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Reference Intervals for the Adult Population of Bahawalpur, Pakistan

LUBNA SARFRAZ, ASMA SHAUKAT, TANVEER H SHAH, *TARIQ M. ARAIN*

ABSTRACT

Aim: To establish reference values of ten commonly requested blood chemistry analytes for the population of Bahawalpur

Methods: A descriptive cross-sectional study was conducted including 800 subjects between the ages of 19-60 years, who were randomly selected according to the guidelines set by National Committee of Clinical Laboratory Standards. The reference values were calculated using 2.5th and 97.5th percentile as lower and upper reference limits, respectively.

Results: This study established the baseline values for ten commonly requested blood chemistry analytes in healthy Pakistani adults of Bahawalpur. In healthy young male adults the values were fasting plasma glucose, 3.7-5.4mmol/L; serum cholesterol, 3.4-5.2mmol/L; triglycerides, 0.7-2.2 mmol/L; urea, 3.8-8.5mmol/L; creatinine, 0.06-0.15mmol/L; uric acid, 209-440 µmol/L; Total bilirubin, 6.5-21.5 µmol/L; Total proteins, 55-76g/L; alanine aminotransferase (ALT), 16-45 u/L and Alkaline phosphatase (ALP), 130-280 u/L.

Conclusion: Age and sex specific reference intervals of some blood chemistry analytes were different from those reported in literature. It is, therefore, recommended that such studies should be carried out in other parts of Pakistan.

Keywords: Reference values, adults, Pakistanis, decision limits, blood chemistry analytes.

INTRODUCTION

Reference values of blood chemistry analytes are the most widely used medical decision making tools as laboratory tests are interpreted in their light[1]. We, therefore, need reference intervals for all the tests performed in a clinical laboratory[2]. These values are also specific to the laboratory that produces the test results. This means each laboratory must establish its own reference values using data from its own equipment and methods[3].

The usual practice in our setup is to use reference intervals given in standard reference books in which the information is based upon European population or the reference values provided by the manufacturers of analyzers and reagent kits. This can be misleading as a study conducted in Rawalpindi, Pakistan, showed that the reference intervals of serum calcium for the residents of that area were 2.03-2.55mmol/L and about 26% of the participants of the study had serum calcium lower than the lower normal limit given in reference books which is 2.15-2.55mmol/L[4]. A study in Kenya showed that their population had higher enzyme and electrolyte levels as compared to population of North America[5]. A similar study in Kuwait showed that reference interval of serum creatinin in Kuwaitis (50-114 µmol/L) was lower and they had relatively higher serum uric acid (141-423µmol/L) and total cholesterol values[6]. Out of 40 analytes studied in turkey, the reference intervals of only creatinine, direct total bilirubin, calcium, Blood Urea Nitrogen and potassium were similar to the reference intervals suggested by the manufacturer[7]. Thus the use of western reference values and intervals in our setup can lead to misdiagnosis and unnecessary medication[8].

In our country few such studies were conducted. A study in Rawalpindi established reference values for serum calcium[4] and a similar study in Multan established baseline values for haematological parameters[9]. There is need to conduct such studies in Pakistan and set reference intervals for our people who are different from those of the western world in many ways. We are not only living in a different geographical area but also have different climate, genetic makeup, customs and eating habits, all of which affect the reference values[10].

Moreover, the value of a variable associated with any particular individual is more likely to fall near the mean value for all the individuals in the particular population[11]. Thus mean values and reference values of blood chemistry analytes are likely to be specific to our population and different from other populations.

Deptt. of Pathology, QAMC, Bahawalpur
Correspondence to Dr. Lubna Sarfraz, Demonstrator, H. 408-C, Chowk Commercial Area, Satellite Town, Bahawalpur e-mail: sarfrazlubna@gmail.com Cell: 03337774607

MATERIALS AND METHODS

The study was a descriptive cross sectional study and was conducted in mohallas, city, slum and perislum areas of Bahawalpur and tests were carried out in Pathology Laboratory, Quaid-e-Azam Medical College. The study started in January 2012 and was finished in July 2012, after about seven months.

NCCLS recommends that for 99% confidence interval 198 samples are required[10,12]. Thus the population was divided into two age groups (19-40 and 41-60 years) and from each group 200 male and 200 female samples were collected, making 400 samples from each group and a total of 800 samples.

Simple random sampling was used in two stages. In the first stage, out of 18 union councils of Bahawalpur, eight were selected by lottery method and from each selected union council 100 house holds were selected, again by lottery method.

