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Validation of Vanillylmandelic Acid (VMA) with Plasma Metanephrine and Normetanephrine for Screening Adrenal Medullary Disorders

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ABSTRACT

Objective: To validate urinary Vanillylmandelic acid (VMA) for screening adrenal medullary disorders, taking plasma-free Metanephrine as the gold standard.

Study Design: Cross-sectional validation study.

Place and Duration of Study: Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi, in collaboration with Armed Forces Institute of Urology, Rawalpindi from Pakistan, Jan 2020 to Mar 2021.

Methodology: One hundred and thirty (130) symptomatic hypertensive patients with adrenal masses on ultrasound were selected Urine and blood samples were collected under specified conditions after taking necessary precautions and subsequently analyzed. Taking plasma Metanephrine as a reference, sensitivity, specificity and predictive values were calculated at predefined cut-off values.

Results: In a young population with a mean age of 28.55±5.54 years, headache, palpitations and sweating were the predominant symptoms having a frequency of 130(100%), 116(89.2%) and 111(85.4%), respectively. Twenty-four hours urinary Vanillylmandelic acid had lower sensitivity (66.3%) than a random urinary VMA/cr ratio (72.1%) but similar specificity (97.7%). On the other hand, plasma-free Normetanephrine had 100% sensitivity but lower specificity (93.2%). ROC curve was plotted, and AUC for 24 hours urinary VMA, urinary VMA/cr ratio and plasma-free Normetanephrine were 0.820, 0.849 and 0.966, respectively.

Conclusion: Plasma-free Metanephrine could be used for screening pheochromocytoma and other adrenal medullary disorders like paraganglionoma. In addition, VMA/cr ratio can be used for biochemical confirmation of the disease owing to the high specificity found in our study.

Keywords: Metanephrine, Normetanephrine, Pheochromocytoma, Vanillylmandelic acid.

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INTRODUCTION

Pheochromocytoma is one of the most common adrenal medullary disorders.[1] Although rare, these neuroendocrine tumours are extremely important because they remain undiagnosed or rarely diagnosed incidentally, often at autopsy.[2] The prevalence is reported to be 0.015% to 0.04% for tumours diagnosed during life, while the autopsy prevalence is 0.09% to 0.12%.[3,4] Considering undiagnosed patients, the prevalence of the disease is estimated to be close to 1 in 1000 or 0.1%. The spectrum of illness ranges from being silent to presentation in shock and death due to hemodynamic instability. The classic triad of symptoms consists of paroxysmal attacks of headache, sweating and palpitations. Morbidity and mortality can be significantly reduced if these are diagnosed timely.[5] The diagnosis is made clinically with biochemical evidence of excess catecholamines supported by radiological investigations, histopathology being the definitive diagnosis.[6] Vanillylmandelic acid (VMA) is the end product of catecholamines breakdown and is secreted in the urine. As the majority of metanephrines are produced within chromaffin cells of the adrenal medulla by a process independent of exocytotic catecholamine release.[7,8]

Plasma and urinary metanephrine or Normetanephrine are usually assayed by high-performance liquid chromatography (HPLC) or a sensitive immunoassay technique such as enzyme-linked immunosorbent assay (ELISA) and chemiluminescence.[9] The analytical techniques for Vanillylmandelic acid (VMA) are usually simple, and photometric assay kits are widely available. However, in a developing country like Pakistan, where medical infrastructure still needs to be fully developed, the testing facilities are very sparse, and high-end, technologically advanced facilities like HPLC and Immunoassay are available.[10] Therefore, there needs to be a cost-effective test with reasonable sensitivity and specificity for use across the country,

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including the far-flung areas where high-end diagnostic facilities are unavailable. This study aimed to validate a cost-effective, easy-to-operate, and widely available photometry-based test for diagnosis in patients with adrenal medullary disorders. The objective of this study was to validate urinary Vanillylmandelic acid (VMA) for screening adrenal medullary disorders by taking plasma-free metanephrine as a reference test.

METHODOLOGY

The cross-sectional validation study conducted at the Department of Chemical Pathology and Endocrinology, Armed Forces Institute of Pathology, Rawalpindi from Pakistan January to September 2020. The study was approved by the Institutional Review Board (IRB) of the Armed Forces Institute of Pathology, Rawalpindi (IRB no. FC-CHP19-7-READ/IRB/20/366 dated 5 May 2020). Pilot study was done on 36 patients on the lines of the study by Guller *et al*.[11] which estimated sensitivity and specificity of 24 hours urinary VMA 56% and 95%, respectively, taken against plasma-free metanephrine as the gold standard test. The sample size was estimated by the WHO sample size calculator, taking a prevalence of 0.1%.[2] A total of 130 patients were enrolled in the study using a consecutive sampling technique.

Inclusion Criteria: Hypertensive patients having adrenal swellings on ultrasound with symptoms of headache, sweating and palpitations were included in the study.

