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Assessment of Utility of Urine Reagent Strips for Analysis of Cerebrospinal Fluid (CSF) In Emergency Settings for Rapid Clinical Interventions

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Abstract

Introduction

Meningitis is an emergency condition, requiring timely diagnosis and prompt clinical intervention. Alacritous availability of reasonably, sound laboratorial setup required for CSF analysis is many times not possible in most small healthcare setups. Urine reagent strips are cheap, easily available and provide rapid analysis of urine samples. These urine reagent strips if found unerring can be utilised for rapid bedside analysis of CSF for provisional diagnosis and initiation of treatment. We have undertaken this study, for assessing the of utility of urine reagent strips in analysis of CSF leucocytes, proteins and glucose and enhance the available evidence for its imbibition in routine clinical practices

Material and methods-

This observational study was done in AVBRH, Sawangi Meghe, SMHRC and Clinical Laboratory division of Department of Pathology DMMC, Wanadongri, Hingna, Nagpur, over a duration of 1 year. A total 100 CSF samples were analysed for Urine protein, glucose and leucocyte counts, using Urine reagent strips bedside and routine biochemical and microscopic investigations were carried out within one hour. Mission expert urinalysis strips were used for index test and Routine biochemistry and microscopy was used as reference test.

RESULTS-

Among the 100 CSF samples of suspected meningitis analysed, male: female ratio was 1.2:1. The sensitivity, specificity and accuracy for leucocyte estimation were 95.95% (71 of 74), 96.15% (25 of 26) and 96.00%. respectively. CSF proteins had sensitivity of 97.29(72 of 74) at +1(<30mg/dl) of urine reagent strips but specificity at +1 was low i.e., 69.23% (18 of 26), which rose to 96.1% (25 of 26) when cut off was kept as +2 (i.e., >100 mg/ dl) in urine reagent strips. The accuracy attained was 90.00%. at cut-off +1 and 97.00% at cut-off +2. The sensitivity for glucose at detecting low glucose levels in CSF on urine reagent strips score 0 i.e., <50 mg/dl is 91.18% (68/74) and specificity is 65.3% (17/26). The accuracy attained was 85%.

Conclusion-

Urine reagent strips can be utilised for testing of CSF for proteins, glucose and granulocytes as a point of care, bedside test. Using a higher cut-off for Proteins improves the specificity and accuracy. Also due to difference between normal range of glucose in urine and CSF the accuracy and specificity for analysis of glucose in CSF is low. Reagent strips specifically designed for CSF and standardised as per normal and diagnostic ranges of granulocytes, glucose and proteins in CSF should be made, which will have better sensitivity, specificity and accuracy and will also be cost effective.

Introduction

Meningitis is the inflammation of meninges and leads to morbidity and mortality in a significant population globally accounting for about 180,000 annual deaths[1]. It results from the infection of the meninges and can be caused by a diverse group of pathogenic organisms like bacteria, fungus or viruses. Globally highest burden faced by the healthcare system is of bacterial meningitis. Haemophilus influenzae, Streptococcus pneumoniae and Neisseria meningitidis are most frequently encountered bacteria in meningitis[2]. Meningitis is an emergency condition, requiring timely diagnosis and prompt clinical intervention. Initiation of treatment in a case of suspected meningitis greatly depends on early recognition of clinical signs and symptoms and rapid analysis of cerebrospinal fluid (CSF) leukocytes, proteins, and glucose[3]. CSF analysis is useful in diagnosis of meningitis as well as determining the type of meningitis into bacterial, viral and tubercular meningitis. The CSF examination is done in the laboratory and requires the availability of technical staff, pathologist and infrastructure. Elevated leucocytes(granulocytes), elevated proteins and lower glucose levels are the diagnostic indicators of meningitis. Alacritous availability of reasonably, sound laboratorial setups is many times not possible in most primary or small healthcare setups. This may lead to delay in CSF analysis and timely clinical action.

Urine reagent strips are cheap, easily available and provide rapid analysis of urine samples. These urine reagent strips if found unerring can be utilised for rapid bedside analysis of CSF for provisional diagnosis and initiation of treatment. The urine reagent strips in correlation with physical examination of CSF can also be used to differentiate between bacterial, viral and tubercular meningitis. Few studies have been done in this regard to evaluate practicality of urine reagent strips for bedside evaluation of CSF proteins, glucose and leucocytes(granulocytes)[4], but quantum of studies and evidence is not sufficient to warrant its employment in regular practice. We have undertaken this study, for assessing the of utility of urine reagent strips in analysis of CSF leucocytes, proteins and glucose and enhance the available evidence for its imbibition in routine clinical practice.

Material and methods-

This observational research was conducted out in AVBRH, Sawangi Meghe, SMHRC and Clinical Laboratory division of Department of Pathology DMMC, Wanadongri, Hingna, Nagpur, over a duration of 1 year. A total 100 CSF samples were analysed for protein, glucose and leucocyte counts, using Urine reagent strips bedside and routine biochemical and microscopic investigations were carried out within one hour in the central clinical laboratory. Bedside examination was carried out using Mission expert urinalysis strips, Routine biochemistry findings analysed on Beckman-Olympus AU480 and Microscopy was carried out by Pathologist using Olympus CX21i microscope.

