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Level of Interleukin 8 and Alpha -1 Antitrypsin in CerebroSpinal Fuid of Iraqi Patient with Bacterial and Viral Meniningitis

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Abstract:
Objective*: Meningitis refers to inflammation of the leptomeninges, the connective tissue layers in closest proximity to the surface of the brain. Meningitis can be caused by bacteria, viruses, parasites, and fungi, as well as by non-infectious conditions, including inflammatory disorders. Interleuk-8 (IL-8) is produced predominantly by monocytes , but also by fibroblasts and endothelial cells . This chemokine may play a role in various inflammatory and infectious diseases. Alpha 1-antitrypsin is also referred to as alpha-1 proteinase inhibitor (A1PI) because it inhibits a wide variety of proteases. It protects tissues from enzymes of inflammatory cells, especially neutrophil elastase .*
Methods: *Forty seven patients with meningitis disease admitted to Medical city hospital and Al- Kadimia Hospital between June –October /2012 , Their age ranged between (11 days to 6.0 years).These patients were divided into group infected with viral and bacterial infections according to the laboratory finding of cells and biochemical tests and microbiology examination .A quantitative sandwich enzyme immunoassay technique was performed . Antibody specific for IL-8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-8 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IL-8 is added.*
Result*: IL-8 concentrations were higher in both bacterial and viral meningitis demonstrating that this molecule may acts as a mediator in meningial inflammation. Alpha -1 Antitrypsin (A1AT) play a major role in laboratory investigations to differentiating viral and bacterial meningitis along with CSF cell count .The level of A1AT in the bacterial meningitis was higher than the level of viral meningitis .*
Keyword*: Interleukin -8 (IL-8) , Alpha -1 Antitrypsin (A1AT).*

I. Introduction

Meningitis, since described first in the year 1805, has been one of the major lethal infectious diseases especially for the neonates and adult people in developing countries (1). Meningitis refers to inflammation of the leptomeninges, the connective tissue layers in closest proximity to the surface of the brain. Meningitis can be caused by bacteria, viruses, parasites, and fungi, as well as by non-infectious conditions, including inflammatory disorders (systemic lupus erythematosus or Kawasaki disease) and neoplasia (leukemic meningitis). (2)

It is not always possible to distinguish between bacterial and viral meningitis according to cerebrospinal fluid (CSF) findings, which leads to unnecessary antibiotic usage.(1) Analysis of cerebrospinal fluid (CSF) remains the key to diagnosis (3) .Most cytokines have multiple cellular sources and targets, and share functions to modulate the levels and effects of each other. (4)

Most humoral factors considered to play a part in the pathogenesis of vasospasms in subarachnoidal haemorrhage-for example, erythrocyte or platetet derived products-are absent in bacterial meningitis. The hallmark of bacterial meningitis is a massive leucocytes infiltration into the perivascular spaces and the CSF (5). These cells act through synthesis of pro-inflammatory cytokines (interleukin-l β (IL-1 β), interleukin-6 (IL-6), and tumour necrosis factor-α (TNF-α), which orchestrate the local and systemic aspects of host response to infection and tissue damage (6). Huge amounts of these cytokines have recently been detected in CSF in bacterial meningitis (7).

Interleukin-8 (IL-8) is produced predominantly by monocytes , but also by fibroblasts and endothelial cells . A variety of cytokine play critical roles in local inflammatory responses in bacterial and aseptic meningitis (8,9) . Previous investigations showed that level of the following cytokines increase in cerebrospinal fluid (CSF) of aseptic meningitis : IL-1, IL-6, Interleukin -10(IL-10) ,interferon-gamma , macrophage inflammatory protein-1 alpha and colony –stimulating factors , but not tumor necrosis factor-α (TNF-α) .Aseptic meningitis is characterized by an initial accumulation of neutrophils in CSF followed by an elevation of mononuclear cells (10,11). .Elevated levels of the cytokines, tumor necrosis factor-alpha interleukin -1, and interleukin -6 have previously been detected in cerebrospinal fluid (CSF) of patients with meningitis (12, 13).

