Efficacy of urine reagent strips for analysis of cerebrospinal fluid cellularity and biochemical parameters

Nikita Singh¹, Suresh Kumar Sutrakar², Uday Raj Singh³, Lokesh Tripathi⁴

¹PG Resident, ²Professor and Head, ³Professor, ⁴Assistant Professor, Department of Pathology, Shyam Shah Medical College, Rewa, Madhya Pradesh, India

Background: Meningitis is a severe infection affecting the central nervous system requiring early management to prevent debilitating consequences and death. Detection of meningitis can be done by the analysis of cerebrospinal fluid cellularity and biochemistry (glucose and protein concentration). Urine reagent strips can be used to assess these variables and reach to the diagnosis of meningitis early, thereby, reducing the delay in the initiation of its treatment. Aims and Objectives: The current study aimed to determine the efficacy of urine reagent strips for the analysis of CSF. Materials and Methods: The current prospective study was done in a tertiary care center in Rewa, M.P., on 100 cerebral spinal fluid (CSF) samples obtained from the children suspected of meningitis, which were, then, analyzed by both the conventional methods of CSF analysis (including routine biochemical and microscopic analysis) the index test, utilizing the Combur-10 urine reagent strips for the estimation of CSF cell, protein, and glucose. These results were, then, correlated to calculate the diagnostic utility of urine reagent strips for the rapid diagnosis of meningitis. Results: The sensitivity, specificity, and diagnostic accuracy of urine reagent strips for the detection of cell count > 10/cumm were 81.81%, 98.21%, and 91%, respectively, and 78.57%, 81.25%, and 79% for detection of protein > 30 mg/dL. Reagent strips showed a sensitivity and specificity of 96.85% and 79.41% for the detection of glucose levels < 50 mg/dL. The two methods showed an almost perfect degree of agreement for the detection of leucocytes with Cohen’s kappa value of 0.86. Conclusion: Reagent strips can be a helpful bedside screening test for the detection of meningitis and also in resource-limited settings.

Key words: Urine reagent strips; Meningitis; Cerebral spinal fluid

ABSTRACT

Background: Meningitis is a severe infection affecting the central nervous system requiring early management to prevent debilitating consequences and death. Detection of meningitis can be done by the analysis of cerebrospinal fluid cellularity and biochemistry (glucose and protein concentration). Urine reagent strips can be used to assess these variables and reach to the diagnosis of meningitis early, thereby, reducing the delay in the initiation of its treatment. Aims and Objectives: The current study aimed to determine the efficacy of urine reagent strips for the analysis of CSF. Materials and Methods: The current prospective study was done in a tertiary care center in Rewa, M.P., on 100 cerebral spinal fluid (CSF) samples obtained from the children suspected of meningitis, which were, then, analyzed by both the conventional methods of CSF analysis (including routine biochemical and microscopic analysis) the index test, utilizing the Combur-10 urine reagent strips for the estimation of CSF cell, protein, and glucose. These results were, then, correlated to calculate the diagnostic utility of urine reagent strips for the rapid diagnosis of meningitis. Results: The sensitivity, specificity, and diagnostic accuracy of urine reagent strips for the detection of cell count > 10/cumm were 81.81%, 98.21%, and 91%, respectively, and 78.57%, 81.25%, and 79% for detection of protein > 30 mg/dL. Reagent strips showed a sensitivity and specificity of 96.85% and 79.41% for the detection of glucose levels < 50 mg/dL. The two methods showed an almost perfect degree of agreement for the detection of leucocytes with Cohen’s kappa value of 0.86. Conclusion: Reagent strips can be a helpful bedside screening test for the detection of meningitis and also in resource-limited settings.

