Accuracy of Cerebrospinal Fluid C– Reactive Protein and Multiplex Polymerase Chain Reaction and Serum Procalcitonin in Diagnosis of Bacterial and Viral Meningitis in Children

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Abstract

Background: Meningitis is one of the most dangerous infection affecting children. The need for rapid and accurate diagnosis is mandatory for improving the outcome.

Aim of the work: to evaluate the role of multiplex polymerase chain reaction (PCR), cerebrospinal fluid (CSF) – C- reactive protein (CRP) and serum procalcitonin (PCT) in diagnosis of meningitis and to detect its accuracy.

Patients and methods: A cross-sectional study was carried out in University Children hospital, Faculty of Medicine, between November 2019 and September 2020. The study was approved by the Ethics Review Board of Faculty of Medicine, Assiut University, and informed written consent was obtained. The committee's reference number is 17200161. Clinicaltrials.gov ID: NCT03387969. 48 Children aged 2 to 18 years with meningitis were included. Detailed history and examination. Blood glucose level at time of admission prior to lumbar puncture, serum CRP level, serum PCT, CSF-CRP level and Multiplex PCR were evaluated.

Funding: The study was supported by Grant Office of Faculty of Medicine, Assiut University with grant NO. 2018-01-04-006-R2.

Results: The mean age of children was 3.27 ± 1.27 years. 35 (72.9%) cases were bacterial meningitis, while 13 (27.1%) cases were viral meningitis. Patients with bacterial meningitis had significantly higher serum CRP, serum PCT and higher CSF-CRP and significantly lower CSF/blood glucose compared to viral meningitis. Multiplex PCR had 94% sensitivity and 100% specificity for diagnosis of bacterial and viral meningitis.

Conclusion: CSF-CRP, CSF/blood glucose, PCT and Multiplex – PCR may help in diagnosis and differentiation of bacterial and viral meningitis.

Keywords: Meningitis, children, Cerebrospinal fluid, Multiplex -PCR, procalcitonin.

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INTRODUCTION

Meningitis is a serious health problem that affects people all over the world, particularly children. The rate of infection varies depending on the nation, area, age group, and pathogen. In developing countries, the incidence of meningitis in children under the age of five varies from 0.07 to 2.5 percent. It is a potentially life-threatening disease with a high rate of morbidity and mortality [1].

Differentiating bacterial from viral meningitis could be done through clinical signs and symptoms, cerebrospinal fluid (CSF) analysis, Gram staining, antigen assays, peripheral white blood cells (WBC), and neutrophil count with some uncertainty and ideally via CSF culture. The usage of sensitive antibiotics in the early stage, which depends on pathogen identification, can improve the outcomes, and reduce the complications [2].

It is often very difficult to distinguish between bacterial and viral meningitis according to CSF findings, which is not accurate 100% and leads to unnecessary antibiotic usage [3].

Besides the atypical history, non-specific physical examination and hesitant and low yield CSF cultures on some occasions, CSF parameters are occasionally non-conclusive and cannot discriminate between bacterial and viral meningitis. Moreover, even insufficient doses of antibiotics can lead to atypical CSF results in patients. Due to the practical problems in discriminating bacterial and viral meningitis, new and strong biomarkers should be identified to help clinicians to start early and appropriate management. Such markers should logically have high sensitivity to prevent any error in diagnosis and management [4].

Blood PCT level is one of the markers that can help to confirm bacterial meningitis and if negative, can effectively rule out it. Blood PCT measurements proved superior to CSF- PCT, with a sensitivity of 95% and a specificity of 97% as a marker for bacterial meningitis [5]. In acute meningitis, serum PCT is an important biomarker for sepsis. It can also be of use in differentiating viral meningitis from bacterial meningitis [6].

In recent years, multiplex polymerase chain reaction (PCR) a rapid and accurate method for diagnosis of acute meningitis has been developed. It has been shown to be fast, and efficient in identifying different microorganisms, such as bacteria, viruses, or fungi [7].

As regard bacterial meningitis, research works reported that evaluation of multiplex -PCR can be sensitive and specific for different organisms and can be used to detect pathogens in CSF samples from patients in whom cultures remain negative or those who received antimicrobials. Also, one of the great advantages of PCR is the need for small volume of sample for the molecular assay [8].

