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Research Article
Measurement of Interleukin-6 in Cerebrospinal Fluid for the Diagnosis of Bacterial Meningitis

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Abstract

Objective: It is assessed whether the measurement of interleukin-6 in the cerebrospinal fluid can serve as a biomarker for the diagnosis of bacterial meningitis. **Methodology:** Cerebrospinal fluid was obtained from 152 patients aged 0-15 years suspected of having meningitis. These patients were classified into the following groups: Bacterial meningitis (n = 85), aseptic meningitis (n = 35) and non-meningitis/control (n = 32) based on leukocyte count and bacterial identification by culture and molecular biology. Interleukin-6 concentrations in cerebrospinal fluid were measured by enzyme-linked immunosorbent assay. **Results:** This study found a significant difference of the mean cerebrospinal fluid interleukin-6 level ($p \leq 0.01$) between patients with bacterial meningitis (3,538.69±2,560.78 pg mL^{-1}) and patients with aseptic meningitis (332.51±470.69 pg mL^{-1}) or those of the control group (205.83±79.39 pg mL^{-1}). There was also a significant difference of the mean cerebrospinal fluid interleukin-6 level between patients with aseptic meningitis and those of the control group. Interleukin-6 had the highest area under the ROC curve: 0.94 (95% confidence interval: 0.901-0.979) compared to that of cerebrospinal fluid glucose and total protein. At a cut-off value of 1,065.96 pg mL^{-1}, interleukin-6 had a sensitivity of 76.2% and specificity of 100%. **Conclusion:** Interleukin-6 is a potential biomarker for the differential diagnosis of meningitis.

Key words: Measurement, diagnosis, interleukin-6, cerebrospinal fluid, bacterial meningitis

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Meningitis remains a public health problem despite advances in the domain of epidemiology, antibiotherapy and vaccination with conjugated vaccines (MenAfrVac®) against meningococcus of the A serogroupe[1-2]. Meningitis epidemics are recurrent in the zone South of the Sahara called "The African meningitis belt[3]" with the emergence of new strains responsible for these epidemics. Epidemics caused by serogroup W[4-6] and serogroup X[7] meningococci have been reported in recent years in the "African meningitis belt". Bacterial meningitis is characterized by high morbidity and mortality necessitating early diagnosis and rapid treatment. Mortality in the absence of treatment can be as high as 70% and one of five of those who survive develop permanent sequelae such as deafness, neurological problems and paralysis of the limbs[8]. Large epidemics lead to considerable problems in caring for patients in developing countries due to limited technical resources and hospital capacity. The establishment of new biomarkers that allow rapid and precise diagnosis and provide information on the severity of the disease may improve patient care and reduce patient mortality. There have been many studies investigating the potential of testing cytokines in the diagnosis of meningitis but they have provided contradictory results. In addition, most have been performed outside the African meningitis belt, the most effected zone. This study assessed whether interleukin-6 (IL-6) in cerebrospinal fluid (CSF) can serve as a biomarker for the differential diagnosis of meningitis.

MATERIALS AND METHODS

This was a non-blinded, observational, transversal, prospective study. Patient recruitment took place at the pediatric emergency department of the National Hospital of Niamey (NHN) during the period from February to April, 2015. Clinical information on the patients as well as the results of microbiological cultures and glucose and protein testing at the Medical Biology and Biochemistry Laboratories of NHN were collected from a data collection form. Aliquots of the CSF supernatant, collected after centrifugation at 4000 rpm for 5 min were conserved at -20°C for IL-6 measurement and bacterial identification by molecular biology at the Medical Research and Health Center (CERMES).

Patients: The study comprised patients presenting clinical signs suggestive of meningitis for whom their medical care included collecting a CSF sample. The decision to collect the CSF sample was made by the medical care team. There were no patients for whom a CSF sample was collected solely for the study. The study was approved by the National Consultative Ethics Committee of Niger. The patients were divided into three groups: Group 1 (controls) consisted of patients for whom the CSF leukocyte counts were <10 cells mm^{-3} and no bacteria was detected in culture or by molecular biology, group 2 consisted of cases of aseptic meningitis (patients for whom the CSF leukocyte count was >10 cells mm^{-3} but the CSF was bacteria-negative by Gram staining, culture and molecular biology and group 3 consisted of bacterial meningitis cases identified by culture and molecular biology.