Key words: Urine reagent strips; Meningitis; Cerebral spinal fluid

INTRODUCTION

Meningitis is a serious infection of the central nervous system involving the meninges which cover the brain and spinal cord.¹ It is a life-threatening disorder that necessitates prompt medical attention.² Delay in diagnosis results in permanent neurological damage and death.³ Examination of cerebrospinal fluid is the gold standard for the diagnosis of meningitis, which includes, samples for routine biochemistry, microscopy, and microbiological diagnosis.⁴ However, these tests require a reasonable laboratory set-up and a trained pathologist, which maybe deficient in resource-limited settings. Furthermore, the turn-around time for cerebral spinal fluid (CSF) analysis is more, which can delay the start of initial treatment. At present, no rapid point-of-care tests are available to detect meningitis.³,⁵,⁷ Several studies have showed the utility of urine reagent strips for the detection of CSF cells, protein, and glucose.³,⁵,¹¹ Urine reagent strips are made of plastic or paper which have pads impregnated with chemical which will change color when it comes in contact with urine or any fluid, and provide a semi-quantitative assessment of CSF cellularity and biochemistry.¹² It is a simple, rapid, and cost-effective method for analyzing CSF that does not require any expertise and can be used for diagnosis of meningitis.

Address for Correspondence:
Dr. Lokesh Tripathi, F 7/1, New Doctor Colony, Rewa - 486 001, Madhya Pradesh, India. Mobile: +91-9755657590.
E-mail: drlokesh99@gmail.com

Access this article online
Website:
http://nepjol.info/index.php/AJMS
DOI: 10.3126/ajms.v13i9.44909
E-ISSN: 2091-0576
P-ISSN: 2467-9100

Copyright (c) 2022 Asian Journal of Medical Sciences
This work is licensed under a Creative Commons Attribution-NonCommercial 4.0 International License.
Aims and objectives
The goal of the present study was to determine the role of reagent strip for CSF analysis at the bedside for rapid diagnosis of meningitis in pediatric age group at tertiary care center.

MATERIALS AND METHODS
The present prospective study was conducted in the Department of Pathology at Shyam Shah Medical College, Rewa, M.P., after obtaining ethical clearance from the Institutional Ethics Committee. One hundred CSF samples were obtained from the patients suspected of meningitis admitted in the Department of Pediatrics, by lumbar puncture. After receiving written informed consent from the guardians of children admitted with clinical suspicion of meningitis, lumbar puncture was conducted under strict aseptic conditions. CSF samples, thus obtained, which were further processed in the pathology laboratory by conventional methods of CSF analysis (reference test), that is, routine microscopy and biochemical analysis followed by index test using the urine reagent strips. The study excluded hemorrhagic samples and those with inadequate quantity to perform both the index and reference tests. For the semi-quantitative examination of CSF, we used Combur-10 (Cobas, Roche diagnostics) urine reagent strips with ten parameters.

Reference test
Improved Neubauer's chamber and differential cell count on centrifuged and Romanowsky stained CSF smears were used for microscopic investigation, which included determining the cell count and type of cells present in the CSF. Biochemical analysis for CSF protein and glucose was performed on CSF sample using an automated analyzer.

Cell count of >10/cumm, protein concentration >45 mg/dL, or glucose <45 mg/dL were considered as positive for meningitis based on the Center for Disease Control and Prevention (CDC).

In our study, the results of the reference test were used as the gold standard for diagnosing meningitis.

Index test
Protein, glucose, and leucocytes were measured in CSF samples using the appropriate reagent strip pads. After waiting for the manufacturer's specified period (i.e., 30 s for glucose, 60 s for protein, and 120 s for leucocytes), the findings were interpreted and compared to the standard values using the change in color of the test pads, as given in Table 1. For leucocytes and protein, samples showing +1 or above on leucocyte or protein test pads were considered positive, whereas samples with no change in color on glucose test pad were considered as positive.

Statistical analysis
Results obtained by both the methods were recorded in a tabulated form and were later analyzed using the Statistical Package for the Social Sciences ver. 22 (Chicago), IL. For each parameter, the results of both methods were compared. For WBC count, samples with count >10 cells/cumm on microscopy and +1 or +2 reading on LE test pad were considered as TP, whereas samples with <10 cells/cumm on microscopy and no change in LE test pad were considered as TN. CSF samples with cell count of >10 cells/cumm on microscopy but showed no change on LE test pad were categorized as FN, while the rest of the samples with count <10 cells/cumm but +1/+2 LE test pad reading were categorized under FP. Similarly for CSF protein, samples with protein concentration >30 mg/dL and +1/+2 reading on protein test pad were considered as TP, whereas those with no change of color on protein test pad were considered as FN. CSF samples with protein concentration <30 mg/dL and no change in color on protein test pad were categorized under TN, while the remaining samples showing +1/+2 reading on protein were regarded as FP. For glucose, CSF samples with glucose concentration <50 mg/dL and showing no color change on glucose test pad were considered as TP, whereas those with +1/+2 reading on glucose test pads were categorized as FN. Samples having >50 mg/dL glucose concentration, and showing +1/+2 reading on glucose pad, were categorized as TN, while rest of the samples which did not change the color of glucose test pad were regarded as FP.