Exclusion Criteria: The patients with thyroid diseases or psychiatric illnesses were excluded from the study.

All the patients were received at the diagnostic endocrine clinic of AFIP, Rawalpindi where detailed history and examination were made. All the patients underwent ultrasonography for both kidneys by the radiologist at the Armed Forces Institute of Radiology and Imaging, Rawalpindi. The patients were given an appointment after briefing them about the nature of the test and the sampling protocol for 24-hour urinary collection, including the precautions to be observed, e.g. avoiding strenuous exercise, medications and dietary restrictions (to avoid caffeine, smoking, beverages containing caffeine and chocolates).

The samples were collected for 24-hour urine specimen for VMA in a large clean container, 3 ml venous blood in an EDTA tube for plasma metanephrine and Normetanephrine and a spot urine specimen for VMA/creatinine ratio. In addition, 1-2 drops of 1M HCl were added to preserve urine till analysis. It was ensured that the sampling for plasma metanephrines was done after resting for at least 30 minutes. The urinary VMA was analyzed on a semi-automated chemistry analyzer Microlab® 300 by the photometric method using a testing kit by FAR ® diagnostics Italy. Urinary creatinine was assayed by the modified Jaffe kinetic method on ADVIA 1800 by Siemens®. Plasma metanephrine and nor-metanephrine were analyzed using 3rd generation competitive ELISA technique using a diagnostic kit by LDN® Germany. Quality control was ensured by running control materials within the run along with the samples.

Statistical Package for Social Sciences (SPSS) version 23.0 was used for the data analysis. The study population was categorised age-wise to determine its prevalence in different age groups. After employing normality tests, the Shapiro Wilk Test, descriptive statistics were applied as frequency and percentages for qualitative variables and mean±SD for quantitative variables. 2x2 tables were constructed to determine sensitivities, specificities, positive and negative predictive values, positive and negative likelihood ratios and total accuracy. The cut-offs for diagnosis of Pheochromocytoma were used from Lenders *et al*.[12] and Pussard *et al*.[13] i.e, 24 hours urinary VMA 40 µmol/day, urinary VMA/Cr ratio 2.6 mmol/mol plasma free metanephrine 0.3 nmol/L and normetanephrine 0.6 nmol/L. False positive and true positive was defined as the test values more than the cut-off values. At the same time, the false negatives and true negatives were defined as values less than the cut-off values. Receiver Operating Characteristics (ROC) curves were plotted between 24 hours of urinary VMA, spot urinary VMA/Cr ratio and plasma Normetanephrine to find out the area under the curve (AUC), taking plasma metanephrine as reference tests. AUC >0.5 was taken as positively significant.

RESULTS

One hundred and thirty (130) subjects were included in the study. 72(55.4%) were males, and 58(44.6%) were females. The mean age was 28.55±5.54 years. All the patients of the study underwent screening tests for Pheochromocytoma, descriptive statistics of whom were summarized in Table-I. ROC curve was plotted between 24 hr urinary VMA, urinary VMA/Cr ratio and plasma-free Normetanephrine, taking plasma-free metanephrine as the gold standard, and the area under the curve (AUC) was calculated.

The results indicate that the VMA/Cr ratio had slightly better performance than 24 hours urinary

VMA levels. Plasma-free Normetanephrine had a close association with plasma-free metanephrine having the highest area under the curve (0.966) (Figure).

Table-I: Descriptive Statistics and Clinical Signs and Symptoms of the Study Participants (n=130)

Parameters	Mean±SD
Age (Years)	28.55±5.54.0
24 hour urinary VMA (µmol/day)	38.29±13.12
Urinary VMA/Cr ratio (mmol/mol)	2.49±0.67
Plasma metanephrine (pmol/L)	0.52±0.31
Plasma Normetanephrine (pmol/L)	0.84±0.43
Sign and Symptoms	n(%)
Headache	130(100 %)
Palpitations	116(89.2%)
Sweating	111(85.4%)
Photophobia	45(34.6%)

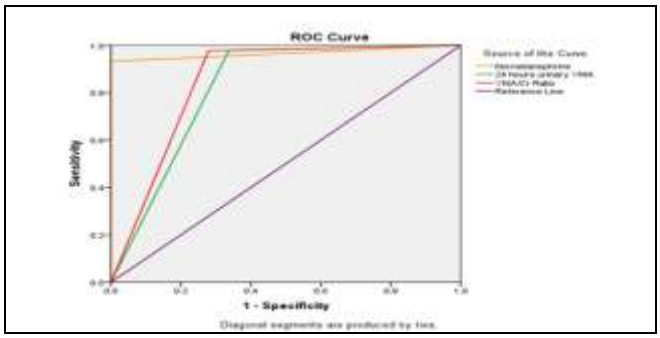

Figure: ROC between Plasma Free Metanephrine and other Related Diagnostic Tests (n=130)

After applying the cut-offs, the patients were categorized as either healthy or diseased. The results showed that out of 130 patients selected for the study, 86 had high plasma-free metanephrine levels (true positives), taken as gold standard/ reference test, and 44 had low levels, taken as true negatives. Therefore, 2x2 tables were constructed between different diagnostic tests taking plasma-free metanephrine as the gold standard. The performance of different tests such as sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), positive likelihood ratio (PLR), negative likelihood ratio (NLR) and total accuracy were calculated and are described in Table-II. Plasma-free Normetanephrine had the highest sensitivity, and urinary VMA had the highest specificity.

