Biochemical and Bacteriological Profiles of Cerebrospinal Fluids of Children with Presumed Sepsis in a Tertiary Hospital in Abeokuta, Ogun State, Nigeria

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Abstract: Cerebrospinal fluid (CSF) analysis is a set of laboratory tests that examine a sample of the fluid surrounding the brain and spinal cord. In this study all cases of suspected meningitis among children less than 5 years of age from February 2013 to May, 2013 were reviewed. A total of 46 Cerebrospinal fluid samples were received. The macroscopic appearance of the CSF was recorded. Biochemical analysis of the protein and glucose were carried out using spectrophotometer while bacteriological analyses were carried out using standard techniques. Antibiotic sensitivity testing was carried out using Kirby-Bauer diffusion method. The protein values of forty one (89.1%) of the cerebrospinal samples fluids range from 10 - 40mg/dl while five (10.9%) of the samples were above 40mg/dl. The glucose values of 31(67.4%) of the cerebrospinal fluids samples range from 45 - 80mg/dl, ten (21.7%) of the samples were above 80mg/dl while the remaining 5(10.9%) were below 45mg/dl. Two (2) *Pseudomonas aeruginosa* were detected from two of the CSF samples. The two *Pseudomonas aeruginosa* isolates showed susceptibility to gentamycin and ceftriaxone while one was sensitive to Ceftazidime. The result of this study highlights the importance of regular surveillance of common pathogens such as *Pseudomonas aeruginosa* in our environment.

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Introduction

Cerebrospinal fluid (CSF) is a clear, colourless bodily fluid produced in the choroid plexus of the brain. It protects the central nervous system from injury, cushions it from the surrounding bone structure, provides it with nutrients, and removes waste products by returning them to the blood (Smith *et al*, 2001). It occupies the subarachnoid space (the space between the arachnoid mater and the pia mater) and the ventricular system around and inside the brain and spinal cord. It constitutes the content of the ventricles, cisterns, and sulci of the brain, as well as the central canal of the spinal cord. The fluid is an ultrafiltrate of plasma. It contains glucose, electrolytes, amino acids, and other small molecules found in plasma, but has very little protein and few cells (Smith et al, 2001).

CSF contains approximately 0.3% plasma proteins, or approximately 15 to 40 mg/dL, depending on sampling site (Felgenhauer, 1974), and it is produced at a rate of 500 ml/day. Since the subarachnoid space around the brain and spinal cord can contain only 135 to 150 ml, large amounts are drained primarily into the blood through arachnoid granulations in the superior sagittal sinus. Thus the CSF turns over about 3.7 times a day. This continuous flow into the venous system dilutes the concentration of larger, lipid-insoluble molecules penetrating the brain and CSF (Saunders *et al*, 1999).

There are quantitative differences in the distributions of a number of proteins in the CSF. In general, globular proteins and albumin are in lower concentration in ventricular CSF compared to lumbar or cisternal fluid (Merril *et al*, 1981). CSF glucose or glycorrhachia is a measurement used to determine the levels of glucose in cerebrospinal fluid (Mohammadi *et al*, 2003: Seehusen *et al*, 2003). It can be useful in distinguishing among causes of meningitis. It is more

likely to be decreased in bacterial meningitis than in viral meningitis (Karen, 2005).

The CSF surrounding the spinal cord normally contains 50-80 mg/dL of glucose. Fluctuations in the CSF glucose level can indicate the presence of disease. Lower levels of glucose in the CSF may indicate increased glucose use, either by white blood cells or bacteria. Lower CSF glucose levels are often found in conjunction with infections of the central nervous system. CSF is withdrawn from the subarachnoid space through a needle by a procedure called a lumbar puncture or spinal tap (Smith et al, 2001).

Meningitis can be caused by many infectious agents like bacteria, viruses and fungi, and non-infectious factors (like trauma). Fever, headache and stiff neck often suggests the diagnosis of meningitis. The clinical manifestations may vary according to age range. In infants, fever and vomiting are frequently the early complaints.

The aim of this study was to determine the biochemical and bacteriological profiles of cerebrospinal fluids of children with presumed meningitis in Abeokuta, Ogun state, Nigeria.

Methods

This a four months laboratory-based retrospective study conducted in Abeokuta. Cerebrospinal fluid samples were received in the laboratory after clinical assessments from the attending clinicians.

Samples

All CSF samples received in the units were processed immediately. A total of 46 Cerebrospinal fluid samples were received, the samples were brought to the laboratory in sterile, clear, transparent, wide mouthed universal bottles. Each sample was brought in duplicate. The macroscopic appearance of the CSF was recorded. Some of the clinical diagnosis was sepsis, meningitis etc.

Processing of Specimens

The specimens were processed according to the guidelines provided by Cheesbrough, 2004 biochemical (protein, glucose) and bacteriological (microscopy, culture) analyses were carried out on all the samples.

