Original article

Extra Domain A Containing Fibronectin Levels in The Cerebrospinal Fluid in Patients with Meningitis

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Background

Meningitis is a life-threatening disease because of the inflammation's proximity to the brain and spinal cord. Untreated bacterial meningitis is almost always fatal; therefore it is important to differentiate bacterial meningitis from aseptic meningitis early.

Objectives: To evaluate the role of Extradomain A (EDA) Fibronectien in diagnosis of meningitis and differentiation between bacterial and aseptic causes.

Methods: This study was conducted on total number of 150 patient aged (18-60years old) suspected to have meningitis, 100 patients were excluded due to trauma, cerebrovascular stroke and CNS tumors. Patients were classified according to the CSF findings into 20 patients as bacterial meningitis group **(group1)** and 20 patients as aseptic meningitis group **(group II)**. In addition to 10 cases of matched age and sex, free from any CNS diseases serving as control group **(group III)**. All subjects were subjected to full history taking, clinical examination, laboratory investigations, and measurement of plasma and CSF EDA- FN levels using ELISA method.

Results: CSF EDA- FN level at a cut off value less than 14.2mg/l have a sensitivity of (90%) and specificity of (85%) to differentiate between bacterial and aseptic meningitis (P-Value <0.001), while plasma EDA- FN level at a cut off value more than 2.45mg/l have a sensitivity of (75%) and specificity of (50%) to differentiate between bacterial and aseptic meningitis (p- value <0.05).

Conclusion: CSF and plasma EDA-Fibronectin level can be used as a diagnostic marker in patients with meningitis. It can also differentiate between bacterial and aseptic meningitis.

Keywords: Central nervous system. Cerebrospinal fluid, Extradomain A Fibronectin,

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Introduction

Meningitis is an inflammation of the meninges (membranes that cover the brain and spinal cord), which is characterized by an abnormal number of white blood cells (WBCs) in the cerebrospinal fluid (CSF). It may be caused by a variety of infectious agents, as well as non-infectious diseases and other etiologies **[1].** Infectious meningitis is then sub-divided into septic (bacterial) and aseptic meningitis which is usually caused by viral or fungal infections **[2].**

It is important to differentiate septic meningitis from aseptic meningitis during the acute phase of the disease, when the clinical symptoms are often similar to reduce the morbidity and mortality related to bacterial meningitis **[3].**

Untreated, bacterial meningitis is almost always fatal .In contrast to aseptic meningitis, which is rarely fatal and tends to resolve spontaneously **[4]**.

Fibronectin (FN) is a multifunctional large dimeric glycoprotein (450–500-kDa) of the extracellular matrix, each monomer of which consists of types I, II, and III of repeating amino acid units **[5].** It is involved in many kinds of cellular activities related to fundamental processes such as embryogenesis, angiogenesis, hemostasis, wound healing, and maintenance of tissue integrity. Extra domain A- FN (EDA-FN) is an alternatively spliced form of fibronectin. It plays a vital role in biological cell function. Also it is directly or indirectly engaged during an inflammatory response to brain injury and/ or neuron regeneration. Determination of fibronectin containing the EDA segment might be considered as an additional diagnostic marker of bacterial meningitis and may play a role in differentiating between bacterial and aseptic meningitis **[6].**

This study aimed to evaluate the role of plasma and CSF level of EDA- Fibronectin in diagnosis of meningitis and differentiation between bacterial and aseptic meningitis.

Subjects and Methods

This case control study was conducted on 150 Egyptian patients (92 males, 58 females; their age ranged between 18-60 years) suspected to have meningitis. Patients enrolled in this study were admittted to Shebin El-Kom fever hospital during the period from May 2017 to April 2018. 100 patients were excluded due to trauma, cerebrovascular stroke andCNS tumors. A written informed consent was obtained from all patients prior to participation in this study. The study was approved by the Ethical scientific committee of Benha University and was conducted according to the guidelines of the Helsinki Declaration. The selected cases were grouped as follows;

- Group 1: included 20 patients diagnosed with bacterial meningitis.
- Group 2: included 20 patients diagnosed with aseptic meningitis.

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- Group 3: included 10 patients suspected to have meningitis but no proved CSF infections serving as control group.

