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Assessment of Reference Values for Selected Plasma Nutrients of Healthy University Students in Oman

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The objective of this study was to assess the reference intervals of specific nutrients in blood of Omani university students. Blood samples were collected from 302 randomly selected apparently healthy students (144 males and 158 females) aged 19-24 years. Plasma concentrations of total protein, albumin, calcium, phosphate, uric acid, glucose, triglycerides, total cholesterol and HDL-cholesterol were measured by Beckman Synchron diagnostic analyzer. Plasma globulin, A/G ratio and LDL and VLDL-cholesterol were calculated by standard methods. Reference intervals were estimated by determining the 5th-95th percentile of the population by truncating values for bottom and top 5% of the data. While there were no differences between mean plasma levels of male and female students for glucose, phosphate and total cholesterol, significant differences ($p<0.001$) were found between male and female subjects for total protein, albumin, calcium, uric acid, triacylglycerol, HDL, LDL and VLDL-cholesterol. However, the reference values were within the published reference ranges for both sexes. The present results revealed a good agreement with the published data from other laboratories for all the parameters analyzed. The data presented here may serve as Gulf population-based reference intervals for the selected analytes in the GCC region.

Key words: Omani students, blood chemistry, nutritional assessment, reference intervals, major nutrients

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INTRODUCTION

Clinical laboratory test results employed in diagnosis, screening, prognosis or nutritional assessment of patients, are usually compared with reference intervals (normal range). These reference values for a particular parameter are defined as the usual values for that parameter in a healthy population (Cembrowski and Martindale, 2005). The term reference interval, as recommended by International Federation of Clinical Chemists (IFCC), is used to denote the normal limits of laboratory data and among other criteria, emphasizes makeup of the reference population with respect to age, sex, genetic and socioeconomic factors (Poulsen *et al*., 1997). Inadequacy and inhomogeneity of the reference population often makes the acquired data of scant scientific quality and useless for studying related diseases (Alimonti *et al*., 2005).

For nutritional assessment, biochemical tests provide the most objective and quantitative data on nutritional status of groups and individuals much earlier than are manifested in anthropometric or appearance of clinical symptoms (Lee and Nieman, 2003). However, the criteria for assessment and use of reference intervals set by IFCC are also used in interpreting laboratory data on nutritional status (Zeman and Ney, 1996).

Illness and injury almost always leads to rapid deterioration in nutritional status. Conversely, changes in nutritional status as a result of malnutrition usually occur at a relatively slower pace. Hence, the laboratory data used to assess these changes must be interpreted differently than the laboratory data used to diagnose disease (Carlson, 2004). Blood chemistry values are essential markers in the diagnosis of disease such as abnormal blood lipid profile which is an important indicator mainly of cardiovascular disease and poorly controlled diabetes. Role of plasma glucose levels in establishing hyperglycemia and onset of diabetes mellitus has long been recognized. Plasma proteins and minerals viz., calcium and phosphorus, are important parameters in clinical conditions such as renal failure and bone disease. They are also very important markers in nutritional assessment of patients (Pagana and Pagana, 2002). Increased plasma uric acid levels have been implicated in gout, kidney failure and certain metabolic disorders and along with elevated levels of calcium and phosphorus, have been shown to increase the risk of renal stones (Rolfes *et al*., 2006). The variations in levels of different analytes in blood of different populations makes it essential to have reference values based on values from the target population itself so as to make a sound diagnosis or assessment. Moreover, any clinical or nutritional intervention would be best served by reference values based on the data from the same community.

At present, we are not aware of any published reference intervals for blood chemistry parameters for Omani population. In fact, reference ranges based on the data from natives of countries of the Gulf Cooperation Council (GCC) are rarely found in the literature (Olusi and Al-Awadhi, 2002). For interpretations, hospitals and clinical laboratories rely on either western reference values or those provided by reagent manufacturing companies.

Due to paucity of published reference intervals based on IFCC recommendations in the GCC region, the present study assessed selected reference blood chemistry parameters for apparently healthy university students in Oman.

MATERIALS AND METHODS

Subjects: This research project was evaluated and approved by the Sultan Qaboos University ethics committee on human subjects and written informal consent was obtained from each study participant. Out of an initial number of 478 students, after applying the given exclusion criteria, a total of 302 healthy student volunteers (144 males and 158 females) aged 19-24 years from Sultan Qaboos University were randomly selected for the study. According to IFCC recommendations (Ritchie and Palomaki, 2004), individuals taking medication and those presenting risk factors such as excessive body weight, smoking and those with a particular physiological state (e.g., pregnancy, lactation and strenuous exercise) and those on special diets were excluded from the study. Subjects with known diseases such as diabetes, jaundice, thalassemia, renal or heart diseases were excluded after a screening of their medical records obtained from the university health center.