This study aimed to evaluate the role of multiplex -PCR, CSF – C-reactive protein (CRP) and PCT in diagnosis of meningitis either bacterial or viral and to detect its accuracy in comparison to traditional methods of diagnosis.

PATIENTS AND METHODS

Study Setting and Design: A cross-sectional hospital-based study was carried out in Assiut University Children hospital, Faculty of Medicine, Assiut University in period between November 2019 and September 2020.

Ethical Approval: The study protocol was approved by the Ethics Review Board of Faculty of Medicine, Assiut University, and an informed written consent was obtained from all participants or their first-degree relatives according to the declaration of Helsinki. The committee's reference number is 17200161. Clinicaltrials.gov ID: NCT03387969

Study Subjects:

- **Inclusion criteria:** Patients admitted to Pediatric Emergency Care Unit were enrolled in the study if they aged 2 to 18 years presented with manifestations suggesting meningitis.

Methodology: All cases will be subjected to:

Detailed history taking included symptoms suggesting meningitis especially fever, convulsion, disturbed conscious level (DCL). History suggestive of sepsis or contact with suspected person was assessed. Full clinical examination before admission and after receiving treatment, then daily follow up with recording progress notes every day.

Investigations included the following:

- Complete blood picture with differential count
- Serum electrolyte. (Na, K, Ca) and kidney function test.
- Blood glucose level at time of admission and prior to lumbar puncture
- Serum CRP level
- Serum LDH level
- Serum PCT level measured by chemilumensce on cobas E411 or by ELISA (enzyme linked immunoassay).
- CSF-CRP level measured by reagent nephelometric method by BN prospec seimense by ELISA (enzyme linked immunoassay)
- Multiplex PCR assay by Biofire film array panel. [9]
- Cutoff points were determined automatically by using receiver operator characteristics curve that determined the best cut off point for each parameter.

Funding:

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Statistical Analysis

Data was collected and analyzed those using SPSS (Statistical Package for the Social Science, version 20, IBM, and Armonk, New York). Continuous data was expressed in form of mean ± SD or median. While nominal data was expressed in form of frequency (percentage). Chi^2-test was used to compare the nominal data of bacterial and viral meningitis in the study while student t-test was used to compare mean of different two groups. ROC curve was used to determine the diagnostic accuracy of serum CRP, LDH, PCT and CSF-CRP and multiplex PCR in diagnosis of bacterial meningitis. Level of confidence was kept at 95% and hence, P value was significant if < 0.05.

RESULTS

The mean age of enrolled patients (48 cases) was 3.27 ± 1.27 years with range between two and seven years. Out of those patients, 34 (70.8%), 11 (22.9%), 3 (6.3%) patients were 2-4, > 4-6 and more than 6 years old, respectively. Twenty-nine (60.4%) patients were males, while 19 (39.6%) patients were females. Urban residence was detected in 79 .2% of cases while 20.8% were rural.

Table (1): Demographic and clinical data of included cases

	N= 48
Age (years)	3.27 ± 1.27
Range	2-7
Age group	
2-4 years	34 (70.8%)
> 4- 6 years	11 (22.9%)
More than 6 years	3 (6.3%)
Sex	
Male	29 (60.4%)
Female	19 (39.6%)
Residence	
Urban	38 (79.2%)
Rural	10 (20.8%)
Positive meningeal signs	48 (100%)
Fever	48 (100%)
High grade	25 (52.1%)
Low grade	23 (47.9%)
Disturbed conscious level	43 (89.6%)
Convulsion	38 (79.2%)
Final diagnosis bases on CSF culture	
Bacterial meningitis	35 (72.9%)
Definite	20 (41.7%)
Probable	15 (31.3%)
Probable viral meningitis	13 (27.1%)
Outcome	
Alive	47 (97.9%)
Died	1 (2.1%)

All patients had positive meningeal signs and fever. High- and low-grade fever presented in 25 (52.1%), and 23 (47.9%) patients, respectively. Low grade fever was considered when body temperature 99.5 °F (37.5°C) and 100.3°F (38.3°C) [10], high grade fever was considered when body temperature 102°F (38.9°C) or higher [11]. Forty-three (89.6%) patients presented with disturbed conscious level (DCL) while 38 (79.2%) patients suffered from convulsions.

Thirty-five children (72.9%) of the patients had bacterial meningitis while 13 (27.1%) patients had viral meningitis. With exception to only one patient, all patients were improved and discharged.