Healthy individuals of both sexes, aged between 19 to 60 years, who were residing in Bahawalpur for at least five years, and were willing to participate, were included in the study. According to IFCC recommendations[13], people with genetic diseases, liver or kidney diseases, recent fevers, recent trauma or transfusion, smokers, oral contraceptive users, drug abusers, alcoholics, pregnant females, overweight (BMI>30) and hypertensives were excluded from the study.

Data collection started after taking permission from Ethical committee, Bahawal Victoria Hospital, the Head of the Pathology department, Administrative officers of selected union councils and EDO. Selected houses were visited by the researcher and lady health workers of the selected union councils. The purpose of the study was explained. A questionnaire was used to interview people who were interested and their weights and blood pressures were recorded. Informed consent was taken from the willing individuals who fulfilled the inclusion criteria. Where no willing/eligible subject was found, the house next in number was selected and visited. When more than one eligible subjects were found, one was selected by lottery method and the names of the rest were recorded in case sufficient number of subjects were not found. In a few instances, the researcher had to contact the persons whose numbers were recorded earlier, to complete the required sample size.

Next morning the selected houses were visited by a phlebotomist and using a sterile syringe about 5ml of blood was drawn from fasting subjects. The collected sample of blood was immediately divided into two volumes. 3ml was put into a plain tube and remaining 2ml was put in a fluoride bottle for glucose analysis. The sample was immediately transferred in a cool box to the clinical chemistry laboratory of QAMC.

In the pathology laboratory, the collected samples were centrifuged according to CLSI instructions at an RCF of 1000g for 10 min and the serum and plasma obtained were separated. Plasma separated from the fluoride bottle was used for glucose analysis. Tests were run on Selectra Excel, which is a fully automated analyzer, using the established techniques[14] summarized in table 1. Measurement bias was controlled by calibration of instruments and repeating each test.

The data was statistically analyzed using SPSS. As the data was already categorized by sex and age, simple descriptive statistics was applied to it to calculate the mean, median, standard deviation and percentiles. Frequencies of variables (the selected analytes) were computed and examined as histograms. Any outliers detected were removed at that point[15]. The data followed a reasonably Gaussian distribution as the skewness and kurtosis co-efficients were between 1 and -1[16]. The intervals were calculated containing 95% values of each analyte, first by parametric (mean ±2SD) and then by non-parametric method (2.5^{th} and 97.5^{th} percentile)[17]. Values obtained by both methods are tabulated in tables 4 and 5. T-test was used to compare the means of various groups and p-value ≤0.05 was taken as significant

RESULTS

A total of 800 apparently healthy subjects between the ages of 19 to 60 years participated in the study. There were 400 males and 400 females. The mean, median and reference values for the blood chemistry analytes both by parametric and non-parametric methods are shown in Table 1 and Table 2. Although blood specimens were drawn from 800 subjects, 200 in each category, the test results for certain analytes in each category were a little less than 200 because of detection and removal of out liers (all values outside mean±3SD)[15].

Statistically significant (p value ≤ 0.05) gender related differences were seen in creatinine, uric acid and ALT values in both age groups. In the younger group, males had higher creatinine ($p<0.0001$), uric acid ($p<0.0001$) and ALT ($p=0.0092$) values. The same trend was seen in the older group, where the males showed higher values of creatinine ($p<0.0001$), uric acid ($p<0.0001$) and ALT ($p=0.001$) than the females of the same age group. No gender related differences were seen in mean values of glucose, cholesterol, triglycerides, urea, bilirubin, total proteins and ALP. Statistically significant differences were seen in different age groups in the values of

creatinin in both male and female groups (p<0.0001 each), with higher values in the older group. Similarly both males and females of the older group had higher cholesterol values (p=0.0002 and p< 0.0001 respectively) than the males and females of the younger group. Older females (41-60 yrs) had higher triglyceride (p=0.0013) and uric acid (p≤0.0001) mean values than the younger females. No such difference was seen in mean values of uric acid and triglycerides in case of males of the two age groups..

Table 1: Mean and reference values of blood chemistry analytes for the young adults (19-40yrs) of Bahawalpur city by parametric (ie mean ±2SD) and non- parametric method (2.5^{th} and 97.5^{th} percentile).

