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Comparitive Biochemical Profile of cerebrospinal Fluid and Serum in the Diagnosis of Pyogenic Meningitis

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Abstract
Meningitis accounts for one of commonest neurological diseases in children and adults. To diagnose the pathogen there is need for reliable biochemical tests on CSF. This difficulty can partly be solved by estimation of CSF LDH, CRP and comparing it with their levels in Serum. The present study was undertaken to evaluate the clinical presentation and CSF analysis in pyogenic meningitis with special reference to LDH & CRP estimation. Study was conducted in Department of General Medicine, Neurology and Biochemistry, Government General Hospital Vijayawada and Bhaskar General Hospital, yenkepally. 32 cases of pyogenic meningitis between 18 years to 65 years were taken and 28 cases of individuals undergoing Lumbar puncture for various surgical procedures were taken as controls. Cases below 18 years, aseptic meningitis, traumatic and dry tap on lumbar puncture were excluded from the study**.** Lumbar puncture was done in all cases where indicated and CSF was collected in sterile bottles and blood was drawn within 1 hour of lumbar puncture. CSF and serum LDH was done by spectrophotometry using Systronics spectrophotometer. CRP was estimated by using Latex Slide Agglutination semi quantitative method (Accurex). Results were analyzed by descriptive statistical analysis. Analysis of variance (ANOVA), CHISQUARE TEST and Z TEST were used. ROC curves, Odds ratio and analysis of logistic regression were done to establish the best diagnostic marker. SSPS 17 and Met cal statistical software was used for statistical analysis. CSF protein was significantly high and CSF/ serum ratio was altered. In majority of cases CSF sugar was less than 2/3 of blood sugar and absent in most of them. LDH was significantly high and CSF/ serum ratio was altered. CRP was positive in almost all cases and was in the range of 0.6 to 9.6 mg/dl and values were corresponding in the serum. CRP and LDH showed a high ODDS ratio and high significance compared to other parameters. CSF CRP is a novel marker with high sensitivity and specificity. LDH and CRP being rapid and cost effective can be used as a supportive evidence of meningitis, along with other routine tests to diagnose pyogenic meningitis.

Keywords: CRP, LDH, Pyogenic meningitis, Cerebrospinal fluid.

Introduction
Acute infections of the nervous system are among the most important diseases in medicine because early recognition, efficient decision making, and rapid institution of therapy can be life saving. Many organisms can cause infections of the central nervous system (CNS). Bacteria are the most important cause of fever associated with signs and symptoms of CNS disease.

Most common infections of CNS are meningitis and encephalitis. Meningitis implies primary involvement of meninges whereas encephalitis indicates brain parenchymal involvement and should be considered to have meningo encephalitis [1]. Nonetheless, specific pathogens are identifiable and are influenced by the age and immune status of the host and epidemiology of the pathogen [2]. Various factors that determine the outcome of disease are age, early diagnosis, early treatment, duration of treatment and type of microorganism [3]. The information yielded by examination of cerebrospinal fluid is often of crucial importance in the diagnosis of neurological disease [4].

Prompt and precise etiological diagnosis remains a challenge and often a thorough cerebrospinal fluid examination may give a precise diagnosis. Many enzymes are known to be present in abundance in the nervous system. Meningitis disturbs the blood brain barrier and is expected to cause rise in enzymatic activity. However the role of various cerebrospinal fluid enzymes needs to be evaluated as not enough work has been carried out and majority of workers have estimated one of these enzymes either in cerebrospinal fluid or serum [5]. It is in this context that the present study was planned to evaluate the diagnostic significance of

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cerebrospinal fluid Lactate Dehydrogenase and CRP in cases of pyogenic meningitis. The current study was conducted among adults 18 – 65 years admitted in the general medicine and neurology wards of Government General Hospital, Vijayawada and Bhaskar General Hospital, Yenkepally, Hyderabad. Patient attendee's were apprised of the purpose of study and written consent was taken prior to commencement of the study. Ethical clearance was obtained by the ethical clearance committee of the institution. The study included the clinical evaluation and CSF analysis of 32 cases of meningitis and 28 cases of normal patients were taken as controls on which LP was performed for various surgical procedures and were admitted in the hospital from period of May 2010 to August 2015.

Materials & Methods
Study group
Selection of cases
Those cases admitted with fever, headache, vomiting, altered sensorium, with or without convulsions in the age group of 18 – 65 years were examined in detail for any clinical evidence of meningitis. After detailed clinical and fundal examination, in stable cases Lumbar Puncture was performed immediately. Blood was drawn for estimation of blood sugar, proteins CRP & LDH within 1 hour of lumbar puncture. 5-6ml of CSF was collected in 3 clean sterile bottles. For culture and sensitivity CSF was directly collected into glucose broth and sent to microbiology lab. One drop of CSF was collected directly over three clean glass slides. One slide was used to study pleocytosis and type of cells. The other two slides were used for preparation of smear by heat fixation for grams stain and ZN stain. Those cases of dry or traumatic tap were excluded from the study.