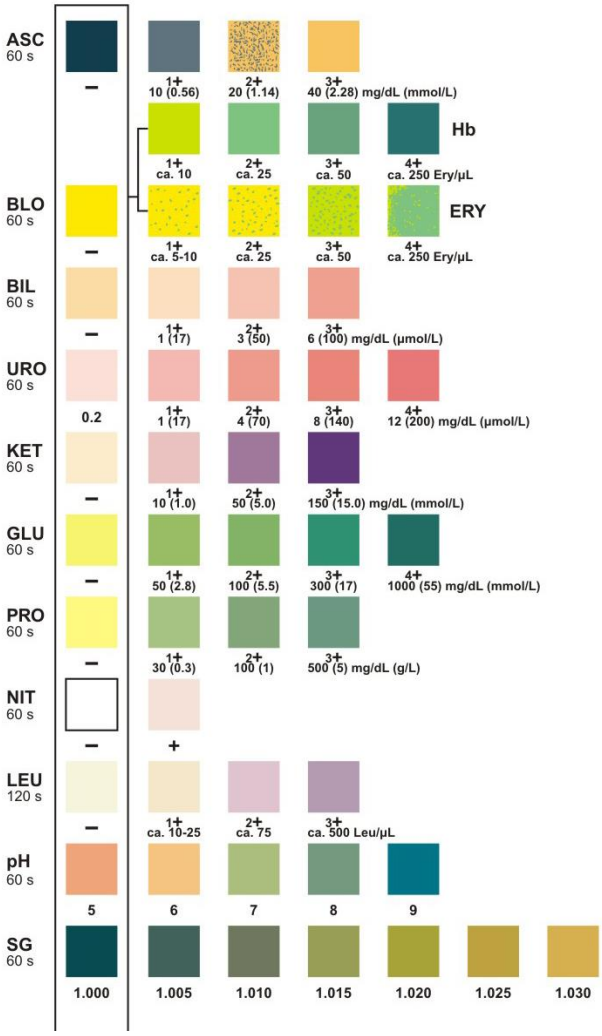
Leucocyte and erythrocyte counts were manually done from undiluted CSF sample within one hour of collection on modified Neubauer chamber, for differential Leucocyte count (DLC). Turk's fluid was used for dilution of CSF at the ratio of (1:1), followed by microscopic evaluation by pathologist. After microscopy, CSF was centrifuged for 10 min at 3000 g. Romanowsky stain was used for staining the smears made from the sediment. In cases of very high CSF cellularity, smears were prepared directly from the CSF sample without centrifugation before staining. Differential leucocyte count was done in the stained smears by the pathologists. In cases where CSF samples were haemorrhagic; the samples were diluted serially by adding normal saline for dilution of the erythrocytes, so as to make

visualisation and analysis of leucocytes effortless. Corrected protein levels and leucocyte counts and in haemorrhagic CSF samples were calculated by the formula-

leucocyte count (Corrected) in CSF = Blood Leucocyte count x RBC count in CSF /RBC count in blood[5]. Gold standard quantitative estimation of glucose and protein were done on the biochemistry analyser AU480 by glucose oxidase-peroxidase (GOD-POD) and pyrogallol red methods respectively. In haemorrhagic samples, correction for CSF protein were done by the formula

CSF Protein (Corrected) = Protein in CSF - (protein in serum x 1000 x (1-Hematocrit/100) x RBC count in CSF (RBC count in blood x 10^6).[6]

Urine reagent strips used were Mission expert urinalysis strips. In the strip's leucocyte esterase estimation method was used for analysis of leucocytes, glucose oxidase-peroxidase method for glucose and pyrogallol red method for proteins. The technicians and pathologists performing the investigations were blinded and unaware of the findings of the gold standard (reference) and index tests. Micropipette was used for mixing and adding 2-3 drops of undiluted CSF sample to urine reagent strips at the patches meant for leucocytes, proteins, glucose analysis and the colour change was observed after 60 seconds, colours of the patches on the test area of the strip were compared and matched with the colour chart provided with the reagent strips as shown in the image below



The values of Urine protein, glucose and leucocyte counts obtained by Mission expert urinalysis strips were compared with those obtained by Routine biochemistry and Microscopy. Routine biochemistry and Microscopic examination were considered as the gold standard. Statistical

tools of sensitivity, specificity, and accuracy were used to assess to the validity of Urine Reagent Strips in comparison to Routine biochemistry and Microscopy.

