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

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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

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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.[2,5-7] 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.

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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).[13,14] 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.

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

Reagent strip test	Gold standard
Leucocytes	Microscopic examination
No color	<10 leucocytes/cumm
1+	10–75 leucocytes/cumm
2+	75–500 leucocytes/cumm
3+	>500 leucocytes/cumm
Glucose	Biochemistry value
No color	<50 mg/dL
1+	50–100 mg/dL
2+	100–300 mg/dL
3+	>300 mg/dL
Protein	Biochemistry value
No color	<30 mg/dL
1+	30–100 mg/dL
2+	100–500 mg/dL
3+	>500 mg/dL

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 considered 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

of agreement between the two methods.[15] 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.

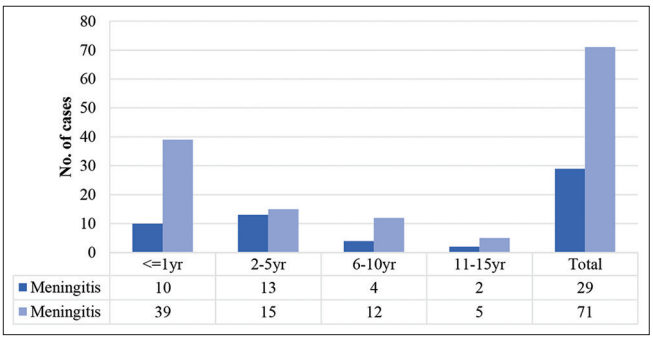

Figure 1: Age-wise distribution of meningitis cases

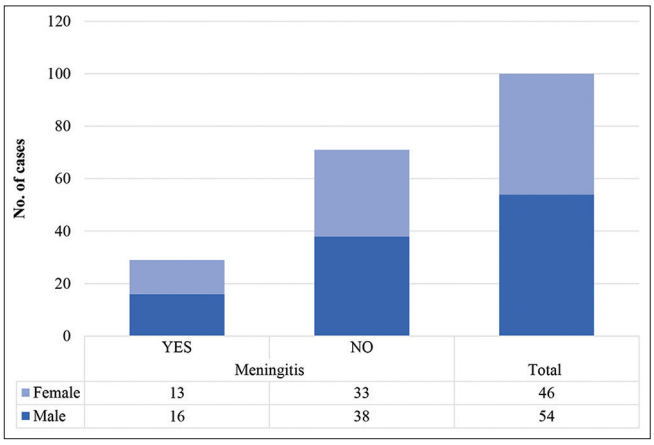

Figure 2: Sex-wise distribution of meningitis cases

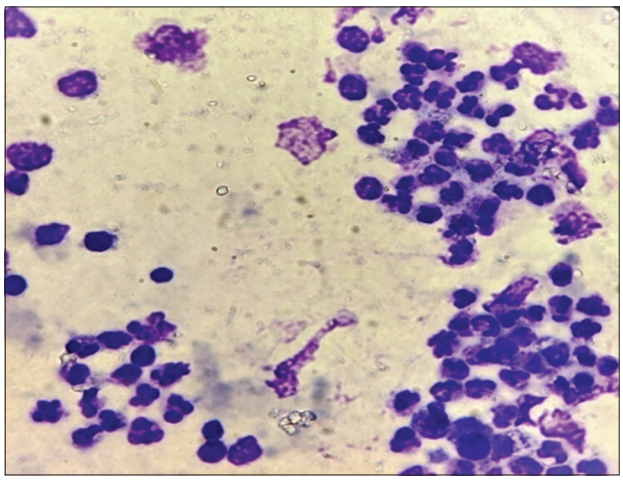

Figure 3: Bacterial meningitis: Cytocentrifuged CSF sample 2-year-old male, showing plenty of polymorphonuclear cells (Leishman stain; ×40)

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 mgdL 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).

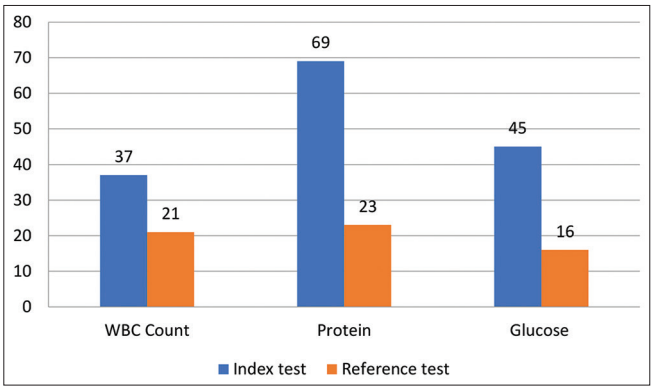

Figure 4: Abnormal WBC count, protein, and glucose in CSF samples by the index and reference test

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 2: Correlation between index and reference test for WBC, protein, and glucose

Index test	Reference test					
	WBC		Protein		Glucose	
	<10cells/cumm	>10cells/cumm	<30 mg/dL	>30 mg/dL	<50 mg/dL	>50 mg/dL
LE						
0	55	08				
+1/+2	1	36				
Protein						
0			13	18		
+1/+2			3	66		
Glucose						
0					31	14
+1/+2					1	54

Table 3: Diagnostic efficacy of urine reagent strips for detection of WBC, protein, and glucose

Index Test	Cut-off value	TP	FP	TN	FN	Sensitivity (%)	Specificity (%)	PPV (%)	NPV (%)	Accuracy (%)	Cohen's kappa value (κ)
Leucocyte esterase	>10 cells/ cumm	36	1	55	8	81.81	98.21	97.29	87.30	91	0.86
Protein	>30 mg/dL	66	3	13	18	78.57	81.25	95.65	41.93	79	0.43
Glucose	<50 mg/dL	31	14	54	1	96.85	79.41	68.88	98.18	85	0.69

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)

Table 4: Diagnostic efficacy of urine reagent strips for WBC, protein, and glucose between various studies

Author	WBC count			Protein			Glucose		
	Sensitivity (%)	Specificity (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)	Sensitivity (%)	Specificity (%)	Accuracy (%)
Mazumder *et al.*,[10] 2018	89.28	98.6	98.7	85.7	95.6	92.9	Low	100	92.8
Wankhade *et al.*,[17] 2018	82.35	100	84.7	78.5	100	93.8	100	78.9	86.5
Rajkumar *et al.*,[11] 2018	90	91.6	90.6	93.2	91.4	97	90	91.6	90.6
Gupta *et al.*,[9] 2019	100	96	91.6	99	54	88.7	100	96	71.4
Sharma and Uradiya.,[20] 2019	94	98.5	97	93	98.5	97	98.5	100	99
Buch *et al.*,[16] 2020	26	100	72	56	85	72	88	73	84
Solanki *et al.*,[18] 2020	94.11	98.79	98	97.2	92.1	94	65.21	100	92
Present study, 2021	81.81	98.21	91	78.57	81.25	79	96.85	79.41	85

researchers using similar methodology for the detection of WBC observed similar diagnostic efficacy of urine reagent strips.[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.,[17] and Mazumder et al.,[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.[9-11,17,18] Similarly, for the detection of glucose <50mg/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.[9,11,16,17] However, Mazumder et al.,[10] Burgoine et al.,[19] Solanki *et al.*,[18] and Chikkannaiah et al.,[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.

Limitations of the study

The drawback of the present study was that no definitive test was used such as culture or polymerase chain reaction (PCR) to confirm the diagnosis of meningitis. Also, the interpretation of urine reagent strips is observer dependent.

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.

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REFERENCES

1. Kumar A, Debata PK, Ranjan A and Gaind R. The role and reliability of rapid bedside diagnostic test in early diagnosis and treatment of bacterial meningitis. Indian J Pediatr. 2015;82(4):311-314.
   http://dx.doi.org/10.1007/s12098-014-1357-z
2. Patil N, Kanetkar S, Agarwal G and Karmakar S. Utility of urine

reagent strips in rapid bedside diagnosis of bacterial meningitis. Int J Res Med Sci. 2020;8(8):2947-2949.
http://dx.doi.org/10.18203/2320-6012.ijrms20203443

3. Bhat A, Roy R, Umashankar T and Jayaprakash CS. Rapid diagnosis of bacterial meningitis by urine reagent strip testing of cerebrospinal fluid. J Med Sci Health. 2018;4(1)15-17.
http://dx.doi.org/10.46347/jmsh.2018v04i01.003
4. Hrishi AP and Sethuraman M. Cerebrospinal fluid (CSF) analysis and interpretation in neurocritical care for acute neurological conditions. Indian J Crit Care Med. 2019;23(Suppl 2):S115-S119.
http://dx.doi.org/10.5005/jp-journals-10071-23187
5. Abdelmotaleb GS, Abdo MK, Behiry EG and Mahmoud MI. Evaluation of using urine reagent strips in the diagnosis of childhood meningitis. Med Res J. 2016;15(2):42-47.
http://dx.doi.org/10.1097/01.MJX.0000511316.35322.38
6. Joshi D, Kundana K, Puranik A and Joshi R. Diagnostic accuracy of urinary reagent strip to determine cerebrospinal fluid chemistry and cellularity. J Neurosci Rural Pract 2013;4(02):140-145.
http://dx.doi.org/10.4103/0976-3147.112737
7. Chikkannaiah P, Benachinmardi KK and Srinivasamurthy V. Semi-quantitative analysis of cerebrospinal fluid chemistry and cellularity using urinary reagent strip: An aid to rapid diagnosis of meningitis. Neurol India. 2016;64(1):50-55.
http://dx.doi.org/10.4103/0028-3886.173641
8. Dhakshinya M and Brundha MP. Use of urinary reagent strips in testing cerebrospinal fluid for meningitis-a review. Indian J Forensic Med Toxicol. 2020;14(4):4933-4940.
https://doi.org/10.37506/ijfmt.v14i4.12409
9. Gupta A and Dwivedi T. Reagent strips test: A simplified method for prompt analysis of cerebrospinal fluid in neurological disorders in emergency. Pract Lab Med. 2019;16:e00124.
https://dx.doi.org/10.1016/j.plabm.2019.e00124
10. Mazumder S, Ramya BS and Biligi DS. Utility of urine reagent strips in cerebrospinal fluid analysis: An aid to bedside diagnosis of meningitis. Indian J Pathol Microbiol. 2018;61(3):356-359.
https://dx.doi.org/10.4103/IJPM.IJPM_821_16
11. Rajkumar D, Ilangumaran L and Umashankar A. Determining the degree of accuracy of Combar-10 reagent strip in diagnosing bacterial meningitis. Int J Contemp Pediatr. 2018;5(6):2320-2325.
http://dx.doi.org/10.18203/2349-3291.ijcp20184304
12. Yetisen AK, Akram MS and Lowe CR. Paper-based microfluidic point-of-care diagnostic devices. Lab Chip. 2013;13(12):2210-2251.
http://dx.doi.org/10.1039/c3lc50169h
13. Poplin V, Boulware DR and Bahr NC. Methods for rapid diagnosis of meningitis etiology in adults. Biomark Med. 2020;14(6):459-479.
http://dx.doi.org/10.2217/bmm-2019-0333
14. Centers for Disease Control and Prevention. Primary culture and presumptive identification of *Neisseria meningitidis*, *Streptococcus pneumoniae*, and *Haemophilus influenzae*, 1998-2000. MMWR Morb Mortal Wkly Rep. 2002;51(11):234-237.
15. Landis JR and Koch GG. The measurement of observer agreement for categorical data. Biometrics. 1977;33(1):159-174.
http://dx.doi.org/10.2307/2529310
16. Buch A, Gupta D, Vasu B and Rathod H. A study of cerebrospinal fluid examination using urine reagent strips for diagnosis of meningitis: Urine strip for diagnosis of meningitis. Recent Adv Pathol Lab Med. 2019;5(4):1-6.
http://dx.doi.org/10.24321/2454.8642.201919
17. Wankhade R and Bhake A. Assessment of efficacy of urine reagent strips for cerebrospinal fluid analysis as emergency workup in critical care setup. J Datta Meghe Inst Med Sci Univ. 2020;15(4):526-32.
http://dx.doi.org/10.4103/jdmimsu.jdmimsu_385_20
18. Solanki J, Shah A, Shah R, Gajjar K and Sumakiya T. Role of urine reagent strips in cerebrospinal fluid analysis an aid to bedside diagnosis of meningitis in paediatric age group. J Dent Med Sci. 2020;19(5):17-21.
http://dx.doi.org/10.9790/0853-1905171721
19. Burgoine K, Ikiror J, Naizuli K, Achom L, Akol S and Olupot-Olupot P. Reagent strips as an aid to diagnosis of neonatal meningitis in a resource-limited setting. J Trop Pediatr. 2019;65(1):9-13.
http://dx.doi.org/10.1093/tropej/fmy003
20. Sharma D and Uradiya I. Semi-quantitative analysis of cerebrospinal fluid chemistry and cellularity using urinary reagent strip in CNS infections. Indian J Res. 2019;8(7):98-101.

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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.

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