.03
Kerning's sign		44	89.8%	9	30%	4	36.4%	32.58	*0.000
Brudzinski's sign		44	89.8%	9	30%	4	36.4%	32.58	*0.000
Photophobia		31	63.3%	6	20%	10	90.9%	21.48	*0.000
Disease outcome	Complete recovery	34	71.43%	30	100%	7	63.4%	11.939	*0.02
	Post complication	2	12.24%	----	0	1	9.09%		
	Death	13	16.33%	---	0	3	27.27%		
Temperature		Mean	SD	mean	SD	Mean	SD	F	*<0.001
		39.2	0.32	38.5	0.26	39	0	59.63	
Hospital stay (days)		median	range	median	range	median	range	H	*<0.001
		11	3-26	7	6-7	10	7-15	41.42	

DCL: deteriorated consciousness level,

GCS: Glasgow coma score: As expected results of CSF analysis showed high statistical difference between groups, with higher CSF WBCs (median= 2564), higher CSF protein (median= 400) & lower CSF glucose (median= 12) in bacterial and tuberculous meningitis compared to viral meningitis ($p<0.01$).

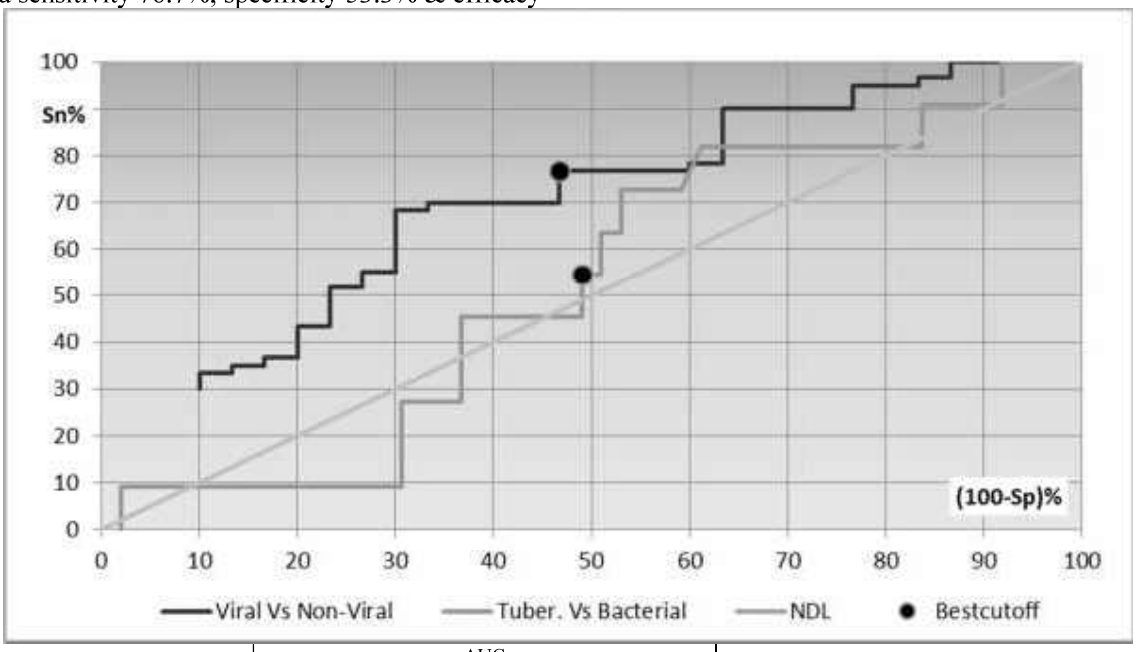
CSF IL-8 level in the studied patients were presented in table 3. CSF IL-8 level was significantly higher in patients with bacterial and tuberculous meningitis in comparison to viral meningitis ($P<0.05$).

Table 3: CSF IL-8 in groups:

	Bacterial meningitis		Viral meningitis		z	p	TB meningitis		Z^1	P^1	Z^2	P^2
	Median	Range	Median	range			median	range				
CSF IL-8 (pg/ml)	145.77	47.03-219.83	120.88	23.87-209.2	-2.69	0.007	142.1	84.76-21902	-2.15	0.032	-0.23	0.82

Z^1tuberculous vs. viral, Z^2 tuberculous vs. bacterial, N.B. Standard concentration of CSF IL-8 (0-125 pg/ml)

ROC curve assessed diagnostic performance of CSF IL-8 levels and its role to differentiate etiologies of meningitis (Fig. 1). At cut off value 121.77pg/ml area under ROC curve was 0.774 to differentiate viral from non-viral meningitis with a sensitivity 76.7%, specificity 53.3% & efficacy 86.9%. At cut off value 154.77 pg/ml area under ROC curve was 0.613 to differentiate tuberculous from bacterial meningitis with a sensitivity 54.5%, specificity 51% and efficacy 51.7% (Tab. 4).



AUC	
Viral Vs Non-Viral	0.774
Tuberculous Vs bacterial	0.613

Fig, 1: ROC curve analysis showing diagnostic performance of CSF-IL8

Table 4: Diagnostic validity test of CSF IL-8.

Study group/Validity test	Viral versus non-viral meningitis	Tuberculous versus bacterial meningitis
Cut off	121.77	145.77
Sensitivity	76.7%	54.5%
Specificity	53.3%	51%
Negative predictive value	53.3%	83.3%
Positive predictive value	76.6%	20%
Efficacy	68.9%	51.7%

Comparing IL-8 CSF levels in patients with complete recovery and those with complication or mortality without significant difference (p>0.05) (Tab. 5).