All patients enrolled in the study were subjected to the following evaluations: Full history taking, complete clinical examination, laboratory investigation and CSF analysis (PMNs, protein, bacteriologic culture and sensitivity).

Sampling

Blood sample

- 6 mL venous blood sample was obtained from anterior cubital vein under complete aseptic conditions, then distributed as follows:
- (a) 1 mL whole blood was placed in an ethylene diamine tetra-acetic salt (EDTA) vacutainer (1.2mg/mL) as an anticoagulant for and 1mL blood was anti-coagulated with 3.2 % sodium citrate in ratio 4: 1 for ESR.
- (b) 2 mL of blood were taken in plain test tubes without anticoagulant, then allowed to clot for 30 mins at room temperature and centrifuged for 15 mins at 1000× g. The separated serum was used for measurement of biochemical tests and CRP.
- (c) 2 ml whole blood anti-coagulated with 3.2 % sodium citrate in a ratio 9:1 and plasma was immediately separated from the blood cells by centrifugation at 2000×g for 10 min. The samples were aliquoted and stored at −80 °C for EDA –fibronectin until analysis.

Lumbar puncture and CSF examination

Samples of CSF were taken by lumbar puncture under complete aseptic conditions. A spinal needle (e.g. 20 gauge) was inserted in the midline between the third and fourth lumbar vertebrae with the patient in either the lateral recumbent (with knees and neck flexed) or sitting position (with the back curved). The CSF then was collected when the needle entered the subarachnoid space **[7].** Approximately (6-8ml) of CSF was withdrawn, it was divided into three parts; one for bacteriological culture; the second one was used for routine CSF analysis while the third one was aliquoted and stored at−80 °C for measurement of EDA –fibronectin by ELISA.

Methods:

- **Complete blood count** measurement by automated hematology system (Sysmex XE 5000)**[8]** .
- **ESR** (by Westergren method) **[9].**
- **Biochemical tests** including random blood sugar, liver and kidney function tests: using Biosystem A15 autoanalyzer (Biosystems S.A., Barcelona, Spain) by appropriate chemical principles.
- **CRP** by latex agglutination method.
- **CSF examination:**

1- Physical examination:

- Color, aspect and tension.

II. Cellular examination:

Total and differential leucocytic count were performed (normal CSF contains only 0 – 5 Leucocytes / mm3) **[10].**

III. Chemical analysis:

- CSF - Protein level (normal CSF contains 15 – 45mg /dL)
- CSF - Glucose level (normal CSF contains 45-70 mg/dl or about 60% of the plasma glucose value).

IV. Bacteriological examination:

- Direct microscopic examination (by gram stain) from Smears prepared from deposit
- Bacterial culture on:

1. Blood agar and chocolate agar plates incubated at 37°C for 72 hours in 5-10% CO2 for evidence of growth N. meningitidis, St. pneumoniae and H. influenzae type B.
2. Mac Conkey's agar, incubated at 37°C for 72 hours for evidence of growth of Escherichia coli, Salmonella enteritidis, Klebsiella pneumonia and Proteus mirabilis.

Three slopes of Lowenstein Jensen medium two at 37°C and one at 25°C for 8 weeks and checked weekly for evidence of growth of M. tuberculosis. The growing organisms were identified by colonial morphology, staining characters and biochemical reactions according to **[11].**

EDA- containing fibronectin measurement in plasma and CSF by ELISA:

Samples of plasma and Cerebrospinal fluid were stored at −80 °C for EDA –fibronectin until analysis.

Frozen samples were thawed for one hour at room temperature (20°C) before use.

Test procedure:

EDA-FN concentrations using a well-defined domain-specific monoclonal antibody directed to an extra domain EDA of FN (DH1, EMD Millipore, Merck KGaA Darmstadt, Germany).

1. The monoclonal antibodies anti-EDA-FN was diluted 1:3000, in TBS, were used as a coating agent in the wells of a microtiter plate (Nalge Nunc International, Naperville, IL, USA) to EDA-FN in the samples.
2. The amount of EDA-FN bound by the monoclonal antibody was quantified by rabbit anti-FN polyclonal antibodies (Sigma Chemical Co, St Louis, MO, USA, diluted 1:2500 in TBS containing 0.1 % Tween-20) and peroxidase-conjugated goat anti-rabbit immunoglobulins (Sigma Chemical Co, St Louis, MO, USA), diluted 1:3000 as the secondary antibodies.
3. The amount of EDA-FN was assayed by a colorimetric reaction using o-phenylenediamine dihydrochloride/ H2O2 as the enzyme substrate and measured in a Stat Fax 2100 Microplate Reader (Awareness Technology Inc, Palm City, FL, USA) at 450 nm with 630 nm as a reference filter.

