C-REACTIVE PROTEIN AND LACTATE DEHYDROGENASE IN SERUM AND CEREBROSPINAL FLUID IN RAPID AND EARLY DIAGNOSIS OF CHILDHOOD MENINGITIS

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Abstract:

Objective

Bacterial meningitis is still a life threatening epidemiological problem especially in many developing countries; considering its dire consequences, its prompt and accurate diagnosis has become a priority for clinicians. Because of the various limitations of conventionally used laboratory techniques, we evaluated and compared the diagnostic utility of C-reactive protein(CRP) and lactate dehydrogenase (LDH)in serum and cerebrospinal fluid (CSF)in the diagnosis of bacterial meningitis and its effectivity in distinguishing it from aseptic meningitis (AP).

Material and Methods

A total of 125 pediatric cases, aged between 1 month and 12 years, including patients with bacterial meningitis (n = 45), aseptic meningitis (n = 42) and a control group (n = 38), were retrospectively analyzed on the basis of data from the initial clinical examinations. Cultures, smears and other common serum and CSF indices were compared with serum and CSF CRP levels and LDH activity.

Results

Compared with each of the other variables, there were significant differences in the mean values of serum-CRP, CSF-glucose, CSF-LDH and CSF/serum LDH ratio between the bacterial and aseptic meningitis groups ($p < 0.001$). Of all the tests applied, the highest sensitivity (95%) and negative predictive value (95%) belonged to CSF-LDH activity and the most specific (100%) test with the highest positive predictive value (100%) was CSF-CRP titration as well as smear and culture. Combination of CSF-CRP serum-CRP, and CSF-LDH yielded the highest sensitivity (100%) and negative predictive value but the combined application of CSF-LDH and CSF-CRP proved to be the most specific and efficient.

Conclusion

In the presence of a normal CRP titration and low glucose level in CSF, bacterial meningitis is excluded, whereas elevated level of CSF-LDH activity is a valid confirmatory predictor of BM. In addition, combination of these three tests with serum CRP is far more effective than the separate determination of any of these parameters.

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Introduction

Bacterial meningitis, still one of the most life-threatening problems worldwide, is more prevalent in children and its timely and early differentiation from viral meningitis (VM) has a huge impact on the treatment of affected patients (1-3). Laboratory investigations play a major role in differentiating viral and bacterial meningitis. The laboratory tests being used include serum and CSF-CRP (4-8), LDH protein and CSF/serum LDH ratio (9,10), albumin (11), alpha 1-antitrypsin, alpha 1- acid glycoprotein, alpha 2-ceruloplasmin and alpha 2-haptoglobulin (12) along with CSF cell count (13). Although many studies have acknowledged the value of CRP measurement of CSF in either diagnosis or prognosis of bacterial meningitis patients (12-14), recent studies however emphasize the fact that absence or low levels of CRP in serum and CSF (especially after 12 hours after manifestation of clinical symptoms) strongly rule out bacterial meningitis (8). Other laboratory tests like measurement of interleukin-6 (7,11), alpha 2-macroglobulin (6,7,11) and neurotrophin-3 (15) along with serum levels of procalcitonin (7,16,17) are considered to be very valuable in the diagnosis of bacterial infections. PCR -either nested PCR (18,19) or real time (RT) PCR (20)- are very effective methods for detecting the bacterial agents responsible even after initiation of antibiotic therapy; they are also helpful in identifying viral agents responsible for meningitis (21).

Considering the importance of early diagnosis and differentiating the bacterial meningitis cases, it is quite understandable to utilize rapid tests, reasonably priced and having the required sensitivity and specificity. Hence this study was conducted to determine and compare the sensitivity, specificity, predictive values and likelihood ratios of such laboratory tests used for diagnosing and differentiating between bacterial and aseptic meningitis and for measurement of LDH activity and levels of CRP in serum and CSF.