Information including name, age, gender, smoking status, any blood disorder, family history of any chronic diseases, any recent illness (over the previous three weeks) and any medications was obtained with the help of a questionnaire. Subjects were familiarized with the questionnaire before coming for blood sampling. Weight and height of subjects was also recorded using standard equipment and protocol.

Sample collection: After a 12 h overnight fasting, blood samples were drawn by a registered nurse using a 21-guage stainless steel vacutainer needle from the antecubital vein of each subject's arm while in a sitting position. Approximately 15 mL of blood was withdrawn from each subject in the morning between 7.00 and

8.00 am and transferred to EDTA treated tubes . One aliquot of each blood sample was transferred into heparin treated tubes for estimation of calcium. Plasma was separated by centrifugation of blood at 3,000 rpm for 5 min and distributed into equal aliquots in eppendorf vials and stored frozen at -70°C till analysis.

Biochemical analysis: One vial each of EDTA treated plasma (heparin treated plasma for calcium) was used and samples were analyzed for levels of glucose, total protein, albumin, calcium, phosphate, uric acid and lipid profile viz., triglycerides, cholesterol and HDL-cholesterol. All measurements were carried out in accordance with the kit's manufacturer instructions on an automated clinical diagnostic analyzer (Beckman Coulter, Synchron CX5 Delta Clinical System, USA). The assay kits, calibrators and standards employed for measurements were all supplied by Beckman for each parameter. A program of internal quality control linked with the university hospital clinical laboratories external proficiency testing protocol was carried out during the study as part of the daily regular routine within the laboratory.

Globulin levels were calculated by difference (Globulin = total protein-albumin) and albumin to globulin ratio (A/G) determined. Similarly, VLDL and LDL were calculated according to the Friedewald formula (Johnson and McNutt, 1997):

$$VLDL = TG/5$$

$$LDL = TC-(HDL+VLDL)$$

Statistical analysis: The data was statistically analyzed using SPSS Statistical Software Package (SPSS Inc., Chicago, IL, USA) for Windows. Simple descriptive statistics was applied to the analytical data obtained to calculate the mean, Standard Deviation (SD), median and percentiles. To determine 5th-95th percentile of the population, values for bottom and top 5% of the data were truncated (Alimonti *et al*., 2005).

RESULTS

Table 1 shows the median, mean and standard deviation of the plasma concentrations of selected analytes from 5th-95th percentile of a total population of 302 healthy male and female Omani university students. The minimum and maximum plasma values (range) estimated for 5th-95th percentile of the population are also shown in Table 1 alongside the published and widely used Tietz Fundamentals of Clinical Chemistry for reference intervals (Painter *et al*., 2001) included in the table for comparison. These values will be referred to here on as the standard reference intervals. The estimated reference intervals are shown for the following

Table 1: Plasma concentrations (5th-95th percentiles) for selected plasma nutrient analytes of male and female Omani University students