Previous studies have shown elevated IL-8 levels in the CSF in meningitis, with the elevation being greater for bacterial meningitis than aseptic meningitis. (14) But results in aseptic meningitis have been variable; IL-8 levels in the CSF were higher than, or similar to those in subjects without meningitis (15) In the CSF

during the course of meningitis, little is known about IL-8 kinetics. Alpha-1Antitrypsin or α_1-antitrypsin (A1AT) is a protease inhibitor belonging to the serpin super family. It is generally known as serum trypsin inhibitor.(16) It protects tissues from enzymes of inflammatory cells, especially neutrophil elastase (17) .

II. Methods

Forty seven patients with meningitis disease admitted to Medical city hospital and Al- Kadimia Hospital between June –October /2012 , Their age ranged between (11 days to 6.0 years) .These patients were divided into group infected with viral and bacterial infections according to the laboratory finding of cells and biochemical tests and microbiology examination .A questionnaire sheet was completed for each patient including personal data (age , sex) and medical history also consent was taken from each patient (fever , headache , nausea) .

Twenty apparently healthy subjects have been included in this study as a control group, who matched the patients study group in age and gender (14 males and 6 females) . Cerebrospinal fluid finding of control subjects were normal, these cases were the patients whose clinical finding indicted the need for lumber puncture to exclude the diagnosis of meningitis.

This assay employs the quantitative sandwich enzyme immunoassay technique. Antibody specific for IL-8 has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IL-8 present is bound by the immobilized antibody. After removing any unbound substances, a biotin-conjugated antibody specific for IL-8 is added to the wells. After washing, avidin conjugated Horseradish Peroxidase (HRP) is added to the wells. Following a wash to remove any unbound avidin-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IL-8 bound in the initial step. The color development is stopped and the intensity of the color is measured.

In single immunodiffution, either antigen and antibody remains fixed or the other reactant is allowed to move and complex with it . Radial immunodiffussion is based on the principle that a quntitative relationship exists between the amounts of antigen placed in a well cut in the agar. Antibody plate and the resulting area circumscribed by the precipitation ring was proportionate to antigen concentration. This end point method requires that the precipitation rings reach the maximal possible size , which often requires 48-72 hours of diffusion as shown in fig (3-4) .(19)

-Statistical analysis

1. ANOVA test
2. Multiple comparisons by LSD
3. Person correlation

All the statistical analysis was done by using pantium 4 computer through the SPSS version 15 and excels application.

III. Results And Discussion

The research with cerebrospinal fluid samples from meningitis patients, 26 had bacterial meningitis, twenty one had viral meningitis and twenty apparently healthy individual (control). Meningitis was diagnosed according to evaluation of history , physical examinations, CSF laboratory finding.The control group was defined by an absence of inflammatory cells in CSF cell count $< 5 \times 10^6$ cell/ l and sterile for bacteriologic and virologic findings .

Of the 47 cases with meningitis , 27 (57.44%) were males and 20 (42.56%) were females , The mean age of the patients was (18±8.0%) , there was difference between bacterial and viral meningitis group in respect to mean age $p < 0.005$ .Clinical characteristic and laboratory findings in both groups are summarized in Table (4-1) .

Table (4-1) :Clinical and prevalence features of all groups

Study group / Variable	Bacterial group No = 26	Viral group No =21	Control group No =20
Age range (months)	(0.40 – 72)	(0.50 -72)	(6 -120)
Mean ± SD	21.25±11.5	15,34±4.5	45.02±30.0
Gender NO (%)			
Female	8 (30.8%)	12(57.1%)	7 (35 %)
Male	18(69.2%)	9 (42.9%)	13 (65 %)
Clinical finding *			
Fever	93.1%	74%	56%
Nausea or vomiting	67 %	69%	71%
Headache	20%	33%	36%

*Percentages do not add to 100% since multiple of clinical manifestations can be found

Generally, this study showed the demographics features which indicated to prevalence of meningitis among males was (57.44%) but not significant. This frequency is comparable to some extent with that of local previous studies in Iraq (20) and that for Turkiye families (65.9%) (21).