DISCUSSION

In our study, we tried to validate a photometry-based test, i.e. urinary Vanillylmandelic acid (VMA), for diagnosing Pheochromocytoma. Two types of urine-based VMA tests were evaluated, i.e. 24, hours urinary VMA and spot urinary VMA/Creatinine ratio. The problem of correct collection of urinary samples and variations in volume subject to water intake & output were catered for by spot urinary VMA/Cr ratio as it was more convenient to the patients. We used plasma-free metanephrine as the gold standard in our study instead of histopathology due to its invasive nature. Weismann *et al*.[8] reported the sensitivity of plasma-free metanephrine as 100% & specificity of 99% for diagnosing Pheochromocytoma and paraganglionoma.

Table-II: Diagnostic Accuracy of 24 Hours Urinary VMA, VMA/Cr ratio and Plasma Free Nor-Metanephrine taking Plasma Free Metanephrine as a Reference (n=130)

	24 hours Urinary VMA	Urinary VMA/Cr Ratio	Plasma Free Normetanephrine
Sensitivity= True Positive/(True Positive +False Negative)	66.3%	72.1%	100%
Specificity= True Negative/(True Negative +False Positive)	97.7%	97.7%	93.2%
Positive Predictive Value=True Positive/ (True Positive+ False Positive)	0.983	0.984	0.966
Negative Predictive Value=True Negative/ (True Negative +False Negative)	0.59	0.64	1.00
Likelihood ratio(+)= Sensitivity/(1-spcificity)	22	24	14.28
Likelihood ratio(-)= (1-Sensitivity)/Specificity	2.85	3.46	0
Diagnostic Accuracy= (TruePositive+True Negative)/All Patients	76.9%	80.77%	97.7%

Similarly, there are several studies,[14-16] which report both the sensitivity and specificity of plasma-free metanephrine as high as 100%. Therefore, in our study, we took it as a reference test for comparison with the index tests, i.e. urinary VMA. In our study, we found that 24 hours urinary VMA had lower sensitivity (66.3%) than a random urinary VMA/cr ratio (72.1%) but similar specificity (97.7%). On the other hand, plasma-free Normetanephrine had 100% sensitivity but lower specificity (93.2%). The area under the curve (AUC) for 24 hours of urinary VMA, urinary VMA/cr ratio and plasma-free Normetanephrine were 0.820, 0.849 and 0.966, respectively.

Earlier studies with similar performance of the VMA test substantiate the results of our study. Eissenhofer *et al*.[17] conducted a study on 195 patients, which reported 65% sensitivity and 96% specificity for 24-hour urinary VMA compared with the biopsy

findings. The high specificity of the 24-hour urinary VMA is an important parameter that can help confirm the diagnosis. In a multicenter cohort study of 1003 European patients for screening of Pheochromocytoma by Similarly, in another study conducted in Japan by Ohno *et al*.[18] the relationship between urinary VMA and metanephrine was analyzed. Urinary VMA was strongly correlated with urinary metanephrine (r=0.87) in patients with Pheochromocytoma. This high correlation suggests a close association between VMA and metanephrine.

Plasma-free metanephrine or plasma-free Normetanephrine could rule out adrenal medullary disorders in patients with high suspicion.[9,19] As plasma-free metanephrine has almost 100% sensitivity and specificity, it can be employed alone for screening. It is advised that there is no need to do both tests simultaneously as it increases the cost without any added advantage. When an assay for metanephrine is unavailable, plasma-free Normetanephrine can be used for screening, followed by urinary VMA. We further recommend using spot urinary VMA/Cr ratio instead of 24 hours urinary VMA due to its patients' convenience, higher sensitivity and specificity and fewer variations associated with 24-hour urinary collection.

CONCLUSION:

Plasma-free metanephrine should be used for screening pheochromocytoma and other adrenal medullary disorders. In addition, VMA/cr ratio can be used for biochemical confirmation of the disease owing to the high specificity found in our study.

Conflict of Interest: None.

Author's Contribution

Following authors have made substantial contributions to the manuscript as under:

UBK & ZHH: Data acquisition, critical review, approval of the final version to be published.

MA & SA: Conception, study design, drafting the manuscript, approval of the final version to be published.

AB & HJ: Data analysis, data interpretation, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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