ORIGINAL RESEARCH PAPER

Medicine

SEMI QUANTITATIVE ANALYSIS OF CEREBROSPINAL FLUID CHEMISTRY AND CELLULARITY USING URINARY REAGENT STRIP IN CNS INFECTIONS

KEY WORDS: Urinary reagent strip, Cerebrospinal fluid, CNS infection

Dr. Deepti Sharma Senior Professor, M.D. Medicine, Department, of Medicine Government Medical College Kota

Dr. Indrajeet Uradiya* Senior Resident, M.D. Medicine, Department of Medicine, Government Medical College Kota *Corresponding Author

ABSTRACT

Introduction: - Rapid diagnosis and prompt treatment remains the cornerstone of management of patients with various CNS infections.

Aim: - The aim of this study is to evaluate role and reliability of urinary reagent strip for the semi quantitative analysis of CSF chemistry and cellularity.

Methods: This prospective, single blinded diagnostical clinical trial was conducted over hundred patients, aged ≥18 years admitted in our hospital with suspected diagnosis of CNS infection. CSF samples were taken for laboratory evaluation and rapid strip analysis.

Results: Highly significant association was observed between CSF examination in routine laboratory method and dipstick method. The strip showed a high sensitivity (94%) and specificity (98.5%) for leukocytes ≥10 cells/mm3. The strip showed a sensitivity of 93% and a specificity of 98.5% for proteins at a cut-off ≥100 mg/dl. With respect to glucose, the strip had 100% specificity and 98.5% sensitive at cut-off ≥50 mg/dl. The Accuracy for leukocytes ≥10 cells/mm3 was 97.00%; for proteins ≥100 mg/dl, it was 97%; and, for glucose ≥50 mg/dl, it was 99.00%.

Conclusions: Urinary reagent strips can reliably predict raised CSF protein (>100 mg/dl), decreased glucose (<50 mg/dl) and increased Neutrophils count (>10/mm3). Urinary reagent strips test is easy to do and would be of particular value in resource limited remote areas where no laboratory facilities exist.

INTRODUCTION

Acute infections of the nervous system are among the most important problems in medicine because early recognition, efficient decision making, and rapid institution of therapy can be lifesaving. These distinct clinical syndromes include acute bacterial meningitis, viral meningitis and encephalitis, focal infection such as brain abscess and subdural empyema, and infectious thrombophlebitis.[1]

Meningitis is a medical emergency as the infection is in the vicinity of the brain and spinal cord.[2]

Rural hospitals and primary health care centres in India frequently lack suited laboratory and pathologist to perform the CSF analysis. This leaves no other choice but to either start empirical treatment or to transfer the patient to a hospital with more resources which might cause further delay.

Even at tertiary centers where sophisticated laboratories are present, time taken from sending of samples to receiving of reports causes further delay in the diagnosis.

Currently, no rapid point-of-care tests are available to detect meningitis.

In India there is also high prevalence of cerebral malaria which might be indistinguishable from bacterial meningitis. The lack of diagnostic facilities further challenges already limited resources in the hospitals by empirical use of antibiotics which are expensive and overburdening the health centres and hospitals.

We designed this study to determine if the use of urinary reagent strip (which are widely available) to make a semi-quantitative assessment of protein, glucose, and presence of leukocyte esterase (as compared to CSF glucose, protein values, and cell count) is accurate to distinguish between a normal and an abnormal CSF sample.

In this study, we reassessed the diagnostic usefulness of Combur-10 urinary reagent strip for the early diagnosis of CNS infection.

MATERIALS & METHODS

A single blinded diagnostic clinical trial (pathologists were not aware of the results of the reagent strip) was conducted in Medicine and Neurology wards of GOVT. MEDICL COLLEGE, KOTA and associated group of Hospital for a period of 1 year.

All patients with age more than 18 years of age who had indication for lumber puncture {Fever, Headache, Intractable vomiting, Altered sensorium, Meningeal signs (neck stiffness, kernig, brudzinski sign), Neurological deficit, Irritability, Behavioural changes and Seizures} were included in the study.

