Journal of Research in Medical and Dental Science
2018, Volume 6, Issue 3, Page No: 393-399

Available Online at: www.jrmds.in
eISSN No. 2347-2367: pISSN No. 2347-2545

Investigate and Comparison of Serum Level Markers Lactate, hsCRP and CDT for Differentiating Bacterial Meningitis from Non-meningitis Patients with Similar Clinical Symptoms

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DOI: 10.24896/jrmds.20186360

ABSTRACT

Meningitis, inflammation of the meninges membrane. Control of meningitis is one of the priorities of the World Health Organization, which based on the status of the disease in different countries, would be in need for different care systems. this study aimed to determine serum levels of biomarkers of hs-CRP, CDT and lactate in patients, and individuals with suspected meningitis and healthy subjects and to compare the value of these markers for differentiating patients with bacterial meningitis from patients with clinical symptoms similar to meningitis .In this fundamental- applied study, blood samples were collected from patients who had meningitis symptoms and referred to Razi medical educational center of Ahvaz. The amount of 5 CC of blood was taken from each patient. Then, in the standard laboratory terms the CDT, hs-CRP and lactate were measured by ELISA, Immunoturbidimetry, and colorimetric methods, respectively. Furthermore, the PCR molecular method was used for confirming the results. the serum levels of hs CRP, CDT and lactate in patients with bacterial meningitis did not show a significant relationship compared with a group of non-meningitis patients with similar clinical symptoms, whilst the rate of the serum levels of lactate and hs CRP in patients with bacterial meningitis compared with the control group without any clinical symptoms similar to meningitis has shown a significant increase .this study showed that the measurement of the serum levels of biochemical markers of lactate, CDT and hs-CRP cannot be useful tool to differentiate bacterial meningitis from non-meningitis patients group with similar clinical symptoms.

Key words: CDT, hs-CRP, Lactate, Meningitis

HOW TO CITE THIS ARTICLE: Samaneh Abyaz, Mohammad Taha jalali, Alireza Ekrami, Gholamabbas Kaydani, Yousef Bashiri, Investigate and Comparison of Serum level markers lactate, hsCRP and CDT for differentiating bacterial meningitis from non-meningitis patients with similar clinical symptoms, J Res Med Dent Sci, 2018, 6(3): 393-399, DOI: 10.24896/jrmds.20186360

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Received: 15/01/2018
Accepted: 10/04/2018

INTRODUCTION

Bacterial meningitis is a serious and lethal infection that affects the nervous system that causes inflammation of the membranes lining the brain and spinal cord. Bacterial meningitis outbreak is less than viral meningitis. But usually it is more serious, and if it cannot be timely diagnosed, is followed by serious complications and a higher mortality. Control of meningitis is one of the priorities of the World Health Organization, and based on the status of the

disease in different countries will require different care systems [1]. The annual incidence of bacterial meningitis is approximately 4 to 6 persons per 100 thousand adult people 80% of its cases are created by Streptococcus pneumoniae and Neisseria meningitidis [2]. Most viral meningitis are recovered without any symptoms. Pneumococcal meningitis leads to deaths up to 30% and in 50% of those who live, it puts the nervous effects such as sensory-motor deficits, convulsions and disorders of learning and memory [3]. Differentiation between bacterial meningitis from other causes of meningitis are usually based on cell counting, measurement of total protein, cerebro-spinal fluid glucose values fluid and ratio of serum glucose to the cerebro-spinal fluid glucose, which is confirmed, of course, with the help of cerebro-spinal fluid culture and isolation of pathogen [3,4].

Although the immediate analysis of the cerebro-spinal fluid can be raised the possibility of meningitis, but sometimes it is unable in distinguishing the bacterial causes from other causes of meningitis [4]. Some bio-chemical parameters can be used in detecting and differentiating these diseases.

A lumbar puncture (LP) is a difficult and aggressive method and the definitive of diagnosis meningitis can be reached only when patients underwent LP, but technical problems and its aggressive nature, sometimes move the mind toward other diagnostic ways. According to some findings, after an acute phase stimulus, a serum CRP value can be increased up to 10000 times [5]. Some studies indicate that other biological indicators such as CSF lactate can be used for accelerating diagnosis of bacterial meningitis. In previous studies, the increased levels of cerebro-spinal fluid lactate have been observed in patients with bacterial meningitis due to anaerobic metabolism of bacteria and brain ischemic [6].

