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Reference Intervals for routine biochemical markers and body mass index; a study based on healthcare center database in northeastern Iran

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Abstract

Objective: Age and sex specific reference intervals (RIs) for some biochemical tests may be useful for their interpretation, due to the variations in lifestyle and genetic, or ethnic factors. The aim of this study was to obtain RIs for some routine biochemical markers including a serum lipid profile, fasting blood glucose (FBG), aspartate and alanine aminotransferase (AST and ALT), uric acid, and body mass index (BMI) in subjects who attended primary healthcare centers.

Methods: The large database of primary healthcare centers use RIs to report results for children, adolescents, young and old adults. RIs were obtained by using the indirect method, recommended by the CLSI Ep28-A3 guidelines.

Results: RIs for FBG, BMI and serum lipid profile, including triglyceride (TG), total cholesterol (TC), high-density lipoprotein cholesterol (HDL-C) and low-density lipoprotein cholesterol (LDL-C) in people aged 18 to 120 years, were obtained without age/sex segmentation. RIs for serum AST, ALT, and uric acid were obtained without age segmentation; though, these RIs were higher in males than females.

Conclusion: The RIs for AST, ALT and uric acid were higher in men; while, the RIs for the other variables were similar in both sexes. This is the first study reporting the use of indirect RIs for BMI.

Keywords: Reference Intervals, biochemical markers, primary healthcare center, CLSI Ep28-A3 guidelines

Introduction

A clinical diagnosis is based on clinical features, the use of laboratory tests and radiological imaging (1-3). Laboratory tests and imaging are used in the management of a disease and in monitoring the patient response to therapy (4, 5). Therefore, the accuracy of reference interval (RI) of laboratory tests is an important parameter for their interpretation (6). The recommended RIs are the central 95% of each laboratory test which are obtained among at least 120 healthy persons per age group of various areas; which is called the “direct method” (7, 8). A new recommendation for establishing RI is the use of a large database, that includes local population samples; which is called as “indirect method” (9). In the indirect method, a large population database may be obtained from outpatients who come to primary healthcare centers; thereby, the recruitment of participants is easier than direct method (10). The limitation of the indirect method is the inclusion of unhealthy people in the dataset (9).

Establishing the RIs for each sex is required because of the hormonal variations between the two sexes (11, 12). During the life course, hormonal status can affect the values of some biochemical tests (11-13). Therefore, the established RIs must be specified for each sex and age range (14-16). Furthermore, specific RIs may be required for each region due to differences in genetic and environmental, ethnic and cultural factors (17, 18). Furthermore, whilst there have been several studies that have reported the pediatric RIs for different biomarkers; the recommended RIs for older people in different regions are scarce (19). Routine laboratory tests include fasting blood glucose (FBG) and lipid profile, that are used to manage cardiometabolic disorders, such as diabetes mellitus and cardiovascular diseases (CVDs) (20, 21). CVD risk differs between men and women, in part attributable to the protective role of estrogen (22). Also, low testosterone concentration in men, especially among young men, can predispose them to the Metabolic Syndrome (MetS) (23). MetS is defined as altered lipid profile (including triglyceride [TG] and high-density lipoprotein cholesterol [HDL-C]), blood pressure, FBG and waist circumference (24). In addition, the prevalence of adiposity and thereby, insulin resistance is developing in children, especially in pubertal period (25). Therefore, the determination of RI for FBG and lipid profile for all age ranges may be useful in the management of diabetes mellitus, MetS and CVD (14).

Liver function tests (LFTs) are some other routinely biochemical assessments which are used to establish the function of the liver and biliary tract (26). According to the pattern of change in LFTs, a diagnosis of hepatic or cholestatic disease may be made (26).

Uric acid is used to assess purine metabolism and the possible diagnosis of gout (27). Uric acid is gathered in the human body by endogenous and exogenous sources; endogenous source is obtained by the purine metabolism in the body organs like kidney, liver, muscles and vascular endothelium, and the exogenous source is obtained by consuming animal protein rich foods (27). The excess

amount of uric acid (named as hyperuricemia) is caused by over-production or impaired excretion of uric acid (28). Most of the excretion of uric acid excretion is via the kidneys (27).