Hemorrhagic CSF samples and Patients on immunosuppressive drugs (anti-neoplastic treatment or steroids) were excluded from the study.

Patient recruitment

All the patients admitted in medicine and neurology ward in whom CSF examination was indicated had been included in the study. Patient's attendant was informed about lumbar puncture procedure and its risks; informed consent was taken.

Once all the formalities had been completed lumbar puncture was performed. This procedure was done at the level of L4–L5, or L5-S1 intervertebral space using standard 22G needle with stylet under all sterile measures. When subarachnoid space was reached, the stylet was removed and 2.5 - 3.5 ml of CSF was collected in four different sterile tubes.

The first tube contained 0.5 ml to 1 ml of CSF to be used for urine reagent strip testing.

The second sterile tube with 1 ml of CSF was used for culture, gram stain, Indian ink for Cryptococcus evaluation and Ziehl neelsen stain if asked.

The third and fourth sterile tubes containing 1 ml were used for testing in haematology and biochemistry department respectively.

Definitive test
For leucocytes, the total count was carried out by Neubauer's counting chamber by a technician who was unaware of the strip reading. The differential count was carried out by cytocentrifuged smears stained with Leishman stain. Definitive values for proteins and sugar were obtained from an automated analyzer (ERBA Mannheim EM-360, Mannheim Germany).

Index test
The combur-10 urinary reagent strip, which can detect 10 parameters such as specific gravity, pH, leucocytes, nitrate, proteins, glucose, ketone body, urobilinogen, bilirubin, and hemoglobin, was used to detect the index tests. With the help of a micropipette, 2–3 drops of undiluted CSF was added to combur-10 urinary reagent strip and the colour changes were recorded.

Combur-10 urinary reagent strip
(Roche Diagnostics, Basel, Switzerland)

A. Cerebrospinal fluid leukocytes:
A normal CSF WBC count is <5cell/mm^3 and the reagent strip needs at least 10 cells for recognition and have an upper limit of 500 granulocytes/mm^3. Undiluted CSF was used and the test result was interpreted using the manufacturer-provided colour grading
<10 granulocytes/mm^3 ------- No shading
10-25 granulocytes/mm^3 -----1+ (Very light Purple)
~25-75 granulocytes/mm^3 ------ 2+ (Light Purple)
~75-500 granulocytes/mm^3 -----3+ (Purple)

B. CSF protein:
Normal CSF proteins range between 15 and 45 mg/dl and the range for protein detection of the strip is between 30 mg/dl to 500 mg/dl. The understanding of most extreme shading on reagent strip was:

< 30 mg/dl	:no adjustment in shading
30 - 100 mg/dl	: 1+ (light Green)
100 - 500 mg/dl	:2+ (Green)
>500 mg/dl	:3+ (olive Green)

C. CSF glucose:
A typical CSF glucose level is 66% of the plasma glucose. The levels of CSF glucose fall in bacterial and contagious meningitis. The interpretation of results on a reagent strip was no change in color if less than 50mg/dl (yellow) and any change in color if more than 50mg/dl.

<50 mg/dl (yellow):	no change in color
~50- 100 mg/dl (green)	:1+ (light Green)
~100- 300 mg/dl	:2+ (Green)
~300-1000 mg/dl	:3+ (dark Green)
~1000 mg/dl	:4+ (Tree Green)

Statistical analysis
Data were collected and statistically analyzed by GraphPad Software, (prism version 6.0). Comparison of qualitative data was performed with chi square (x^2) test. The validity of screening tests were measured and expressed as sensitivity, specificity, accuracy, positive predictive value, and negative predictive value (in comparison to standard laboratory tests).

RESULTS
Our study included 100 cases dispersed in a wide range of age from 18 years to 85 years, with the maximum number of cases (26 %) in the age group of 51-60years. The mean age of the study population was 46.03 +/- 15.28 years. Males outnumbered the females with the male: female ratio being 1.86:1.

