Chattogram Maa-O-Shishu Hospital Medical College Journal
Volume 21, Issue 2, July 2022

Original Article

Diagnostic Accuracy of Urinary Reagent Strip to Determine CSF Chemistry and Cellularity

Shemanta Waddadar[1*]
A.N.M. Manzurul Kader Chowdhury[2]
Rajsree Chowdhury[3]
Priyatosh Das[4]
Mohammad Shamchul Alam[2]
Md. Rubel[1]
Shiuly Majumder[1]
Md. Hassanuzzaman[1]

[1]Department of Neurology
Chittagong Medical College Hospital
Chattogram, Bangladesh.

[2]Department of Forensic Medicine
Chittagong Medical College
Chattogram, Bangladesh.

[3]Boalkhali Upazilla Health Complex
Chattogram, Bangladesh.

[4]Department of Microbiology
Chittagong Medical College
Chattogram, Bangladesh.

[5]Khagrachari Sadar Hospital
Khagrachari, Bangladesh.

*Correspondence to:
Dr. Shemanta Waddadar
Junior Consultant
Department of Neurology
Chittagong Medical College Hospital
Chattogram, Bangladesh
Mobile: +88 01816 80 12 59
Email- drshemanta@yahoo.com

Date of Submission : 02.06.2022
Date of Acceptance : 28.06.2022

www.banglajol.info/index.php/CMOSHMCJ

Abstract

Background: *Cerebrospinal Fluid (CSF) examination is a common investigation to diagnose and evaluate different neurological diseases. The gold standard method of CSF examination is microscopy, biochemistry and culture in a standard laboratory, which require an experienced microscopist and laboratory support. Urinary reagent strip can be a point of care test in such condition. The study aimed to document the diagnostic accuracy of urinary reagent strip for the semi-quantitative analysis of CSF chemistry and cellularity.*

Materials and methods: *One hundred hospitalized patients from the Department of Neurology, Chittagong Medical College Hospital were included in this study. CSF samples of the patients were subjected for the de nitive test (CSF microscopy and laboratory biochemical values) and the index test [Combur-10 urinary reagent strip was used as the index test for protein, glucose, leukocytes and erythrocytes. The diagnostic accuracy of each index test was calculated using different cut-off levels (Glucose 1+ vs. CSF glucose >50 mg/dL, protein 1+ vs. CSF protein >30 mg/dL, leukocyte esterase 1+ positivity vs. >10 granulocytes/mm^3 and erythrocyte 1+ positivity versus >5 RBC/mm^3 in CSF sample).*

Results: *The diagnostic accuracy of protein estimation by reagent strip shows 100% sensitivity, 84.21% specificity for detection of CSF protein at level 30mg/dl. Glucose reagent strip positivity had 98.8% sensitivity and 75% specificity for detection of CSF glucose at level 50 mg/dl. Leukocyte esterase positivity by test strip had sensitivity of 60% and specificity of 100% for the detection of CSF granulocyte of >10 granulocytes/mm^3. The diagnostic accuracy of erythrocyte estimation by reagent strip show 92% sensitivity, 92% specificity for detection of CSF RBC >5 cells/mm^3.*

Conclusion: *Urinary reagent strip can be used routinely for rapid analysis of CSF.A resource-limited hospital might find it useful as a point-of-care laboratory workup of CSF.*

Key words: Cerebrospinal fluid; Diagnostic accuracy; Urinary reagent strip.

INTRODUCTION

Cerebrospinal fluid (CSF) diagnostics emerged as a valid tool for a variety of neurological diseases. It is routinely used to detect neuronal diseases such as acute or chronic meningitis or encephalitis and is even able to distinguish between an acute viral and a bacterial intrathecal infection.[1,2] Besides infections normal CSF chemistry and cellularity including protein, sugar & leukocytes are altered in many other neurological conditions which are also not uncommon like Encephalities, subarachnoid haemorrhages, Guillain-Barré syndrome, Chronic inflammatory demyelinating polyradiculoneuropathy, Leptomeningeal Metastases, Acute disseminated encephalomyelitis, Transverse myelities, Multiple sclerosis etc.[3,4]

In these situations, examination of the CSF for leukocytes, glucose, protein and culture are the cornerstones and combination of gram staining and cultures of CSF is the gold standard. However, the gold standard tests are time consuming, costly and labor intensive. In low resource settings, the trained personnel, supplies and laboratory equipment are not always available to perform CSF analysis. Even when they are available, substantial delays in receiving results can occur, and in turn, this can delay the initiation of appropriate treatment.[5,6] Bangladesh, which is a densely populated resource limited developing country, there is a need for affordable, rapid and accurate method for CSF analysis to aid in the diagnosing neurological diseases. Urine reagent strips are widely available, able to yield result within seconds to minutes which could be a promising point of care test in such situations. Different studies have investigated the utility of urinary reagent strips for semi-quantitative analysis of CSF in neonates, older children and adults, found it to be a rapid and relatively reliable method for diagnosing bacterial meningitis and also to rule out blood in CSF.[7-10] However, this test was not validated in our setting till now and not used in our clinical practice. So, this study was conducted to investigate the diagnostic accuracy of urinary reagent strip to determine CSF chemistry and cellularity in neurological disease.

MATERIALS AND METHODS

This hospital based cross-sectional study was conducted in the Department of Neurology, Chittagong Medical College Hospital, Chattogram, Bangladesh from February 2018 to January 2019. The study was approved by Ethical Review Committee of Chittagong Medical College and informed consent was obtained from the patients or the guardian of patients as required.

One hundred hospitalized patients of all ages and both sex requiring diagnostic lumbar puncture were included. Patients with localized infection at puncture site, suspicion of focal mass lesion of brain, suspected haemorrhagic disorder, on anticoagulant or thrombolytic therapy, having pathological localized deformity at puncture site; haemorrhagic CSF samples were excluded from the study.

After performing gross examination of CSF for appearance and color, both definitive test and the index test were carried out as described below. Total cell count for leucocytes and erythrocytes were done manually from undiluted CSF sample. Other reference standard tests like CSF protein and glucose were performed in the biochemistry analyzer.

The combur-10 (Roche Diagnostics) urinary reagent strip (Figure 1) was used as index test to detect CSF leucocytes by leucocyte esterase estimation, glucose by glucose oxidase-peroxidase method, protein levels by pyrogallol red method and erythrocytes by peroxidase method. The technicians, who performed the index test, were blinded to the results of definitive tests. Undiluted CSF was mixed with the micropipette and 2–3 drops of CSF was then added to patches of leucocytes, proteins, sugar and erythrocytes and reaction was noted after 60–120 second. Then, reaction colors of the test area were compared with the color chart on the label as shown in Figure 2. Reference standard for all the parameters as shown on the label of strip are shown in Table I.

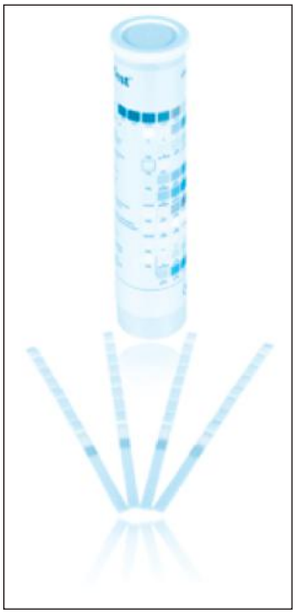

Figure 1 Combur-10 urinary reagent strip

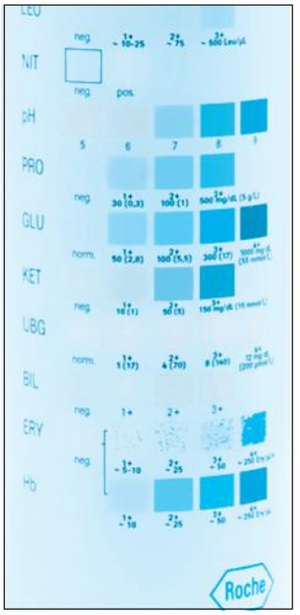

Figure 2 Showing color chart scale as shown on the label of combur-10 urinary reagent strip

Table I Reference standard for all the parameters as shown in strip label of combur-10 urinary reagent strip

Parameter	Normal value	Principle of strip test	No color	1+	2+	3+	4+
CSF Leucocyte (cells/mm^3)	<5	Leucocyte esterase estimation	<10	10-75	75-499	>500	-
CSF Protein (mg/dl)	15-45	Protein error of pH indicator	<29	30-99	100-499	>500	-
CSF Glucose (mg/dl)	40-70	Glucose oxidase-peroxidase method	<50	50-99	100-299	300-999	1000
CSF Erythrocyte (cells/ mm^3)	Absent	Peroxidase method	<5	5-10	25	50	250

Statistical analysis was performed to derive the specificity, sensitivity, positive predictive value, negative predictive value, positive likelihood ratio and negative likelihood ratio. We also constructed Receiver Operating Curves (ROC) to evaluate overall performance of index tests and estimated Area Under the Curve (AUC). Agreement between two testes was assessed by calculating kappa value. Data were analyzed and graphed using SPSS 23 software, Microsoft excel and Medcalc software.

RESULTS

The study included a fairly equal distribution of males (46%) and females (54%). Seventy two percent of cases were from 11-40 years of age group and 9% of cases were below 10 years (Table II).

Table II Distribution of the study patients according to age and sex

Age	Sex of the patients		
	Female	Male	Total
0-10 years	3 (5.6%)	6 (13.0%)	9(9.0%)
11-20 years	14 (25.9%)	10 (21.7%)	24 (24.0%)
21-30 years	17 (31.5%)	7 (15.2%)	24 (24.0%)
31-40 years	6 (11.1%)	6(13.0%)	12 (12.0%)
41-50 years	5 (9.3%)	3 (6.5%)	8 (8.0%)
51-60 years	2 (3.7%)	6 (13.0%)	8 (8.0%)
≥61 years	7(13.0%)	8 (17.4%)	15 (15.0%)
Total	54 (54.0%)	46 (46.0%)	100 (100%)

Data are expressed as frequency and percentage.

At the time of CSF collection most of the cases were provisionally diagnosed as peripheral neuropathy (32%), encephalitis (28%) and TBM (20%) (Table III).

Table III Provisional diagnosis of the patients

Provisional Diagnosis	Frequency	Percentage
Peripheral neuropathy (GBS, Beriberi)	32	32%
Encephalitis	28	28%
Tubercular meningitis	20	20%
CIDP	5	5%
Meningitis	5	5%
ADEM	5	5%
ATM	2	2%
SAH	2	2%
Multiple sclerosis	1	1%

We estimated the diagnostic accuracy of key indicator tests with rapid strip-based testing as index tests and microscopy or laboratory-based measurements, as reference standards. Table IV shows that, there is concordance in majority of the cases (90%) between strip test and lab results regarding CEF glucose. Concordance between index test and reference test in detecting CSF protein was 91%, 95% have leukocyte count <10 cells/mm^3 by the reference laboratory test. Index test result is 100% concordance with this result, and with the cut off value <5 RBC/mm^3 index test shows 6 (8%) false negative results.

Table IV Comparison of strip test and standard test of CSF for glucose

Screening test	Reference standard test			Kappa value
CSF glucose, mg/dl	**<50**	**50-100**	**100-300**	
No color (<50 mg/dl)	15 (75.0%)	1 (1.3%)	0 (0%)	0.742
1$^+$ (50-100 mg/dl)	5 (25.0%)	72 (96.0%)	0(0%)	
2$^+$ (100-300 mg/dl)	0 (0%)	2 (2.7%)	3 (60.0%)	
3$^+$ (300-1000 mg/dl)	0 (0%)	0 (0%)	2 (40.0%)	
CSF protein , mg/dl	**<30**	**30-100**	**100-500**	
No color (<30 mg/dl)	32 (84.2%)	0 (0%)	0 (0%)	0.656
1$^+$ (30-100 mg/dl)	6 (15.8%)	53 (98.1%)	0 (0%)	
2$^+$ (100-500 mg/dl)	0 (0%)	1 (1.9%)	6 (75.0%)	
3$^+$ (>500 mg/dl)	0 (0%)	0 (0%)	2 (25.0%)	
CSF leukocyte, cells/mm^3	**<10**	**10-75**	**75-500**	
No color (<10 cells/mm^3)	95 (100%)	2 (66.7%)	0 (0%)	
1$^+$ (10-75 cells/mm^3)	0 (0%)	1 (33.3%)	1 (50%)	
2$^+$ (75-500 cells/mm^3)	0 (0%)	0 (0%)	1 (50%)	
CSF RBC, cells/mm^3	**<5**	**50-250**	**>250**	
No color (<5)	69 (97.2%)	4 (57.1%)	2 (9.1%)	0.71
1$^+$ (5-25 cells/mm^3)	1 (1.4%)	0 (0%)	1 (4.5%)	
2$^+$ (25-50 cells/mm^3)	1 (1.4%)	0 (0%)	0 (0%)	
3$^+$ (50-250 cells/mm^3)	0 (0%)	1 (14.3%)	0 (0%)	
4$^+$ (>250 cells/mm^3)	0 (0%)	2 (28.6%)	19 (86.4%)	

The reagent strip showed high sensitivity and specificity (92%) for an overall RBC count ≥ 5 cells/mm^3. For glucose<50 mg/dl, the strip was highly sensitive (98.8%) and moderately specific (75%). The strip showed a high sensitivity (100%) for protein 30 mg/dl, although it was less specific (84.2%). The reagent strip showed low sensitivity (60%) and high specificity (100%) for an overall leukocyte count ≥10 cells/mm^3 (Table V). According to the ROC curve, CSF leucocytes, Glucose, and protein had similar high AUC value of 0.98. CSF erythrocytes had an AUC value of 0.95 as shown in Table V.

Table V Diagnostic accuracy of urinary reagent strip test when compared with reference standard values of parameters

	CSF leucocyte	CSF Protein	CSF Glucose	CSF Erythrocyte
True positive	3	62	79	23
False positive	0	6	5	6
False negative	2	0	1	2
True negative	95	32	15	69
Sensitivity	60.0%	100.0%	98.8%	92.0%
Specificity	100.0%	84.2%	75.0%	92.0%
PPV	100.0%	91.2%	94.1	79.3%
NPV	97.9%	100.0%	93.8	97.1%
PLR	-	6.3	3.9	11.5
NLR	0.4	0	0.02	0.1
Overall accuracy	94.0%	94.0%	98.0%	96.0%
AUC	0.98	0.98	0.98	0.95

PLR: Positive Likelihood Ratio, NLR: Negative Likelihood ratio, PPV: Positive Predictive Value, NPV: Negative Predictive Value, AUC: Area Under the Curve.

DISCUSSION

When doing a lumbar puncture, it is often useful to be able to examine the CSF at the time of the procedure. This is especially so in busy outpatient departments of hospitals and more so at night, or in rural setting. Rapid handling of the patient or obtaining some idea of the diagnosis, without waiting many hours for the result, can speed up treatment considerably. In this context, we have evaluated the urinary reagent strip to determine the CSF cellularity and biochemistry at bedside.

After categorizing the laboratory value as per the cut-off values for urinary test strip the concordance (Agreement) between test strip result and laboratory results were in 90%, 91%, 97%, 98% cases respectively for glucose, protein, leukocyte and RBC. The results of our study suggest that Combur 10 strips can determine CSF protein levels more than 30 mg/dL, neutrophil count more than 10/mm^3, RBC count more than 5/mm^3 and glucose levels less than 50 mg/dL with reasonable accuracy. Results of our study and those of a few other published studies suggest that there is good agreement between the strip method and laboratory methods of determining CSF protein, glucose, leukocyte and RBC. Romanelli et al Moosa et al, Salvador et al, and Joshi et al also used previous version of Combur-10 strips concluding that this method is also useful in making a rapid bedside diagnosis of CSF analysis.[11-14] Gupta et al drew similar conclusion with the version used in the present study.[10]

High sensitivity is essential to rule in the disease for treatment or for other diagnostic evaluation.[15] Sensitivity of the test strip in this study were 98.8%, 100%, 60% and 92% for detecting glucose <50mg/dl, protein ≥30 gm/dl, leukocyte ≥10 cells/mm^3 and red blood cell ≥5 cells/mm^3 respectively in comparison to laboratory findings. It indicates that, except leukocyte for other three parameters there are a very few cases of false negative results by the urinary test strip. However, for leukocyte out of 100 specimens in only 5 cases have leukocyte count ≥10 cells/mm^3 and test strip failed to detect 2 cases of these.

On the other hand, specificity defines proportion of the CSF specimen which has negative results on test strip that have negative result on laboratory test.[15] High specificity is essential to rule out the disease. Specificity of the test strip in this study were 75%, 84.21%, 100% and 92% for detecting glucose <50mg/dl, protein ≥ 30 gm/dl, leukocyte ≥10 cells/mm^3 and red blood cell ≥5 cells/mm^3 respectively in comparison to laboratory findings.

Negative predictive value defines the proportion of people with negative test that do not have the disease or probability that the disease is not present when the test is negative.[15] In our context, NPV defines the proportion of the specimens with negative test that were negative in laboratory test. Urinary test strip has high NPV (93.8%, 100%, 97.9% and 97.1% for detecting glucose <50mg/dl, protein ≥30 gm/dl, leukocyte ≥10 cells/mm^3 and red blood cell ≥5 cells/mm^3 respectively in comparison to laboratory findings) in the present study.

Positive predictive value defines probability that the laboratory result is positive when the urinary test strip test is positive. In the present study the test strip has high PPV (94.1%, 91.18%, 100% and 79.3% for detecting glucose <50mg/dl, protein ≥30 gm/dl, leukocyte ≥10 cells/mm^3 and red blood cell ≥5 cells/mm^3 respectively in comparison to laboratory findings).
This procedure is easy to perform, does not require any technical expertise and is a help to the primary health-care centers and rural setups where no laboratory facilities exist. The operators can be trained very easily and competency of the operators can be monitored prior to routine use. The only shortcoming is the slight variation in the cut-off normal values of protein and sugar in CSF as compared to urine which has added to the few false negatives and false positives in our study. This can be overcome by designing strips specific for CSF analysis, at the manufacturer's level.