Analytes (units)	Gender	Mean	Median	Reference values by Parametric method	Reference values by Non-Parametric method
p. glucose	M	4.49	4.5	3.6-5.4	3.7-5.4
(mmol/l)	F	4.17	4.5	3.7-5.3	3.7-5.3
s.cholesterol	M	4.16	4.1	3.3-5.0	3.4-5.2
(mmol/l)	F	4.14	4.1	3.3-5.0	3.4-5.1
s.tryglycerides	M	1.49	1.5	0.8-2.2	0.7-2.2
(mmol/l)	F	1.44	1.45	0.7-2.1	0.6-2.1
s.urea	M	5.9	5.9	3.3-8.5	3.8-8.5
(mmol/l)	F	5.83	5.8	3.1-8.5	3.6-8.5
s.creatinine	M	0.09	0.09	0.06-0.14	0.06-0.15
(mmol/l)	F	0.08	0.08	0.05-0.12	0.06-0.13
s.uric acid	M	303.4	300	190-410	209-440
(umol/l)	F	260.8	265	137-380	140-370
s.bilirubin	M	13.3	13	5.4-21.2	6.5-21.5
(umol/l)	F	13.3	13	5.4-21.2	6-21
T.proteins	M	65.35	65	55.1-76.6	55-76
(g/l)	F	65.09	65	59.4-76.0	53-76
ALT	M	27.74	26.5	14-41	16-45
(iu/l)	F	25.94	26	12-40	13-40
ALP	M	206	205	135-277	130-280
(iu/l)	F	202.5	200	73-278	130-275

Table 2: Mean and reference values of blood chemistry analytes for the middle aged group (41-60 yrs) of Bahawalpur city by parametric (i.e., mean ±2SD) and non- parametric method (2.5^{th} and 97.5^{th} percentile).

Analytes (units)	Gender	Mean	Median	Reference values by Parametric method	Reference values by Non-Parametric method
p. glucose	M	4.67	4.65	3.9-5.4	4-5.6
(mmol/l)	F	4.68	4.7	3.9-5.4	4-5.6
s.cholesterol	M	4.31	4.3	3.6-5	3.7-5.2
(mmol/l)	F	4.31	4.3	3.6-5	3.7-5.2
s.tryglycerides	M	1.52	1.5	0.88-2.2	0.98-2.2
(mmol/l)	F	1.55	1.5	0.19-2.2	1.05-2.2
s.urea	M	5.9	5.9	3.6-8.3	3.8-8.5
(mmol/l)	F	5.9	5.9	3.6-8.4	3.8-8.4
s.creatinine	M	0.12	0.10	0.08-0.16	0.07-0.15
(mmol/l)	F	0.09	0.10	0.05-0.13	0.06-0.13
s.uric acid	M	306.7	300	210-430	220-430
(umol/l)	F	286.5	290	184-390	180-380
s.bilirubin	M	13.4	13	5.9-21	6.5-21
(umol/l)	F	13.3	13	5.8-21	6.5-21
T.proteins	M	65.5	66	55.2-76	55-75
(g/l)	F	65.1	65	54.8-75.6	54-75
ALT	M	28.6	28	15-43	16-44
(iu/l)	F	26.5	26	15-38	16-38
ALP	M	206.5	205	141-271	139-275
(iu/l)	F	205.7	205	141-270	139-270

Table 3: Suggested reference values of selected blood chemistry analytes for healthy adults (19-60 yrs) of Bahawalpur

Analytes (units)	Gender	19-40 yrs	41-60 yrs
p. glucose (mmol/l)	M F	3.7-5.4 3.7-5.3	4-5.6 4-5.6
s.cholesterol (mmol/l)	M F	3.4-5.2 3.4-5.1	3.7-5.2 3.7-5.2
s.tryglycerides (mmol/l)	M F	0.7-2.2 0.6-2.1	0.98-2.2 1.05-2.2
s.urea (mmol/l)	M F	3.8-8.5 3.6-8.5	3.8-8.5 3.8-8.4
s.creatinine (mmol/l)	M F	0.06-0.15 0.06-0.13	0.07-0.15 0.06-0.13
s.uric acid (umol/l)	M F	209-440 140-370	220-430 180-380
s.bilirubin (umol/l)	M F	6.5-21.5 6-21	6.5-21 6.5-21
T.proteins (g/l)	M F	55-76 53-76	55-75 54-75
ALT (iu/l)	M F	16-45 13-40	16-44 16-38
ALP (iu/l)	M F	130-280 130-275	139-275 139-270

DISCUSSION

This study reports age and sex specific mean and reference values for ten selected blood chemistry analytes in apparently healthy adults of Bahawalpur. There is no significant difference between the parametric and non parametric reference intervals, but as IFCC recommends the use of non-parametric method[18], it is suggested that these intervals should be used. Non parametric reference intervals are given in Table-3.