Biochemical analysis

Glucose: 10µl of the CSF sample and 1000µl of glucose oxidase reagents were mixed together in a sterile test tube and incubated at 37ºC for 10 minutes. This was read using spectrophotometer.

Protein: 25µl of the CSF sample and 1000µl of sulphur salicylic acid, were mixed together and incubated at room temperature for 10 minutes. This was then read using Absorbance mode on spectrophotometer at 567nm wavelength. Controls were also set up for the tests.

$$\textbf{Concentration of protein} = \frac{\text{Absorbance of Test} \times \text{Concentration of standard}}{\text{Absorbance of standard}}$$

Microbiology analysis

Culture

CSF specimens were inoculated onto sterile MacConkey, Blood agar and Chocolate agar plates. MacConkey, Blood agar plates were incubated at 37ºC in ambient air while the Chocolate agar plates were incubated at 37ºC under microaerophillic condition (in a CO^2 jar). The organisms isolated were identified by gram stain, and standard bacteriological techniques (Cheesbrough, 2004). Antibiotic susceptibility was done by standard disc diffusion method of Kirby and Bauer (Cheesbrough, 2004), and results were interpreted accordingly.

Results

A total of 46 cerebrospinal fluids samples were received, twenty four of the samples were from male children while twenty one of the samples were from female children. The age and sex distribution is shown below (Table 1).

Table 1*:* Age and Sex of children tested.

Table 1: Age and Sex of children tested

Age (years)	No. Tested (%)	No. Males (%)	No. Females (%)
≤ 1	24(53.3)	16(66.7)	8(33.3)
≤ 2	9(20.0)	4(44.4)	5(55.6)
≤ 3	6(13.3)	2(33.3)	4(66.7)
≤ 4	4(8.9)	1(25.0)	3(75.0)
≤ 5	2(4.4)	2(100.0)	0(0.0)
Total	**45(100.0)**	**24(53.3)**	**21(46.7)**

Macroscopic examination showed that out of the 46 cerebrospinal fluid specimens received, 27 were clear while 19 were turbid. Twenty seven of the samples were colourless, 12 were xanthochromic while the remaining 7 were bloody in table 2.

Table 2: Macroscopic examination of the cerebrospinal fluid

Parameters	Number of samples (%)
Appearance	
Clear	27(58.7)
Turbid	19(41.3)
Total	**46(100.0)**
Colour	
Colourless	27(58.7)
Xanthochromic	12(26.1)
Bloody	7(15.2)
Total	**46(100.0)**

The protein values of forty one (89.1%) of the cerebrospinal samples fluids range from 10 - 40mg/dl while five (10.9%) were above 40mg/dl. The glucose values of 31(67.4%) of the cerebrospinal fluids samples range from 45 - 80mg/dl, ten (21.7%) of the samples were above 80mg/dl while 5(10.9%) were below 45mg/dl in Table 3.

Table 3: Values of proteins and glucose

Parameters	Range	No. Occurrence (%)
Protein:	**10-40 mg/dl**	41 (89.13)
	Above 40mg/dl	5 (10.87)
Glucose:	**45-80 mg/dl**	31 (67.4)
	Above 80mg/dl	10 (21.7)
	Below	5 (10.9)

Out of the 46 CSF samples cultured, 2(4.4%) were culture positive while 44(78.3%) were bacteriologically sterile. The two culture positive samples were from CSF samples of two children in age bracket ≤ 1 year. The bacteria isolates was identified as *Pseudomonas sp* using various biochemical tests. Figure 1 showed the distribution of the *Pseudomonas aeruginosa* isolates detected.