Cerebrospinal fluid finding of patients with bacterial and viral meningitis and of the control groups are presented in Table (4-2). CSF neutrophil (438.2308 cell/ mm^3) and lymphocyte (1538.6250 cell /mm^3) of bacterial meningitis were higher than in viral meningitis group for neutrophil and lymphocyte (6.000 cell/ mm^3) and (55.8571 cell/ mm^3) respectively with significant difference at ($P < 0.05$). Results of mean CSF protein of bacterial meningitis patients was (170.8846 mg / l) higher than in viral meningitis patients and control groups (40. 2857 mg / l and 24.5455 mg / l) consecutively at ($P <0.005$) , but there was no significant differences among studied groups regard to CSF glucose concentration at ($P >0.005$) .

Table (4-2) : The laboratory findings of studied groups

Study group / Variable	Bacterial meningitis NO=26	Viral meningitis NO=21	Control group NO=20	P- value
CSF Neutrophil count (cells /mm^3)	438.2308	6.0000	1	(S) 0.039
CSF Lymphocyte count (cells /mm^3)	1538.6250	55.8571	1.3333	(S) 0.045
CSF Protein (mg / l)	170.8846	40.2857	24.5455	H.S (0.001)
CSF Glucose (mg/dL)	42.6923	39.1429	46.5000	N.S (0.6)

The typical CSF findings may not present in every patient who has bacterial meningitis and may even show a normal WBC in the CSF and/or lymphocyte predominance, especially if early in the disease (2) .Occasionally, if the CSF is examined in the first 12 to 24 hours of the viral meningitis, a transient predominance of neutrophils can be seen, which converts to a lymphocytic predominance the next day. (22,23)

Protein level in CSF in bacterial meningitis patients was higher than viral meningitis and control groups with significantly differential at $p = 0.001$, present result corresponding with other previous study.(24) while disagree with study of Togas M. PE , *et al.* 2004 , protein level in CSF may vary depending on age , number of cell , etiology , and damaged cells . High protein level may be found in either bacterial meningitis or other conditions. (25)

The CSF glucose concentration in bacterial meningitis patient was lower than the glucose level in both viral and control group since the glucose concentration was <40mg /ml in approximately 50%-60% of patients of bacterial meningitis (87), although viral meningitis usually has normal glucose level , a few viruses (mumps and lymphocytic chorio-meningitis) may produce a mildly depressed CSF glucose level , typically 30 to 40 mg / dl (23).

The Cerebrospinal fluid level of IL-8 was assessed in three study groups. As shown in table (4-3) and Fig (4-1) , the mean CSF IL-8 concentration in bacterial meningitis's patients (825,3250 pg / ml) was significantly higher than both viral meningitis (142,8143 pg / ml) and control group(56,3409 pg / ml) while CSF IL-8 of viral meningitis group was higher than that for control group but with no significant differences .

Table (4- 3) : Cerebrospinal fluid levels of IL-8 in studied group

Study group / IL-8	Bacterial group No=26	Viral group No = 21	Patients group NO=47	Control group No = 20
Range (pg/ ml)	(1.38-2566.80)	(20-554.10)	1.38-2566.80	(22-130.841)
Mean ±SD	825.3250±674.37109	142.8143±188.3388	484.0695	56.3409±130.84145
P- value	Bacterial * viral = 0.002 S* Bacterial * Normal =0.00 H.S* Viral * Normal = 0.679 N.S*			

S* =Significant differences at P< 0,05 level
H.S*=High significant differences at P< 0.01 level
N.S* =Non significant differences

The present study , showed that IL-8 concentrations were higher in both bacterial and viral meningitis groups demonstrating that this molecule may acts as a mediator in meningial inflammation , this is in agreement with other studies which also compared the levels of IL-8 in cases of bacterial and viral meningitis , and studies revealed that in control CSF (without inflammatory central nerves system disease) , little if any IL-8 was detectable , but during both bacterial (26,27) and viral meningitis(28) , significant up regulation of IL-8 has been demonstrated .