Specific RI for anthropometric parameters like body mass index (BMI) is required due to variations in body composition of men and women, as well, variations among normal physiologic puberty and aging process (13, 29, 30). A high BMI is a CVD risk factor (20); whilst a low BMI may be associated with malnutrition (31). The World Health Organization (WHO) recommends the 18.5 - 25 kg/m² as the normal range for BMI in adults of Asia (31). Although, the prevalence of obesity and malnutrition is different among countries of Asia (32); thereby, specific BMI range for every regions is required. Recently a systematic review and meta-analysis showed the overall prevalence of under-nutrition as 13.5% and over-nutrition as 21.2% in 26 countries of Asia; while, the prevalence of under-nutrition was higher in low- and lower-middle-income countries and the prevalence of over-nutrition was higher in upper-middle-income countries (32).

In the current study, we aimed to establish RIs for lipid profile, FBG, two of the LFTs (AST and ALT), uric acid and BMI in a large database of primary healthcare center with a wide age range (including pediatrics samples, adolescents and older individuals) of a population sample from Mashhad, in the northeastern of Iran, by usage of indirect method.

Method:

Study population

In this study we used the large database of primary healthcare center that all of 5000000 data sets are related to subjects aged between birth and 120 years that were gathered from 2017-2019. The sample population for each parameter was different according to available data; thereby, the sample size and age range of variables were different to each other. All participants signed the consent form, which was approved by the Human Research Ethics Committee of University of Medical Sciences.

Laboratory tests and assessment of body mass index

Collection of blood samples were done after 14-hours fasting over-night in Vacutainer® tubes. Blood samples were centrifuged at 5,000 g for 15 min at 4 degree centigrade for separation the serum in the clinical laboratories of health centers in Mashhad and its suburbs. Enzymatic method was utilized to measure total cholesterol (TC), TG, uric acid, FBG and low-density lipoprotein cholesterol (LDL-C) and HDL-C by using Pars Azmoun, Man and Biotech kits on an auto analyzer (Selectra XI). The intra- and inter-assay coefficients of variation (CV) were as 2% for TC, uric acid and FBG, 4% for TG, and 5% for LDL-C and HDL-C.

BMI was calculated by dividing weight (Kg) by the square of height (m²); weight was measured using electronic scales to the nearest 0.1 Kg and height was measured by a tape measure to the nearest millimeter.

Statistical analysis

The scatter plots of distribution of each factor were made based on age and sex; thereafter, age/sex partitioning was done through the Harris and Boyd method (7). Outliers were excluded by utilizing the Tukey's method (7). The central 95% was calculated for each factor to define the lower and upper limits of RI, by performing the indirect method, according to CLSI Ep28-A3c guidelines (9). The lower and upper limits of RIs were presented with 90% confidence intervals (CIs).

Results

Baseline characteristics of the population are shown in Table 1. Table 2 shows the RIs with 90% CI for upper and lower limits, for each variable for the sample population. RIs of FBG and BMI were obtained in people aged 0 to 30 years old, without age/sex partitioning. RIs of all fasted lipid profile parameters (TG, TC, HDL-C and LDL-C) were calculated in people aged 18 to 120 years, without age/sex division. RIs for serum AST and ALT were obtained in persons aged 10 to 30 years, without age differentiation; though, these RIs were higher in males than females. The RI for serum uric acid was obtained in people aged 1 to 96 years, with higher level in men than women; although, age partitioning was not applied.

Discussion

This study was conducted to assess the 95% RIs for some laboratory tests and BMI in a large database of men and women from a primary healthcare center by using the statistically indirect method based on the CLSI Ep28-A3c guidelines. A comparison of the results from the current study are compared to the previous reported RIs and are presented in Table 3. Although, there have been few studies which have reported RIs based on the indirect method. These studies compared RIs which were obtained by direct and indirect methods, and concluded that most of the RIs obtained by indirect method were near to the International Federation of Clinical Chemistry and Laboratory Medicine (IFCC) recommendations (33); while, Shaw *et al.* have explained that indirect RIs were useful in some cases, but we should not replace these RIs for healthy populations (34). Though, they used the large database of hospitalized pediatrics, which is the tertiary healthcare center, that affects on the results due to sickness of the population sample (34). Although, den Elzen *et al.* have suggested that the use of large data of primary healthcare centers is useful to improve unequivocal interpretation of biological tests; since, the clientele of primary healthcare centers are not severely ill, and also, may consult the general practitioner for routinely

observations (35). As well, we considered the people who came to primary healthcare center of Mashhad.