In Iran as in other developing countries, highly developed and expensive laboratory tests are still unavailable, and because of their financial status, many patients simply cannot afford such tests. Hence our aim is to improve and upgrade the diagnostic efficacy of tests presently available

Materials & Methods

Subjects

This retrospective study was jointly carried out between June 2002, and July 2003 in the pediatric departments of the Mofid and Shohada hospitals, both affiliated with the Shaheed Beheshti University of Medical Sciences, Iran. During the said period, 125 children, aged 1 month to 12 years, requiring CSF examinations were enrolled for the study. Of these, 87 children clinically suspected of having meningitis were considered as the case group; based on the results of their LP results, subjects of this group were divided into two distinct groups of bacterial and aseptic meningitis. The bacterial meningitis group which consisted of 45 pediatric cases were patients whose CSF culture yielded one or more known causative bacteria, positive gram stain of CSF, similarity of organisms found in CSF and blood or positive latex agglutination test for bacterial antigens. The causative bacteria were Streptococcus pneumoniae (n = 17), Haemophilus influenzae (n=12), Neisseria meningitidis (n=4), group B Streptococcus (n=2), Salmonella paratyphi A, Escherichia coli and Klebsiella (one case in each group).The aseptic meningitis group comprised 42 cases, including those with negative CSF culture and gram stain, negative latex test and absence of polymorphonuclear pleocytosis in CSF. It was assumed that viruses were the causative agents in these patients, although virus isolation was not performed. The control group consisted of 38 children who presented with fever, dehydration or electrolyte disturbances and had undergone LP to exclude the presence of meningitis. Based on results of CSF examination and clinical findings, they were found to be free of any such infections. Both groups were screened for hepatic, renal, musculoskeletal and cardiovascular disorder along with leukemia and all the results were negative.

Biochemical assays

In the first 24 hours of hospitalization, prior to administration of antimicrobial agents, all cases underwent lumbar puncture for which. 3-5 ml of CSF was sampled. Variables analyzed were the smear, culture and common CSF tests including white cell count, polymorphonuclear cell count, latex agglutination, protein value and glucose concentration. Routine methods were used to measure these variables. LDH activity measurement and CRP titration of CSF were also performed.

Simultaneously with performance of LP, 2 cc of basilic vein blood were obtained for either quantitative measurement of serum CRP, or documenting of serum glucose and LDH levels.

LDH activity was measured by observation of the increase in optical density at 340 nm that occurs as diphosphopyridine nucleotide is reduced in the presence of lactic dehydrogenase. This procedure was performed using the SIGMA kit. Serum CRP was measured by laser immunonephelometry or turbidometric assay using a RA 1000 auto analyzer and semi quantitative measurement of CRP in CSF was performed by the reversed passive agglutination method. Antigen detection was performed by the latex agglutination test.

Analytical and statistical methods

Data were expressed as mean ± SD and range. Differences between groups were analyzed for statistical significance by the independent t-test or one way ANOVA. The correlation between variables was assessed by Pearson's correlation coefficient. A P-value of < 0.05 was considered to be statistically significant. Formulas used for calculation were as follows: Sensitivity = TP/ (TP + FN), Specificity = TN / (TN + FP), Positive predictive value (the likelihood that a patient with a positive test result has the disease) = TP / (TP + FP) and Negative predictive value (the likelihood that a patient with a negative test result does not have the disease) = TN / (TN + FN), in which TP=true positive, FP=false positive, TN=true negative and FN=false negative. The study was approved by the university ethical board and parents gave written consent.

Results

The mean values and ranges for CSF and serum markers in both groups are shown in table 1. The differences in the mean values were statistically significant for CSF protein levels, CSF leukocyte count, LDH activity in CSF and CSF/serum glucose between both the bacterial and aseptic meningitis groups and the control group ($p<0.001$). While values of serum CRP, glucose levels of CSF, CSF/serum LDH had significant differences in the bacterial meningitis group, compared to the other two groups ($p <0.001$), the differences between the aseptic meningitis group and control group were not significant.

In the bacterial meningitis group, a total of 24 (63.2%) gram smear results were positive in contrast to other two groups in which no positive results were recorded. In this regard, 27 (71%) positive cultures from CSF specimens in bacterial meningitis group were recorded; the other two groups did not have positive culture results. Latex agglutination test was positive in 26 cases (68.4%) of bacterial meningitis group.

Values of measurements of LDH and CRP in CSF and serum of bacterial meningitis group are shown in table 2; as shown different bacterial agents had different effects on the level of measured parameters. In comparison with other bacterial agents, the most changes in serum CRP, LDH activity of CSF-0 and CSF/serum LDH ratio were caused by Salmonella paratyphi A and H.influenza, respectively.

Serum CRP levels were elevated in 31 patients of bacterial meningitis group, whereas CRP titration in CSF was positive in 32 cases of this group and its results were as follows: 1 in 3 patients, ½ in 8, ¼ in 8, 1/8 in 12 and 1/16 in one patient respectively.

