Urinary reagent strips a rapid diagnostic tool for cerebrospinal fluid analysis: A study from Western India

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Abstract

Background: Meningitis is a common infection of the brain and spinal cord which has significant short-term and long-term complications. Early diagnosis and treatment are essential to prevent these complications. For diagnosis of meningitis, cerebrospinal fluid (CSF) microscopy and biochemistry are required.

Objectives: To compare results of the urinary reagent strip method in estimation of CSF leucocytes, proteins and sugar with the standard method of CSF microscopy and biochemistry and to compare the utility of the reagent strip method for CSF analysis with the standard laboratory method for diagnosis of meningitis in the form of sensitivity, specificity, positive predictive value and negative predictive value.

Method: A prospective single-blinded study was conducted in the paediatric department of a tertiary care teaching hospital. We studied 111 consecutive CSF samples received in our laboratory within an hour of the tap. We recorded the age and sex of all samples. CSF samples were immediately processed for microscopy by an independent researcher. CSF proteins and glucose estimation were done in the biochemistry laboratory. The Combur-10 patch strip was used as an index test for CSF analysis (cells, proteins and glucose). Index tests and definitive tests were performed and diagnostic accuracy was compared between the two.

Results: This study was conducted on 111 consecutive CSF samples. Leucocyte positivity by test strip had a sensitivity of 100% and specificity of 99% for detection of CSF granulocytes of more than 15/ cu mm. For detection of CSF proteins greater than 30 mg/dL, the reagent strip method had a sensitivity of 97% and specificity of 89.7%. Specificity improved when we used a higher cut-off of CSF protein (greater than 100 mg/dL). Diagnostic accuracy of both leucocyte and protein strips was improved by using the higher cut off values. Glucose strips had a sensitivity of 82.9% and specificity of 89.7%.

Conclusions: Our study demonstrates that the urinary reagent strip method can be used for estimation of CSF leucocytes, proteins and sugar in resource-limited settings for early diagnosis and management of meningitis.

(Key words: Urinary reagent strip, CSF, Diagnostic, Meningitis)

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Introduction

Meningitis is a common central nervous system (CNS) infection in children associated with high short-term and long-term complications like convulsions, hearing impairment, death, and permanent neurological deficit. Early diagnosis and treatment are essential to prevent such complications[1,2]. For diagnosis of meningitis, cerebrospinal fluid (CSF) analysis is essential[3]. A qualified and competent pathologist and a well-equipped laboratory setup are required for estimating the protein and sugar levels. In a rural setup, such facilities may not be available or feasible[4]. The Combur-10 patch strip was used as an index test for CSF analysis (cells, proteins and glucose). This Combur-10 patch strip is routinely used to test urine for specific gravity, protein, glucose, leucocytes, nitrites, pH, haemoglobin, ketones and bilirubin. Many studies have reported the utility of the urinary reagent strip (widely used semi-quantitative method for urine analysis) for the diagnosis of meningitis[5-14]. This reagent strip method can be used as a bedside diagnostic test for CSF analysis[15]. In this study, we have analysed the accuracy of the urinary reagent strip method for CSF analysis.

Objectives

- To compare results of the urinary reagent strip method in estimation of CSF leucocytes, proteins and sugar with the standard method of CSF microscopy and biochemistry.
- To compare the utility of the reagent strip method for CSF analysis with the standard laboratory method for diagnosis of meningitis in the form of sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV).

Method

We conducted a prospective single blinded study in the paediatric department of a tertiary care teaching hospital from January 2019 to June 2021. The study centre caters to heterogeneous populations representing both urban and rural patients.