Measurement of CSF biomarkers: Glucose (cypress diagnostics, langdorp, Belgium) and protein (Sprinreact, Sant Esteve de Bas, Spain) contents of the CSF supernatant were determined using an enzymatic, colorimetric method[9-14].

An enzyme-linked immunosorbent assay (ELISA) method using the human IL-6 ELISA (EH2IL65) kit (Thermo Scientific, USA) was used to measure IL-6 in the CSF supernatant. Each sample was analyzed in duplicate and the absorbance of the plates measured at 450 nm. The test has a sensitivity <1 pg mL^{-1} and requires 50 µL of CSF supernatant. The results are expressed in pico gram per milliliter.

Bacteriological and molecular identification: Bacteriological identification involved culture on polyvitex chocolate agar or blood agar using an API NH gallery for *Neisseria meningitidis* and *Haemophilus influenzae* and the Alere BinaxNOW® *Streptococcus pneumoniae* Antigen Card Kit (Alere Inc, USA) for *Streptococcus pneumoniae.*

A conventional multiplex Polymerase Chain Reaction (PCR) was used to identify the three principle bacteria responsible for bacterial meningitis: *Neisseria meningitidis* (*crg*A gene), *Streptococcus pneumoniae* (*lyt*A gene) and *Haemophilus influenzae* (*bex*A gene). The genogroup of *Neisseria meningitidis* was determined by multiplex PCR that first identifies the A, X and W genogroups and then the Y and C genogroups for those that are negative in the first PCR[15-17].

Statistics: Statistical analysis were performed using IBM SPSS Statistics vs 20 software. The Kolmogorov-Smirnov test was used to determine the normality of the distribution and the non-parametric Kruskal-Wallis test to compare biological parameters between the various groups. The diagnostic power of the CSF biomarkers was determined using a ROC curve. Inverse values were used for the glucose levels. The $p \leq 0.05$ is considered significant.

RESULTS

In total, 152 patients, of which 50.8% were female, with a mean age of 6.8±4.7 years were included in this study. Eighty five (70.8%) patients were diagnosed with bacterial meningitis and 35 (29.2%) with aseptic meningitis. Thirty two (21%) patients comprised the control group. The principle agent identified for bacterial meningitis was *Neisseria meningitidis* (97.6% of the cases). The distribution by serogroup was 77.6% for sero group C, 11.8% for serogroup W and 8.2% not determined. Only two cases of *S. pneumoniae* (2.4%) were detected. The IL-6 concentration for patients with bacterial meningitis was significantly higher than that for patients with aseptic meningitis (3,538.69±2,560.78 pg mL^{-1} versus 332.51±470.69 pg mL^{-1} at $p \leq 0.01$) and that of patients of the control/non-meningitis group (3,538.69±2,560.78 pg mL^{-1} versus 79.39±205.83 pg mL^{-1}, $p \leq 0.01$) (Table 1). For the glucose and protein levels, a significant difference was observed only between patients with bacterial meningitis and those with aseptic meningitis and the control group. The ROC curves (Fig. 1) were analyzed. The Area Under Curve (AUC) was higher for the IL-6 concentration (0.94 CI 95%: 0.901-0.979) than for other CSF biomarkers: glucose level (AUC: 0.762 CI 95%: 0.670-0.853) and protein level (AUC: 0.77 CI 95%: 0.681-0.859). A cutoff for the CSF IL-6 concentration of 1,065.96 pg mL^{-1} gave the best values for sensitivity (76.2%) and specificity (100%) (Table 2). Atypical IL-6 values were observed (Fig. 2) for 10 patients with aseptic meningitis (between 1,027.69 and 4,059.60 pg mL^{-1}) five of these patients had a leukocyte count greater than 100 cells per microliter with a predominance of neutrophils for four patients and lymphocytes for one patient who had an IL-6 concentration of 3,173.88 pg mL^{-1}.

DISCUSSION

This study coincided with the most important meningitis epidemic caused by serogroup C *Neisseria meningitidis* recorded in the African meningitis belt[18]. As of June 30, 2015, 8,500 suspected cases of meningitis with a mortality rate of 6.8% have been reported in Niger. The region of Niamey, where this study was performed, was the most highly affected, accounting for 61.9% of the reported cases in the country. The principle biological agent of the epidemic was *Neisseria meningitidis* (92.65% of the positive cases) of which most were of serogroup C (80.58%)[19].