4. The samples were analyzed in two different sample dilutions, each in duplicate. EDA-FN concentrations are given in milligram per liter and presented as the mean ± standard deviation (SD).
6. A cellular fibronectin from human foreskin fibroblasts (Sigma, St. Louis, MO, USA, from 3.125 to 50 ng/ well) was used as a standard for EDA-FN-ELISA determination.
5. To determine non-specific binding, two controls were included in the tests: minus primary antibody and minus secondary antibody.

Data management:

The collected data were tabulated and analyzed using SPSS version 16 software (SpssInc, Chicago, ILL Company. Categorical data were presented as number and percentages while quantitative data were expressed as mean ± standard deviation median, inter quartile range (IQR) and range. Fisher's exact test (FET) was used to analyze categorical variables. Quantitative data were tested for normality using Shapiro-Wilks test, assuming normality at P>0.05. Non parametric variables among 2 independent groups were analyzed using Man Whitney U test, while parametric ones were analyzed using Student "t" test. Difference among 3 independent means was analyzed using ANOVA for parametric variables or Kruskal Wallis test (KW) for non-parametric ones. ANOVA and KW tests were followed by post hoc multiple comparisons using Bonferroni tests to detect the significant pairs. Spearman's correlation coefficient (rho) was used to assess linear association between nonparametric variables. ROC curve was used to determine cutoff value of fibronectin with optimum sensitivity and specificity in prediction of bacterial and aseptic meningitis. Matching CSF and plasma EDA-fibronectin was conducted using Wilcoxon test. The accepted level of significance in this work was stated at 0.05 (P <0.05 was considered significant)

Results

There was no statistically significant difference between the studied groups regarding the age and other sociodemographic data (P value>0.05) **(Table 1).** WBC count were significantly higher in bacterial meningitis than aseptic meningitis (P<0.001). And there was no statistically significant difference as regard Hb Concentration and platelet count between the studied groups (P>0.05).

A high tension, greyish colour and turbid aspect of CSFwere more frequent in bacterial than aseptic and control groups with high significant difference (P<0.00) **(Table 2)**.

There was highly significant increase of the CSF protein and CSF leucocytes with neutrophil predominance in bacterial than aseptic groups,

while CSF lymphocytes were more predominant in aseptic than bacterial groups. Sugar was highly significant lower in bacterial than aseptic groups (P<0.001) **(Table 3)**.

Table (1) Socio-demographic data of the studied group

Variable		Bacterial group (n=20)		Aseptic (n=20)		Control group (n=10)		ANOVA test	P	P values of multiple comparisons
Age (ys)	Mean ±SD	39.1±12.3		37.8±11.8		33.8±11.5		0.65	0.52 (NS)	P1=1.0 P2=0.79 P3=1.0
	Range	20-60		22-56		20-54				
		No.	%	No.	%	No.	%	Fisher's Exact Test	P	Multiple P
Sex	Male	13	65.0	13	65.0	6	60.0	0.19	1.0 (NS)	P1=1.0 P2=1.0 P3=1.0
	Female	7	35.0	7	35.0	4	40.0			
Marital status	Married	17	85.0	16	80.0	7	70.0	1.09	0.6 (NS)	P1=1.0 P2=0.37 P3=0.65
	Single	3	15.0	4	20.0	3	30.0			
Occupation	Farmer	7	35.0	8	40.0	3	30.0	0.35	0.92 (NS)	P1=0.74 P2=1.0 P3=0.89
	Non farmer	13	65.0	12	60.0	7	70.0			
Smoking	No	10	50.0	9	45.0	5	50.0	0.21	1.0 (NS)	P1=0.75 P2=1.0 P3=1.0
	Yes	10	50.0	11	55.0	5	50.0			

P1→ Bacterial ≠ aseptic, P2→ Bacterial ≠ Controls, P3→ aseptic≠ Control

The mean value of CSF EDA-Fibronectin level was statistically significant higher in aseptic meningitis group when compared with other groups and in control group when compared with bacterial meningitis group **(Table4) (Figure1)**.

