SERUM PROCALCITONIN IN THE DIAGNOSIS OF BACTERIAL MENINGITIS

Thesis

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By

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ABSTRACT

Background

Bacterial meningitis is a common and serious infection of the central nervous system, which necessitates rapid diagnosis and initiation of antibiotic therapy for a favorable outcome. On the other hand, viral meningitis is a benign self-limited disease. Differentiating both diseases is of great importance. Clinical findings and conventional laboratory data were said to have a modest accuracy rate for differentiating both diseases. However, even these basic diagnostic facilities may be lacking or inadequate in many developing countries including Egypt.

Earlier studies using urine reagent strips and procalcitonin (PCT) for diagnosis of meningitis had given a high sensitivity and specificity rates reaching ~ 100%. However, later ones yielded a much lower accuracy rates.

Objectives

The main objective of this study was to evaluate the usefulness of two bedside diagnostic tests in the diagnosis of meningitis. These were urine reagent strips and semi-quantitative procalcitonin (PCT-Q) test.

Also, the value of clinical and conventional laboratory studies (blood and cerebrospinal fluid) were evaluated for their ability to differentiate meningitis from non-meningitis cases, and also bacterial from aseptic meningitis.

Methods

All patients admitted to Shebin El-kom fever hospital during a three years period from first of July 2008 to 30 June 2011 (they were 1712 patients) with clinical data suggesting meningitis were included in this study.

Clinical manifestations; laboratory examination of cerebrospinal fluid (CSF) (glucose, protein, leukocyte count, Gram stain and bacterial cultures); and serum inflammatory markers (peripheral blood leukocyte count and C-reactive protein) were evaluated for their ability to differentiate meningitis from non-meningitis cases, and also, in differentiating bacterial from aseptic meningitis.

CSF samples (number = 1587) were tested using urine reagent strips for protein, glucose and leukocytes; results were compared with the same CSF parameters obtained from routine laboratory study.

A total of 100 cases of meningitis (64 bacterial and 36 viral) were selected for PCT-Q testing. Bacterial meningitis cases were either confirmed (number = 27) with positive bacterial culture and/or Gram stain; or presumed bacterial meningitis (number = 37) with neutrophilic CSF (leukocyte count >4,000/mm^3). Presumed viral meningitis (number = 36) were cases with negative CSF Gram stain, negative CSF and blood cultures for bacteria, CSF pleocytosis (≤ 100 cells/mm^3) with a predominance of mononuclear cells, blood leukocyte count <10,000/mm^3, negative CRP, no seizures and recovery without antibiotic therapy. The ability of PCT-Q testing to differentiate bacterial from viral meningitis was assessed.

Results

Clinical manifestations were of moderate assistance in differentiating meningitis (number = 623) from non-meningitis patients (these were 297 encephalitis cases and 792 cases with meningism). Also, clinical symptoms and signs were of little assistance in differentiating bacterial from aseptic meningitis.

Laboratory results and especially bacteriological examination of CSF (positive bacterial culture is the gold standard in the diagnosis of bacterial meningitis) yielded a very low positivity rate (combined Gram stain and cultures were positive in only 4.3%). Although CSF protein concentration and leukocyte count and serum CRP were significantly higher in bacterial than aseptic meningitis, there was a wide area of overlapping results between the two groups.

The number of reagent strip results coinciding with the laboratory results were 1134 (71.45%), 802 (50.54%) and 1016 (64.02%) out of 1587 patients for leukocytes, protein and glucose respectively. Sensitivity rates were moderate and the degree of agreement between CSF strip and laboratory results were increased with higher grades.

PCT-Q had a good discriminating ability in differentiating bacterial from viral meningitis. It was positive in 87.5% of patients with bacterial meningitis and negative in 91% of cases of viral meningitis. Serum PCT with cutoff value

0.5 ng/mL showed sensitivity, specificity, accuracy, positive predictive value, and negative predictive value of 88%, 92%, 89%, 95% and 80% respectively for the diagnosis of bacterial meningitis.

Conclusion

Caution is needed in the use of reagent strips for the diagnosis of meningitis. They can be used only as a preliminary screening test till the laboratory results becomes available.

Although PCT-Q test was more sensitive in differentiating bacterial from viral meningitis (compared with other laboratory parameters), it cannot be used alone for this diagnosis. No single test will be sufficiently sensitive to conclusively distinguish bacterial from viral meningitis.