Table (2): Culture of the cerebrospinal fluid among enrolled patients: It was noticed that 28 (58.4%) sample showed no growth. The most frequent organisms were Streptococcus pneumoniae (18.8%), Neisseria meningitides (8.3%), and Hemophilus influenza (6.3%). Two samples revealed Klebsiella pneumoniae and two other samples showed Staph. Aureus.

Type of organism based on result of multiplex PCR among enrolled patients: It was noticed that 21 (43.8%) sample showed undetectable growth. The most frequent organisms detected by multiplex PCR were Streptococcus pneumoniae (16.7%), Enteroviruses (12.5%), Herpes simplex virus (12.5%), Neisseria meningitides (8.3%), and Hemophilus influenza (6.3%).

Table (3): shows the characteristics of patients based on final diagnosis: It was noticed that mean age of patients with viral meningitis was significantly lower than those with bacterial meningitis (2.65 ± 1.06 vs. 3.50 ± 1.28 (years); P= 0.03). All patients with viral meningitis presented with low grade fever while majority (71.4%) of patients with bacterial meningitis suffered from high grade fever. All patients with bacterial meningitis and 8 (61.5%) patients with viral meningitis presented with DCL.

Patients with bacterial meningitis had significantly higher serum CRP (43.46 ± 12.45 vs. 17.03 ± 10.97 (mg/dl); P= 0.03), lactate dehydrogenase (232.65 ± 57.09 vs. 123.30 ± 19.71 (u/l); P= 0.04) and serum PCT (1.98 ± 0.14 vs. 1.10 ± 0.20 (ng/dl); P< 0.001) in comparison to those with viral meningitis.

As regarding CSF examination; patients with bacterial meningitis had significantly higher protein, total cells and neutrophil with lower glucose and lymphocytic count in comparison to those with viral meningitis. Also, patients with bacterial meningitis had significantly higher CSF-CRP (0.71 ± 0.37 vs. 0.14 ± 0.06 (pg/ml); P< 0.001) and significantly lower CSF/blood glucose ratio (0.25 ± 0.09 vs. 0.58 ± 0.25; P< 0.001).

Table (4) and figure (1): Accuracy of serum and CSF biomarkers in differentiation between bacterial and viral meningitis: at cutoff > 29.9 mg/dl, serum CRP had 60% sensitivity and 100% specificity for diagnosis of bacterial meningitis with overall accuracy was 70.8% and area under curve was 0.66 while CSF-CRP at cutoff > 0.18 mg/dl had 80% sensitivity and 92.3% specificity for diagnosis of bacterial meningitis with overall accuracy was 83.2% and area under curve was 0.91.At cutoff > 0.23 ng/dl, serum PCT had 69% sensitivity and 85% specificity for diagnosis of bacterial meningitis with overall accuracy was 73.3% and area under curve was 0.67.

At cutoff < 0.33, CSF/blood glucose ratio had 80% sensitivity and 84% specificity for diagnosis of bacterial meningitis with overall accuracy was 81.1% and area

Table (2): Types of the organisms based on culture of the cerebrospinal fluid and multiplex PCR among enrolled patients.

Results of the cerebrospinal fluid culture	N= 48
Streptococcus pneumoniae	9 (18.8%)
Neisseria meningitides	4 (8.3%)
Hemophilus influenza	3 (6.3%)
Klebsiella pneumoniae	2 (4.2%)
Staph. Aureus	2 (4.2%)
No growth	28 (58.4%)
Results of multiplex PCR	**N= 48**
Streptococcus pneumoniae	8 (16.7%)
Enteroviruses	6 (12.5%)
Herpes simplex virus	6 (12.5%)
Neisseria meningitides	4 (8.3%)
Hemophilus influenza	3 (6.3%)
Undetectable growth	21 (43.8%)

Data expressed as frequency (percentage).