The plasma EDA-Fibronectin level was statistically significant in bacterial meningitis group when compared with aseptic group and no significant when compared with control group **(Table 5)**.

CSF EDA FN level at a cut off value less than 14.2mg/l have a sensitivity of (90%) and specificity of (85%) to differntiate between bacterial and aseptic meningitis with AUC=0.958, Positive predictive value was (85.7%) and Negative predictive value was (89.5%) **(Figure 2)**

Table (2) Physical properties of CSF in the studied groups

Variable		Bacterial group (n=20)		Aseptic group (n=20)		Control group (n=10)		Fisher's Exact test	P	Multiple comparisons
		No.	%	No.	%	No.	%			
Tension	Normal	4	20.0	17	85.0	9	90.0	22.1	<0.001 (HS)	P1<0.001(HS) P2<0.001(HS) P3=1.0
	High	16	80.0	3	15.0	1	10.0			
Color	Colorless	5	25.0	20	100.0	10	100.0	32.6	<0.001 (HS)	P1<0.001(HS) P2<0.001(HS) P3=1.0
	Greyish	15	75.0	0	0.0	0	0.0			
Aspect	Clear	1	5.0	17	85.0	10	100.0	42.05	<0.001 (HS)	P1<0.001(HS) P2<0.001(HS) P3=0.53
	Hazy	5	25.0	3	15.0	0	0.0			
	Turbid	14	70.0	0	0.0	0	0.0			

P1→ Bacterial ≠ aseptic, P2→ Bacterial ≠ Controls, P3→ aseptic≠ Controls

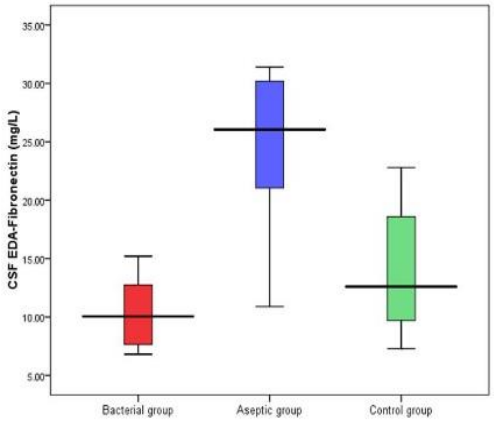

Figure (1): Box plot showing median and IQR of CSF EDA-Fibronectin.

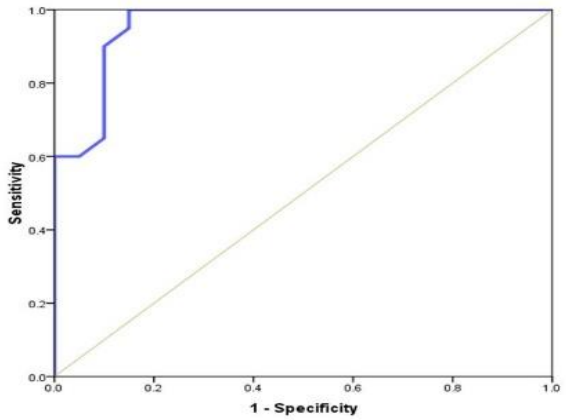

Figure (2): ROC curve for CSF EDA-Fibronectin to differentiate between bacterial and aseptic meningitis

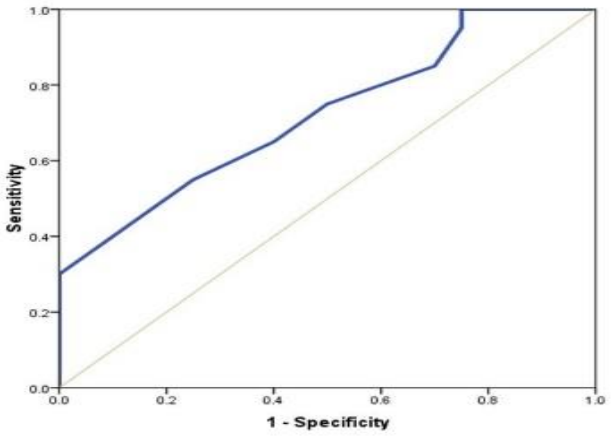

Figure (3): ROC curve for plasma EDA-Fibronectin to differentiate between bacterial and aseptic meningitis