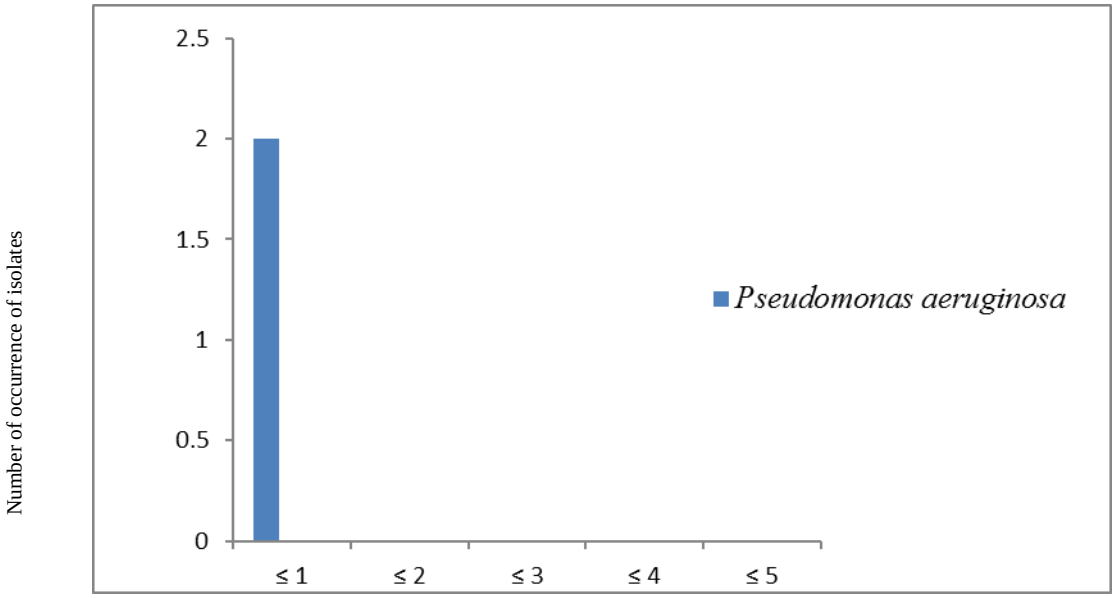

Figure 1. Showed the distribution of the *Pseudomonas aeruginosa* isolates detected

The susceptibility studies showed that the two *Pseudomonas aeruginosa* isolates were 100% resistance to cloxacillin, ampicillin, augmentin, streptomycin, cefuroxime, cotrimoxazole and erythromycin. Ceftazidime was sensitive to one of the *Pseudomonas aeruginosa* (P1) isolates while gentamycin and ceftriaxone were sensitive to the two *Pseudomonas aeruginosa* isolates.

Table 4: *In - vitro* susceptibility patterns of *Pseudomonas aeruginosa* isolates from Csf samples Antibiotics

Isolates codes	Cxc	Amp	Aug	Gen	Str	Caz	Cxm	Ctr	Cot	Ery
P 1	R	R	R	S	R	S	R	S	R	R
P 2	R	R	R	S	R	R	R	S	R	R

Keys: Gen = Gentamycin, Ery = Erythromycin, Amp = Ampicillin, Aug = Augmentin,
Ctr = Ceftriaxone, Cot = Cotrimoxazole, Str = Streptomycin, Cxc = Cloxacillin,
Cxm = Cefuroxime, Caz = Ceftazidime. S – Sensitive R – Resistant
P – *Pseudomonas aeruginosa*

Discussion

The present study investigated the biochemical and bacteriological profiles of cerebrospinal fluids (Csf) of children with presumed meningitis in a tertiary hospital in Abeokuta, Ogun state. Our results showed that 10.87% of the Csf obtained from the children had protein level above 40mg/dl, (the normal range is between15 to 40 mg/dl protein) while 89.13% were within the normal range. According to Cheesbrough, 2004 total Csf protein is normally between 15 to 40 mg% while values up to 100mg% is said to be normal for newborn infants. CSF from the lumbar region according to Dimitri and Agamanolis, (2013) contains 15 to 45 mg/dl protein (lower in children). Dimitri and Agamanolis, 2013 observed that a more moderate increase (150-200 mg/dl) of CSF protein occurs in inflammatory diseases of meninges (meningitis, encephalitis), intracranial tumors, subarachnoid hemorrhage, and cerebral infarction. Increased Csf total protein with pandy's test: occurs in all forms of meningitis, in amoebic and trypanosomiasis meningoencephalitis, cerebral malaria, brain tumours, cerebral injury, spinal cord compression, poliomyelitis, the Guillain-Barre syndrome and polyneuritis Cheesbrough, 2004. Thirty one (67.4%) of the Csf analyzed were within the normal glucose level for children while 21.7% of the cerebrospinal fluid had glucose level above (80mg/dl), the normal range is between 50-80 mg/dl**.** The high trace of glucose in the CSF observed in this study may be a reflection of higher blood glucose, otherwise known as hyperglycemia. In this study also, five (10.9%) of the samples had low Csf glucose level, according to Cheesbrough, 2004, Csf glucose reduced in most forms of meningitis, except viral meningitis. Mohammadi *et al*, 2003 also stated that the levels of glucose in the cerebrospinal fluid (CSF) can be used to differentiate bacterial meningitis from viral meningitis. Children with bacterial meningitis typically have low levels of Csf glucose because of glycolysis by both white cells and the pathogen and impaired CSF glucose transport Seehusen *et al*, (2003). *P. aeruginosa* was detected in two of the Csf samples with low glucose level in this study, which might suggest that the glucose reduction in the two samples might be due to the presence of the organisms. Gentamycin, ceftriaxone and Ceftazidime were the only antibiotics that showed sensitivity to *P. aeruginosa* detected in this reviewed, this is in agreement with a research carried out in south west Nigeria by Akingbade *et al.* (2012) and a research on multi drug *P. aeruginosa* in Abeokuta, Ogun, Nigeria by Akingbade *et al.* (2013), who recorded high susceptibility activities of gentamycin against *P. aeruginosa.* In conclusion, it is advised that cerebrospinal fluids samples be taken to the laboratory immediately after collection to prevent glycolysis which can result in falsely low result of Csf glucose.

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