EVALUATION OF REFERENCE INTERVALS FOR PLASMA ELECTROLYTES, UREA AND CREATININE IN APPARENTLY HEALTHY STUDENTS OF NIGER DELTA UNIVERSITY, BAYELSA STATE, NIGERIA

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Abstract:
Background: A locally-derived population reference interval is the backbone for correct interpretation of laboratory results. Reference values from American and European population are used for African population despite previous studies showing significant differences.

Objective: This study was to determine clinical laboratory reference values for plasma electrolytes, urea and creatinine of apparently healthy students.

Setting: College of Health Sciences, Niger Delta University, Bayelsa state, Nigeria

Study design: Cross-sectional prospective study.

Methods and Materials: 102 apparently healthy students aged between 20-40 years were selected by random sampling technique for the study. Electrolytes were assayed using Ion Selective Electrode (ISE) method, urea by enzymatic endpoint method and creatinine through enzymatic kinetic method on the plasma using Randox kits. Reference range was constructed using non-parametric method to estimate 2.5 and 97.5 percentile of distribution as lower and upper reference intervals respectively.

Result: The derived reference intervals for analytes in this study are: sodium (128.5-144.5)mmol/L, potassium (3.0-4.9)mmol/L, chloride (96-109)mmol/L, Bicarbonate (13.5-26)mmol/L, urea (1.9-4.6)mmol/L and creatinine (63 -108.5)μmol/L. There was no significant gender difference in all the studied analytes.

Conclusion: The developed reference intervals in this study are generally lower than the Caucasian (kit) values commonly used in our laboratories. Adoption of its use for patients’ management within Bayelsa and southern Nigeria is advised.

KEYWORDS: Plasma Electrolytes, Urea and Creatinine, Reference intervals, Healthy young students
INTRODUCTION:

Reference intervals of biochemical constituents can be defined as the concentration of analytes found in group of clinically healthy persons that are suited for the respective population. They determine whether disease is present or absent and if any individual is at risk from future disease state. Clinical care requires adequate laboratory reference intervals for appropriate assessment of patients, monitoring disease progress as well as reporting adverse events. Interpretation of laboratory data is a decision making process that compares patients result with reference intervals from a local population or validates the use of those obtained from different setting. The best reference interval for use is individual baseline biochemical values that had been documented all through their physiological and developmental milestone in healthy states. It is against these that pathological values are compared. However, due to difficulties in obtaining these, it becomes imperative to develop population reference intervals. Certain specified factors are needed when reference intervals are to be established: population make up, ethnicity, genetic factor, socioeconomic factors, analytical methods, diet, lifestyle, physiologic conditions among other controllable factors. These factors has led to differences in the analytical outcomes of reference values.

The population reference intervals are established according to the recommendation of the Expert Panel on Theory of Reference values (EPTR) and of the International Federation of Clinical Chemistry(IFCC) who use the terms “normal range, reference range or reference interval” interchangeably to correspond to health associated (central 95%) reference intervals. These values are taken to be between 2.5 and 97.5 percentile of the said population following strict quality assurance program and standard statistical methods.

Establishing reference intervals continues to be a major challenge in many parts of the world because it is time and effort consuming and requires a lot of funds. These limitations have led clinical laboratories to adopt manufacturers' (reagent kit) values mainly from Caucasian population which are often inappropriate for the diverse African population.

Currently, in Nigeria there are very few studies on reference intervals for electrolytes, urea and creatinine especially in the younger age groups and relevant clinical reference data are required for clinical practise and research in our region. In view of this, we embarked in this study to evaluate population reference intervals for electrolytes, urea and creatinine amongst healthy students of Niger Delta University, Bayelsa state, South-south Nigeria. The reported values may also as source of reference and comparison for laboratories in our environment.

Materials and Methods:

Study area and Design: The study consisted 102 apparently healthy students aged between 20-40 years. The participants were recruited randomly. Participants with hypertension, diabetes, renal disease, liver disease or those on drugs for any illness were excluded from the study. Also excluded were those with history of smoking, alcohol intake, exercise excess, pregnant women, recent history of fever, trauma or blood transfusion. Relevant information demograph like age and sex were obtained alongside physical examination to rule out palor and jaundice.

Ethical Consideration: Ethical approval was sought and obtained for this study from the Niger Delta University ethics committee. Written and informed consent was sought and obtained before participation in the study.

Methods: Participants weight and height
were taken, basal metabolic index (BMI) was calculated, each of them was allowed 5 minutes rest before blood pressure measurement at sitting position was done.

Specimen Collection: 4mls of blood was collected from each participant through an aseptic cubital venepuncture at sitting position and after a 10 minutes rest period and transferred into lithium heparinised tube. This was centrifuged at 3000rpm using a bench centrifuge within 30 mins of collection and plasma was separated with clean Pasteur pipette into plane bottles and stored frozen (at -20°C) before analysis. Analysis was done in batches within one week of specimen of collection.

Measurements of Biochemical parameters: Na⁺, K⁺, Cl⁻ and HCO₃⁻ were analysed using direct electrochemical method using Ion Selective Electrode (ISE analyser LW E60E). Urea was analysed using enzymatic endpoint assay (urease-Berthelot method) with commercially available Randox kits where urea was hydrolysed to ammonia in the presence of urease. The ammonia is then measured photometrically by Berthelot's reaction. Creatinine was analysed using enzymatic kinetic method (modified Jaffe method) with Randox kits whereby creatinine in an alkaline solution reacts with picric acid to form a colored complex which is directly proportional to the creatinine concentration read photometrically.