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LIST OF ABBREVIATIONS

AA	Amino Acid
ACE	Angiotensin Converting Enzyme
AIDS	Acquired Immunodeficiency Syndrome
BBB	Blood Brain Barrier
C5	Complement factor 5
CALC-1 gene	Calcitonin-1 gene
CDC	Center for Disease Control and Prevention
C. immitis	*Coccidioides immitis*
C. neoformans	*Cryptococcus neoformans*
CNS	Central Nervous System
CO_2	Carbon dioxide
COPD	Chronic Obstructive Pulmonary Disease
CRP	C-reactive protein
CSF	Cerebrospinal fluid
CT	Computed Tomography
DNA	Deoxyribonucleic Acid
E. coli	*Escherichia coli*
H. capsulatum	*Histoplasma capsulatum*
H. influenzae	*Haemophilus influenzae*
HIV	*Human Immunodeficiency Virus*
HSV	*Herpes Simplex Virus*
Ig G	Immunoglobulin-G
IL-6	Interleukin-6
L. monocytogenes	*Listeria monocytogenes*
LP	Lumbar Puncture
MIC	Minimum Inhibitory Concentration
MMWR	Morbidity and Mortality Weekly Report
MODS	Multiple Organ Dysfunction Syndrome
MOH	Ministry Of Health
mRNA	messenger Ribonucleic Acid
M. tuberculosis	*Mycobacterium tuberculosis*
N. meningitidis	*Neisseria meningitidis*
P. acnes	*Propionibacterium acnes*
PCR	Polymerase Chain Reaction
PCT	Procalcitonin

PMN	Polymorphonuclear
R. rickettsia	*Rickettsia rickettsii*
S. agalactiae	*Streptococcus agalactiae*
S. aureus	*Staphylococcus aureus*
S. epidermidis	*Staphylococcus epidermidis*
SIRS	Systemic Inflammatory Response Syndrome
SLE	Systemic Lupus Erythematosus
S. pneumoniae	*Streptococcuc pneumoniae*
TNF-a	Tumor Necrosis Factor-alpha
s-TREM	soluble-Triggering Receptor Expressed in Myloid cells
USA	United States of America
VDRL	Venereal Disease Research Laboratory
VZV	*Varicella zoster virus*
WBCs	White Blood Cells
WHO	World Health Organization
Z-N	Ziehl-Neelsen stain

INTRODUCTION

Meningitis is an inflammation of the membranes (leptomeninges) surrounding the brain and spinal cord and the intervening cerebrospinal fluid ***(Chanteau et al., 2006).***

Acute meningitis is caused by a variety of infectious agents. The most serious form is caused by pyogenic bacteria, such *as S. pneumoniae, N. meningitidis and H. influenzae* ***(Tunkel and Scheld, 2005).*** Aseptic meningitis, in which no bacterial pathogen can be isolated by routine cultures, can mimic bacterial meningitis. Although viruses are the most common cause of aseptic meningitis (primarily enteroviruses), there are numerous non-viral and non-infectious etiologies ***(Tunkel, 2001).***

Acute bacterial meningitis is a major cause of death and disability worldwide. It affects over one million people each year, and it is much more common in developing countries and in specific geographic areas such as the meningitis belt of Africa ***(Pfister and Roos, 2003).*** It is an endemic disease in Egypt. *S. pneumoniae* was the leading cause of bacterial meningitis in Egypt (42%), followed by *H. influenzae* (20%), *N. meningitidis* (16%) and *M. tuberculosis* (16%) ***(Afifi et al., 2007).***

Typical CSF findings in patients with bacterial meningitis include neutrophil pleocytosis, decreased glucose concentration and an increased protein concentration. Diagnosis is established by identification of the bacterial pathogen by microscopy of a Gram stained smear and by a positive CSF culture ***(Bannister at al., 2006).***

Gram stain is positive in identifying the meningeal pathogen in 36 - 90% ***(Brouwer et al., 2010).*** A wide range of CSF culture positivity ranged from 8 - 62% ***(Afifi et al., 2007).*** Bacterial culture takes at least 36 hours to obtain, and is adversely affected by preadmission treatment with antibiotics.