In this study, it is assessed whether there was a significant difference in the IL-6 concentration in CSF as a function of the type of meningitis. This study report that there is, indeed, a significant difference. Furthermore, IL-6 gave higher AUC for the ROC curve than other potential biomarkers for the diagnosis of bacterial meningitis. Rapid diagnosis of meningitis in children is essential to improve the prognosis[20,21]. Currently, diagnosis is primarily based on the leukocyte count and assaying glucose and protein in the CSF[22-25] but these techniques are not sufficiently precise for the diagnosis of meningitis[26]. The role of cytokines in inflammation in the central nervous system in response to an infection is well known[27,28]. This inflammatory reaction is partially responsible for the physiopathological consequences of bacterial

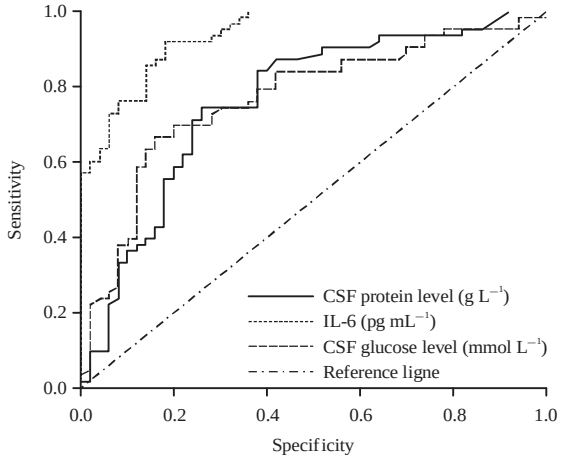

Fig. 1: Comparison of the diagnostic power of CSF Il-6 concentrations with those of glucose and total protein for bacterial meningitis

Table 1: CSF IL-6, glucose and total protein values in the different groups

Parameters	IL-6 (pg mL^{-1})	Glucose (mmol L^{-1})	Protein (g L^{-1})
Bacterial meningitis	3,538.69±2,560.78**[ab] (n = 85)	1.31±1.59**[ab] (n = 65)	3.09±2.79**[ab] (n = 65)
Aseptic meningitis	332.51±470.69**[c] (n = 35)	3.01±2.09 (n = 18)	1.70±1.79 (n = 18)
Non meningitis	79.39±205.83 (n = 32)	2.75±1.38 (n = 32)	1.08±2.02 (n = 32)

Results are expressed as the Mean±SD, n: Number of patients, **$p \leq 0.01$, [a]Significant difference between the bacterial meningitis group and that of aseptic meningitis, [b]Significant difference between the bacterial meningitis group and that of non meningitis, [c]Significant difference between the aseptic meningitis group and that of non meningitis