Sample size: n = (Z α/2)2 *2*Specificity* (1-specificity)/ standard error *2*(1- prevalence of meningitis)

Z is constant 1.96; specificity of reagent strip for CSF protein analysis was reported as 45.8% in a previous study by Chikkannaiah P, *et al*[4]; standard error was taken as 10%; 10% prevalence of meningitis reported in study by Bonev V, *et al*[6]. n=1.96* 1.96*2*0.572*0.0.428/0.1*0.1*2*0.1=104.49

We studied 111 consecutive CSF samples. CSF samples were processed within half an hour of tapping and received in our laboratory within an hour of the tap. We recorded the age and sex of all samples. We excluded the samples with haemorrhagic tap or if the quantity was not sufficient to perform an index test. CSF samples were immediately processed for microscopy by an independent researcher. CSF proteins and glucose estimation were done in the biochemistry laboratory. Index test was performed by an independent researcher and then to compare diagnostic accuracy of index and definitive tests different statistical tests were performed.

Index test was done by one of the investigators using urinary reagent strips. This Combur-10 strip can detect 10 different parameters like leucocytes, proteins, and sugar. CSF was withdrawn with all aseptic precautions and with the help of the pipette 2-3 drops of undiluted CSF was added to the patches of leucocyte esterase, proteins and sugar and colour changes were compared with the manufacturer's given colour coding.

- *CSF leucocytes:* The reagent strip is designed to detect the range of leucocytes from 15 to 500 cells/ cu mm. By estimating leucocyte esterase, we can find the number of leucocytes. Leucocytes are graded on the basis of colour change:
  Negative: <10 cells/cu mm
  1+: 10-75 cells/cu mm
  2+: 75-500 cells/cu mm
  3+: >500 cells/ cu mm
- *CSF proteins***:** The normal range of CSF protein is 15 - 45 mg/dL; the range for protein detection of the strip is 30-500mg/dL. The interpretation of colour change on reagent strips for proteins was as follows:
  Negative: <30 mg/dl
  1+: 30–100 mg/dl
  2+: 100–500 mg/dl
  3+: >500 mg/dl
- *CSF sugar:* We used the reagent strip method to find out whether CSF glucose level is below or above 50 mg/dL The interpretation of results on the reagent strip was:
  No colour change: <50 mg/dL
  Any change: >50 mg/dL.

Definitive test: Definitive tests were done by an independent investigator. Neubauer chamber cell count of all tests was done and differential counting was done on two centrifuged smears by staining with haematoxylin and eosin stain and Leishman stain. Proteins and sugar were estimated by an automated analyser.

Ethical issues: Approval for the study was obtained from the Institutional Ethics Committee of Smt Kashibai Navale Medical College and General Hospital, Maharashtra, India (No. SKNMC/ Ethics/ App/2018/414).

Statistical analysis: Standard statistical tests like sensitivity, specificity, positive predictive value (PPV) and negative predictive value (NPV), and positive and negative likelihood ratios were used to compare the diagnostic accuracy of the reagent strip method with the standard laboratory method of CSF estimation. A receiver operating curve (ROC) curve showing the relationship between sensitivity and specificity as a function of the strip colour cut-off was plotted. SPSS software package was used to find out the area under the curve (AUC) along with its standard error.

Results

This study was conducted on 111 consecutive CSF samples. The age and sex distribution of the suspected cases of meningitis are shown in Table 1.

Table 2 shows the comparison of results of CSF analysis by laboratory and urinary reagent strip method.

Table 1: ***Age and sex distribution of cases (n=111)***

Age	Male	Female	Total
0-1 month	21	17	38 (34.2%)
1 month - 5 years	21	20	41(36.9%)
5-10 years	12	10	22 (19.8%)
10-15 years	04	06	10 (09.0%)
Total	58 (52.3%)	53 (47.7%)	111 (100.0%)

Table 2: ***Comparison of results of CSF analysis by laboratory and urinary reagent strip method***

CSF analysis by strip method	CSF analysis by laboratory method				
	One test normal	Two tests normal	Three tests normal	All abnormal	Total
One test normal	12	0	0	0	12
Two tests normal	01	55	03	0	59
Three tests normal	0	0	12	0	12
All abnormal	0	0	01	27	28
Total	13	55	16	27	111

There were 12 cases of bacterial meningitis and 18 cases of non-bacterial meningitis. In the non-bacterial meningitis cases, 13 patients had viral meningitis, 3 had autoimmune encephalitis and one had hydrocephalus.