Table (3): Characteristics of included patients based on final diagnosis

	Viral meningitis (n= 13)	Bacterial meningitis (n= 35)	P value
Age (years)	2.65 ± 1.06	3.50 ± 1.28	0.03
Sex			
Male	6 (46.2%)	23 (65.7%)	0.18
Female	7 (53.8%)	12 (34.3%)	
Fever			
High grade	0	25 (71.4%)	< 0.001
Low grade	13 (100%)	10 (28.6%)	
Disturbed conscious level	8 (61.5%)	35 (100%)	< 0.001
Convulsion	11 (84.6%)	27 (77.1%)	0.44
Blood glucose (mg/dl)	91.38 ± 40.21	121.08 ± 55.12	0.08
Leucocytes (10^3/ul)	13.76 ± 6.28	8.97 ± 6.54	0.79
Platelets (10^3/ul)	349.07 ± 128.36	387.58 ± 125.08	0.99
Neutrophil (10^3/ul)	7.21 ± 1.60	5.56 ± 1.23	0.35
Lymphocytes (10^3/ul)	2.3 ± 1.14	3.19 ± 1.42	0.30
Sodium (mmol/l)	138.16 ± 2.43	138.30 ± 4.31	0.39
Potassium (mmol/l)	4.45 ± 0.33	4.54 ± 0.64	0.80
Calcium (mg/dl)	8.43 ± 1.54	8.54 ± 1.54	0.62
Urea (mg/dl)	6.64 ± 2.80	5.77 ± 2.30	0.98
Creatinine (mg/dl)	0.98 ± 0.12	1.31 ± 0.14	0.57
Serum CRP (mg/dl)	17.03 ± 10.97	43.46 ± 12.45	0.03
Serum LDH (u/l)	123.30 ± 19.71	232.65 ± 57.09	0.04
Procalcitonin (ng/dl)	1.10 ± 0.20	1.98 ± 0.14	< 0.001
CSF examination			
Protein (mg/dl)	40.19 ± 24.85	94.65 ± 23.45	< 0.001
Glucose (mg/dl)	50.50 ± 9.72	38.28 ± 15.98	0.01
CRP (pg/ml)	0.14 ± 0.06	0.71 ± 0.37	< 0.001
Total cells (cells/mm^3)	817.53 ± 45.78	2565.87 ± 543.12	< 0.001
Neutrophil (cells/mm^3)	253.07 ± 102.34	2122.85 ± 385.45	< 0.001
Lymphocytes (cells/mm^3)	575.23 ± 185.13	437.14 ± 213.45	0.37
Predominant cells in CSF			
Polymorphonuclear cells	0	35 (72.9%)	< 0.001
Lymphocytes	13 (27.1%)	0	
CSF/blood glucose ratio	0.58 ± 0.25	0.25 ± 0.09	< 0.001

Data expressed as mean (SD), frequency (percentage). CSF: cerebrospinal fluid; CRP: C-reactive protein; LDH: lactate dehydrogenase

under curve was 0.89.

At cutoff >205serum LDH had 69% sensitivity and 85% specificity for diagnosis of bacterial meningitis with overall accuracy was 73% and area under curve was 0.67.

Table (5) and figure (2) showed the accuracy of multiplex PCR in differentiation between bacterial and viral meningitis: It was noticed that multiplex PCR had 94% sensitivity and 100% specificity for diagnosis of bacterial meningitis with overall accuracy was 95.6% and area under curve was 0.94.

Table (4): Accuracy of CRP, PCT and CSF/blood glucose ratio in differentiation between bacterial and viral meningitis

	Serum LDH	Serum PCT	Serum CRP	CSF-CRP	CSF/blood glucose ratio
Sensitivity	69%	69%	60%	80%	80%
Specificity	85%	85%	100%	92.3%	84%
PPV	92.3%	92%	100%	97%	93%
NPV	50%	52%	48.1%	63%	61%
Accuracy	73%	73.3%	70.8%	83.2%	81.1%
Cutoff	> 205	> 0.23	> 29.9	> 0.18	< 0.33
AUC	0.67	0.67	0.66	0.91	0.89

LDH: lactate dehydrogenase; CRP: C-reactive protein; PCT: procalcitonin; CSF: cerebrospinal fluid; PPV: positive predictive value; NPV: negative predictive value; AUC: area under curve