The diagnostic efficacy of urine reagent strips for the diagnosis of meningitis was calculated and the Cohen's Kappa (κ) value was also calculated to evaluate the degree.

### Table 1: Study parameters with reagent strip test values and their corresponding biochemical values and microscopy

<table>
<thead>
<tr>
<th>Reagent strip test</th>
<th>Gold standard</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leucocytes</td>
<td>Microscopic examination</td>
</tr>
<tr>
<td>No color</td>
<td>&lt;10 leucocytes/cumm</td>
</tr>
<tr>
<td>1+</td>
<td>10–75 leucocytes/cumm</td>
</tr>
<tr>
<td>2+</td>
<td>75–500 leucocytes/cumm</td>
</tr>
<tr>
<td>3+</td>
<td>&gt;500 leucocytes/cumm</td>
</tr>
<tr>
<td>Glucose</td>
<td>Biochemistry value</td>
</tr>
<tr>
<td>No color</td>
<td>&lt;50 mg/dL</td>
</tr>
<tr>
<td>1+</td>
<td>50–100 mg/dL</td>
</tr>
<tr>
<td>2+</td>
<td>100–300 mg/dL</td>
</tr>
<tr>
<td>3+</td>
<td>&gt;300 mg/dL</td>
</tr>
<tr>
<td>Protein</td>
<td>Biochemistry value</td>
</tr>
<tr>
<td>No color</td>
<td>&lt;30 mg/dL</td>
</tr>
<tr>
<td>1+</td>
<td>30–100 mg/dL</td>
</tr>
<tr>
<td>2+</td>
<td>100–500 mg/dL</td>
</tr>
<tr>
<td>3+</td>
<td>&gt;500 mg/dL</td>
</tr>
</tbody>
</table>
of agreement between the two methods. Statistical analysis was performed to derive the specificity, sensitivity, positive predictive value, and negative predictive value.

**RESULTS**

**Reference test**

The CDC criteria for diagnosing meningitis were used to interpret the reference test, according to which the prevalence of meningitis in our study was 29%. Most common age group for meningitis was 2–5 year with 13% (13/100) of the total cases, followed by infants with 10% (10/100) cases. The age showed a significant correlation between age and occurrence of meningitis (P < 0.05) (Figure 1).

There were almost equal number of cases in males and females with 16% and 13%, respectively; however, statistically no significant correlation was seen between the occurrence of meningitis and gender (P > 0.05) (Figure 2).

Most of the samples of CSF were clear in appearance with meningitis in 19.7% (17/86) of samples and 85.7% (12/14) in turbid samples, and a significant correlation was seen between turbidity and meningitis with P < 0.001, which implies that turbidity of the samples can indicate toward the presence of high cell count or a higher protein concentration which, in turn, can be suggestive of meningitis.

About 21% of the CSF samples showed an abnormal WBC count on reference test (Figure 3), while 23% samples showed protein concentration >45 mg/dL. Abnormal glucose concentration <45 mg/dL was seen in 16% of the CSF samples (Figure 4).

**Index test**

Urine reagent dipstick for the evaluation of CSF was the index test in this study. Based on the values provided by the manufacturer, cutoff for the diagnosis of meningitis in our study was as follows:
- Leucocyte esterase: +1 or more (with prior age correlation)
- Protein: +1 or more
- Glucose: Negative.

The index test for WBC using the leucocyte esterase was positive in 37% of the samples, whereas abnormal protein concentration was seen in 69% of the samples. Abnormal glucose concentration suggestive of meningitis, that is, <45mg/dL was seen 45% of the samples (Figure 4).