Gold standard reference methods were considered as confirmatory diagnostic and sensitivity, specificity and accuracy calculated accordingly

The formula used for calculation of specificity, sensitivity and accuracy were -

Sensitivity = TP (True Positive)/ {TP (True Positive) + FN (False Negative)}

Specificity = TN (True Negative)/ {TN (True Negative) + FP (false Positive)}

Accuracy = {TN (True Negative) + TP (True Negative)}/ {TN (True Negative) +TP (True Positive) +FN (False Negative) +FP (false Positive)}

True Positive (TP)- Positive by urine reagent strips (index test) as well as Gold standard (reference test)

True Negative (TN)- Negative by urine reagent strips (index test) as well as Gold standard (reference test)

False Positive (FP)- Positive by urine reagent strips (index test) as but negative by Gold standard (reference test)

False Negative (FN) - Negative by urine reagent strips (index test) but positive by Gold standard (reference test)

RESULTS

A total of 100 CSF samples, of suspected meningitis were included in our study. Among the 100-study population 58 were males and 48 were females, so male: female ratio was 1.2:1. The study was aimed at the analysis of three parameters i.e., leucocytes, proteins, and glucose which are of paramount importance for diagnosis of meningitis. The findings of the reagent strips were compared with that of routine biochemistry analyser findings and microscopic examination. 74 (74%) CSF sample were diagnosed as bacterial meningitis CSF as per the reference (gold standard), while 26 samples were normal.

The sensitivity and specificity for leucocyte estimation by the urine reagent strips in comparison to reference method for diagnosis of meningitis i.e., leucocyte counts >10 cells/mm^3 were 95.95% (71 of 74) and 96.15% (25 of 26) respectively. The accuracy we got was 96.00%. CSF proteins had sensitivity of 97.29(72 of 74) at +1(<30mg/dl) of urine reagent strips but specificity at +1 was low i.e., 69.23% (18 of 26), which rose to 96.1% (25 of 26) when cut off was kept as +2 (i.e., >100 mg/ dl) in urine reagent strips. The accuracy attained was 90.00%. at cut-off +1 and 97.00% at cut-off +2. The sensitivity for glucose at detecting low glucose levels in CSF on urine reagent strips score 0 i.e., <50 mg/dl is 91.18% (68/74) and specificity is 65.3% (17/26). The accuracy attained was 85%.

Discussion-

Meningitis is the inflammation of meninges and is diagnosed using laboratory and imaging studies. Early diagnosis and initiation of treatment is very important for favourable prognosis, but not always possible due to unavailability of adequate diagnostic facilities. Reagent strips available for urine analysis can be easily utilized for emergency analysis of CSF protein, glucose and leucocyte count in health centres scantily resourced as it requires minimal skill and time for achieving a provisional diagnosis and initiation of early treatment. In our study we found bacterial meningitis more commonly encountered in paediatric population similar findings were seen in studies done by other authors[3,7,8,9].

We used Mission expert urinalysis strips as the index test and found high sensitivity (95.95%), specificity (96.15%) and accuracy 96% in determining leucocyte count for diagnosis of meningitis.

Similar findings were found in the studies of kumar et al, Scarborough et al and mazumdar et al [3,10,11]. The sensitivity(97.29%) for protein was high, but specificity(69.23%) was low as the urine strips are meant for urine analysis and ranges standardised fell in normal range of CSF protein. Increasing the cut-off to +2 solved the problem and specificity(96.1%) as well as accuracy(97%) sharpy increased to satisfactory levels. Similar observations were noted in the studies of Chikkannaiah et al, Joshi et al and Romanelli et al[7,12,13]. In case of glucose we got sensitivity of 90.18% and low specificity 65.3% this was due to lowest cut-off of glucose values in urine reagent strips is 50mg/dl which may also be seen in normal CSF. Similar low specificity due to high cut-off values for glucose in urine reagent strips were seen in the studies of and mazumdar et al, Joshi et al and Romanelli et al [3,12,13.]

The three parameters analysed in the study yielded satisfactory results in diagnosing meningitis with high sensitivity and we found the strips can be utilized in emergency cases where laboratory resources are scarce. Meningitis in patients of hypoxic seizures and/ or febrile convulsions can be ruled our or confirmed and treated earlier with specific protocols. These tests can be of great help in avoiding unnecessary over treatment in such cases. The test is easy and rapid to perform and can be utilized as a bedside test for rapid diagnosis of bacterial meningitis. It can be extremely helpful in low resource rural and tribal set ups where laboratory facilities are scarce or non-existent. The only limitation of this method is variation in the cut off normal values for protein and sugar in CSF as compared to urine. This can be overcome by standardising strips specific for CSF analysis, moreover, the CSF strips can be made to include only these three parameters so as to save on cost of the strips, reagent, quantity of sample required and simplify the interpretation and avoid confusion if interpretation done by less skilled personal.

Conclusion

Urine reagent strips can be utilized for testing of CSF for proteins, glucose and granulocytes as a point of care, bedside test. Using a higher cut-off for proteins improves the specificity and accuracy. Also due to difference between normal range of glucose in urine and CSF the accuracy and specificity for analysis if glucose in CSF is low. Reagent strips specifically made for CSF and standardised as per normal and diagnostic ranges of granulocytes, glucose and proteins in CSF should be made which will have better sensitivity, specificity and accuracy and will also be cost effective.

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