Quality Control Procedure: Commercial quality control (QC) sera (normal, low and pathological ranges were analysed in each batch. Mean, standard deviation and the intra and inter assay Coefficient of Variations (CVs) were calculated and found to be within acceptable quality values of the method for each analyte.

Statistical Analysis: Continuous variables were summarized as mean±SD while categorical variable were expressed as frequency (normal Gaussian) distribution curves. Reference intervals were constructed using non-parametric method to estimate 2.5 and 97.5 percentiles of distribution as lower and upper reference intervals respectively. The statistical analysis were done using Statistical Package for Social Sciences (SPSS) software, (IBM SPSS Statistics version 20 Armonk, New York.)

RESULTS:
There were 102 apparently healthy participants aged between 20-40 years comprising of 55 (54%) males and 47 (46%) females. The mean age is 23.8 ±3.67 years. Table 1 depicts the mean ± SD of participants according to gender. There was no significant gender differences in sodium, potassium, chloride, bicarbonate, urea and creatinine values.

Table 1: Comparison of mean plasma electrolytes, urea and creatinine in participants by gender.

<table>
<thead>
<tr>
<th>Analytes (unit)</th>
<th>Total pop(n=102) Mean ± SD</th>
<th>Males (n=55) Mean ± SD</th>
<th>Females (n=47) Mean ±SD</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium(mmol/L)</td>
<td>134.7 ± 4.12</td>
<td>134.01 ±3.65</td>
<td>134.37 ±5.20</td>
<td>0.12</td>
</tr>
<tr>
<td>Potassium(mmol/L)</td>
<td>3.75 ± 0.49</td>
<td>3.93 ± 1.35</td>
<td>3.75 ± 0.42</td>
<td>0.36</td>
</tr>
<tr>
<td>Chloride(mmol/L)</td>
<td>101.0 ± 3.56</td>
<td>100.79±3.56</td>
<td>101.29±3.55</td>
<td>0.13</td>
</tr>
<tr>
<td>Bicarbonate(mmol/L)</td>
<td>20.7 ± 3.13</td>
<td>20.47 ± 3.79</td>
<td>20.52 ± 4.02</td>
<td>0.95</td>
</tr>
</tbody>
</table>
Table 2 shows reference intervals derived in this study for Sodium(128.5 -144.5)mmol/L, Potassium(3-4.9)mmol/L, Chloride(96-109.4)mmol/L, Bicarbonate(13.5-26)mmol/L, Urea(1.9-4.6)mmol/L and Creatinine (63-108)μmol/L with the assay methods used. Results obtained are lower than most standard reference quoted in Caucasians (kit) values.

Table 2: Reference Intervals of Electrolytes, Urea and Creatinine (2.5- 97.5 percentiles) in comparison with Adopted (kit) values.

<table>
<thead>
<tr>
<th>Analytes (unit)</th>
<th>Total pop(n=102)</th>
<th>Males (n=55)</th>
<th>Females (n=47)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urea (mmol/L)</td>
<td>2.84 ± 0.73</td>
<td>2.84 ± 0.73</td>
<td>2.82 ± 0.75</td>
<td>0.91</td>
</tr>
<tr>
<td>Creatinine (μmol/L)</td>
<td>88.2 ± 13.2</td>
<td>88.16±12.98</td>
<td>87.28±14.20</td>
<td>0.74</td>
</tr>
</tbody>
</table>

Students T-test, p<0.05=Significant (S), Not Significant (NS)

All the analytes maintain normal (Gaussian) distribution curves as depicted in figures 1-6.
Discussion:

The assessment of health status of individuals is made on clinical grounds by medical history, physical examination, laboratory tests and other special investigations using reliable reference data. In the absence of locally-derived reference values, clinicians and researchers had to use reference data from European and American population. Previous studies showed variation in reference intervals with age, ethnic and socio demographic characteristics in different population hence, the establishment of regional specific reference values is essential for efficient patient management and proper conduct of clinical trials. The reference intervals derived in this study differ slightly from the manufacturers (kit) reference values which were adopted from the Caucasian population. The differences in values may be related to methodologies of analysis, racial differences and geographical location. Lifestyle, diet and genetic makeup may also play crucial role in these existing differences.

In this study, 15.7% of study population have lower potassium than 3.3mmol/L, 57.8% have sodium <135mmol/L, 29.4% have bicarbonate lower than 20mmol/L and 34.3% had urea values < 2.6mmol/L. This result is similar to reports in Abeokuta and LAUTH-Ogbomoso both of south western Nigeria whose geographical location and other...
demographic characters are comparable to ours here in south-south Nigeria. This corroborates with studies carried out in other African countries like Kenya, Khartoum-Sudan and Tanzania which reported that derived reference intervals differed from the Caucasian (kit) values. This further underscores the need to derive population reference intervals.

This study also revealed no significant gender differences in values which is similar to a study done on medical students of Denmark University, which reported no significant gender and age dependency in values. The story is however different in Plateau University study and in Kericho, Kenya, which reported gender differences in some biological analytes most especially urea and creatinine which is probably due to muscular build-up variation of both gender.

The limitation of this study are small sample size due to poor funding and not conducting a thorough clinical examination including urinalysis in the selection of healthy patients. However, the result in this study is tenable and highlights the importance of deriving local reference interval for our population.

Conclusion:
The developed reference intervals in this study are generally lower than the Caucasian (kit) values but similar to studies in areas of close geographical location. This underscores the need for individual laboratories to establish local population reference intervals rather than relying on manufacturers' (kit) values from another population. We recommend the use of these derived values in Niger Delta University Teaching Hospital, Okolobiri, other areas of Bayelsa state and Southern Nigeria.

References:
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