**Correlation between index and reference test**

The values of the index test and the reference tests were compared for each parameter.

**Figure 1: Age-wise distribution of meningitis cases**

**Figure 2: Sex-wise distribution of meningitis cases**

**Figure 3: Bacterial meningitis: Cytocentrifuged CSF sample 2-year-old male, showing plenty of polymorphonuclear cells (Leishman stain; ×40)**

**WBC count**

Out of 56 cases having a WBC count <10 cells/cumm (by reference test), LE strip was able to correctly identify 55 cases with a negative result in the test pad, whereas 44 samples with count >10 cells/cumm, LE strip pads correctly identified 36 samples with a +1/+2 reading. Eight samples were misdiagnosed by the LE strip with a negative result. The sensitivity and specificity of LE for cell count >10/cumm were 81.81% and 98.21%, respectively, while
the diagnostic accuracy was 91%. Cohen’s kappa (κ) showed an almost perfect agreement between the two methods for the detection of WBC, with a value of 0.86 (Tables 2 and 3).

**Protein**

From the comparison of protein values between the index test and reference test for protein, it was seen that for protein concentration <30 mg/dL, the test strip was correctly able to identify 13 cases out of 16. Out of the remaining 84 samples with protein concentration >30 mg/dL, protein test pads identified 66 samples correctly with a positive result; however, rest 18 samples were misdiagnosed with a negative result. The sensitivity, specificity, and diagnostic accuracy of protein detection by urine reagent strips for >30 mg/dL were 78.57%, 81.25%, and 79%, respectively and the Cohen’s kappa (κ) showed moderate agreement between the two methods for the detection of protein, with a value of 0.43 (Tables 2 and 3).

**Glucose**

Glucose test pads in the urine reagent strips showed negative results in 31 out of 45 cases with a glucose concentration of <50 mg/dL. For glucose concentration >50 mg/dL in 68 samples, reagent strips showed a result of 1+ in 52 samples and 2+ in 2 samples. The sensitivity, specificity, and diagnostic accuracy for the detection of glucose by reagent strips for glucose concentration <50 mg/dL were 96.85%, 79.41%, and 68.88%, respectively. Cohen’s kappa (κ) showed a substantial agreement between the two methods for the detection of glucose, with a value of 0.69 (Tables 2 and 3).

**DISCUSSION**

Meningitis is a severe infection, which can have a wide spectrum of clinical manifestations ranging from fever, headache, nausea, vomiting, photophobia, neurological deficits, altered and sensorium to coma and death.

The sensitivity, specificity, and diagnostic accuracy for the detection of WBC >10 cells/cumm in our study were 81.81%, 98.21%, and 91%, respectively. The previous

<table>
<thead>
<tr>
<th>Table 2: Correlation between index and reference test for WBC, protein, and glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Index test</strong></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td></td>
</tr>
<tr>
<td>LE</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
<tr>
<td>+1/+2</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table 3: Diagnostic efficacy of urine reagent strips for detection of WBC, protein, and glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Index Test</strong></td>
</tr>
<tr>
<td>Leucocyte esterase</td>
</tr>
<tr>
<td>Protein</td>
</tr>
<tr>
<td>Glucose</td>
</tr>
</tbody>
</table>

Sensitivity=TP/(TP+FN)
Specificity=TN/(TN+FP)
PPV=TP/(TP+FP)
NPV=TN/(TN+FN)
Diagnostic Accuracy=(TP+TN)/(TP+FN+FP+TN)
Cohen’s Kappa value (κ)
researchers using similar methodology for the detection of WBC observed similar diagnostic efficacy of urine reagent strips.\textsuperscript{9-11,16-18} For the detection of protein >30 mg/dL, the sensitivity, specificity, and diagnostic accuracy of urine reagent strips were 78.57\%, 81.25\%, and 79\%, respectively. Wankhade et al.,\textsuperscript{17} and Mazumder et al.,\textsuperscript{10} observed a similar result for the sensitivity of protein detection; however, some studies showed a higher sensitivity, specificity, and diagnostic accuracy of urine reagent strips for protein detection.\textsuperscript{9-11,17,18} Similarly, for the detection of glucose <50 mg/dL, we observed high sensitivity (96.85\%) and moderate specificity (79.41\%) of urine reagent strips, which were in accordance with the results observed in the previous similar studies.\textsuperscript{9,11,16,17} However, Mazumder et al.,\textsuperscript{10} Burgoine et al.,\textsuperscript{19} Solanki et al.,\textsuperscript{18} and Chikkannaiah et al.,\textsuperscript{7} reported very low sensitivity and high specificity of urine reagent strips for the detection of glucose <50 mg/dL (Table 4).