Table 3 shows the comparison between the index test and definitive test and the accuracy of the index test alone and in combination with the definitive test.

Table 3: ***Comparison between index test and definitive test and accuracy of index test alone and in combination with definitive test***

Index Test	Cut-off value	True positive	False positive	False negative	True negative	Sensitivity	Specificity	Positive predictive value	Negative predictive value	Accuracy
Leucocytes /cu mm	>15	10	01	0	100	100	99	90.9	100	99.1
	>70	11	0	0	100	100	100	100	100	100
	>125	02	0	0	109	100	100	100	100	100
Protein mg/dL	>30	32	08	01	70	97	89.7	80	98.6	91.9
	>100	11	06	0	94	100	94	64,7	100	94.6
	>300	05	0	0	106	100	100	100	100	100
Glucose mg/dL	<50	68	03	14	26	82.9	89.7	95.8	65	84.7

Leucocyte positivity by test strip had a sensitivity of 100% and specificity of 99% for detection of CSF granulocytes of more than 15/cu mm. For detection of CSF proteins greater than 30 mg/dL, the reagent strip method had a sensitivity of 97% and specificity of 89.7%. Specificity improved when we used a higher cut-off of CSF protein (greater than 100 mg/dL). Diagnostic accuracy of both leucocyte and protein strips was improved by using the higher cut-off values. Glucose strips had a sensitivity of 82.9% and specificity of 89.7%.

Table 4 shows the diagnostic accuracy of the urinary reagent strip.

Table 4: ***Diagnostic accuracy of urinary reagent strip***

Index Test	Cut-off value	Positive likelihood ratio	Negative likelihood ratio	Accuracy
Leucocytes /cu mm	>15	101	-	99.1
	>70	-	-	100
	>125	-	-	100
Protein mg/dL	>30	9.45	0.033	91.9
	>100	16.67	-	94.6
	>300	-	-	100
Glucose mg/dL	<50	8.016	0.19	84.7

Receiver operator curves (ROCs) for the performance of test strip at different cut-off levels of CSF leucocyte (>15), protein (>30) and sugar (<50) AUC (area under curve) and their 95% confidence interval are: a) 0.845 (0.747-0.943) b) 0.9 (0.832-0.969) c) 0.863(0.783-0.943). This is shown in Figures 1-3.

Table 5 shows the comparison of diagnostic accuracy of urinary reagent strip method with various studies.

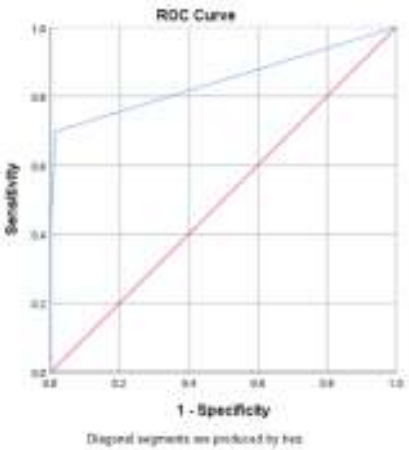

Figure 1: ***Receiver operated curve for leucocyte >15/cu mm Area under the curve 0.845 (p<0.001)***