In the current study Diagnostic value of biochemical markers of hs CRP, CDT and serum lactate in patients with suspected bacterial meningitis and their significance in differentiating patients with bacterial meningitis from patients with clinical symptoms similar to meningitis were studied as well as diagnostic value of molecular methods of PCR in the differentiation and detection of bacterial meningitis in serum of patients were studied

MATERIALS AND METHODS

Sampling

This study was conducted among patients who were with meningitis symptoms (headache, fever, vomiting, and neck stiffness) and referred to Razi medical educational center of Ahvaz on April 2013 to April 2014. Patients with the conditions listed, were admitted in the emergency of the Razi Hospital of Ahvaz and initial diagnostic measures such as Blood culture, Cr, Bun, CBC diff and so on were done. Relying on the results of the analysis of the cerebro-spinal fluid (CSF) by Lab of the Medical Center, the patients were divided into two groups: 1) patients with bacterial meningitis and 2) patients without meningitis. After the initial arrangements for diagnosis and treatment, and with obtaining written consent, 5 CC of blood clot was taken from each patient. Blood clots for 20-30 minutes at ambient temperature were kept and then were transferred to a hospital lab. Blood clots then were centrifuged by centrifuge machine with the speed of 3000 rpm. After this point the serum-containing tubes were kept at temperatures of 20 degrees below zero and moved to the laboratory of the University.

Among serum samples 22 patients with a definitive diagnosis of bacterial meningitis diagnosis randomly were selected as group1 and 22 people with clinical symptoms similar to meningitis and a definitive diagnosis of the lack of catching bacterial meningitis, were selected as group2. Moreover, 22 people without any clinical symptoms and no history of diabetes and other inflammatory diseases were considered as the control group (group3).

Methods

In this study, the CDT was measured quantitatively by ENZYME IMMUNOASSAY (EIA); in addition, serum lactate was measured by enzymatic method and hs CRP was measured by immunoturbidimetry method by Hitachi 911 device.

Biochemical tests

The hs CRP measurement by immunoturbidimetric method for a quantitative measurement of hs CRP in serum a quantitative diagnostic kit of the hs CRP by immunoturbidimetric method (made by PISHTAZ TEB Co., Iran) was used. Normal values of hs CRP were considered 0 -5 mg/ml.

Measurement of lactate by Colorimetric method

Serum lactate levels were evaluated by the Enzymatic Colorimetric method and using a measurement Kit of lactate (Randox Company, England) that intranasal CV was 4.1% AND sensitivity was 4.0 mg/dl. Method of test was Enzymatic that in this test, the color intensity of colored complex produced is proportional to the amount of lactate contained in the sample.

Measurement of CDT by sandwich Elisa method

CDT was measured using the antibody Sandwich ELISA Kit, HRP- labelled (made by Glory Science Co., USA). This kit has been designed for measuring human samples in the CDT.

PCR molecular method

since one of the diagnostic methods of meningitis disease is to check the presence of the micro-organisms in the blood as well as the presence of the micro-organisms in the cerebrospinal fluid, so this study has tried to investigate the presence of the bacteria in the serum of subjects (patients) using PCR techniques the presence of three major bacteria causing meningitis, i.e., Neisseria meningitides, Haemophilus influenzae and Streptococcus pneumoniae. To do this, the DNA extraction operation was done on patients' serum and then, PCR test was performed using the selected Primers (as described below). In the research, DNA extraction Kit (DNA CinnaPure) was used. In order to confirm the bacteria in serum samples, CtrA gene replication, bexA gene replication, and ply gene replication to identify were used to identify the genes of Neisseria meningitides, Haemophilus influenza type b, and pneumonia. Sequence of the used primers is given in table 1 [7].

Table1: Sequence of primers

Primer's name	Primer sequence	Size of Amplicon
F-CtrA	5-GCT GCG GTA GGT GGT TCAA-3'	110bp
R-CtrA	5'-TTG TCG CGG ATT TGC AAC TA-3'	
F-bexA	5'-TAT CAC ACA AAT AGC CGT TGG-3'	181 bp
R-bexA	5'-GGC CAA GAG ATA CTC ATA GAA CGT-3'	
F-ply	5'-TGC AGA GCG TCC TTT GGT CTA T-3'	80 bp
R-ply	5'-CTC TTA CTC GTG GTT TCC AAC TTG A-3'	

The standard strain of every three bacteria was prepared from the collection center of fungi and bacteria, Iran. These strains included Hae of all the buck names of the mophilus influenza with a strain number of PTCC1766 (ATCC 49766), Stlep to cocci pneumonia with a strain number of PTCC1240 (NCTC 7465), Neisseria meningitides with a strain number of PTCC1760 (ATCC 13090). After the preparation of the material, for each sample the PCR reaction was done in Thermocycler machine (PeqStar, Germany) under the specified temperature terms. A temperature program given to the machine to perform PCR included one cycle 95 °C for 2 minutes, 30 cycles 95 °C for 30 seconds, cycles 59.8 °C for 45 seconds, and cycles 72°C for 90 seconds and also one cycle 72 °C for 5 minutes. Finally, the evaluation of PCR products was performed using 1.5% agarose gel.

RESULTS

Table 2 shows the results of a review of the role of the three bio-markers of lactate, CDT and hs CRP in differentiating patients with bacterial meningitis from patients with clinical symptoms similar to meningitis.

Table2: A comparison between the means of serum levels of lactate, CDT and hs CRP in the three groups

Groups	Lactate	CDT	hsCRP
Patients (Mean±SD)	33.42±32.95	278.73±72.76	8.14±3.62
Suspected meningitis (Mean±SD)	42.19±38.76	272.00±60.23	6.55±3.99
Control (Mean±SD)	18.94±5.58	288.64±80.31	1.68±2.75
P-value	0.133	0.779	0.001

As can be seen in table2, the average serum level of hs CRP in the patient group was 14.8, in the group of suspected meningitis was 55.6 and in the control group was equal to 1.68. Using the Kruskal Wallis test, the difference of the average serum level of CRP hs in the three groups has been compared and the difference was a significant in the level of the 95% (pvalue< 0.001). And the mean serum level of lactate in the patient group, the group of patients with suspected meningitis, and the control group was 42.33, 42.19, and 18.94, respectively. Using the Kruskal-Wallis test, the mean serum level of lactate in the three groups was compared and there was not a significant difference at a significance level of 95% (P-value =0. 133). The average serum levels of lactate between groups of patient and suspect do not

make a huge difference, but there is a lot of difference in the control group with the other two groups, the greater the difference. As well as the average serum levels of CDT in the patient group was 278.73, in the group of suspected meningitis was 272, in the control group was equal to 288.64. Using the Kruskal Wallis test, also difference of the average serum level of CDT in the three groups has been investigated and at the significance level of 95%, the difference is not significant (p-value=0.779). The results of performing PCR on serum of patients with bacterial meningitis PCR test was performed on serum samples of patients with bacterial meningitis (Group 1) to confirm the presence of the bacteria in the serum of these patients. A bex gene was used for detecting Haemophilus influenza type b, ctrA gene for detecting Neisseria meningitis and ply gene was used to identify the pneumococcus .After creating favorable conditions for the PCR reaction and detection of bacterial genomes in this study, the reaction product was transferred on 1.5% agarose gel and electrophoresed with 140 volts for 35 minutes. Gels were stained with ethidium bromide solution for 20 minutes and then imaging was performed in the duct gel under ultraviolet light radiation (Figure 1). After staining and observing the gel, but bands of positive control samples (Direct strains of bacteria under study) there was no other band. In total, the PCR test results in the present study can indicate that unlike the CSF sample, serum samples are not appropriate for finding bacteria genome under study and thus confirming their presence. And PCR molecular method on the serum could not be an appropriate method for detecting bacterial meningitis disease, as well as to differentiate bacterial meningitis from the group of non-meningitis patients with similar clinical symptoms.

DISCUSSION

Bacterial meningitis is one of the most important diseases in the emergency ward with high mortality that should be quickly diagnosed and treated. The prognosis of this disease will depend on a quick diagnosis and treatment. Normally differentiating bacterial meningitis from viral meningitis can be done with the analysis and cultivation of the cerebro-spinal fluid; however, in 70% of cases of suspected clinically, cerebro-spinal fluid remains negative.

In many cases, before getting a spinal fluid an imaging of the central nervous system such as the CT-SCAN id necessary, which delays detection as well as imposes costs to the health system [5, 6]. The aim of the study was to investigate the diagnostic value of biochemical markers of hs CRP, CDT and serum lactate in patients suspected to bacterial meningitis and their importance in differentiating bacterial meningitis patients from patients with clinical symptoms similar to meningitis. In this study it has been tried that without the use of the analysis of the cerebro-spinal fluid, the assessment of serum markers mentioned is done only in serum of the patients. In this research the worthiness of Biochemical markers of hs CRP and lactate in differentiating patients with bacterial meningitis than healthy people has been discussed. CDT refers to the low-carbohydrate formats of transferrin, including asialo-, monosialo- and disialo- transferrin; in order to diagnose heavy alcohol consumption so far the evaluation and measurement of this marker have been used often for reasons of higher sensitivity and specificity (95%, 95%) compared to other markers such as Alanine transaminase (ALT) and Aspartate transaminase (AST) [8]. So far no studies have mentioned the measurement and evaluation of this marker in meningitis. Due to the specificity of asialo- transferrin form of the cerebro-spinal fluid, and the fluid leak due to damage to the blood brain barrier in meningitis, the factor for the evaluation and assessment was used in this study that the information derived from the analysis of the results achieved did not show any significant relationship. In 2012 in a similar study of the diagnostic value of serum levels of hs CRP as a marker in differentiating viral meningitis from bacterial meningitis on the 95 newborn, Abdollahi et al. evaluated 16 newborn as the control group and without clinical symptoms, 30 newborn with positive blood culture and the definitive diagnosis of sepsis and 49 people with suspected sepsis. This study showed that this biomarker with sensitivity of 48% can be a useful tool for early diagnosis of neonatal sepsis and differentiation of the control group from infants with sepsis with positive blood culture (P-value<0.001) [9]. This study showed that this biomarker with sensitivity of 48% can be a useful tool for early diagnosis of neonatal sepsis and differentiation of the control group from infants with sepsis with positive blood culture (P-value<0.001) [9]. In 2012 in a study conducted by Panjeta et al. of the 20 patients in age groups of 1 to 50 years with a diagnosis of bacterial meningitis, the control group consisted of 25 cases without bacterial infection. The average value of

the hs CRP of cerebrospinal fluid in the control group was 25.0 mg/lit while its value in patients with bacterial meningitis was 21.4 mgr/lit. An increase of hs CRP was seen in 95% of bacterial meningitis [10]. In the present study, the average hs-CRP in patients with bacterial meningitis was 8.14 mgr/lit and in patients with suspected meningitis was 6.55 mgr/lit and in the normal group was 1.68 mgr/lit. Data from the present study confirm the results of previous studies [9, 10] indicating the increase of serum levels of hs-CRP in patients with bacterial meningitis that can be a beneficial diagnostic tool to differentiate bacterial meningitis from healthy people. In 2012 Hamedi et al. performed the simultaneous assessment of his CRP in serum and cerebrospinal fluid of 81 children suspected meningitis referred to the emergency ward of the Mashhad Qaem hospital. Among these children, 27 people were with bacterial meningitis, 27 people were with viral meningitis, and 27 people were with normal cerebro-spinal fluid. Finally, the results of this study have not shown a substantial increase in the concentration of hs-CRP in cerebro-spinal fluid and serum of patients with bacterial meningitis (P = 0.46; P = 0.29) [11]. In most of the studies, hs-CRP level on the cerebro-spinal fluid has been measured, and, as mentioned in some studies [11], the level of hs-CRP of cerebro-spinal fluid has a high diagnostic value of differentiating bacterial meningitis from non-bacterial meningitis while the serum levels of hs-CRP has a lack of high sensitivity and specificity for this goal. It seems that since the hs-CRP is an acute phase protein, the increase in its serum levels after inflammation of the meninges curtains in the bacterial and non-bacterial meningitis are nonspecificity cause and the lack of ability of this biochemical marker in differentiating them from each other. And also probably the intensity of the blood brain barrier damage has not been to an extent that can make the molecular passing as big as his-CRP protein .This study showed that these factors can cause an inability of this biochemical marker in differentiating patients with bacterial meningitis from patients with clinical symptoms similar to meningitis. While the difference in the amount of the increase of serum levels of hs-CRP is not significant between the two groups, because of more severity of bacterial meningitis infections, the amount of its increased serum levels in this type of meningitis is higher than non-bacterial meningitis. In any case, where the amount of hs-CRP is high and medium level, it may pose bacterial meningitis diagnosis, in addition, the inflammation and infections in other parts of the body should be considered and the patient should be fully assessed. In 2014, Mekitarian et al. conducted a study on 451 children with meningitis in which 40 patients (9%) were diagnosed with bacterial meningitis, and diagnostic value of CSF lactate in differentiating bacterial meningitis from non-bacterial meningitis was examined. The results showed that this biomarker with sensitivity of 95% and specificity of 94% can be useful in differentiating bacterial meningitis from non-bacterial meningitis [12]. In 2011 in a similar study of 254 patients with meningitis conducted by Viallon et al., the results showed that the level of CSF lactate with 94% sensitivity and 92% specificity is an appropriate parameter to differentiate bacterial meningitis from non-bacterial meningitis [13]. Considering that in our study, the average serum lactate in patients with bacterial meningitis, patients with suspected meningitis, and normal group was 33.42mg/dl, 42.19mg/dl, and 18.94; the analysis of results showed that while the serum levels of lactate is not a useful tool to differentiate bacterial meningitis from non-bacterial meningitis; however, it is useful in the differentiation of healthy individuals from patients with suspected meningitis. The mentioned studies emphasized to increase in the level of lactate in the cerebro-spinal fluid of patients with bacterial meningitis than patients with non-bacterial meningitis. Anaerobic conditions and hypoxia in the central nervous system due to inflammation of the walls of the blood vessels of the brain is a cause for increase the level of lactate in the cerebro-spinal fluid. Based on the results of this study, it appears that the severity of the damage to the blood brain barrier is not to an extent that can cause the penetration of lactate cerebro-spinal fluid into the blood. To confirm the bacterial meningitis in the serum of patients with bacterial meningitis, this study investigated the presence of three major bacteria causing meningitis, i.e., Neisseria meningitis, haemophilus influenzae and Streptococcus pneumoniae using the PCR technique. The purpose of doing PCR in research of replication is a specific sequence of DNA from among a heterogeneous set of the DNA sequences [14]. In 2003 in a study by Lisa Louie et al., and also in 2015 in another study by Lee et al., to diagnose meningococcal meningitis in the CSF, they used and confirmed the PCR as a useful and invaluable technique in diagnosing this disease [15, 16]. Using primers prepared from the genes of bex, ply, and ctrA, in 2005 Tsopanomichalou et al.

designed and evaluated the PCR molecular method for detecting Neisseria meningitidis, Streptococcus pneumoniae and haemophilus influenzae. The study was conducted on 157 plasma samples, 36 CSF samples, 31 serum samples and 31 full blood samples of patients with meningitis. In this study the sensitivity of PCR molecular methods for diagnosis of meningococcal meningitis was assessed as follows: in the CSF sample, 9.88%; in the plasma sample, 90.4%; in serum sample, 80.6%; and in complete blood sample, 80% [17]. Since cerebro-spinal fluid is a sterile environment and devoid of any microorganisms, so it has been used in most studies of CSF samples in order to carry out molecular methods such as PCR for detection and differentiation of bacterial meningitis. In the present study this technique (PCR) was used to identify the major factors causing bacterial meningitis in serum samples of bacterial meningitis patients in order to remove the need for an aggressive method to take cerebro-spinal fluid from individuals with suspected meningitis, but the results of this study showed that unlike the cerebro-spinal fluid sample, serum of patients is not appropriate sample for identification and detection of the studied bacteria.

CONCLUSION

Based on the data obtained from this study, the measurement of serum biochemical markers of lactate, hs-CRP, and CDT is a useful tool for differentiating patients with bacterial meningitis from patients with clinical symptoms similar to meningitis. It also showed that unlike the samples of cerebro-spinal fluid, serum of patients is not an appropriate sample for identification and detection of the studied bacteria by PCR molecular method.

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