Etiologically study population divided into: - 32% cases with Bacterial meningitis, 12% with viral meningitis, 20% with cerebral malaria, 16% with viral encephalitis, 12% with tubercular meningitis and 8% patients with Meningoencephalitis. (Figure 1)

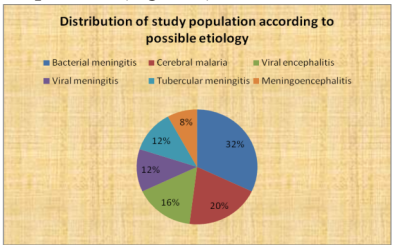

Figure 1 Distribution of study population according to possible etiology

We have analyzed three cardinal parameters of CSF to diagnose various CNS infection namely leucocytes, proteins, and glucose. The findings of the reagent strip were compared with the reference standard.

Leucocytes
Leucocytes were detected by the esterase activity. The sensitivity and specificity for leucocytes by the strip method for >10 cells/cumm were 94% and 98.5% respectively, with a very high accuracy of 97% and p value less than 0.0001 (<0.0001) which was statistically significant. (Table no. 1)

Table no.1

CSF Neutrophils	TP*	FP†	FN‡	TN§	Sensitivity	Specificity	ACCURACY	Chi-square	P-Value
≥10 cells/mm^3	32	2	1	65	94%	98.5%	97%	87.03	<0.0001
* True positive. † False positive. ‡ False negative. § True negative.									

Proteins
The urinary reagent strip had a high sensitivity of 100% and a low specificity of 50% for the **CSF protein** levels ≥30 mg/dl. If the cut-off level of the **CSF protein** was increased to ≥100 mg/dl, specificity was increased to 98.5% and the sensitivity was 93%, with a very high accuracy of 97% and p value less than 0.0001 (<0.0001) which was statistically significant. (Table no. 2)

Table no.2

CSF protein	TP*	FP†	FN‡	TN§	Sensitivity	Specificity	ACCURACY	Chi-square	P-Value
≥30 mg/dl	98	0	1	1	100%	50%	99%	49.49	<0.0006
≥100 mg/dl	31	2	1	66	93%	98.5%	97%	82.64	<0.0001
* True positive. † False positive. ‡ False negative. § True negative.									

Glucose
At the cut-off value of 50mg/dl of CSF glucose, the urinary test strip showed high sensitivity (98.5%) and specificity (100%) with a very high accuracy of 99% and p value less than 0.0001 (<0.0001) which was statistically significant. [Table no.3]

Table no.3

CSF protein	TP*	FP†	FN‡	TN§	Sensitivity	Specificity	ACCURACY	Chi-square	P-Value
≥50 mg/dl	65	1	0	34	98.5%	100.0%	99%	95.67	<0.0001
* True positive. † False positive. ‡ False negative. § True negative.									

DISCUSSION

CNS infection is a life threatening disease. Prompt diagnosis and treatment are important predictors of patient outcome. Many studies have been done in attempt to diagnose and differentiate between various CNS infections using simple methodology based on clinical signs and symptoms to very complex and expensive tests such as latex agglutination tests, real time PCR and estimation of cytokine levels in CSF.

In resource limited areas most of these tests are not available and diagnosis can be very difficult. Often the doctors find themselves in dilemma when dealing with unconscious patients with no laboratory resources and with high burden of malaria in our country.

In the present study, population classified according to aetiology; - 32% cases were found with Bacterial meningitis, 12% with viral meningitis, 20% with cerebral malaria, 16% with viral encephalitis, 12% with TBM and 8% patients with Meningoencephalitis.