Analysis of CSF & Serum
Biochemical analysis for sugar, proteins, CRP and LDH for CSF & Serum were performed in the department of biochemistry. CSF & Serum Glucose Estimation was done by Glucose Oxidase Method (Marks & Lloyd 1963). CSF protein estimation was done by turbidimetric method using 3% sulphosalicylic acid (kingsbury etal 1926). Serum protein estimation was done using Biuret method [6, 7]. CSF & Serum Lactate Dehydrogenase estimation was done by Woblewski & La Due (1955) [8, 9] method (Kit method).CSF & Serum C-Reactive protein estimation was done by Semi quantitative Latex Agglutination test[10, 11] CSF cytology was studied in the department of pathology.

Two slides and CSF collected in media were taken to the department of microbiology for identification of organism. In all the cases the smears were stained with gram and ZN stain for the identification of organism. The specimen collected in the glucose broth was sub cultured in the appropriate media and incubated at 37^0C for 24 hours. In all cases one sample was kept for cob web formation. The diagnosis of pyogenic meningitis was made on the basis of CSF leucocytic pleocytosis with >90% Neutrophils and CSF glucose less than $1/3^{rd}$ of corresponding blood glucose with or without positive CSF culture or gram stain.

Results were analyzed by descriptive statistical analysis. Analysis of variance (ANOVA), CHISQUARE TEST and Z TEST were used. The Sensitivity, specificity, Positive predictive value (PPV) and negative predictive value (NPV) has been defined for each test. ROC curves, Odds ratio and analysis of logistic regression were done to establish the best diagnostic marker. SSPS 17 and Met cal statistical software was used for statistical analysis.

Results
Diagnosis and protein status
1. CSF protein: CSF protein was increased in all the 32 cases (100%) of pyogenic meningitis. The mean CSF protein in cases was 368.875 ± 494.808 mg/dl with Z value 38.387(p<0.0001) and is statistically significant when compared with controls. The mean CSF protein in controls was 47.357 ± 26.630 mg/dl. The CSF protein showed sensitivity of 90.6%, specificity 92.8%, positive predictive value of 93.5% and negative predictive value of 89.7% at cut off value of >88 mg/dl.
2. Serum protein: no significant difference was seen in serum proteins between cases and controls. The mean serum total proteins in cases was 5859.375 ± 535.73 mg/dl and in controls was 5635.714 ± 693.47 mg/dl with Z value 1.503(p >0.05) which is statistically not significant.

Diagnosis and glucose status
1. CSF glucose: CSF glucose was decreased in 24 cases (75%) and was normal in 8 cases (25%). The mean CSF glucose in cases was 33.656 ± 20.185 mg/dl with Z value 7.599 (p<0.0001) and is statistically significant when compared with controls. The mean CSF glucose in controls was 70.642 ± 17.51 mg/dl. The CSF glucose showed sensitivity of 71%, specificity 100%, positive predictive value of 92.3% and negative predictive value of 92.8% at cut off value of ≤ 45mg/dl.
2. Serum glucose: no significant difference was seen in serum glucose between cases and controls. The mean serum glucose in cases was 96.937 ± 22.997 mg/dl and in controls was 104.892 ± 22.521 mg/dl with Z value 1.697(p >0.05) which is statistically not significant.
3. CSF/serum glucose ratio: a significant difference was seen in CSF/serum glucose between cases and controls. The mean CSF/serum glucose in cases was 0.348 ± 0.196 mg/dl with Z value 8.177 (p<0.0001) which is statistically significant when compared to controls. The mean CSF/serum glucose in controls was 0.678 ± 0.110 mg/dl. CSF/serum glucose ratio was <0.6 in 29 cases (91%) and less than 0.4 in 25 cases (78%).