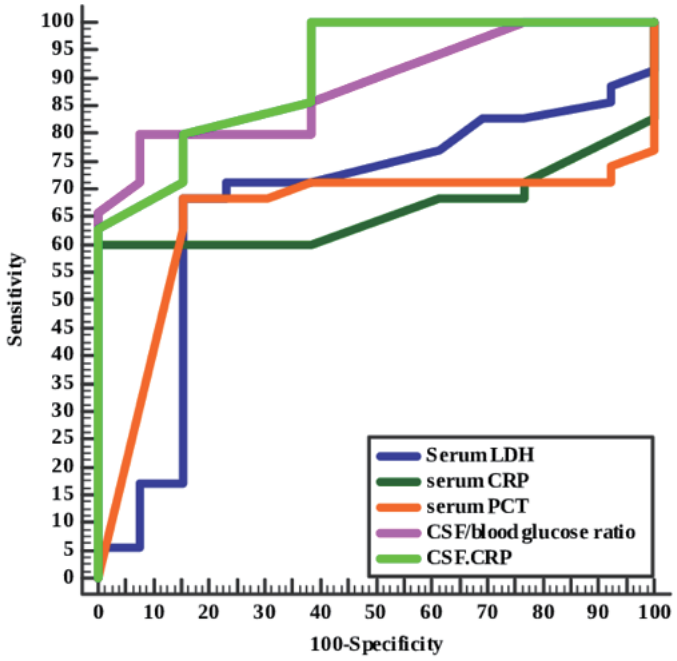

Fig. 1. Accuracy of serum and CSF biomarkers in differentiation between bacterial and viral meningitis.

Table (5): Accuracy of multiplex PCR in differentiation between bacterial and viral meningitis

	Value
Sensitivity	94%
Specificity	100%
Positive predictive value	100%
Negative predictive value	96%
Accuracy	95.6%
Area under curve	0.94

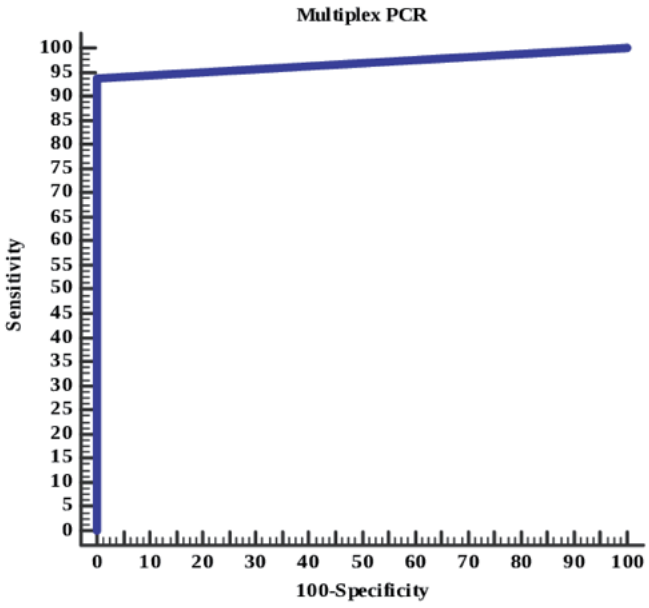

Fig. 2. Accuracy of multiplex PCR in differentiation between bacterial ana viral meningitis.

DISCUSSION

The current study assessed the diagnostic accuracy of CRP-CSF, multiplex PCR and serum PCT in differentiating bacterial and viral meningitis in children. The study enrolled 48 patients presented to emergency pediatric care unit with clinical manifestations suggestive of meningitis. Their mean age was 3.27 ± 1.27 years with range between two and seven years. The majority (70.8%) of them was 2-4 years old. 29 (60.4%) patients were males.

In line with the current study, Chaudhary (2017) [12], studied 60 children with clinically suspected meningitis. (61.67%) of those patients was males and majority of the patients was 2-5 years old. Also, a previous study done by Yadhav obtained the same results [13].

It was reported that > 66% of cases of meningitis occur in the first years of life, owing to decreased immunity and high vascularity of the brain [13]. But generally, the frequency of bacterial meningitis in children has declined. The introduction of vaccines against S pneumoniae and serogroup C meningococcus has substantially reduced the incidence of meningitis in children. During a 1998-2007 survey, the incidence of meningitis declined by 31%, a decrease that can be credited to vaccination programs [14]].

Our study is consistent with other studies regarding male predominance [15,13,12,16]. This predominance may be explained by the fact that females have stronger humoral and cellular immune response than males during infancy and childhood. This increased level of immunity can be of benefit in protection against, and clearance of, a proportion of pathogens [17].