Analyte	C.F[1]	Sex	n	Range[2]	Mean[2]	SD	Median	Published intervals[3]
Total protein (g L^{-1})	10.0	M	117	75.0-88	81.70*	3.70	82.00	64.0-83
		F	143	69.0-87	79.50	4.50	79.00	
Albumin (g L^{-1})	10.0	M	115	43.0-52	48.00*	2.40	48.00	35.0-52
		F	142	40.0-51	45.30	2.70	45.00	
Globulin (g L^{-1})	10.0	M	114	28.0-39	32.90	2.70	33.00	29.0-31
		F	142	28.0-41	34.00	3.10	34.00	
A/G ratio (albumin:globulin)	--	M	114	1.19-1.71	1.46	0.14	1.40	1.20-1.7
		F	142	1.07-1.59	1.33	0.14	1.30	
Calcium (mmol L^{-1})	0.25	M	122	2.3-2.6	2.47*	0.08	2.48	2.15-2.5
		F	142	2.2-2.6	2.40	0.09	2.40	
Phosphate (mmol L^{-1})	0.323	M	125	0.8-1.3	1.06	0.14	1.07	0.87-1.45
		F	143	0.8-1.3	1.08	0.12	1.10	
Glucose (mmol L^{-1})	0.0555	M	121	3.6-5.4	4.50	0.46	4.40	4.10-5.9
		F	137	3.6-5.3	4.47	0.44	4.50	
Uric acid (mmol L^{-1})	0.059	M	112	0.2-0.4	0.32*	0.05	0.32	0.26-0.45
		F	139	0.16-0.30	0.23	0.04	0.23	0.13-0.39
Triglycerides (mmol L^{-1})	0.0113	M	128	0.3-1.5	0.70*	0.27	0.62	0.50-2.27
		F	142	0.28-1.1	0.58	0.21	0.51	0.41-1.63
Cholesterol (mmol L^{-1})	0.0259	M	130	3.1-5.6	4.20	0.59	4.20	3.21-5.64
		F	144	3.1-5.8	4.30	0.61	4.18	3.16-5.59
HDL-C (mmol L^{-1})	0.0259	M	122	0.6-1.3	0.92*	0.17	0.91	0.78-1.63
		F	139	0.8-1.6	1.16	0.19	1.16	0.85-2.04
LDL-C (mmol L^{-1})	0.0259	M	116	2.1-4.3	2.95	0.55	2.90	1.71-3.81
		F	136	1.8-4.2	2.83	0.54	2.80	1.48-4.12
VLDL-C (mmol L^{-1})	0.0114	M	128	0.06-0.29	0.14*	0.05	0.13	0.10-0.45
		F	142	0.06-0.22	0.12	0.04	0.10	0.08-0.33

[1]Conversion factor: For conventional units divide by CF; *Significant difference between males and females for each parameter; $p<0.001$ analysis of variance; [2]Values represent 5th-95th percentile of a total population of 302 male and female university students estimated by truncating values for 5% of the subjects at top and bottom; [3]Reference intervals from Painter *et al*. (2001) for comparison

parameters: total protein, albumin, globulin, A/G ratio, calcium, phosphate, uric acid, glucose, triacylglycerol, total cholesterol, HDL, LDL and VLDL-cholesterol. Plasma levels of the analytes are documented in international units (SI units) which can be converted into conventional units using the conversion factors provided for each parameter. Mean plasma levels of total protein, albumin, calcium, uric acid, triacylglycerol, HDL, LDL and VLDL-cholesterol were significantly different ($p<0.001$) between male and female students. However, no gender difference in mean plasma levels of glucose and total cholesterol were observed. Total protein values were higher at both minimum and maximum levels, while as the globulins were higher at upper level compared to the standard reference values. Albumin values were higher at lower level but similar to the standard reference values at upper level.

Estimated values for calcium were found to be slightly higher at both ends while the glucose measured lower at both ends of the intervals compared to the standard reference intervals. Phosphate and uric acid values were nearly the same as the standard reference intervals.

Triglycerides, HDL-cholesterol and VLDL-cholesterol in both males and females were markedly lower at the upper levels and slightly lower at minimum levels compared to their respective standard reference intervals. Estimated values for cholesterol were the same, but LDL-cholesterol values were higher at both ends of the intervals when compared to the standard reference intervals.

DISCUSSION

Reference intervals are the most widely used medical decision-making tools. These values are central to assessing an individual's health status (Horn and Pesce, 2003). These values are commonly based on the measurements in reference population representative of a defined group of individuals (Painter *et al*., 2001; Horn and Pesce, 2003; Sasse, 1996). The present study reports reference intervals for specific plasma nutrients in apparently healthy Omani university students aged 19-24 years. The basic statistical treatment of data on 302 subjects as 5% trimmed mean, i.e., mean of the data in the 5-95% percentiles, standard deviation, median and minimum and maximum values (range) reveal a general agreement with the published data (Zeman and Ney, 1996; Painter *et al*., 2001). The percentile method was preferred because it requires no assumptions concerning the frequency distribution (Ash *et al*., 1983). While significant differences ($p<0.001$) were found between mean plasma levels of male and female students for total protein, albumin, calcium, uric acid, triacylglycerol, HDL, LDL and VLDL-cholesterol, the plasma concentrations were within the published reference ranges for both sexes.