Interleukin-8 is produced in monocytes and vascular endothelial cells in response to stimulation with bacteria or lipopolysaccharides, and is released from these cells into blood stream or tissue fluid.(29) Not only TNF, IL-I and IL-6 but also IL-8 activate neutrophils in inflammatory sites.(30) IL-8 increases the expression

of the integrins on neutrophils. Adhesion molecules of the integrin family on neutrophils bind to immunoglobulin superfamily adhesion receptors on the endothelial cells. IL-8 appears to be closely related to the switching of adhesion molecule expression from the early selectin adhesion to the integrin-mediated adherence of neutrophils to vascular endothelium. (121) However, how closely IL-8 is related to bacterial meningitis and so-called aseptic meningitis due to viral infections in humans remains to be clarified.

Bacteria induce the production of cytokines by many cell types including leukocytes and endothelial cells (32 ,33) that form the vascular component of the blood-brain barrier (BBB). Additionally, astrocytes, which are the predominant cell type on the brain side of the BBB, and microglial cells (contributing up to 20% of the brain cells of the BBB (34) produce a wide variety of cytokines and chemokines (35) when activated. Cytokines appear to either play a protective role or initiate an irreversible over-reaction of the immune system ultimately leading to cell death (36). Both IL-1 β and TNF-α are produced during meningeal inflammation (37) and have been implicated in cellular damage (38). Furthermore, they have been found to initiate an inflammatory cascade (39) that includes the release of IL-8 (40). Although the role of IL-8 and the specific cells that produce it during meningeal inflammation are unknown, studies have shown that IL-8 is a potent chemoattractant and activator of neutrophils (41).

This activation may be exhibited by increased respiratory burst activity, the production of bioactive lipids, and the release of lysosomal enzymes, potentially contributing to tissue injury (42). IL-8 also regulates neutrophil adhesion to endothelial cells (43), possible mediating the large influx of these cells into the subarachnoid space that is seen in patients with bacterial meningitis. (44) The release of IL-8 by cells comprising component in the BBB (specifically , astrocytes and microglia) may be a key component in this influx .

Interleukin-8 synthesis by human microglia adds further support to the hypothesis that IL-8 is involved in the pathogenesis of acute bacterial meningitis and other CNS diseases including Alzheimer's disease (45). The production of IL-8 during inflammation is fairly well established (46).Table (4-4) showed a significant correlation between CSF IL-8 in bacterial meningitis patients and neutrophils counts (R^2= 0.179; P<0,05). CSF IL-8 concentration of viral meningitis and neutrophilic counts results no correlation was appeared. Levels of IL-8 in bacterial meningitis group correlated with lymphocytes counts and non significant positive correlation (R^2=0.424 , P>0.05) Fig. (4-2) .

IL-8 in viral meningitis patients exhibited the same trend as in bacterial meningitis and had linear correlation between IL-8 and lymphocytes which reach significantly positive correlation (R^2=0.007, p<0.05) figure (4-3) .

Table (4-4): correlation between IL-8 and cell counts in studied patient groups

Groups / Variable	CSF IL-8 pg / ml	
	Bacterial meningitis	Viral meningitis
Neutrophil		
R^2 Liner	0.179	0.05
P value	0.01	0.23
Sig.	S	N.S
Lymphocytes		
R^2 Liner	0.424	0.07
P value	0.056	0.02
Sig.	N.S*	S*

S* =significant
N.S*= non significant

Interleukin-8 , a chemotactic cytokine , has inflammatory and growth - regulating properties . It recruits neutrophils to the brain parenchyma (47) and is potent chemoattractant and activator of neutrophils (48)The present study showed high concentration of IL-8 in CSF of bacterial meningitis which corresponded with count of neutrophiles . Recent study agree previous researches of Ishiguro *et al.,1997* (85) , Singh *et al.*,1993(49) and lopez *et al.*, 1995 (50) .

Although a transient increase in the level of IL-8 in CSF has been observed in patients with viral aseptic meningitis (85) , bacterial pyogenic meningitis (51) , or self – resolving aseptic meningitis (50) .The data suggest a possible role of IL-8 as PMNL chemotactic factor in different infectious of the subarachnoid space , not only in pyogenic meningitis .