Diagnosis and LDH status:
1. CSF Lactate Dehydrogenase: CSF LDH was increased in 31 cases (96%) of pyogenic meningitis. the mean CSF LDH in cases was 159.062 ± 124.562 IU/L with Z value 13.564 (p<0.0001) and is statistically significant when compared to controls. The mean CSF LDH in controls was 31.571 ± 40.175 IU/L. The CSF LDH showed sensitivity of 96.8%, specificity 85.7%, positive predictive value of 88.6% and negative predictive value of 96.0% with cut off value of >34IU/L.
2. Serum Lactate Dehydrogenase: Serum LDH was increased in 29 cases (91%) of pyogenic meningitis. the mean serum LDH in cases was 274.25 ± 131.347 IU/L with Z value 20.473 (p<0.0001) and is statistically significant when compared to controls. The mean serum LDH in controls was 58.928 ± 52.317 IU/L. The serum LDH showed sensitivity of 90.6%, specificity 89.2%, positive predictive value of 90.6% and negative predictive value of 89.2% with cut off value > 96 IU/L.
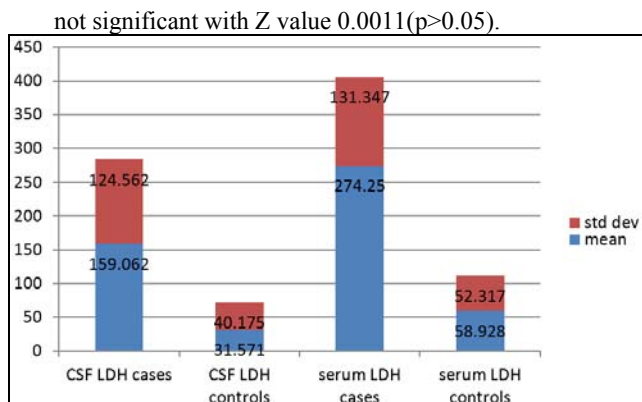3. CSF/Serum LDH ratio between cases and controls was

not significant with Z value 0.0011(p>0.05).

Fig 11: CSF & Serum LDH values in cases and controls

Diagnosis and CRP status:
1. CSF C Reactive Protein: CSF CRP was increased in 31 cases (96.8%) of pyogenic meningitis. the mean CSF CRP in cases was 2.25 ± 2.021 mg/dl with Z value 25.464 (p<0.0001) and is statistically significant when compared to controls. The mean CSF CRP in controls was 0.064 ± 0.188 mg/dl. The CSF CRP showed sensitivity of 96.8%, specificity 89.2%, positive predictive value of 91.1% and negative predictive value of 96.1%with cut off value >0.6 mg/dl.
2. Serum CRP: Serum CRP was increased in 30 cases (93.7%) of pyogenic meningitis. The mean serum CRP in cases was 1.725 ± 2.008 mg/dl with Z value 4.64 (p<0.001) and is statistically significant when compared to controls. The mean serum CRP in controls was 0.064 ±0.249 mg/dl. The serum CRP showed sensitivity of 93.7%, specificity 92.8%, positive predictive value of 93.7% and negative predictive value of 92.8%.
3. CSF/Serum CRP ratio: CSF/Serum CRP ratio was increased in 30 cases (93.7%) of pyogenic meningitis. The mean CSF/serum CRP ratio in cases was 1.73 ± 1.097 mg/dl with Z value 8.5 (p<0.001) and is statistically significant when compared to controls. The mean CSF/serum CRP ratio in controls was 0.0535 ±0.208 mg/dl. The CSF/serum CRP ratio showed sensitivity of 93.7%, specificity 92.8%, positive predictive value of 93.7% and negative predictive value of 92.8%.
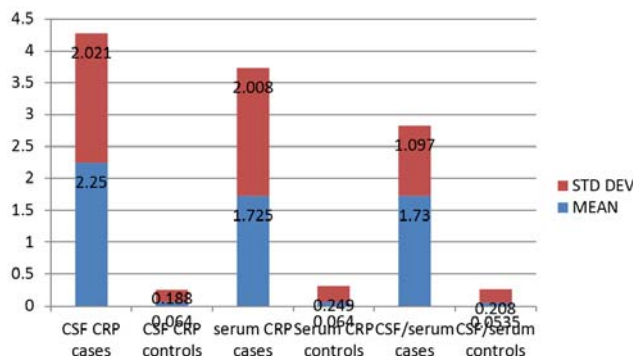

Fig 2: CRP values in cases and controls

Table 1: regression analysis

Parameter	AUC	Best cut off	Odds ratio	Sensitivity	Specificity	PPV	NPV	P VALUE
CSF protein	0.980	>88	125.6	90.62	92.86	93.5	89.7	<0.0001
CSF glucose	0.871	≤ 45	39	71.87	100	92.3	76.4	<0.0001
CSF LDH	0.941	>34	186	96.87	85.71	88.6	96.0	<0.0001
CSF CRP	0.973	>0	258.33	96.87	89.29	91.2	96.2	<0.0001
serum proteins	0.616	>5.4	3.7	81.2	50.0	65	70	0.132
serum glucose	0.624	≤94	2.6	59.38	64.29	65.5	58.1	0.089
serum LDH	0.958	>96	80.5	90.62	89.2	90.6	89.2	<0.0001
serum CRP	0.941	>0	192	93.75	92.86	93.7	92.8	<0.0001