In the current study, fever and meningeal signs present in all patients. Forty-three (89.6%) patients were presented with DCL, while 38 (79.2%) patients suffered from convulsions (79.2%). As the included cases were more than two years so meningeal signs and manifestations of increased intracranial pressure are the most expected which is not the role in infants and children less than two years[18].

In contrast, Dashti et al (2017) reported that the most frequent presentations among their patients were poor feeding and loss of appetite. They noticed that only 17 (34%) patients had meningeal signs [2]. This discrepancy between the two studies may be explained by the younger age of their enrolled patients less than one year.

Most cases were from urban residence (79.2%)

which may be the cause of very low mortality rate in this study just one case, early admission and treatment, high educational and socioeconomic levels in urban resident may be the factors that decreased the mortality rate in addition to availability of well-equipped emergency units in a tertiary care hospital.

Based on the clinical manifestations, and CSF analysis and culture; 35 (72.9%) patients were diagnosed as bacterial meningitis while 13 (27.1%) patients were considered viral meningitis. Culture of CSF was positive in 20 (41.7%) patients with bacterial meningitis while undetectable growth found in 28 (56.3%) patients. This result is consistent with many previous studies that revealed limited diagnostic yields of CSF culture[19] and [2].

The cause of decreased detection of bacterial isolates on culture noticed in different studies may be due to inaccurate diagnosis of meningitis, unnecessary lumbar puncture, unavailable media for specific pathogen isolation, use of antibiotic treatment before lumbar puncture and the difference in study population [1].

In agreement with the current work, Chaudhary et al (2017) reported that frequency of bacterial meningitis was higher than viral meningitis (75% vs. 13%) [12]. Also, Shokrollahi et al (2018) found that most children (52.6%) had bacterial meningitis [20]. But in contrast to our results, it was found that out of 50 cases of meningitis, 38 (76%) patients had viral meningitis and 12 (24%) had bacterial meningitis [2]. The common incidence of bacterial meningitis in this study may be related to low socioeconomic status which favors poor personal hygiene and sanitation leading to spread of infection[21].

Regarding the causative organism in current study Streptococcus pneumoniae (18.8%), Neisseria meningitides (8.3%), and Hemophilus influenza (6.3%) were the most common, respectively. It was recorded that Neisseria meningitides was the commonest organism in Egypt [22], which is changed now to be Streptococcus pneumoniae, this change should be taken into consideration when giving empirical treatment [23].

In comparison of bacterial with viral meningitis, the current study revealed that mean age of patients with viral meningitis was significantly lower than those with bacterial meningitis (2.65 ± 1.06 vs. 3.50 ± 1.28 (years); P= 0.03). Also, Patients with bacterial meningitis had significantly higher serum CRP, lactate dehydrogenase and serum PCT compared to those with viral meningitis (1.98 ± 0.14 vs. 1.10 ± 0.20 (ng/dl); P< 0.001).

PCT and CRP have commonly been used in the diagnosis of children at risk for serious bacterial infection. In addition, PCT and CRP have been used successfully in distinguishing serious bacterial infections from viral illnesses. PCT offers some advantages in detection of patients with invasive bacterial infections, specifically patients with meningococcal diseases [24].

The importance of PCT and CRP in diagnosing bacterial meningitis and the differentiation between bacterial and viral infections was recorded in many previous studies [19,25] , and [9].

In this study, PCT sensitivity and specificity were 69% and 85% respectively with 92 % PPV, in diagnosis and differentiation between bacterial and viral meningitis, so the use of serum PCT assay in addition to gold standard combination of clinical history, physical examination, basic laboratory tests and CSF analysis could increase the accuracy of diagnosis and differentiation of the etiology of suspected meningitis. Also, it may help in cases that conventional methods of diagnosis are not conclusive such as non-conclusive CSF findings.

Lactate dehydrogenase (LDH) is a sensitive measure of bacterial meningitis. It appears to help distinguish bacterial from non-bacterial meningitis and is more sensitive as an early predictor of bacterial meningitis than glucose. However, some researchers believe that CSF-LDH may not be useful in distinguishing between septic and aseptic meningitis, but it may be useful as a marker [27].

As regard CSF analysis we found that patients with bacterial meningitis had significantly higher protein, total cells and neutrophil with lower glucose and lymphocytic count in comparison to those with viral meningitis. Also, patients with bacterial meningitis had significantly higher CSF-CRP (0.71 ± 0.37 vs. 0.14 ± 0.06 (pg/ml); P< 0.001) and significantly lower CSF/blood glucose ratio (0.25 ± 0.09 vs. 0.58 ± 0.25; P< 0.001).

Nadeem et al (2018) and Attia et al (2020). reported similar results[28,29]. The increased total leucocytic count (TLC) in bacterial meningitis may be due to increased release of leukocytes from the bone marrow in response to bacterial infection. This increased TLC in bacterial meningitis may be due to increased release of leukocytes from bone marrow storage pools in response to bacterial

infection [30].

Levels of CRP in serum and CSF increase due to invasive central nervous system infection. Increased CRP production is an early and sensitive response to most forms of microbial infections and the value of its measurement in the diagnosis of various infective conditions was established in previous studies [31].

In this study, we detected significantly increased levels of CRP in blood and CSF of children with meningitis compared to viral meningitis It was detected in a similar study that CRP was positive in 100% of initial lumbar puncture cerebrospinal fluid samples with culture positive bacterial meningitis, as compared to 6% patients with aseptic meningitis [28].

Serum CRP had 60% sensitivity and 100% specificity for diagnosis of bacterial meningitis with overall accuracy was 70.8%, while CSF-CRP had 80% sensitivity and 92.3% specificity for diagnosis of bacterial meningitis with overall accuracy was 83.2%. High sensitivity may prove the role of CRP as a screening tool in diagnosis of bacterial meningitis, with high specificity as an evaluation of accurate diagnosis of bacterial and viral meningitis. CSF- CRP is more sensitive than serum CRP in diagnosis of meningitis.

CSF - CRP has been reported to be one of the most reliable and early indices to differentiate between bacterial and non-bacterial meningitis[32].

This indicates that CSF-CRP is a better marker than serum CRP in differentiating bacterial meningitis from viral meningitis. The same observation was detected by other researchers who found raised levels of CRP above the normal range in CSF to be a better indicator of bacterial meningitis. It also served to distinguish bacterial meningitis from viral and tubercular infections, and other central nervous system disorders [32].

In the current study we found that CSF/blood glucose ratio was significantly lower among patients with bacterial meningitis. This study was consistent with a previous study reported that this ratio was decreased among patients with bacterial meningitis [33].

However, there is paucity in studying role of this ratio in differentiation between bacterial and viral meningitis, in the current study, it was found that at cutoff < 0.33, CSF/blood glucose ratio had 80% sensitivity and 84% specificity for diagnosis of bacterial meningitis with overall accuracy was 81.1% and area under curve was 0.89, which may offer an accurate method in prediction of bacterial meningitis than other routinely measured markers in CSF. Since the CSF glucose levels and blood glucose levels are promptly available, the CSF/blood glucose ratio should be examined as a timely diagnostic indicator for bacterial meningitis.

The multiplex-PCR was simple, affordable, sensitive, and specific, detecting small amount of DNA samples, and did not cross-react with fungi or other bacterial pathogens [34].

Based on the current work, it was noticed that multiplex PCR had 94% sensitivity and 100% specificity for differentiation of bacterial and viral meningitis with overall accuracy was 95.6% and area under curve was 0.94. This means that it fails to detect only one case from those with Streptococcus pneumoniae. At the same time, it was able to detect viral etiology of patients with suspected viral meningitis (6 cases with enteroviruses and another 6 cases with herpes simplex virus). Also, this technique failed to detect Klebsiella pneumoniae and Staph. Aureus but these organisms are outside its panel of detection.

Multiplex PCR is more accurate than CSF culture in detection of organisms even in negative culture results and in cases received antibiotics for several days[35,34].

Overall, the multiplex-PCR diagnostic test demonstrated an excellent diagnostic accuracy, with a good correlation with the conventional culture routine testing, detecting nearly all bacterial pathogens included to the test.

Conclusion: CSF-CRP, CSF/blood glucose, PCT and Multiplex — PCR are important biomarkers that may help in diagnosis and differentiation of bacterial and viral meningitis. Multiplex — PCR may be used as initial and single method for cases with suspected meningitis if available.

The main limitations of the current study; 1) relatively small sample size which was related to the number of multiplex PCR kits restricted to the available fund. 2) the study was done in a single center and 3) this study does not include patients younger than two years old.

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