While the finding of the current study disagree with (85) who reported that the correlations were significant between IL-8 levels and counts of mononuclear cells in viral meningitis while non significantly with bacterial meningitis.

Ishiguro and his co –workers (85) have also shown the production of IL-8 in CSF during meningitis in children . IL-8 induces chemotaxis of neutrophils, releases intracellular enzymes from neutrophils and up-regulates cell adhesion molecules, potentially aiding leucocytes trafficking across the blood – brain barrier

(BBB) (52) once neutrophils have crossed the BBB , it induces degranulation of neutrophils to release chemo-attractants for T-lymphocytes (53) and primes them for superoxide production (54) . Thus an access of activated neutrophils may have a detrimental effect and regulation of IL-8 chemotactic activity may be necessary to reduce the negative effects of neutrophil influx.

The interactions between monocyte /macrophages, IL-8 and neutrophils appear to be important in understanding the pathogenesis of inflammation in meningitis . (55) The alpha- 1 antitrypsin was screening in the study groups. In three study group (bacterial , viral , and control group) no distinctive difference was found in mean of α 1 antitrypsin in CSF , but little tangible deference between the bacterial group separately from the viral and control group , as in table (4-5). Results of this study showed no relationship between the A1AT and both IL-8 and cells in meningitis patients.

Table (4-5) : Distribution of studied groups according to A1AT level

Study group / A1AT varies	Bacterial meningitis N=26	Viral meningitis N=21	Control meningitis N=20
No. (% of positive test)	13 (50%)	6 (25%)	15 (75%)
Mean (mg / dl)±SD	21.6462±9.6	16.4000±4.5	0.6 ±0.11
P value	0.693*		

N.S*= non significant

The archetype of the serpin family is α 1 antitrypsin, the inhibitor present at highest concentration in human plasma. Although named α-1 antitrypsin, its physiologic target is leukocyte elastase rather than trypsin and for this reason, it is alternatively called α-proteinase inhibitor; in this study, the historical name will be retained and sometimes abbreviated to antitrypsin.(56)

Alpha-1 Antitrypsin (A1AT) play a major role in laboratory investigations to differentiating viral and bacterial meningitis along with CSF cell count (57), in this study we noticed that level of A1AT in the bacterial meningitis (21.6462 mg / dl) was higher than the level of viral meningitis (16.4000 mg / dl) and that agree with Pearl GS *et al*.(1985) (58) but in little variance with no significant correlation is detected .

Neutrophil elastase (NE) a 29-Kd glycoprotein is a potent serine protease capable of cleaving a wide range of substrates, including all of the major protein components of connective tissue matrices. (59,60) circulating neutrophils likely release little, if any, of the stored NE. After ingesting particulates, the neutrophil disgorges the enzyme into phagosomes (61) Simultaneously, however, as well as following perturbation of the plasma membrane via specific receptors or surface interaction with tissue components, NE is released into the local environment .(61, 62)Although extracellular NE may be beneficial in infectious loci or in wounds . It is so potent that its presence in the extracellular milieu is highly dangerous and can lead to marked damage of normal tissue architecture (63)

A1AT is the most abundant, endogenous serine protease inhibitor (Pi) in the circulation. Serum A1AT concentrations in healthy subjects are 150–350 mg / dl and can increase fourfold during inflammation indicating that A1AT is an acute-phase protein and in cerebrospinal fluid are 0.8 mg / dl (64) . The primary function of A1AT is thought to be inactivation of neutrophil elastase and other endogenous serine proteases.(65)

The physiological role of A1AT is the maintenance of the protease/anti-protease balance. The primary site of activity of A1AT is in the lower respiratory tract, where it prevents damage from proteolytic destruction by inhibiting neutrophil elastase. Interaction of A1AT with a bacterial virulence factor has not been described so far should be investigated in more detail in future studies. One would expect that A1AT as a serpin would preferentially bind to serine proteases . Knappstein S. *et al.* (2004) show that A typical Enteropathogenic *Escherichia coli* (EPEC) grow in the presence of A1AT without any growth impairment or other obvious defect. (66)

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