Table (3) Chemical and cytological properities of CSF between bacterial and aseptic meningitis groups

Variable	Bacterial group (n=20)			Aseptic group (n=20)			ZMWU Test	P
	Mean	± SD	Range	Mean	± SD	Range		
TLC	6012.5	7892.1	950-35000	282.5	134.6	75-500	5.41	<0.001 (HS)

Neutrophil %	87.0	10.0	72-100.0	19.2	17.1	5-85	5.2	<0.001 (HS)
Lymphocytes %	11.5	8.80	0-28	80.7	17.18	15-95	5.25	<0.001 (HS)
Protein	198.4	56.1	129-320	73.1	12.9	40-90	5.4	<0.001 (HS)
Glucose	29.8	8.08	16-45	59.7	11.72	45-90	9.38†	<0.001 (HS)

ZMWU→Z value of Mann Whitney U test, †→ Student "t "T test was used.

Table (4) CSF EDA-Fibronectin in the studied groups

Group	n.	CSF EDA-Fibronectin (mg/L)			KW test	P	P of multiple comparisons
		Mean	± SD	Range			
Bacterial group	20	10.30	2.79	6.8-15.2	28.5	<0.001 (HS)	P1<0.001 (HS) P2=0.22 P3<0.001 (HS)
Aseptic group	20	24.62	6.67	10.9-31.4			
Control group	10	13.95	5.38	7.3-22.8			

P1→ Bacterial ≠ aseptic, P2→ Bacterial ≠ Controls, P3→ aseptic≠ Controls

Table (5) Comparing the studied groups regarding plasma EDA-Fibronectin.

Group	n.	Plasma EDA-Fibronectin (mg/L)			ANOVA test	P	P of multiple comparisons
		Mean	± SD	Range			
Bacterial group	20	2.65	0.41	2-3.4	3.97	0.025 (S)	P1=0.041 (S) P2=1.0 P3=0.11
Aseptic group	20	2.31	0.33	1.8-2.8			
Control group	10	2.31	0.54	1.7-3.3			

P1→ Bacterial ≠ aseptic, P2→ Bacterial ≠ Controls, P3→ aseptic≠ Controls

Plasma EDA- FN level at a cut off value more than 2.45mg/l have a sensitivity of (75%) and specificity of (50%) to differntiate between bacterial and aseptic meningitis with AUC=0.721, Positive predictive value was (60%) and Negative predictive value was (66.7%) **(Figure 3)**.

There was significant negative correlation between CSF EDA-FN with ESR and CRP in the bacterial group (p<0.05) **(Table 6)**.

There was no statistically significant correlation between both CSF, plasma EDA-FN and CSF physical properities in bacterial meningitis (P>0.05).

There was a significant negative correlation between CSF EDA-FN and CSF TLC in bacterial meningitis **(Table 7)**.

There was a significant positive correlation between plasma EDA-FN and CSF neutrophils in bacterial meningitis ($p<0.05$) **(Table 8)**.

There was no statistically significant correlation between CSF EDA-FN and CSF properities in aseptic meningitis ($P>0.05$).

Table (6) Correlation between CSF EDA-fibronectin and age, laboratory data among bacterial group.

With	CSF fibronectin Bacterial group (n=20)	
	Rho	P
Age (ys)	0.076	0.75
Hb%	0.03	0.90
WBCs	0.109	0.64
PLTs	0.014	0.95
Creat	-0.297	0.20
Urea	-0.385	0.093
ALT	-0.004	0.98
AST	0.066	0.78
RBS	-0.387	0.094
ESR	-0.589	0.006 (S)
CRP Titre (n=14)	-0.639	0.014 (S)
Plasma fibronectin	0.174	0.46

P1→ Bacterial ≠ aseptic, P2→ Bacterial ≠ Controls, P3→ aseptic≠ Controls

Table (7), Correlation between CSF EDA-fibronectin and CSF Chemical and cytological properities among bacterial group.