Sensitivity, specificity, positive and negative predictive values, efficacy and likelihood ratios of each test are shown in table 3. The highest sensitivity and negative predictive value belonged to measurement of LDH activity in CSF and the test that showed the highest positive predictive value was titration of CRP in CSF along with smear and culture. It must be mentioned that the two latter tests have higher efficacy (93%) as compared to other tests. Glucose concentration of CSF was revealed to have the highest negative likelihood ratio (0.5). Positive likelihood ratios for CSF/serum glucose (30.5) and CSF/serum LDH (15.2) were higher than for those other tests applied, although it seems that LDH activity in CSF (LR+ = 9.5) and serum CRP (LR+ = 8.4) have more practical utility in clinical evaluations. As shown in table 4, simultaneous use of more than one of these measurements would increase the diagnostic efficacy for differentiating between bacterial and aseptic meningitis.

Our analysis revealed a statistically significant correlation between LDH activity in CSF and protein levels of CSF ($p <0.001$) LDH activity of CSF and CSF/serum LDH ($p <0.001$) and glucose levels in CSF and CSF/ serum glucose ($p <0.01$). There was also a significant correlation between serum CRP with either glucose level of CSF ($p <0.01$), or CSF/ Serum Glucose ratio ($p <0.001$).

Table 1. The differences in the mean and range Values in Bacterial meningitis, Aseptic meningitis and control groups

Variables \ Group		Bacterial meningitis group (N=45)	Aseptic meningitis group (N=42)	Control group (N=38)
Age (months)	Mean ± St.deviation	49 ± 44.9	67.8 ± 46.5	53.6 ± 43.9
	Range	15 days –12 years	11 days-12 years	13days–12years
Serum Glucose (mg/dl)	Mean ± St.deviation	108 ± 15.6	103.8 ±17.8	100.7 ±11.6
	Range	67 - 139	65 – 128	75 – 120.5
CSF Glucose (mg/dl)	Mean ± St.deviation	21.3 ± 17.5	65.8 ± 18.8	72.3 ±11.8
	Range	0 – 81	20 – 102	51 – 100
CSF glucose Serum glucose	Mean ± St.deviation	0.29 ±0.25	0.63 ±0.13	0.72 ± 0.08
	Range	0 – 0.72	0.24 – 0.84	12 –38
WBC count of CSF	Mean ± St.deviation	3698 ± 3003.1	415 ± 231.4	2.03 ± 1.9
	Range	30 – 12300	21 – 2600	0 – 8
CSF protein (mg/dl)	Mean ± St.deviation	295 ± 204.4	57.5 ± 51.9	19.4 ± 5.87
	Range	20 – 2088	10 – 378	12 – 38
LDH activity in serum (IU/L)	Mean ± St.deviation	352.9 ± 125	206.43 ± 61.9	147.2 ± 52.3
	Range	146 – 720	87 –362	70 –320
LDH activity in CSF(IU/L)	Mean ± St.deviation	273.8 ± 198	37.9 ±13.5	17.9 ± 6.6
	Range	13 – 1360	8 – 62	5 – 37
Serum LDH CSF LDH	Mean ± St.deviation	0.59 ± 0.54	0.14 ± 0.08	0.12 ± 0.02
	Range	0.04 – 3.64	0.04 – 0.49	0.06 - 0.17
Serum CRP (mg /L)SERUM	Mean ± St.deviation	131.32 ± 79.55	19.19 ± 18.02	5.7 ± 2.7
	Range	10 - 325	8 - 91	< 8

Table 2: Frequency and precentage of different bacteria causing bacterial meningitis with average, (SD) standard division,LDH CSF, LDH CSF/serumLDH, serumCRP, Positive titer of CRP according each kind of bacteria

Kind of bacteria	Frequency	Percent	LDH activity in serum (Iu/ml)	CSF LDH Serum LDH	Serum CRP (mg/dl)	Positive titr of CRP in CSF
Pneumococus	17	44.7	112.94 ± 64.61	0.4 ± 0.22	120.53 ± 76.5	14
H. influenza	12	31.6	275.5 ± 348.6	0.8 ± 0.93	151.8 ± 88.5	12
N. meningitidis	4	10.6	129.5 ±74.67	0.36 ± 0.21	149.5 ±0.5	3
Streptocucos	2	5.3	167 ± 24	0.31 ± 0.05	134 ± 16.9	2
Salmonella .typhi	1	2.6	1254	1.74	155	1
E. coli	1	2.6	83	0.21	10	0
Klebsiella	1	2.6	107	0.39	89	0

