Reference Values of Common Blood Chemistry Analytes in Healthy Population of Rawalpindi-Islamabad Area

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Abstract
The reference values of common blood chemistry analytes in healthy population, aged newborn to 80 years, of Rawalpindi Islamabad area were determined at AFIP, Rawalpindi. A total of 2115 healthy subjects, 1206 males and 909 females, were included in the study. Plasma glucose was analysed by GOD/POD, serum cholesterol by CHOD/PAP, triglycerides by GPO/PAP, urea by urease/GLDH, creatinine by Jaffe’s rate reaction, uric acid by uricase, total bilirubin by Jendrassik and Grof, total protein by biuret, alanine transaminase (ALT) by optimized IFCC and alkaline phosphatase (Al’) by optimized DGKC method. The between batch CVs of all the parameters were within acceptable quality goals. The reference values were calculated using 2.5 and 97.5 percentiles as lower and upper limits (95% CI). In healthy adult males the reference values were: fasting plasma glucose, 3.6-6.0 mmol/l; serum cholesterol, 3.2-6.6 mmol/l; triglycerides, 0.6-2.3 mmol/l; urea, 2.8-6.4 mmol/l; creatinine, 65-132 umol/l; uric acid, 164-430 umol/l; total bilirubin, 5-18 umol/l; total protein, 57-83 g/l; ALT, 15-45 U/I and Al’, 185-620 UI. The values in adult females, children and elderly subjects were slightly different than adult males. The reference values of our population show mild to moderate differences from the other Asian, European and American populations. It is recommended that reference values of different biochemical investigations should be established in various areas of Pakistan to make appropriate use of such investigations. (JPMA 47:156,1997).

Introduction
Any measured value of the laboratory test from an individual should be compared with the reference values of that analyte for the purpose of making a medical diagnosis, physiological assessment of therapeutic management decision. The interpretation of clinical laboratory data is, therefore, a comparative decision making process. Hence, the reference values are required for all the analytes tested in clinical laboratory. Although, defining reference values for a particular population is an expensive and time consuming project, each laboratory should arrange for its own reference ranges. The usual practice in Pakistan is to interpret the patient’s laboratory results in the light of reference values given in the standard text books or as mentioned in the literature of manufactured kits which are actually derived from European or American populations. Different populations have different genetic make-up, dietary habits, life style, socio-economic and many other environmental and biological factors which can affect the analytical outcome. The reference values for each population and each laboratory are to be established according to the recommendations of Expert Panel on Theory of Reference Values (EPTR) of International Federation of Clinical Chemistry (IFCC). A proper and well defined selection of reference individuals is a major consideration. Inclusion and exclusion criteria need to be clearly defined and practically followed. The avoidable variables such as exercise, smoking, specific foods, alcohol and drugs should be strictly taken care of. The controllable variables, such as, time of blood collection, individual’s posture, tourniquet collection containers, serum separation and storage should be thoroughly monitored. As the reference values are affected by the analytical system (instruments and methods) also, these should be kept uniform. The quality assurance
prograni.me has to be strictly followed and standard statistical methods should be applied to the
reference data to derive reference values\(^7,8\). There is little information on the reference values of blood
chemistry analytes of Pakistani population. Only limited studies have been reported from Karachi\(^9,10\).
Therefore, a study was designed to ascertain the reference values in healthy population of Rawalpindi-
Islamabad area.

**Subjects and Methods**

The project was started in April, 1993 and completed in September, 1994 in the Department of
Chemical Pathology and Endocrinology, AFIP, Rawalpindi.

**Subjects**

A total of 2115 individuals comprising 1206 males and 909 females, aged newborn to 80 years,
residing in Rawalpindi- Islamabad area for the last 5 years were included in the study by random
selection. The subjects comprised: newborn (n=90); children (n=370); adults (n=1452) and elderly
(n=203). At the time of preliminary inquiry regarding health state of individuals, medical history and
physical examination were carried out. Any individual with positive history of liver, kidney or thyroid
disease, genetic factor (twin), recent fever/trauma/transfusion, smoking, alcohol intake, drugs of abuse
and physical exercise were excluded.

**Blood collection**

The blood samples were collected between 0730 to 0930 hours in fasting state (8-16 h fast). Venous blood
(10 ml) was collected in sitting position with a clean venipuncture from an antecubital vein, with
minimal stasis. For newborn, the cord blood was collected. The blood was allowed to clot at room
temperature for one hour and serum was separated by centrifugation at 8000 g for 5 min. The serum was
then transferred to small sterile tube and stored at -20°C prior to analysis.