With	CSF fibronectin Bacterial group (n=20)	
	Rho	P
TLC	-0.587	0.006 (S)
Neutrophil	0.250	0.29
Lymphocytes	-0.260	0.26
Protein	-0.270	0.25
Glucose	0.088	0.71

Table (8) Correlation between plasma EDA-fibronectin and CSF Chemical and cytological properities among bacterial group.

With	Plasma fibronectin Bacterial group (n=20)	
	Rho	P
TLC	-0.113	0.63
Neutrophil	0.457	0.043 (S)
Lymphocytes	-0.380	0.098
Protein	-0.011	0.96
Glucose	0.142	0.55

Discussion

Meningitis is a dreadful inflammation in the meninges and cerebrospinal fluid (CSF) of human central nervous system [12].

Distinguishing bacterial from aseptic meningitis is not always easy due to considerable overlap in clinical symptoms, signs and laboratory findings [13].

Septic (bacterial) meningitis is almost always fatal disease that defined as an inflammation of the meninges, in particular the arachnoid and the pia mater and the underlying subarachnoid CSF caused by bacterial agents [14]. While the term aseptic

meningitis refers loosely to all cases of meningitis in which no bacterial infection can be demonstrated and usually caused by viral infection [15].

A quick, sensitive, and specific diagnostic methods are essential to initiate early treatment against the meningococcal meningitis patients during an outbreak while traditional methods of diagnosis involving culture, latex agglutination (LA) and biochemical tests may be non-confirmatory or have some limitations[16].

The aim of this study was to assess the efficacy of EDA- Fibronectin levels in CSF in diagnosis of meningitis and differentiating between bacterial and aseptic meningitis. For this purpose, 150 patients aged18-60 years old (92 males and 58 females) with clinical presentations of meningitis and admittted to Shebin El-Kom fever hospital during the period from May 2017 to April 2018 were included in this study. 100 patients were excluded due to trauma, cerebrovascular stroke and CNS tumors .These patients were classified according to the results of CSF findings into 20 patients had bacterial meningitis (group1) and 20 patients had aseptic meningitis (group II). In addition to 10 cases of matched age and sex, suspected to have meningitis but no proved CSF infections serving as control group (groupIII).

As regard laboratory data, there was a highly statistically significant increase of peripheral leucocytic count in group1 with a mean value (11.1±3.16 x 103) than in group II with a mean value (8.1±1.35x10 3 c/cm), ($p < 0.001$).This result was agreed with those of Makoo and his colleagues (2010) [17] and Alkholi and his colleagues(2011) [18] who stated that patients with bacterial meningitis had a higher significant increase in the the mean peripheral leucocytic count than those with aseptic meningitis. However, Singhi and Bansal (2006) [19] stated that, there was no significant difference in peripheral blood WBCs between the patients with bacterial and aseptic meningitis ($p = 0. 36$).

Regarding both ESR, CRP, there was a high statistically significant increase in the frequency of positive CRP in group I patients when compared to group II ($p < 0.001$).This result was agreed with those of Yetkin and his colleagues (2010) [20] who found that, there were statistically significant increase in ESR, CRP ($p<0.001$) in patients with bacterial meningitis than those with aseptic meningitis. Similar results were reported by Dubos and his colleagues (2008) [21].

In terms of physical characters of CSF, there were highly significant differences between patients with bacterial meningitis and those with aseptic meningitis as regard tension, colour and aspect of CSF. High tension and cloudy CSF were more frequent in bacterial meningitis group than aseptic meningitis group ($p<0.001$). This result was similar to El- Kapany (2011) [22] who stated that there was statistically significant difference in aspect of CSF between patients of bacterial and aseptic meningitis.

These were explained by presence of WBCs, red blood cells and bacteria, or protein in CSF of BM [23].

The present study showed a highly significant increase in CSF–total leucocytic count (TLC) with neutrophil predominance and CSF- protein and highly significant decrease in CSF – glucose in goup I patients when compared to group II (p <0.001),This result is similar to those of Makoo and his colleagues (2010) [17] and Alkholi and his colleagues(2011) [18] who stated that, there were high statistically significant differences(P<0.001) between bacterial and aseptic meningitis patients regarding CSF leukocyte count, CSF protein and CSF glucose.