Concerning symptoms viz. fever (93.75%), headache (92%) and altered mental status (81.25%) were the prominent features in all the patients with bacterial meningitis which were in agreement with **Ibrahim et al.** [3] who found fever in 95% patients. **Abdul rab et al**. found headache and vomiting in 56.1% and 76% patients, respectively.[4]

Our study showed the signs of meningeal irritation included neck rigidity, positive Kerning's and Brudzinski's sign present in 81.25%, 71% and 68% of cases of bacterial meningitis respectively which were in agreement with **Ndreu et al.**[5] who found these sign in73.1%, 55.2% and 56.7% of the cases of bacterial meningitis respectively. **Van de Beek et al.,** observed headache, neck stiffness, fever, and altered mental status in 87%, 83% 77% and 69% cases. None of these was specific as 95% patients had two symptoms and 1% had none.[6]

Fever and headache being the most frequent symptoms of viral meningitis were present in 83.3% and 58% patients. Signs like neck rigidity, positive Kerning's and Brudzenisky sign were present in 91.66%, 83.33% and 58.33% of cases respectively. **Choi et al.** found fever, headache, vomiting, and decreased consciousness in 92.9%, 57%, 54.8% and 16.7% respectively. Other symptoms included seizure (9.5%), motor weakness (7.1%), and dysuria (2.4%).[7]

High grade fever and altered sensorium were the most common symptoms of cerebral malaria 60%, 50% and 95% patients had vomiting, seizure and Splenomegaly respectively. **Gopinath V.P**. (1981) observed altered sensorium, neck stiffness, convulsion and papilloedema in 8.8%, 6.1%, 3.9%, and 1.1% patients respectively.[8] **Dhamija et al** (1994) reported headache, vomiting, focal neurological sign, papilloedema, abnormal movements, neck rigidity and neuropathy in 100%, 50%, 26%, 17%, 17%, 20% and 1% respectively.[9]

Dhamija et al (1994) reported headache in 100%, vomiting in 50%, focal neurological sign in 26%, papilloedema in 17%, abnormal movements in 17%, neck rigidity in 20% and neuropathy in 1% of the patients of cerebral malaria.[9]

At the cut-off value of 10 cells/mm^3 of Neutrophils, the urinary test strip showed the sensitivity of 94% and specificity of 98.5%. Also, urinary strip had a very high accuracy of 97% and results were statistically significant with p value less than 0.0001 (<0.0001).

Joshi et al.[10] used the same reagent strip, they observed the sensitivity of 85.2% and specificity of 89.6% for leucocytes >10 cells/mm3. Our results are comparable to this.

Abdelmotaleb GS, Abdo MK, Behiry EG, et al. in their study observed sensitivity of 100% and specificity of 50% for leucocytes >10 cells/mm3. Results were statistically significant with p value less than 0.001.[11]

Chikkannaiah P, Benachinmardi KK, Srinivasamurthy V., et al. in 2016 in their study showed that urinary strip had high sensitivity (96.6%) and specificity (94.5%) for leucocytes ≥10 cells/mm3.[12]

At the cut-off value of 30mg/dl of CSF protein, the urinary test strip showed the sensitivity of 100% and specificity of 50%. Results were statistically significant with p value less than 0.0006 (<0.0006).

Joshi et al. observed that detection of CSF proteins greater than 30 mg/dl (1 + or more on reagent strip) showed high sensitivity (98.1%) and low specificity (57.1%) with AUC of 0.97.[10] **Abdelmotaleb GS, Abdo MK, Behiry EG, et al.** in their study also observed high sensitivity of 100% and low specificity of 50% for CSF proteins greater than or equal to 47 mg/dl. Results were statistically significant with p value less than 0.001.[11] **Chikkannaiah P, Benachinmardi KK, Srinivasamurthy V., et al.** in 2016 in their study showed that urinary strip had high sensitivity of 94.9% and low specificity of 45.8% for CSF proteins greater than or equal to 30 mg/dl.[12]

At the cut-off value of 100mg/dl of CSF protein, the urinary test strip showed the sensitivity of 93% and specificity of 98.5%. Also, urinary strip had a very high accuracy of 97% and results were statistically significant with p value less than 0.0001 (<0.0001).