**Analytical methods**

Plasma glucose was analyzed by glucose oxidase/peroxidase method of Trinder (Scm Pak, England);
serum cholesterol by enzymatic end point method of Allain (CHOD-PAP) (Chromatest, Knicker
Brocker, Spain); serum triglycerides by enzymatic end point method (GPO-PAP) (Merck, Genuany);
urea by enzymatic UV method (urease/GLDH) (Cromatest, Knicker Brocker, Spain); creatinine by
Jaffe’s kinetic method Cromatest, Knicker Brocker, Spain, uric acid by enzymatic Trinder method
(Uricase) with reagent kits of Menagent, Menarini Diagnostic, France. These analyses were carried out
on autoanalyzer RA-1000 (Technicon). Serum total bilirubin was analyzed by Jendrassik and Gmf
method, total protein by biuret method, ALT by optimized IFCC method and AP by optimized DGKC
method using reagent kits of Mercktest, Merk, Gennany. These analyses were carried out on
photometer-4010 (Boehringer).

**Quality control procedures**

Commercial controls, normal (Test Point I) and abnormal (Test Point II) of Miles - USA, were run
within and between the batches. Mean, SD and CVs were within acceptable quality goals of the
methods for respective analytes.

**Statistical analysis**

Statistical analysis was processed by the computer programme - Special Package for Social Sciences
(SPSS). The mean, median and percentile values of the analytes were calculated and recorded. The
upper and lower limit of the reference ranges were taken from the 97.5 percentile and 2.5 percentile
values of each analyte. Student’s ‘t’ test and Wilcoxon Rank sum test were used to compare different
groups.

**Results**
Table gives reference values of different analytes in different age groups of males and females. The fasting plasma glucose values rose from newborn to the middle aged subjects (upto 60 years) and fell in elderly individuals (61-80 years). There was no significant difference in values of both sexes. Serum cholesterol increased with advancing age from newborn to the middle aged subjects and fell in elderly subjects. The values were slightly higher in males than females. The reference values of serum triglycerides were lower in newborn than those of children and adults. The values were slightly higher in males than in females of all age groups. Serum urea was lowest in newborn and there was a progressive rise with advancing age. Serum urea remained slightly higher in males than in females in all age groups. Serum creatinine in younger children was significantly lower than all other age groups. The values increased with advancing age. The values were equal in both sexes upto 14 years of age but were significantly lower in females than males with the advancing age from 2nd to 8th decade of life. The levels of serum uric acid rose from 1st to 6th decade and fell in 7th and 8th decade. There was no difference in values of both sexes in newborn and younger children but the values were found significantly higher in males than females of all Other groups. Total serum bilirubin in newborn was much higher than the other age groups. The levels were slightly higher in males than females. Total serum protein values in newborn were lowest and increased with age upto 14 years. The values were slightly higher in females than male children group and vice versa in adults and elderly. The levels of semm alanine transaminase (ALT) were highest in newborn. The values in males were slightly higher than females in all age groups. Serum alkaline phosphatase (AP) in newborn was lower than in children. With advancing age there was decrease in adults. There is no significant difference between males and