Several rapid diagnostic tests have been developed. Latex agglutination test detects bacterial antigens in 64% of cases ***(Narkeviciute et al., 2006).*** A broad range bacterial PCR assay is available for the early detection of bacterial meningitis. It has a sensitivity of 100% and a specificity of 98.2%. PCR assays also are available to detect DNA of all the common meningeal pathogens ***(Saravolatz et al., 2003).***

At fever hospitals in Egypt, CSF analysis may be delayed, and culture facilities may be lacking. Urine reagent strips has been used to test urine, ascetic fluid and pleural aspirate to evaluate infection in these biologic fluids. These reagent strips may be a valuable rapid bedside diagnostic test for CSF pleocytosis, glucose and protein till the laboratory results are available ***(Rommanelli et al., 2001).***

In viral meningitis, the CSF shows a lymphocytic pleocytosis, a normal glucose concentration, and a normal or slightly elevated protein concentration ***(Chadwick, 2006).*** Again, in Egypt, important diagnostic tools of viral meningitis e.g. viral culture, serologic testing and PCR are not available in our fever hospitals.

Differentiating bacterial from viral meningitis is very important. Bacterial meningitis is a life-threatening neurological condition and needs prompt parentral antibiotics, while viral meningitis is a benign condition with a good outcome. However, this differentiation is not always easy due to considerable overlap in clinical symptoms and laboratory findings. Uncertainty in diagnosis resulted in prolonged hospitalization and unnecessary use of antibiotics ***(Ray et al., 2007).***

Additional diagnostic tests are necessary to distinguish between bacterial and viral meningitis. The peripheral WBC count, CRP and ESR are usually elevated in patients with bacterial meningitis ***(Meynaar et al., 2011).***

Procalcitonin is a prohormone of the hormone calcitonin. It is produced by several cell types and many organs in response to pro-inflammatory stimuli, in particular by bacterial products ***(Giunti et al., 2010).*** A significant elevation of plasma PCT is found during sepsis and in bacterial meningitis. PCT expression is only slightly induced, if at all, by viral infections, autoimmune, neoplastic diseases, and trauma of surgical intervention ***(Arkader et al., 2006).***

The high diagnostic accuracy of serum PCT level has been suggested for differentiating between acute bacterial and viral meningitis. The use of a bedside PCT rapid assay can be used to guide appropriate therapy for meningitis cases i.e. to give or withhold antibiotic therapy ***(Oh et al., 2009).***

Aim of the work

The primary objective of the present study was to assess the utility of two bedside diagnostic tests in the diagnosis of meningitis:

i - The usage of urine reagent strips (combur-10) for the rapid determination of CSF glucose, protein and leukocytes.

ii - The diagnostic accuracy of serum procalcitonin (PCT-Q) for differentiating bacterial from viral meningitis.

Also, the following objectives were thought:

1 - To define the diagnostic accuracy of clinical symptoms and signs in patients with meningitis.

2 - To evaluate the accuracy of CSF analysis: protein, glucose, and leukocytes (total and differential), Gram stain and bacterial culture, and of plasma inflammation markers (WBC and CRP) in differentiating bacterial from aseptic meningitis.

CHAPTER I

MENINGITIS

Definition

Meningitis is an inflammation of the leptomeninges (the arachnoid and pia mater) with infection of the cerebrospinal fluid (CSF) within the subarachnoid space of the brain and spinal cord, and the ventricular system ***(Warrell et al., 2000).*** It is identified by an abnormal number of white blood cells in cerebrospinal fluid ***(Tunkel and Scheld, 2005).***

Anatomical considerations

- Meninges and subarachnoid space:

The brain and spinal cord are enveloped by three membranes -the meninges- which provide support and protection. From without inwards, these comprise the dura mater (pachymeninx), the arachnoid mater, and the pia mater. The arachnoid mater and pia mater are sometimes referred to collectively as the leptomeninges ***(Standring et al., 2005).***

The dura mater is a thick dense and fibrous membrane. It is separated from the arachnoid by a narrow subdural space. The arachnoid is much thinner than the dura and is mostly translucent. The cerebral part of the arachnoid mater invests the brain but does not enter the sulci or fissures, except for the longitudinal fissure. The arachnoid mater and the pia mater are separated by the subarachnoid space and joined by trabeculae ***(Standring et al., 2005).***

The subarachnoid space contains the CSF and the larger arteries and veins which traverse the surface of the brain. Arachnoid and pia mater are at closest association over the summits of the gyri. Wherever the brain and the cranium are not closely applied to each other, the arachnoid is separated from the pia by a wide interval to form subarachnoid cisterns. The cerebral subarachnoid space is connected with the fourth ventricle of the brain by three openings; the median aperture or foramen of Magendie and the two lateral apertures or foramina of Luschka. The subarachnoid space extends along the optic nerves to the back of the globe. There is also, a connection between the subarachnoid space and the ear through the cochlear duct ***(Zhang et al., 1992).***