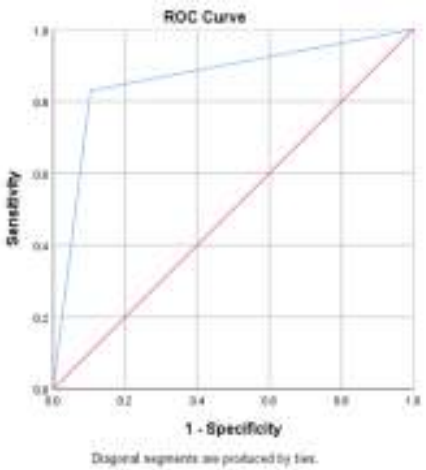

Figure 3: ***Receiver operated curve for glucose <50mg/dL Area under the curve 0.865 (p<0.001)***

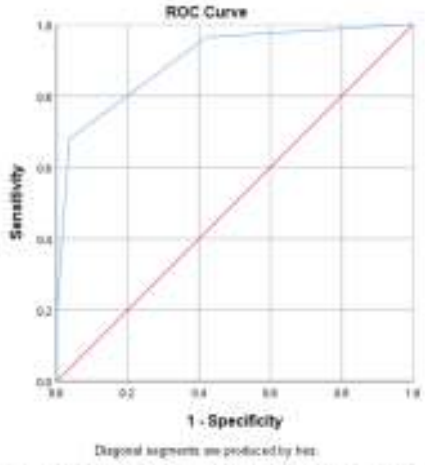

Figure 2: ***Receiver operated curve for protein>30mg/dL Area under the curve 0.900 (p<0.001)***

Table 5: ***Comparison of diagnostic accuracy of urinary reagent strip method with various studies***

Using reagent strip method for CSF analysis	Present study	Wankhade R, *et al*[20]	Mazumder S, *et al*[21]	Chikkannaiah P, *et al*[4]	Joshi D, *et al*[22]
Sensitivity, specificity, positive predictive value, negative predictive value in % for leukocytes	100, 99, 90.9, 100 for CSF granulocyte >15/cu mm	82.53, 100, 100, 47.1 for CSF granulocyte >15/cu mm	89.3, 98.6, 96.61, 95.9% CSF granulocyte >15/ cu mm	96.6, 94.5, 87.8, 98.5 CSF granulocyte >10/ cu mm	85.2, 89.6, 82.1, 91.5 CSF granulocyte >10/cu mm
Sensitivity, specificity, Positive predictive value, Negative predictive value in % for proteins	97, 89.7, 80, 98.6 for CSF proteins >30 mg/ dl	78.6, 100, 100, 33.3 for CSF proteins >30 mg/ dl	85.71, 95.7, 98.5, 66.7 for CSF proteins >30 mg/ dl	94.9, 45.8, 85.2, 73.3 for CSF proteins >30 mg/ dL; 96.9. 1, 70. 5, 98.5 for proteins at a cutoff ≥100 mg/dl; strip less specific at cutoff of ≥30 mg/dl.	98.1, 57.1, 85.5, 92.3 for CSF proteins >30 mg/ dl
Sensitivity, specificity, Positive predictive value, Negative predictive value in % for glucose	82.9, 89.7, 95.8, 65 <50mg/dL sugar	100,78.95, 92, 100 <50mg/dl sugar	48.2, 100, 100, 82.55 <50mg/dl sugar	14.2,100, 100, 75.7 for <50mg/dl sugar. specific (100%) and less sensitive at both cutoff levels (<40mg/dl and <50mg/dl)	48.2, 98, 92.3, 77.4 <50mg/dl sugar

Discussion

Meningitis is a common emergency in the neonatal and paediatric age groups and it is associated with significant post-meningitic sequelae due to the damage caused to the brain structure[15]. Early diagnosis and treatment are important for neonates and infants to prevent complications like hearing loss, developmental delay, weakness and issues with cognition, vision and behaviour[16,17]. To prevent such morbidity it is important to initiate treatment within 3 hours[18]. Diagnosis of meningitis is mainly by estimating CSF cellularity, protein and sugar[19].