The mean values of glucose for all the four groups of this study are similar. Both upper and lower limits reported in this study are lower than the published intervals (4.1-5.9mmol/L)[19]. However our reference values are similar to those reported from a study on male and female Omani university students[20]. Our upper limit values are also lower than Kenyan (2.8-6.8mmol/l)[21], Kuwaiti values (3.7-6mmol/L)[6] and those reported from a study in Rawalpindi-Islamabad area (3.6-6mmol/L)[22]. Diet and geographical differences may be responsible for such a difference[23].

In case of cholesterol upper reference limits are more significant from clinical point of view. Upper reference values in this study in both age groups are lower than those of published values (3.21-5.64mmol/L)[9]. These values are also lower than those of Omani students (3.1-6.6mmol/L)[22]. Dietary and genetic differences may be responsible for this difference[23].

The upper reference values of this study are similar to published values in case of males (0.50-2.27mmol/L)[19] but the values for females are slightly higher than the published values (0.41-1.63mmol/L)[19]. Both male and female Omani students have lower triglyceride values which are 0.3-1.5mmol/L and 0.24-1.1mmol/L respectively. The triglyceride values reported from Rawalpindi-Islamabad study (0.6-2.2mmol/L) are similar to our values[22]. Dietary and genetic factors may be responsible for these similarities and differences[23]. Also triglyceride levels are affected by fasting state of the individual and tend to be higher in non-fasting state, so an issue of not fully complying with the instructions of the researcher may be there. Triglyceride levels are also higher in people living in areas where water is hard.

The mean values of urea are almost same in all the four groups of this study. The upper reference value of urea in this study is higher than the values reported from Rawalpindi (2.8-6.4mmol/L)[22]. Very hot climate of Bahawalpur resulting in a little dehydration of subjects may be a cause of this difference[23]. However the electrolyte values of the study participants were normal.

Our creatinine values are higher than those of Kenyan (0.06-0.12mmol/L)[21], Kuwaiti values (0.063-0.115mmol/L)[6] and African reference values (0.047-0.109mmol/L)[24]. Our values are closer to those reported from people of Rawalpindi (0.065-0.132mmol/L)[22]. As creatinine values are directly

related to muscle mass genetic factors may be responsible for this difference[23].

Our uric acid values are higher than those of the Omani students[20], Kenyans[21] and Kuwaitis[6], but similar to the published values[19] and to the people of Rawalpindi[22].

Upper reference limit of bilirubin values in this study are lower than the upper limit of published intervals (0-34umol/L)[19] but it is a little higher than the upper reference limit reported from Rawalpindi (5-18umol/L)[22]. The study from Eastern and Southern Africa[24] reports higher values (29-37umol/L). Environmental and genetic factors may be responsible for this difference[23].

Mean values of total proteins are similar in all the groups of this study, however it is lower than the Kenyan (57-89g/L)[21], Kuwaiti (63-78.8g/L)[6], African (58-88g/L)[24], Omani students (75-81g/L)[20], published values (60-78g/L)[19] and Rawalpindi values (57-83g/L)[22]. The difference may be due to geographical, environmental or genetic factors[23].

Upper limit of ALT in this study is higher than the Kenyan values (0-39 IU/L)[21]. It is similar to those of Rawalpindi study (15-45 IU/L)[22] and published values (<45 IU/L)[19], however, it is very much lower than those of Kuwaiti values (3-95 IU/L)[6] and African values (8-61 IU/L)[24]. Environmental and genetic factors may be responsible for this difference[23].

Mean ALP values are almost similar in the four study groups. The upper reference limits are higher than the Kenyan (63-201 IU/L)[21] and Kuwaiti values (3-95 IU/L)[6]. These values are a little lower than those of Rawalpindi study (196-297 IU/L)[22]. Genetic factors may be responsible for this difference[23].

CONCLUSION

The reference intervals presented in this study provide information about the ten commonly requested blood chemistry analytes, for the people of Bahawalpur. These values show mild to moderate differences from the published values and other Asian and Pakistani populations. These differences may be due to geographical, climatic, dietary, environmental and genetic differences or may be due to difference of methods of analysis. Whatever the cause is, it definitely shows that there is a need to do such studies and reference values should be established in various areas of Pakistan. Moreover, reference intervals should be set through an organisational structure which enables appropriate intervals to be set taking all relevant factors into account, including the opinions of expert clinicians. Medical decision limits may be more appropriate in case of certain analytes.

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