LIMITATIONS

Limitations of our study were smaller sample size and only few cases of neurological disorders, other than meningitis were noted, so studies with larger sample size are required for elaborative analysis in future. Secondly, despite matching reagent strip very carefully, color grading was subjective, so there could be an inter observer variation in reading the results.

CONCLUSION

The result of our study confirms that Combur 10 urinary reagent strip has a good sensitivity and specificity for quantitative analysis of CSF protein, glucose, leukocytes and erythrocytes. Hence these strips has a great value to clinicians working in resource constraint settings to reliably take a rapid decision for further management of various neurological diseases like meningitis, encephalitis and also to rule out SAH and ultimately help to initiate appropriate treatment and to make decision about referral.

RECOMMENDATIONS

Although standard laboratory evaluation practices clearly remain the gold standard, urine reagent strips could be considered as a rule-out test to prevent irrational use of multiple chemotherapeutics and to make decision about proper referral. Future studies should evaluate newer versions of reagent strips with cut-off value specific for CSF by addressing cost-effective analysis in specific disease condition.

DISCLOSURE

All the authors declared no competing interest.

REFERENCES

1. Aminoff MJ, Boller F, Bruyn GW, Klawans HL, Swaab DF, Vinken PJ, editors. Handbook of clinical neurology. North-Holland Publishing Company. 1968.
2. Studahl M, Lindquist L, Eriksson BM, Günther G, Bengner M, Franzen-Röhl E, et al. Acute viral infections of the central nervous system in immunocompetent adults: Diagnosis and management. Drugs. 2013;73(2):131-158.
3. Levine GJ, Cook JR. Cerebrospinal fluid and central nervous system cytology. Cowell and Tyler's Diagnostic Cytology and Hematology of the Dog and Cat. 2020:210.
4. Bianchine JR, McConnell H. Cerebrospinal fluid in neurology and psychiatry. Springer. 2013.
5. Taniguchi T, Tsuha S, Shiiki S, Narita M. Point-of-care cerebrospinal fluid Gram stain for the management of acute meningitis in adults: A retrospective observational study. Annals of Clinical Microbiology and Antimicrobials. 2020;19(1):1-1.
6. Burgoine K, Ikiror J, Naizuli K, Achom L, Akol S, Olupot-Olupot P. Reagent strips as an aid to diagnosis of neonatal meningitis in a resource-limited setting. Journal of tropical pediatrics. 2019;65(1):9-13.
7. Mazumder S, Ramya BS, Biligi DS. Utility of urine reagent strips in cerebrospinal fluid analysis: An aid to bedside diagnosis of meningitis. Indian Journal of Pathology and Microbiology. 2018;61(3):356.
8. Bortcosh W, Siedner M, Carroll RW. Utility of the urine reagent strip leucocyte esterase assay for the diagnosis of meningitis in resource limited settings: meta analysis. Tropical Medicine & International Health. 2017;22(9):1072-1080.
9. Wankhade R, Bhake A. Assessment of efficacy of urine reagent strips for cerebrospinal fluid analysis as emergency workup in critical care setup. Journal of Datta Meghe Institute of Medical Sciences University. 2020;15(4):526.
10. Gupta A, Dwivedi T. Reagent strips test: A simplified method for prompt analysis of cerebrospinal fluid in neurological disorders in emergency. Practical laboratory medicine. 2019;16:e00124.
11. Romanelli R, Thome EE, Duarte F, Gomes RS, Camargos PA, Freire H. Diagnosis of meningitis with reagent strips. Jornal de Pediatria. 2001;77(3):203-208.
12. Moosa A, Ibrahim MD, Quortum HA. Rapid diagnosis of bacterial meningitis with reagent strips. The Lancet. 1995;345(8960):1290-1291.
13. Salvador JM, Zarallo CG. Usefulness of reactive strips in the diagnosis of suppurative meningitis, at the patient's bedside. Anales Espanoles de Pediatria. 1988;29(2):105-108.
14. Joshi D, Kundana K, Puranik A, Joshi R. Diagnostic accuracy of urinary reagent strip to determine cerebrospinal fluid chemistry and cellularity. Journal of neurosciences in rural practice. 2013;4(02):140-145.
15. Akobeng AK. Understanding diagnostic tests 1: Sensitivity, specificity and predictive values. Acta paediatrica. 2007;96(3):338-341.