Table 3.CSF spesific characteristics in differential diagnostis tests of septic and aseptic meningitis

	Accepted criter for diagnosis of meningitis	Sensitivity	Specificity	Positive predictive value	Negative predictive value	efficacy	Positive likelihood ratio	Negative likelihood ratio
CSF Glucose	< 40 mg/dl	58 %	90 %	85 %	70 %	75%	5.8	0.5
CSF glucose / Serum glucose	< 0.3	61 %	98 %	96 %	73 %	80 %	30.5	0.4
CSF protein	> 100 mg/dl	73 %	93 %	90 %	80 %	84 %	10.4	0.3
WBC count	> 500 / mm3	58 %	90 %	85 %	70 %	75 %	5.8	0.5
Neutrophil count of CSF	> 60 %	79 %	83 %	81 %	81 %	81 %	4.6	0.25
Latex agglutination of CSF	Positive	68 %	93 %	90 %	76 %	81 %	9.7	0.34
Gram smear	Positive	63 %	100 %	100 %	75 %	83%	-	0.4
Culture	Positive	71 %	100 %	100 %	79 %	86 %	-	0.3
CSF titration for CRP	Positive	84 %	100 %	100 %	88 %	93 %	-	0.16
Serum CRP	> 40 mg/lit	84 %	90 %	89 %	86 %	88 %	8.4	0.17
LDH activity in CSF	> 50 Iu/lit	95 %	90 %	90 %	95 %	93 %	9.5	0.05
CSF LDH / Serum LDH	>0.3	86 %	95 %	94 %	82 %	86 %	15.2	0.25

Table 4: CSF specific characteristics for diagnostic efficacy in bacterial and aseptic meningitis

	Accepted criteria for diagnosis of meningitis	Sensitivity (percent)	Specificity (percent)	Positive Predictive Value (percent)	Negative Predictive Value (percent)	Efficacy (percent)	Positive likelihood ratio	Negative likelihood ratio
CSF LDH & Serum CRP & CSF CRP	> 50 Iu/lit > 40 mg/lit positive	100	81	83	100	90	5.2	-
CSF LDH & Serum CRP	> 50 Iu/lit > 40 mg/lit	100	81	83	100	90	5.2	-
CSF LDH & CSF CRP	> 50 Iu/lit positive	97	90	90	97	94	9.7	0.03
CSF CRP & Serum CRP	> 40 mg/lit positive	92	90	90	93	91	9.2	0.08

Table 5: Correlation between laboratory tests used in bacterial and aseptic meningitis

Correlation	CSF glucose	Neut. Count of CSF	WBC count of CSF	CSF protein	CSF Gluc. Serum Gluc.	CSF CRP	Serum CRP	CSF LDH Serum LDH
CSF LDH	- 0.2	0.11	0.2	0.5**	- 0.2	0.12	- 0.06	0.9**
CSF LDH Serum LDH	- 0.2	0.14	0.3	0.2	- 0.3	0.13	- 0.14	
Serum CRP	0.4*	- 0.1	- 0.1	0.06	- 0.4*	0.2		
CSF CRP	- 0.2	0.16	0.2	0.13	- 0.2			
CSF/Gluc Serum/Gluc.	0.98**	0.1	- 0.13	- 0.08				
CSF protein	- 0.04	0.05	- 0.2					
WBC count of CSF	- 0.12	0.2						
Neut. Count of CSF	- 0.07							

* P< 0.01
** P<0.001

Discussion

Rapid and definite differentiation of bacterial meningitis is an important and demanding issue in emergency situations. A number of studies have shown the effectiveness of tests to differentiate between bacterial and aseptic meningitis. These tests utilize of various common and uncommon laboratory measurements including CSF variables and markers of peripheral blood (4-8, 11-17). Some of these tests despite their low costs and individual dependency have more potential diagnostic accuracy than CSF-WBC, peripheral WBC and serum CRP; they also can compete with recently studied expensive tests such as INF, IL-1, IL-6, IL-8 (22). In more recent studies procalcitonin has been found to be more beneficial with least overlap in differentiating bacterial from viral meningitis (16.17). In some cases special indices such as Alpha-2 macroglobuline have been applied to evaluate the possibility of destruction of blood-brain barrier (6,11).

Our study was performed on three groups, the bacterial meningitis, the aseptic meningitis and the control. There was no significant difference in distribution of age and sex between these groups. As expected, levels of glucose, protein, WBC and neutrophil count were elevated in CSF of the BM group as compared to the other two groups. These results are in agreement with those of other studies (8,13,16).