According to Solberg (1987, 2006), reference interval is an arbitrary but common convention defined as the central interval bounded by the 2.5 and 97.5 percentiles. Another size or an asymmetrical location of the reference interval may be more appropriate in particular cases and thus the techniques are easily adapted to other locations of the limits. Horn and Pesce (2003) suggested that the 95% reference region would consist of a single value (the 95th percentile), representing the upper limit cutoff which is a more appropriate tool because the 97.5th percentile essentially wastes 2.5% at the lower end, which is of no interest in most cases. Similar principles would be applicable to the 5% lower limit cutoff as of importance in clinical diagnosis with specific parameters such as in the case of albumin and other protein fractions, calcium, phosphate and glucose (hypoglycemia). Five percent trimmed means and medians used in the present study for each analyte further strengthen a good central validity for all analytes as they are scarcely affected by the influence of the skewness in both directions (Alimonti *et al*., 2005). This gives more supportive evidence of having symmetric distribution for the values obtained and the data come from a Gaussian or normal population.

The reference values obtained from the subjects in the present study are in general agreement with those reported by Olusi and Al-Awadhi (2002), Painter *et al*. (2001), Reed *et al*. (1972), Khan *et al*. (1997), Furruqh *et al*. (2004) and Ashavaid *et al*. (2005). Even though there are well-known differences between the values generated with different analytical systems, some investigators have used manual methods for deriving such values (Khan *et al*., 1997; Furruqh *et al*., 2004). Painter *et al*. (2001) presented detailed and comprehensive tables of reference intervals but did not specify analytical systems used. However, their values were derived from different referred quality controlled laboratories in recognized clinical centers. Quality control in automated analytical systems can be maintained by use of accuracy control specimens, at both normal and pathological levels of concentration. Use of a number of control specimens included at fixed or random positions in each analytical run can further ensure precision and reliability of data obtained (Ashavaid *et al*., 2005). Moreover, during sample collection and measurement of different analytes, it is also important to maintain conditions as similar as possible to those prevailing in clinical practice. The present study was also conducted under conditions very similar to those of usual clinical practice. The analytical system (Beckman Coulter Synchron CX5) used in this study is similar to Beckman Coulter Synchron LX-20 (Olusi and

Al-Awadhi, 2002) and also similar to Beckman Coulter Synchron CX7 (Ashavaid *et al*., 2005). Hence, the data estimated by using Beckman Coulter Synchron LX-20 systems can also be applied to other Beckman Coulter generations, since identical analytical procedures for measurement of chemistries and serum indices are used in these systems (Vermeer *et al*., 2007).

Comparing the relative lower and the upper limits (two-sided reference intervals) of students with the standard published reference intervals (Painter *et al*., 2001), for total proteins, both the upper and lower limits were higher than the published values, whereas the reference values for albumin coincided with the upper limit but the lower limit was higher. However, for globulin, while the values for lower limits were relatively similar, the upper limits tended to be higher compared to published values. Calcium and phosphate levels overlapped the published values on both upper and lower limit, uric acid tended to be higher on both ends. Glucose appeared markedly lower on both upper and lower limit compared to published values. However, the lipid fractions exhibited lower values on both ends of the range except for LDL-C that showed higher values on both ends of the range, respectively, compared to published reference intervals (Painter *et al*., 2001).

For clinical purposes, only the one-sided reference regions are considered instead of the traditional two-sided reference interval, although by its very nature reference interval is two-sided, i.e., values that are too large or too small are considered abnormal and in case of commonly measured enzymes, the upper limit of the reference interval is used in medical decision making. Based on this, Horn and Pesce (2003) in their review concluded that the 95% reference region would consist of a single value, i.e., the upper limit cutoff, which in their view, is a more appropriate tool in clinical chemistry. However, the investigators of this study believe that this conclusion can not be applied for all estimated blood chemistry parameters. For evaluated data in the present study, the upper limit cutoff may be used for uric acid and lipid profile except HDL-cholesterol, but not for albumin and other protein fractions, nor for calcium, phosphate and glucose, since the lower values are as important as the higher intervals for clinical diagnosis.

In conclusion, the reference intervals presented in this study provide information about major blood chemistry parameters in university students as a uniform population. These values may serve as reference intervals for healthy individuals in the GCC countries, since similarities exist in the populations of these countries in food habits, climate, socioeconomic status, genetic makeup as well as religious and cultural values. Moreover, these reference ranges are in general overlapping with the reported published data from Kuwait (Olusi and Al-Awadhi, 2002), USA (Painter *et al*., 2001), Pakistan (Khan *et al*., 1997) and India (Furruqh *et al*., 2004; Ashavaid *et al*., 2005) and thus can be considered in establishing needed reference intervals of blood chemistry for Middle East in general and the Gulf region in particular.

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