Since, the specificity of urine reagent strips for detection of WBC is very high, a negative result by LE test pad will rule out the likelihood of meningitis. Protein detection by urine strips, on the other hand, had a moderate sensitivity and specificity, so the likelihood of meningitis is low if the test result is negative. Similarly, high sensitivity of reagent strips for the detection of glucose concentration below 50 mg/dL, which helps in the identification of true positives, with negative results on glucose test pads.

<table>
<thead>
<tr>
<th>Author</th>
<th>WBC count</th>
<th>Protein</th>
<th>Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Sensitivity (%)</td>
<td>Specificity (%)</td>
<td>Accuracy (%)</td>
</tr>
<tr>
<td>Mazumder et al.,\textsuperscript{10} 2018</td>
<td>89.28</td>
<td>98.6</td>
<td>98.7</td>
</tr>
<tr>
<td>Wankhade et al.,\textsuperscript{17} 2018</td>
<td>82.35</td>
<td>100</td>
<td>84.7</td>
</tr>
<tr>
<td>Rajkumar et al.,\textsuperscript{11} 2018</td>
<td>90</td>
<td>91.6</td>
<td>90.6</td>
</tr>
<tr>
<td>Gupta et al.,\textsuperscript{9} 2019</td>
<td>100</td>
<td>96</td>
<td>91.6</td>
</tr>
<tr>
<td>Sharma and Uradiya.,\textsuperscript{20} 2019</td>
<td>94</td>
<td>98.5</td>
<td>97</td>
</tr>
<tr>
<td>Buch et al.,\textsuperscript{16} 2020</td>
<td>26</td>
<td>100</td>
<td>72</td>
</tr>
<tr>
<td>Solanki et al.,\textsuperscript{18} 2020</td>
<td>94.11</td>
<td>98.79</td>
<td>98</td>
</tr>
<tr>
<td>Present study, 2021</td>
<td>81.81</td>
<td>98.21</td>
<td>91</td>
</tr>
</tbody>
</table>

CONCLUSION

The present study concluded that urine reagent strips can be used to detect WBC >10/cumm, protein >30 mg/dL, and glucose <50 mg/dL in CSF samples. The diagnostic efficacy was best for LE and moderate for protein and glucose detection. This knowledge can be of value for rapid diagnosis of meningitis in resource-limited settings and in emergency situations, thereby reducing the delay in initiation of the treatment. However, it can only be used as a screening test and must be followed by the definitive tests for the detection of meningitis.

ACKNOWLEDGMENT

The authors would like to acknowledge gratitude toward the Department of Pathology and Biochemistry, along with all the technical and non-technical staff for their constant support and coordination throughout the duration of the study.

REFERENCES


Authors Contribution:
NS- Concept and design of the study, prepared first draft of manuscript; SSK- reviewed the literature; URS- Concept, coordination, statistical analysis and interpretation; LT- Preparation of manuscript, interpretation and revision of the manuscript.

Work attributed to:
Shyam Shah Medical College and hospital, Rewa-486001, Madhya Pradesh, India.

Orcid ID:
Dr. Nikita Singh - https://orcid.org/0000-0002-3809-5354
Dr. Suresh Kumar Sutrakar - https://orcid.org/0000-0002-1892-2770
Dr. Uday Raj Singh - https://orcid.org/0000-0003-0551-2024
Dr. Lokesh Tripathi - https://orcid.org/0000-0002-3674-0751

Source of Support: Nil, Conflict of Interest: None.