Measurement of LDH in CSF (10) and its ratio to serum LDH (CSF LDH / serum LDH) (9) were described for the first time in 1967 and 1987 respectively. Since then various studies have been performed showing the alteration in levels of LDH in CSF and serum and also variation of their ratios in cases with bacterial meningitis. What has been unanimously accepted is the increased CSF and serum LDH activity among BM patients (23-25). This marker, in part, reflects the extent of brain injury (26). In this regard, the only diagnostic tool to differentiate the BM patients is the pattern of LDH isoenzyme alteration in CSF (27). In our study, LDH activity of CSF was significantly higher among BM patients compared to the aseptic ones and the control group. Because of our limitations we did not quantify the LDH isoenzymes in CSF of our cases. Semi-Quantitative measurement of CRP in CSF is a more accurate test not only in differentiating the BM from viral meningitis but also in discrimination of causative infectious agents, compared to serum CRP (12,17,28). Nevertheless, the latter test has been proved to have diagnostic accuracy than WBC count, ESR and other standard CSF parameters (8,13). In our study there was a considerable difference between the BM and AM groups in their serum CRP levels, despite there being an overlap in ranges of this value between these two groups. In this regard, we found a group of 12 BM patients whose serum CRP levels were in the range of values of the AM group, a phenomenon previously been reported in numerous studies (13, 16, 29). Based on the results of a related meta-analysis, central tendency was calculated 94% for true positive and 6% false negative cases of bacterial meningitis, respectively. The post test probability of not having bacterial meningitis given a negative CRP test was calculated to be 97–99%, in the range of a pre-test probability from 10 to 30%. A positive result of the CRP test as a sole marker is not an absolute confirmatory method, nor a predictor of bacterial meningitis, whereas negative results do however confirm this (30). Thus, it would be reasonable to conclude that a negative result of CRP measurement, rather than a positive one, is a more useful and valuable indicator. In our study we found overlapping values of serum CRP among bacterial and viral meningitis groups. This concept is in agreement with other latest reports (13, 16, 29). Nevertheless, considering the high values of CRP concentration either in serum or in CSF, diminished levels of CRP particularly in CSF may ordinarily be associated with situations in which bacterial meningitis is unlikely. In some reports the CRP concentration of CSF of less than 10 mg/L has been considered as a cut off level, more likely associated with conditions of absence of bacterial meningitis (31, 32). In contrast this probability would be increased when its concentration exceeds 100 mg/L (17).

Ordinarily, a low CSF glucose concentration strongly suggests bacterial meningitis, although Sormunen et al found this situation in only one third of bacterial meningitis patients (13). In addition, negative correlation between CSF glucose and serum CRP in our study revealed the important role of reduced CSF-glucose levels in confirming the diagnosis of bacterial meningitis.

Among all the tests applied, CSF-LDH had the highest sensitivity rate, whereas the highest specificity rate belonged to gram smear along with semi-quantitative measurement of CRP in CSF. Serum CRP had a positive predictive value of 89 %. In addition, CSF-LDH revealed to have the highest predictive value of negative test (95%).

In combination, the three tests of serum CRP, CSF titration of CRP and CSF-LDH yielded a 100% negative predictive value. So it can be reasonably concluded that the negative results of these tests would significantly decrease the probability of presence of BM. This value was calculated 97% for combination of CSF-LDH and CSF-CRP and 93% for simultaneous performance of CSF-CRP and serum CRP, while the positive predictive values of these parameters were 90%. In our study, accordingly, negative results of such tests, rather than the positive ones, in confirming the diagnosis is of considerably greater value in ruling out of BM. Paradowski et al. proposed the simultaneous determination of serum CRP concentration, alpha 1-antitrypsin and alpha 1- glycoprotein for prediction of bacterial meningitis with area under the ROC curve of 0.96, along with serum CRP and CSF CRP with area under the curve of 0.97 (12).

To conclude simultaneous measurement of CSF-LDH, serum CRP and CSF-CRP is the best method that could be utilized to distinguish between bacterial and aseptic meningitis. Considering the need for rapid, accurate and inexpensive diagnostic tools for distinguishing between bacterial and viral meningitis,

and consequently initiating treatment of BM patients and also to limit the unnecessary use of antimicrobial agents in patients with viral meningitis, we recommend the combined application of serum CRP, CSF-CRP, CSF-LDH and CSF glucose tests as the method of choice.

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