<table>
<thead>
<tr>
<th>Analytes (Units)</th>
<th>Sex</th>
<th>Newborn (age:0)</th>
<th>Children (1-10 yrs)</th>
<th>Children (11-14 yrs)</th>
<th>Adults (15-40 yrs)</th>
<th>Adults (41-60 yrs)</th>
<th>Geriatric (61-80 yrs)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose (mmol/L)</td>
<td>M</td>
<td>3.1-5.7</td>
<td>3.1-5.8</td>
<td>3.5-6.0</td>
<td>3.6-6.0</td>
<td>3.7-6.3</td>
<td>3.4-5.2</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>3.1-5.7</td>
<td>3.2-5.9</td>
<td>3.6-6.0</td>
<td>3.5-6.2</td>
<td>3.6-6.2</td>
<td>3.1-6.1</td>
</tr>
<tr>
<td>Cholesterol (mmol/L)</td>
<td>M</td>
<td>2.5-5.1</td>
<td>2.8-5.6</td>
<td>3.0-5.9</td>
<td>3.2-6.6</td>
<td>3.3-6.7</td>
<td>3.1-6.4</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2.5-5.1</td>
<td>2.7-5.5</td>
<td>2.8-5.8</td>
<td>3.1-6.4</td>
<td>3.5-6.9</td>
<td>3.2-6.5</td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>M</td>
<td>0.3-2.0</td>
<td>0.45-2.1</td>
<td>0.5-2.2</td>
<td>0.6-2.3</td>
<td>0.55-2.25</td>
<td>0.45-2.1</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>0.3-2.0</td>
<td>0.4-2.05</td>
<td>0.45-2.2</td>
<td>0.55-2.2</td>
<td>0.6-2.2</td>
<td>0.4-2.0</td>
</tr>
<tr>
<td>Urea (mmol/L)</td>
<td>M</td>
<td>2.4-5.2</td>
<td>2.6-5.8</td>
<td>2.7-6.2</td>
<td>2.8-6.4</td>
<td>3.0-6.8</td>
<td>3.2-6.9</td>
</tr>
<tr>
<td></td>
<td>F</td>
<td>2.4-5.2</td>
<td>2.5-5.7</td>
<td>2.6-6.1</td>
<td>2.7-6.3</td>
<td>2.9-6.5</td>
<td>3.1-6.8</td>
</tr>
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<td>Creatinine (umol/L)</td>
<td>M</td>
<td>55-120</td>
<td>53-80</td>
<td>59-128</td>
<td>65-132</td>
<td>68-138</td>
<td>70-142</td>
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<tr>
<td></td>
<td>F</td>
<td>55-120</td>
<td>31-78</td>
<td>50-110</td>
<td>54-119</td>
<td>57-120</td>
<td>58-125</td>
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<tr>
<td>Uric acid (umol/L)</td>
<td>M</td>
<td>135-390</td>
<td>125-351</td>
<td>150-410</td>
<td>164-430</td>
<td>174-450</td>
<td>167-435</td>
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<tr>
<td>Bilirubin, total (umol/L)</td>
<td>M</td>
<td>14-52</td>
<td>4-17</td>
<td>5-18</td>
<td>4-17</td>
<td>5-16</td>
<td>4-17</td>
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<td>4-16</td>
<td>4-17</td>
<td>3-15</td>
<td>4-15</td>
<td>3-16</td>
</tr>
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<td>Protein, total (g/l)</td>
<td>M</td>
<td>55-73</td>
<td>56-79</td>
<td>57-83</td>
<td>58-82</td>
<td>59-83</td>
<td>58-80</td>
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<tr>
<td></td>
<td>F</td>
<td>55-73</td>
<td>55-80</td>
<td>56-82</td>
<td>58-80</td>
<td>58-81</td>
<td>56-78</td>
</tr>
<tr>
<td>Alanine transaminase (U/L)</td>
<td>M</td>
<td>20-65</td>
<td>16-52</td>
<td>15-45</td>
<td>11-42</td>
<td>11-41</td>
<td>10-40</td>
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<td></td>
<td>F</td>
<td>20-65</td>
<td>14-49</td>
<td>13-43</td>
<td>10-41</td>
<td>9-40</td>
<td>10-40</td>
</tr>
<tr>
<td>Alkaline phosphatase (U/L)</td>
<td>M</td>
<td>206-630</td>
<td>186-690</td>
<td>185-620</td>
<td>126-297</td>
<td>101-287</td>
<td>105-280</td>
</tr>
</tbody>
</table>
females in all age groups.

Discussion

Reference range is an essential component of any laboratory report which has to be established for each population. There is limited information on laboratory values of various parameters on healthy population of different areas of Pakistan. The values reported by Molla et al.\textsuperscript{10} for Karachi area are not representative of the entire Pakistani population. Most of the studies reported from Pakistan are not of much help for practical laboratory reporting because these do not cover more than a few biochemical analytes\textsuperscript{11,12}. In some studies the standard methods for selection of subjects, sample processing and analytical system are not given which do not help to reproduce or compare data with future studies\textsuperscript{14}. While giving the reference ranges of different enzymes, temperature, pH and other optimized parameters are not mentioned\textsuperscript{9,15}. Moreover, the sample size is not as per IFCC recommendations as minimum of 120 subjects are required for a group to obtain reliable estimates\textsuperscript{13,14}. The reference range of newborns in the present study is different from other studies in paediatric age group from other areas of Pakistan\textsuperscript{10-14}. The reference values differ in geriatric age group from developed nations of Asia, Europe and America\textsuperscript{20-24,27-30}. No study on reference range in geriatric age group has so far been published from any Pakistani population except in the present study.

For exclusion of individuals from the reference sample group, modified physiological states (pregnancy, exercise, stress) intake of pharmacologically active agents (drug abuse, alcohol, smoking) and pathophysiological states (obesity, hypertension, heart, liver, kidney disease) have been taken care of in the present study to make the whole exercise useful\textsuperscript{5,6}. The values of plasma glucose, serum cholesterol, triglycerides and total protein were higher; urea, creatinine, ALT, AP were lower and uric acid and total bilirubin were comparable with those of Karachi population\textsuperscript{10}. It may be due to selection criteria of population, geographical or analytical factors. The values of plasma glucose serum cholesterol, triglycerides, urea, creatinine, uric acid, total bilirubin were lower and total protein ALT and AP were higher than American population\textsuperscript{17-19}. Similarly there were mild to significant differences, in various analytes when compared with different age and sex groups of Chinese\textsuperscript{19}, Indonesian\textsuperscript{21}, Australian\textsuperscript{22}, Dutch\textsuperscript{23}, Japanese\textsuperscript{24,25}, Italian\textsuperscript{26}, Spanish\textsuperscript{27}, Brazilian\textsuperscript{28} and Canadian\textsuperscript{29,30} populations. These differences may be due to racial and genetic differences, dietary habits and socioeconomical and analytical variables.

References

25. Handa, N. and Kojima, Y. Reference values of serum creatinine, uric acid and total protein. In: