Assessment of Some Inflammatory Biomarkers in Children with Febrile Seizure and Bacterial Meningitis

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Abstract

Introduction: Bacterial meningitis (BM) is a serious infection characterized by inflammation of the meninges, causing a high rate of acute complications in children. This research aimed to explain the effect of procalcitonin (PCT) in BM and its correlation with the others some inflammatory biomarkers.

Method: The case-control study included Fifty children who were diagnosed with suspected bacterial meningitis associated with febrile seizure according to the clinical investigation by specialized pediatrician and analysis of CSF and Forty children were enrolled as healthy control. The inflammatory biomarkers procalcitonin, Erythrocyte sedimentation rate (ESR), C-reactive protein (CRP) were estimated for each participants in addition to measurement of CBC(complete blood count) by using an automated instrument.

Results: Patients have significantly higher serum levels of PRO, CRP, ESR, total WBC count, ANC, and NLR than the control group. Elevating serum PRO and CRP levels in patients group were significantly associated with increased serum ANC levels. A significant relationship was also observed between PRO and NLR, indicating that the PRO, CRP and NLR had a suitable biomarkers in the diagnosis of BM.

Conclusion: Serum procalcitonin is more specific and sensitive inflammatory biomarkers than the NLR and CRP for the evaluation of suspected bacterial meningitis, so the recommended to use PCT assays in the early diagnosis of children with BM.

Keywords: *Bacterial meningitis; biomarkers; Health; inflammatory.*

Introduction

Bacterial meningitis (BM) is a serious infectious disease characterized by inflammation of the meninges, producing a high rate of morbidity and mortality in children[1]. BM could be fdeadly for about half of untreated cases. Even with early diagnosis and adequate treatment, more than 15% of the patients die, especially within 1-2 days of starting symptoms.[1] Furthermore, 10– 20% of the survivors are susceptible for persistent sequelae, including CNS damage, hearing loss and difficulties in learning[1]. Three primary organisms account for more than 90% of the cases of meningitis globally, which are S. pneumoniae, N. meningitides and H. influenzae type b[2].

Bacterial meningitis remains a source of mortality and morbidity in spite of new and effective antibiotics use, whereas aseptic meningitis generally has a benign course requiring only supportive care.[3] Early diagnosis of BM and its differentiation from aseptic meningitis should be made to terminate the needful for antibiotic use and hospital admissions[4].

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Cerebrospinal fluid (CSF) assay is a preferable for predicting of BM, together with inflammatory biomarkers such as CRP and WBC count. Furthermore, it provide enough specificity without giving a good sensitivity for the diagnosis of BM[5]. Also, CSF analysis requires considerable time to prepare its results and most children with CSF pleocytosis are treated with powerful antibiotics in the hospital before the CSF culture results was obtained.[6] Besides atypical history, no distinctive physical examination and irresolute and low yield CSF cultures on some occasions, Sometimes CSF parameters cannot differentiate between bacterial and aseptic meningitis[7] while CRP and WBC count can discriminate between bacterial and nonbacterial meningitis[8]. Procalcitonin (PCT) is the best candidate to supersede CRP due to its high diagnostic accuracy in different infectious pathologies, including meningitis[9]. Procalcitonin (PCT) is a procalcitonin peptid, is produced in C-cells of the thyroid gland in physiological conditions and released from WBC on the peripheral circulation in bacterial infections. [10,11].

This study aimed to determine the diagnostic ability and using of PCT for estimating of BM and its correlation with the studied some inflammatory biomarkers (e.g., C-reactive protein, ESR, total WBC count, neutrophil/ lymphocyte ratio) which are required to predict the presence of BM ifLP is contraindicated or difficult to be performed.

Method

The case control study was conducted from September 2018 to March 2019, at the Children Welfare Hospital, Medical City, Baghdad, Iraq. The Ethics approved the research of the University of Baghdad, Faculty of Pharmacy IEC (UBCP-RECA-562018). The information about the patients was gained from the parents of each child for study participation and undergoing the assigned investigations. Ninety children aged between 6 months to 5 years, age – sex-matched children were included in the study. Fifty of themwere diagnosed with suspected bacterial meningitis according to the clinical investigation by specialized pediatrician and analysis of CSF. Forty children were enrolled as healthy control. The clinical diagnostic criteria of bacterial meningitis included fever (>38 C°) with signs of meningeal irritation, bulging fontanels, unexplained irritability, disturbed level of consciousness with or without heat, complex febrile seizure, lethargy and various skin manifestations such as petechial or an erythema[12].

Every child who presented with suspected meningitis underwent lumbar puncture by pediatric specialist or resident in the absence of any contra indications, to obtain CSF for Gram stain and culture, which is the most important step in the diagnosis of BM.

The samples of CSF were evaluated for simple cell analysis (pleocytosis with neutrophilia), an elevated protein, reduced concentration of CSF glucose and CSF culture for Streptococcus pneumonia, Neisseria meningitides, Haemophilus influenza. Furthermore, Blood samples were collected from each patient and healthy controls included in the study and divided into two parts, one was put in EDTA tubes for at once CBC(complete blood count) estimation using autoanalyzer instrument (Celltac-G, Nihon Kohden/ Japan) which included measurement of WBC count, absolute neutrophil count(ANC), absolute lymphocyte count (ALC) and lymphocyte counts and hemoglobin (Hb). Also, ESR was measured by using automated instruments (Vistal X20, Siemens/Italy).

The other part was allowed to clot. It centrifuged at 3500-4000 rpm for 10 minutes to obtain serum then set over pyrogen-free Eppendorf tubes to measure C-Reactive Protein) CRP by heterogeneous enzymatic immunoassay (Vitro 950 analyzer, USA), It is a latex agglutination test for qualitative examination of CRP in serum. Also frozen serum sample at - 20°C was kept until assayed of PCT using ready to use human PCT ELISA kit supplied by Mybiosource (MBS760892/USA).

The normal CSF analysis includedWBC counts below 8 cells/mm3, glucose levels at (60- 80)% of the serum glucose level and protein levels between 16- 46 g/L. Abnormal CSF results wereclassified as bacterial profile (elevated cell count with a prevalence of neutrophil, increased protein levels and decrease glucose levels).

Statistical analysis: it was performed by SPSS-21. Independent t-test and one-way ANOVA were used to assess the differences between means. The receiver operation characteristic curve (ROC curve) was used to identify the validity of markers as an indicator of disease. The markers were compared according to area under curve. The analysis was performed by using MedCalc Software. $P \leq 0.05$ is considered significant.

Results

Laboratory and demographic characteristics of patients with bacterial meningitis and control groups are listed in Table 1. Lumbar puncture was done for all patients and the results of CSF analysis include: polymorph nuclear cell (PMN cell/mm^3) 44.96±110.13, protein levels (g/l) 70.16±44.66, glucose levels (mg/dl) 53.16±17.94. In additionto the positive CSF cultures for patients with bacterial meningitis (15 cases of N. meningitides, 18 cases of S. pneumonia, 10 cases of H. influenza and 7 cases of E.coli). These results with clinical investigations were used as diagnostic criteria for the identification of bacterial meningitis.

Children with bacterial meningitis have significantly higher serum levels of PRO, CRP, ESR and NLR than the control group.

Table 1: Laboratory and demographic characteristics of patients and control groups

Parameters	Control No. 40	Patients No. 50	p-value
Sex (male vs female)	28:12	40:10	
Age (months)	33.60±18.41	33.55±15.28	0.96
Body Temperature (C°)	36.98± 0.23	38.37±0.36	<0.0001**
Hb (g/dl)	11.05±1.62	10.35±1.19	0.02*
WBC (*10^3/µ l)	6630.00±1450.76	13408.80 ±6356.48	<0.0001**
ANC (*10^3/µ l)	3583.00± 1130.14	5122.89±4077.34	0.01*
ALC (*10^3/µ l)	1787.50 ± 430.52	2292.90± 1530.84	0.03*
NLR	2.09 ±0.72	2.56 ± 1.38	0.04*
ESR (mm/1 hr)	8.60±4.10	31.80 ±32.71	<0.0001**
PRO (pg/mL)	395.56±663.59	918.34 ±633.91	<0.0001**
CRP(mg/l)	4.32± 0.86	11.72± 14.49	0.0007*
RBS (mg/dl)	95.00±8.23	110.86± 16.86	<0.0001**

Data was expressed as Mean± Standard Deviation (SD), *statistically significant at p*<0. 05 or* ** statistically highly significant at *p*-value ≤ 0.01. Hb: Hemoglobin, WBC: White Blood Cell, ANC: Absolute Neutrophil Count, ALC: Absolute Lymphocyte Count, NLR: Neutrophil Lymphocyte Ratio, ESR: Erythrocyte sedimentation rate, CRP: C-Reactive Protein, PRO: Procalcitonin, RBS: Random Blood Sugar.

Data were subjected to Receiver Operation Characteristic curve (ROC curve) to establish the preferable inflammatory biomarkers for estimation of BM in children with febrile seizure [Fig-1]. Curves of ROC studies confirmed that the ANC was a preferable diagnostic marker of bacterial meningitis, followed by PRO and NLR which are highly specific as compare to CRP. The sensitivity, specificity and cut-off point of inflammatory biomarkers for patients with bacterial meningitis were estimated by ROC [Table-2].

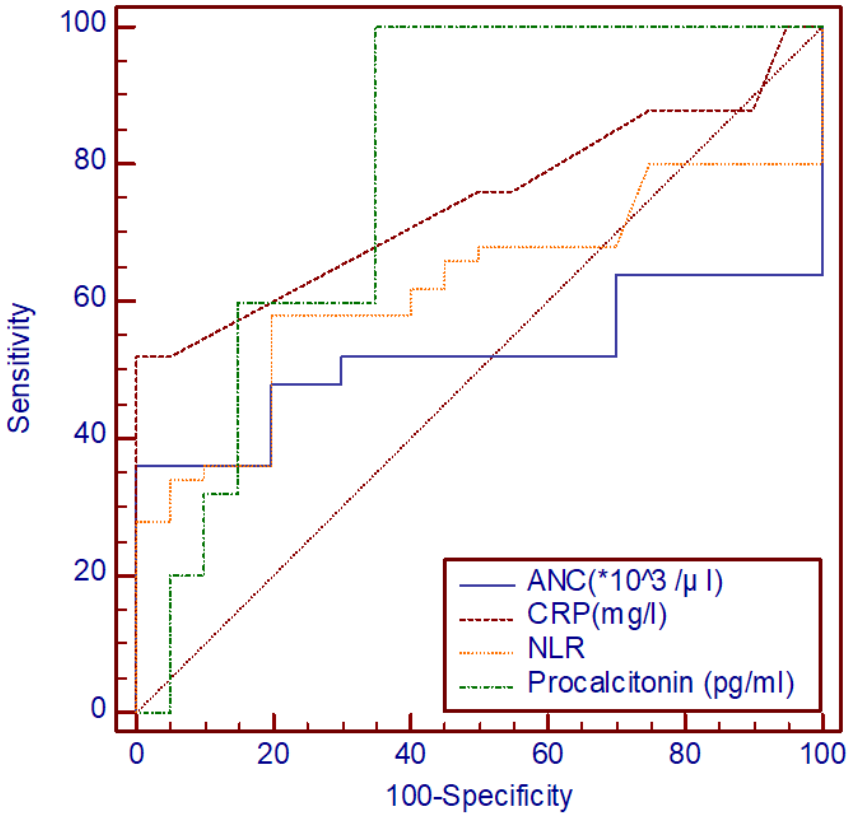

Fig 1: ROC curves for ANC, CRP, NLR and Procalcitonin

Table 2: Area under the curve (AUC), Standard error (SE), Sensitivity, Specificity, cut-off point and 95% confidence intervals (95%CI) of ANC, CRP, NLR and procalcitonin for bacterial meningitis patients.

Variable	AUC	SE[a]	Sensitivity	Specificity	Cut –off point	95% CI[b]
ANC (*10^3/µ l)	0.902	0.0303	72.00	100.00	>8600	0.821 to 0.955
CRP (mg/l)	0.729	0.0541	56.00	90.00	>15	0.625 to 0.817
NLR	0.820	0.0603	52.00	100.00	>2.5	0.512 to 0.720
Procalcitonin (pg/mL)	0.945	0.0522	85.00	100.00	>5.5	0.642 to 0.831

[a]Hanley & McNeil, 1982, [b]Binomial exact

Elevating serum levels of PRO and CRP in patients group were significantly associated with increased serum ANC levels according to the AUC. A significant relationship was also observed between PRO and NLR, indicating that the PRO, CRP and NLR had better biomarkers in the diagnosis of BM table 3.

Table 3: Pairwise comparison of ROC curves according to the AUC.

Comparison between parameters	AUC	p-value
ANC ~ CRP	0.225	0.0008*
ANC ~ NLR	0.1000	0.0763
ANC~ Procalcitonin	0.276	0.0001*
CRP ~ NLR	0.125	0.0774
CRP ~ Procalcitonin	0.0510	0.4399
NLR ~ Procalcitonin	0.176	0.0012*

ANC: Absolute Neutrophil Count, NLR: Neutrophil Lymphocyte Ratio and CRP: C-Reactive Protein.*p<0.05 Statistically Significant, p<0.001 Statistically highly significant.

Discussion

A reliable markers for bacterial disease should carry out the following criteria: early prognostic diagnostic and values and should be additionally helpful for therapeutic antimicrobial decisions[13]. BM is a pediatric emergency with increase morbidity and mortality, so it must be diagnosed and treated immediately. But identical clinical presentation often creates it tricky to discriminate bacterial and non-bacterial etiologies in children [14]

CSF analysis was considered the standard for estimation ofBM, together with WBCcount and CRP. Also, clinical criteria, gram staining and bacterial antigen testing of CSF as well or CSF (glucose, protein, WBC and neutrophil count) used alone lack the specificity and sensitivity for the diagnosis of meningitis and can only providea clinical probability[15,16].

Procalcitonin has been considered to be a typical marker with the highest rigor for bacterial infections due to giving an early diagnosis, give information about the course and prognosis of the disease and rapid therapeutic decisions[17].

PCThave good sensitive and specific properties for diagnosis of meningitis.[18]. In this study, serum PCT significantly higher in children with BM than healthy control groups. This result documented with other researcheswhere PCT concentration increased in bacterial meningitis as compare to other sources of infection with bacterial meningitis [19-21].

This elevation in serum PCT of BMdue to the release of PCT from all differentiated cell types and parenchymal tissues throughout the body in response to elevated calcitonin gene (CALC-I gene) expression in the presence of bacterial lipopolysaccharides and cytokines associated with severe bacterial infections. PCT secretion during inflammation depends from bacterial endotoxins and inflammatory cytokines, interleukins 6 (IL-6) and tumor necrosis factor (TNF alpha)[22]. In contrast to this, there is a transient increase of PCT in patients with viral infection.Furthermore, a rapidresponse characterized by increase in serum PCT levels after a bacterial stimulus which done by giving a single endotoxin injection makes PCT level a sensitive marker for BM[23].

The results of this study display that the procalcitonin was more sensitive and specific inflammatory biomarkers in patients group than the CRP and ANC according to the ROC analysis, these results in agreement with other findings which observed that serum PCT level was a good biomarker for differentiation of bacterial meningitis from other causes[5].

Morales Casado et al. was concluded that serum PCT performs better than CRP in the detection of bacterial meningitis. Measuring the CRPreleased in response to inflammation and bacterial infection in BM is less sensitive and specific than measuring PCT[24].

Serum PCT level dramatically increasedin early stage during the bacterial infection, making it more sensitive and specific than CRP as a marker of systemic bacterial infection in children. Also, increase in PCT concentrations begin from about few hours after a single endotoxin injection, peak at about 6 hours and persistant elevation for over 24 hours[25]. WBC count can differentiate bacterial and non-bacterial meningitis[5]. In this study, total WBC count was significantly higher in children with BM than the healthy control. The results of this research documented by other studies in that leukocytosis were valuable in distinguishing between bacterial and non-bacterial infections but not an independent predictor of serious bacterial infection like PCT and CRP, [28,29]. PCT is more worthy than CRP or and total WBC count [25]. The results of ROC analysis found that the PCT and ANC were more sensitive and specific biomarkers than CRP and NLR for predicting the diagnosis of BM in febrile children with seizure.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq

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