Table 5: Comparison of CSF IL-8 levels in patients in relation to disease outcome.

	CSF IL-8 (pg/ml) (Median/ range)			Z	P value
	Complete recovery	Post infectious complications	Death		
Bacterial meningitis	148.83/47.03-219.83	141.62/ 52.41-193.42	140.82/ 52.41- 187.53	-0.32	0.74
Tuberculous meningitis	143.67/84.76– 165.8	153.5	156.96/155.32-219.22	-0.32	0.75

No correlation of CSF IL-8 level and indicators of disease severity as Hospital stay; ICU admission or deteriorated consciousness (Tab.6).

Table 6: Correlation between IL-8 and different variables among groups

CSF IL-8 / Variable	Bacterial meningitis		Viral meningitis		TB meningitis	
	r	p	r	p	r	p
Age	-0.085	>0.05	0.09	>0.05	0.54	>0.05
DCL (GCS<15)	0.016	>0.05	-0.09	>0.05	-0.03	>0.05
Hospital stay	0.05	>0.05	0.078	>0.05	-0.42	>0.05
Duration of antibiotic use	0.054	>0.05	0.18	>0.05	0.47	>0.05
ICU admission	0.05	>0.05	-	-	-0.775	>0.05

Discussion

In the present study, the median CSF IL-8 levels were significantly higher in patients with bacterial meningitis (145.77pg/ml) & TB meningitis (142.1pg/ml) than in patients with aseptic meningitis (120.88pg/ml).

These results agreed with many authors who reported that higher concentrations of IL-8 were found in the CSF of patients with bacterial meningitis in comparison to aseptic meningitis (Gerber and Nau, 2010; Makis *et al,* 2010; Pinto Junior *et al,* 2011; Bociąga-Jasik *et al,* 2012; Abdelmoez *et al,* 2014). A study done on 57 children with bacterial meningitis and 15 children with viral meningitis concluded the same result (Prasad *et al.,* 2014).

In the present work, CSF IL-8 level at a cutoff value of 121.77pg/ml was useful in distinguishing viral from non-viral meningitis as area under ROC curve was 0.774 with a sensitivity of 76.7% and specificity of 53.3%, positive predictive value 76.6% and negative predictive value 53.3%.

In the present study, to differentiate TB from bacterial meningitis, CSF IL-8 level at a cutoff value of 145.77pg/ml showed area under ROC curve 0.613 with a sensitivity of 54.5% and specificity of 51%, PPV 20% and NPV 83.3%. Others evaluated the role of CSF IL-8 level in differentiating bacterial from aseptic meningitis with different cut off values. Abdelmoez *et al.* (2014) in Egypt studied 80 patients (40 with bacterial meningitis & 40 with aseptic meningitis). They concluded that CSF IL-8 cut off value of 3.6ng/ml distinguished bacterial from aseptic meningitis with a sensitivity of 82.5% and a specificity of 85%. El-Bahnasawy *et al*. (2016) in Egypt stated that meningitis can be life-threatening because of inflammation's proximity to the brain and spinal cord; therefore, the condition is classified as a medical emergency. The commonest symptoms of meningitis are headache and neck stiffness associated with fever, confusion or altered consciousness, vomiting, and an inability to tolerate light (photophobia) or loud noises (phonophobia). Children often exhibit only nonspecific symptoms, such as irritability and drowsiness. If a rash is present, it may indicate a particular cause of meningitis; for instance, meningitis caused by meningococcal bacteria may be accompanied by a characteristic rash. A broad variety of allergic, infectious, neoplastic, and idiopathic diseases were associated with increased blood and/or tissue eosinophilia and range in severity from self-limited conditions to life-threatening disorders. Although accepted upper limits of normal blood eosinophil varied, above 600 eosinophils/microL of blood was abnormal in vast majority of cases.

Also, Bociąga-Jasik *et al*. (2012) in Poland found CSF IL-8 cut off value 773.5pg/ml helped in diagnosing bacterial from aseptic meningitis. Other studies revealed different CSF IL-8 cut off values 4000ng/ml (Kleine *et al*, 2003), 1.685ng/ml (Pinto Junior *et al*, 2011), 1.14pg/ml (Chen *et al*, 2012) and 75 pg/ml (Prasad *et al,* 2014), These variable cut off values might be attributed to different essay techniques for measuring CSF IL-8 levels, different countries with variable incidence of endemic diseases that might affect cytokines levels, and small sample size in some studies (Pinto junior's study included only 9 cases of bacterial meningitis). Meta-analysis studied the efficacy of CSF IL-8 in diagnosis of bacterial meningitis reported that area under Roc curve was 0.95 & diagnostic odd ratio was 154.25 providing a high

discriminatory ability of CSF IL-8 levels (Yao *et al,* 2015).

In the present study, CSF IL-8 levels were not correlated with disease severity or outcome. This contradicted Prasad *et al.* (2014) who reported that CSF IL-8 levels were higher in non survivors suggesting it might be related to prognosis.

Conflict of interest: None

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Conclusion

The CSF IL-8 levels can be used as a rapid method for differentiating bacterial and tuberculous from aseptic meningitis in association with traditional methods to limit unnecessary antibiotic use and hospital admissions. It is not related to prognosis or severity of disease.

Further study to confirm the cut off value of CSF IL-8 in the diagnosis of meningitis to confirm either its 'role in predicting the prognosis or not is ongoing and will be published in due time.

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