Heckmann JG, Engelhardt A, Druschky A. et al. obsereved that Twenty-one samples out of twenty seven samples with elevated protein were correctly diagnosed as right positive (sensitivity 77.8%; specificity 100%) in patients with bacterial meningitis.[13]

Joshi et al. used the same reagent strip and observed sensitivity of 92.6% and specificity of 87.5% for proteins at a cut-off of ≥100 mg/dl.[10]

Abdelmotaleb GS, Abdo MK, Behiry EG, et al. in their study also observed sensitivity of 100% and low specificity of 3.7% for CSF proteins greater than or equal to 100 mg/dl.[11]

Chikkannaiah P, Benachinmardi KK, Srinivasamurthy V., et al. in 2016 showed that urinary strip had sensitivity of 96.0% and specificity of 87.1% for higher cut-off level of CSF proteins greater than or equal to 100 mg/dl.[12]

At the cut-off value of 50mg/dl of CSF glucose, the urinary test strip showed the sensitivity of 98.5% and specificity of 100%. Also, urinary strip had a very high accuracy of 99% and results were statistically significant with p value less than 0.0001 (p <0.0001).

Joshi et al. in 2016, was used the same reagent strip and observed sensitivity of 46.2% and specificity of 98% for CSF glucose at a cut-off value greater than or equal to 50 mg/dl.[10] **Abdelmotaleb GS, Abdo MK, Behiry EG, et al.** in their study also observed sensitivity of 98% and specificity of 100% for CSF glucose greater than or equal to 50 mg/dl.[11] **Chikkannaiah P, Benachinmardi KK, Srinivasamurthy V., et al.** in 2016 showed that urinary strip had sensitivity of 14.2% and specificity of 100% for cut-off level of CSF glucose greater than or equal to 50 mg/dl.[12]

Kumar et al.,[14] **Parmar et al.,**[15] and **Romanelli et al.,**[16] also tested the Combur-10 urinary reagent strip for a rapid CSF analysis.

Kumar et al.,[14] in their study, observed a positive correlation

between the strip and laboratory values for the diagnosis of meningitis with k =0.94, 0.819, 0.819 for cells, protein and glucose respectively, with P< 0.0001, which was statistically significant. **Parmar et al.,** [15] in their study on 63 CSF samples observed a high sensitivity and specificity for the diagnosis of bacterial and tubercular meningitis, both being 100% and 96.55%, respectively. For the diagnosis of aseptic meningitis, the strip was more specific than sensitive.

Romanelli et al., [16] observed the sensitivity of 90.7%, specificity of 98.1%, PPV of 95.1%, NPV value of 96.4%, and accuracy of 96.1% for the results of reagent strip analysis in the diagnosis of bacterial meningitis. The P value observed by them was <0.0001 and was 0.9. These values were statistically significant.[17,18]

An issue in utilizing these strips is the distinctive shorts for protein and glucose values for CSF analysis. These strips can be designed to indicate clinically meaningful cut-offs for CSF analysis (i.e. a low CSF glucose is clinically more meaningful).[11] The strips may be designed to include only three parameters for proteins, glucose, and granulocytes, as opposed to the 10 for urinary investigation, in this manner the expense of these tests would be chopped down and the making of CSF strip analysis would be more cost effective.[(11)]

The study of **Koulaouzidis**[19] notified that caution was needed in the use of reagent strips for the diagnosis of meningitis, because this test is subjective, especially in cases with slightly altered CSF; therefore, the method is considered qualitative or semi-quantitative.

Our study results show that the quick diagnosis of meningitis can be made with the Combur-10 reagent strips with a high specificity and sensitivity. It is easy to do and would be of particular value in resource limited remote areas where no laboratory facilities exist. Also, Urine reagent strips may help as a complementary method for guiding the physicians in deciding management till the standard results are available.

CONCLUSION

Urinary reagent strips can reliably predict raised CSF protein (>100 mg/dl), decreased glucose (<50 mg/dl) and increased Neutrophils count (>10/mm). Hence, reagent strips can be of value to clinicians working in resource-constrained settings to reliably make a rapid diagnosis of meningitis and even at tertiary centers it shorten the time lag between sending of samples and receiving of reports.