In our study, the accuracy of estimation of CSF leucocytes, protein and sugar by using a urinary reagent strip is comparable with laboratory CSF analysis with significant sensitivity and specificity and positive predictive value. Receiver operator curves for the performance of test strips at different cut-off levels of CSF leucocyte (>15/cu mm), protein (>30mg/dL) and sugar <50mg/dL showed a good correlation.

In the present study, by using the strip method for estimation of CSF proteins we could diagnose 105 cases correctly and only 6 cases were false positive. All these false positive cases had protein values above 45mg/dl which was above the biochemical cut-off used to interpret CSF proteins. Specifically designed strip for this cut-off will have more yield. Similarly, the strip method used to diagnose CSF leucocytes, could diagnose 110 cases out of 111cases. There was one false positive case that may be due to presence of RBCs on cytological examination. As the leucocyte esterase is specific for identification of leucocytes, the strip that can identify all types of leucocytes will be of better use.

Present study results are comparable with previously reported studies (Table 5). Wankhade R, *et al*[20], Mazumder S, *et al*[21], Chikkannaiah P, *et al*[4] and Joshi D, *et al*[22], reported high sensitivity, specificity, PPV and NPV in the range of 85-100% by using the urinary reagent strip method for estimation of CSF leucocytes except in a study by Wankhade R, *et al*[20] in which the NPV was low. These findings are consistent with our study findings. This strip method has higher sensitivity and PPV, so this can be a useful tool to screen CSF at the bedside for early diagnosis of meningitis. Good specificity and NPV make this test more useful in ruling out meningitis.

Table 5 shows various studies which demonstrated significant diagnostic accuracy of the reagent strip method for estimation of CSF proteins with a few exceptions like Wankhade R, *et al*[20] who reported less sensitivity and NPV, Chikkannaiah P, *et al*[4], who showed lesser specificity and NPV and Joshi D, *et al*[22] who showed lesser specificity with good NPV. Our study also showed similar results. These studies showed lower specificity and NPV but had better sensitivity and PPV which will help to diagnose the cases of meningitis with some false positive cases but at least cases will not be missed which in turn prevent the morbidity and mortality due to meningitis.

As shown in table 5 various reported studies and our study had comparable results for the estimation of CSF glucose by urinary reagent strip method. A study by Romanelli RM, *et al*[14] showed a significant correlation between results of reagent strips and standard cytological and biochemical analysis. Sensitivity, specificity, PPV, and NPV were 90.7%, 98.1%, 95.1%, and 96.4%, respectively for the diagnosis of bacterial meningitis. This shows that this urinary reagent strip method can qualify to be a good screening test for early diagnosis meningitis.

In our study, we observed that the diagnostic accuracy of both leucocyte and protein strip was improved on using the higher cut-off values and it reached 100% accuracy for very high-test results. In bacterial meningitis, CSF cell counts and protein levels are very high with low sugar levels as compared to aseptic meningitis and thus the reagent strip method is very useful in the early identification and treatment of bacterial meningitis in resource-limited settings. This will help to avoid the use of unnecessary antibiotics in the case of febrile seizures or neonates with hypoxic seizures which are common differential diagnoses of meningitis. This will prove to be useful in the setting of the primary healthcare centres and rural setups where no laboratory facilities exist.

The strengths of our study are that it was carried out in a blinded fashion and that definitive and index tests were carried out within half-hour of CSF collection. The shortcomings in our study are the small sample size and that these reagent strips are mainly made for urine analysis so that there is a slight variation in cut-off normal values of proteins and sugar in CSF compared with urine. The main problem in identifying whether 1+ CSF protein is normal or abnormal. This we can overcome by asking the manufacturer to make a CSF specific strip. This will prove to be a useful tool to the clinician in the management of meningitis. Further studies with larger numbers and comparisons with different manufacturers' strips are recommended.

Conclusions

Our study demonstrates that the urinary reagent strip method can be used for estimation of CSF leucocytes, proteins and sugar in resource-limited settings for early diagnosis and management of meningitis.

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