This study revealed presence of organisms in gram stain in (55%) and no organisms in (45%) of bacterial meningitis patients. This result was agreed with those of Mani and his colleagues (2007) [24] who found the causative organisms in 65.7% of patients by Gram stain.

This study showed that, culture results in bacterial meningitis group were positive in 15 patients (80%) and negative in 5 patients (20%). The most frequently detected organisms were St. pneumoniae (Gram positive cocci) in 6 patients (30%), Meningococci(Gram negative diplococci) in 3 patients (15%), Hemophilus influenza in 2 patients (10%), Klebsiella Pneumoniae in 2 patients (10%) ,Staph aureus in 2 patients (10%) and E Coli (gram negative bacilli) in 1 patient (5%). This is in accordance with Alam and his colleagues (2013) [25], who demonstrated that S. pneumoniae was the most common organism isolated (47.4%) followed by N. meningitides (33.9 %), H. influenzae (10.2%) and other Gram negative bacteria (8.5%) among Egyptian populations.

In Egypt, Shaban and Siam (2009) [25] found that pneumococcal meningitis is currently the leading cause of meningitis.

In the present study it was found that, plasma EDA- FN level was significantly increased in bacterial meningitis group than the aseptic meningitis group with mean values of 2.65mg/l versus2.31 mg/l, (p value < 0.05).and no significant differences were found when compared with control group 2.31 mg/l (p value > 0.05). This is in agreement with Peters and his colleagues (2011) [27] who found that, EDA-FN can appear in plasma and its presence may constitute a predictive marker for distinct clinical outcomes in critically ill patients.

However, in a study performed by Małgorzata and his colleagues (2013) [28] it was revealed that EDA-FN was almost undetectable in most of plasma collected from children suffering from bacterial and aseptic meningitis. Only 30% of the plasma samples from the bacterial meningitis group had EDA-FN at a significant level (from 0.04 to 0.09 AU).

The present study showed that CSF EDA FN level at a cut off value less than 13.5 mg/l have a

sensitivity of (80%) and specificity of (76.7%) in diagnosing of bacterial meningitis with AUC=0.875. Positive predictive value was (69.6%) and Negative predictive value was (85.2%). However, the cut off Value of CSF EDA FN level more than 13.5 mg/l have a sensitivity of (90%) and Specificity of (80%) in diagnosing of aseptic meningitis with AUC=0.935 Positive predictive value was (75%) and Negative predictive Value was (92.3%).

The present study showed that CSF EDA FN level at a cut off value less than 14.2mg/l have a sensitivity of (90%) and specificity of (85%) to differentiate between bacterial and aseptic meningitis with AUC=0.958, Positive predictive value was (85.7%) and Negative predictive value was (89.5%). This result is similar to Małgorzata and his colleagues (2013) [28] who demonstrated that CSF EDA-FN level of 0.17 AU as an optimal cut-off value to differentiate between bacterial and aseptic meningitis with a sensitivity of 83% and a specificity of 89% (AUC 0.92).

This study showed that plasma EDA- FN level at a cut off value more than 2.45mg/l have a sensitivity of (75%) and specificity of (50%) to differentiate between bacterial and aseptic meningitis with AUC=0.721, Positive predictive value was (60%) and Negative predictive value was (66.7%).

Regarding the correlation between CSF, plasma EDA-FN and other studied parameters among the bacterial group , there was significant negative correlation between CSF EDA-FN and inflammatory markers such as ESR and CRP ($p < 0.05$). While Shiozawa and his colleagues (2001) [29] in a study performe in rheumatoid arthritis patients, no significant correlations were detected between the concentration of EDA-Fn in synovial fluid and the inflammatory indices, such as CRP and ESR. Also there was significant positive correlation between plasma EDA-FN and CSF neutrophils ($p < 0.05$). These results disagree with Małgorzata and his colleagues [28] who found, A negative correlation ($r = -0.584$, $p<0.000$) between the cerebrospinal EDA-FN level and the cerebrospinal polymorphonuclear cell percentage.

Regarding the correlation between CSF, plasma EDA-FN and other studied parameters among the aseptic group there was statistically significant positive correlation between plasma EDA-FN and CSF protein level ($p<0.05$).While there was no significant correlation with other parameters ($p > 0.05$)

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