REFERENCES

1. 19th edition Harrison's principles of Internal medicine by Karen L. Roos, Kenneth L. Tyler Meningitis, Encephalitis, Brain Abscess, chapter 164, page 883-887.
2. Kumar A, Debata PK, Ranjan A, Gaind R. The role and reliability of rapid bedside diagnostic test in early diagnosis and treatment of bacterial meningitis. Indian J Pediatr 2015; 82:311-4.
3. Ibrahim KA, Abdel-Wahab AA, Ibrahim AS (2011) Diagnostic value of serum procalcitonin levels in children with meningitis: A comparison with blood leukocyte count and Creactive protein. J Pak Med Assoc (JPMA) 61:346-351.
4. Abdulrab A, Algobaty F, Salem AK, Mohammed YA (2010) Acute bacterial meningitis in adults: A hospital based study in Yemen. Jpn. J Infect Dis 63: 128-131.
5. Ndreu AH, Shytaj KM, Harxhi AK (2009) Dexamethasone efficacy on bacterial meningitis – A retrospective analysis of Albanian adult patients. J Infect Dev Ctries 3:849-855.
6. Van de Beek, D., de Gans, J., Spanjaard, L., et al., 2004. Clinical features and prognostic factors in adults with bacterial meningitis. N. Engl. J. Med. 351, 1849–1859.
7. Choi CS, Choi YJ, Choi UY, Han JW, Jeong DC, et al. (2011) Clinical manifestations of CNS infections caused by Enterovirus type 71.Korean J Pediatr 54:11-16.
8. Gopinath VP, Ratle PK, Bhopte AG. Falciparum malaria in north eastern sector JAPI Ind, Vol. 29 Dec. 1981.
9. Dhamija RM. Cerebral malaria: Indian scence, Medicine Update, Vol. 4 (APICON-94) ED. Walial PK P:11-19
10. Joshi D, Kundana K, Puranik A, Joshi R. Diagnostic accuracy of urinary reagent strip to determine cerebrospinal fluid chemistry and cellularity. J Neurosci Rural Pract 2013; 4:140-5.
11. Abdelmotaleb GS, Abdo MK, Behiry EG, et al. Evaluation of Using Urine Reagent Strips in the Diagnosis of Childhood Meningitis. Ann Clin Lab Res. 2016, 4:3.
12. Chikkannaiah P, Benachinmardi KK, 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;64:50-5
13. Heckmann JG, Engelhardt A, Druschky A, Muck-Weymann M, Neundorfer B. Urine test strips for cerebrospinal fluid diagnosis of bacterial meningitis. Med Klin (Munich) 1996;91:766–8.
14. Kumar A, Debata PK, Ranjan A, Gaind R. The role and reliability of rapid bedside diagnostic test in early diagnosis and treatment of bacterial meningitis. Indian J Pediatr 2015; 82:311-4.
15. Parmar RC, Warke S, Sira P, Kamat JR. Rapid diagnosis of meningitis using reagent strips. Indian J Med Sci 2004; 58:62-6.
16. Romanelli RM, Thome EE, Duarte FM, Gomes RS, Camargos PA, Freire HB. Diagnosis of meningitis with reagent strips. J Pediatr (Rio J) 2001; 77:203-8.
17. Moosa AA, Quortum HA, Ibrahim MD. Rapid diagnosis of bacterial meningitis with reagent strips. Lancet 1995; 345:1290-1.
18. Oses Salvador JM, Zarallo Cortés L, Cardesa García JJ. Usefulness of reactive strips in the diagnosis of suppurative meningitis, at the patient's bedside. An Esp Pediatr 1988; 29:105-8.
19. Koulaouzidis A (2011) Diagnosis of spontaneous bacterial peritonitis: an update on leucocyte esterase reagent strips. World J. Gastroenterol. 17: 1091-1094.