Discussion
In this study of CSF analysis in pyogenic meningitis with special reference to proteins, glucose, CRP and LDH estimation, 60 cases were studied. Out of 60 cases, 32 had pyogenic meningitis, and 28 cases posted for various surgeries were taken as controls.
There is very significant elevation in levels of CSF protein in cases of pyogenic meningitis with a mean of 368.875 ± 494.808 (p<0.0001) when compared to controls with a mean of 47.357 ± 26.630. The CSF protein showed sensitivity of 90.6%, specificity 92.8%, positive predictive value of 93.5% and negative predictive value of 89.7%. Serum proteins showed no significant difference p>0.05.
CSF glucose was very much decreased in 75% of cases with mean of 33.656 ± 20.185 and was statistically significant p < 0.0001compared to controls with mean of 70.642 ± 17.514. The CSF glucose showed sensitivity of 71%, specificity 100%, positive predictive value of 92.3% and negative predictive value of 92.8%. There was no significant difference between the serum glucose levels of cases and controls. The CSF glucose in controls was about 67% of serum glucose whereas in cases it was 35 %. CSF/serum glucose ratio corrects for hyperglycemia which can mask a decrease in CSF glucose concentration. The CSF glucose concentration is considered decreased when the CSF/serum ratio is less than 0.6. A ratio of <0.4 is highly suggestive of pyogenic meningitis [12]. In our present study CSF/serum glucose ratio was <0.6 in 29 cases (91%) and less than 0.4 in 25 cases (78%).
CSF LDH was increased in almost 96% cases with mean of 159.062 ± 124.562 and was statistically significant (p<0.0001) when compared to controls with mean of 35.571 ± 40.175. The CSF LDH showed sensitivity of 96.8%, specificity 85.7%, positive predictive value of 88.6% and negative predictive value of 96.0%. Serum LDH was also increased in majority 91% (29 cases) with a mean of 274.25 ± 131.347 and was statistically significant p<0.0001compared to controls with mean of 58.928 ± 52.317. The serum LDH showed sensitivity of 90.6%, specificity 89.2%, positive predictive value of 90.6% and negative predictive value of 89.2%. The LDH CSF: serum ratio though elevated was not significant p>0.05.
CSF CRP was elevated in 97% of cases with mean of 2.25 ± 2.021 and was statistically significant p<0.0001when

compared to controls with mean of 0.064 ± 0.188. The CSF CRP showed sensitivity of 96.8%, specificity 89.2%, positive predictive value of 91.1% and negative predictive value of 96.1%. Serum CRP was also increased in 94% of cases with mean of 1.725 ± 2.008 and was statistically significant p<0.0001 when compared to controls with mean of 0.064 ± 0.249. The serum CRP showed sensitivity of 93.7%, specificity 92.8%, positive predictive value of 93.7% and negative predictive value of 92.8%.. The CSF: serum CRP ratio was elevated in 94% of cases and is statistically significant p<1.001.

Analysis of logistic regression
Analysis of logistic regression to identify the best diagnostic marker in pyogenic meningitis:
Logistic regression analysis showed that CSF CRP cut off value is associated with258 fold chance of diagnosing pyogenic meningitis (Odds ratio: 258.3, p<0.0001). Then for CSF LDH, CSF protein, CSF glucose cut off values were associated with 186, 126, 39 fold chance respectively of diagnosing pyogenic meningitis (Odds ratio: 186, 126.6, 39 and p<0.0001 for all).

Conclusion
Meningitis is one of the commonest central nervous system diseases in children and adults. Although majority of cases manifest typically with fever, convulsion, headache, vomiting and altered sensorium, atypical presentations are not uncommon. In majority of the cases, detailed history and clinical examination and analysis of cells was helpful in diagnosing meningitis, CSF analysis was helpful in majority of the patients for diagnosis of meningitis.
The biochemical profile observed in the present study which help in diagnosing pyogenic meningitis is: low CSF glucose with mean of 33.65 ± 20.18(p<0.0001) and glucose CSF/serum ratio <0.6 in 91% cases and increased CSF protein with mean 368.875 ± 494.808 (p<0.0001). The CSF LDH was increased with mean 159.065 ± 124.56 (p<0.0001), serum LDH was increased with mean 274.25 ± 131.34 (p<0.0001) and CSF CRP was increased with mean of 2.25 ± 2.021(p<0.0001), serum CRP was increased with mean of 1.725± 2.008 (p<0.0001) and CSF/serum CRP ratio of 1.737 ± 1.09 (p<0.001) was observed and may be a sensitive parameter along with them. To conclude in majority of cases the CSF glucose was decreased and CSF proteins were elevated. A corresponding increase in CSF and serum LDH and CRP was seen and CSF CRP appeared to be the best marker amongst them.

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