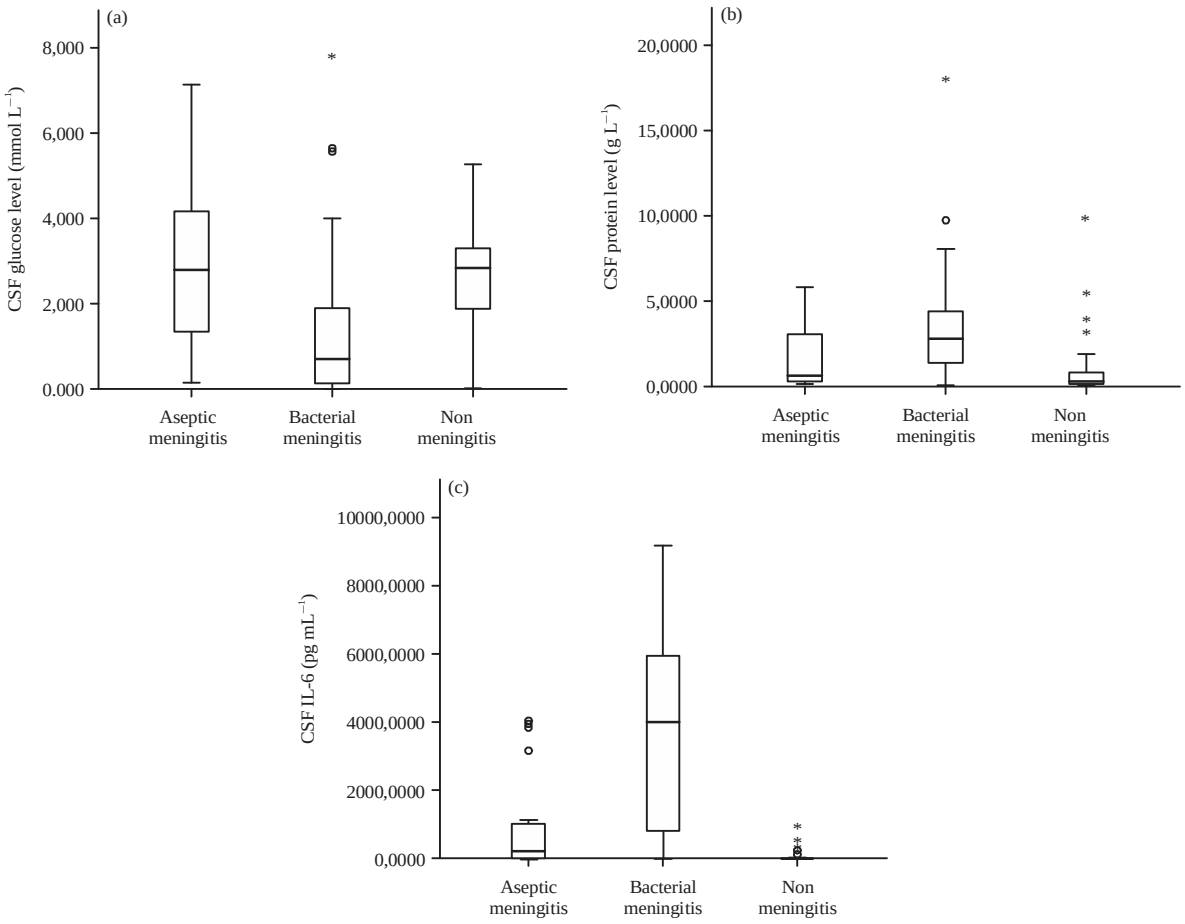

Fig. 2(a-c): Distribution of (a) CSF glucose level, (b) CSF protein level and (c) CSF IL-6 levels

Table 2: Cutoff values for each marker showing best sensitivity and specificity

CSF biomarkers	Cutoff	Sensitivity	Specificity
Glucose (mmol L^{-1})	0.13	98.4	100
	1.51	98.4	98
Protein (g L^{-1})	0.09	100	92
	0.10	98.4	90
IL-6 (pg mL^{-1})	1,065.96	76.2	100
	1,006.20	76.2	80

meningitis such as the stimulation of protein secretion (exudation), fever, increase of leukocyte numbers[29] and nerve damage. The presence of bacterial particles in the CSF stimulates cytokine secretion[30] either by blood monocytes due to edema of the brain or by endothelial cells and astrocytes[31]. Studies of the value of IL-6 for the diagnosis of bacterial meningitis have provided contradictory results. Most of these studies were performed outside of the meningitis belt and on small sample sets. Some studies, such as that of Pinto *et al.*[32] did not show significantly high CSF IL-6 concentrations in meningitis patients whereas others reported higher than control values[33-35].

The potential of IL-6 as a diagnostic biomarker for bacterial meningitis is known[26,34,36]. The AUC for IL-6 obtained in this study is comparable to those reported by Garcia-Hernandez *et al.*[36] and Takahashi *et al.*[26] found an AUC of 0.937 and 0.962, respectively. Garcia-Hernandez *et al.*[36] obtained a sensitivity of 95.5% and a specificity of 77.5% with an IL-6 concentration cutoff of 1,418 pg mL^{-1}. In contrast, Takahashi *et al.*[26] obtained a sensitivity of 92.3% and a specificity of 89.5% with a lower cutoff of 644 pg mL^{-1}. The cutoff value proposed in this study is between these two values, but the sensitivity that we obtained is lower than that of these earlier studies.

CONCLUSION

The IL-6 is a potential biomarker for the differential diagnosis of meningitis. However, further studies to evaluate the consequences of measuring IL-6 levels on the treatment of bacterial meningitis in sub-Saharan Africa are required.

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