RIs of lipid profile

For TC and HDL-C three reports exist, two of them were for adults living in Turkey (33, 36) and one from a pediatric sample from Canada (34). The widest age range was in our population (18 – 120 years); while, the recommended RIs were similar in adults from Mashhad and Turkey. Although, sex differentiation was applied in two studies of Turkey, in contrast to our RIs. As serum HDL-C and TC range were higher in women in the study of Ilcol *et al.* (33); while, TC was higher in men of Bakan *et al.* study and HDL-C was higher in women in this study (36). The differences of two studies of Turkish people may be due to the differences in populations over time; since, Ilcol *et al.* recruited participants in 2004 – 2005 (33), while, Bakan *et al.* gathered participants in 2012 (36). In addition, the age range of Bakan *et al.* study was wider than Ilcol *et al.* study (18 – 65 vs. 18 – 45 years old). In the two studies from Turkey, the statistical method was the modified Bhattacharya method (33, 36). Also, in children in Canada, serum HDL-C and TC were not different among two sexes, except for HDL-C in children aged 13 to 18 years old (34). Also, there is a rising trend in children aged <13 years for HDL-C (34). Additionally, RI of TC was equal in pediatrics aged 1 to 19 years old in Canada (34). The only reported RI for LDL-C was from Turkish people in the study of Bakan *et al.* (36), which was similar to our RIs; although, in the Turkish population RIs were higher in males, in contrast to our population. Additionally, RIs of TG of two studies of Turkey were different to each other; while, the RI of Ilcol *et al.* study was nearer to our RI. It is implied that the serum TG range was higher in Turkish people during 2004 to 2012 (33, 36). Also, sex division was applied for Turkish persons, disagreeing to RI of TG in healthcare center of Mashhad.

The similar RI for all components of lipid profile in all age range of our population is in contrast to previous studies, which showed the increasing in TC and TG through aging process; as TC is elevated in human blood and accumulates in tissues by increasing in age (37). Furthermore, TG metabolism is altered by aging; since, fasted serum TG concentration is higher, and clearance rate of TG after eating foods is lower, and also, the lipoprotein lipase enzyme activity is reduced by aging process (38).

RIs of fasting blood glucose

The only previous indirect RI for FBG was obtained in middle-aged adults in Turkey (33); which showed that FBG was higher in males. Although, the available data of primary healthcare center of Mashhad was from children, adolescents and young adults (0 to 30 years old). This finding shows the aging process did not influence FBG-RI in Iranians younger than 30 years old; in contrast to this result, a recently published paper from United States demonstrated that the prevalence of prediabetes was higher in young adults compared to adolescents (24% vs. 18%,

respectively) (39). This variation, may be related to lifestyle factors (e.g. diet and activity level) and genetic differences; as high-fat diets can cause insulin resistance and impaired glucose through mammalian sterile-20₃ (MST₃/STK₂₄) pathway (40). While, Iranian diets are mostly rich in carbohydrates; the positive association of low carbohydrate and low FBG was resulted in a study of Tehran (capital of Iran) (41).

RI of liver and kidney function tests

The only previously reported indirect RI for uric acid is from Turkish people in 2004 – 2005 (33). In Turkish population, the uric acid range was higher in females; while, in population of Mashhad it was higher in males. As well, previous studies showed the higher levels of uric acid in males (42-44). Also, we observed a null difference in uric acid RI between various age ranges; while, previous studies have shown the increased uric acid level in elderly; according to muscle mass decline (sarcopenia), elevated oxidative stress and enhanced prevalence of CVDs and MetS, during aging process (44, 45).

Among LFTs, we obtained RIs for AST and ALT; with higher range in males for both. As seen in Table 3, all previously reported indirect RIs for AST and ALT were higher in men than women (33, 35, 36); except for in Canadian children, in which both RIs were equal among both sexes (except for adolescents aged 12 – 18 years for AST and 13 – 18 years for ALT) (34). Both of these enzyme levels are used to evaluate the liver function status; while, ALT is more specific than AST, because of the presence of AST in some other tissues, like muscles, kidneys, pancreas and cardiac cells (46). Therefore, some other situations like the muscle mass atrophy/hypertrophy and disturbances in heart, kidney and pancreas can alter AST level (46). Also, the rising trend was observed in populations of Netherlands (35) and Canada (34); in contrast to our population. Bussler *et al.* have demonstrated that in children and adolescents aged below 16 years old, the peak of ALT activity level was defined in infancy (in two sexes), and between 7.5 – 9.5 years in girls, and 11.5 years in boys; while, in early adolescent ALT was fallen (47). Furthermore, Brussler *et al.* reported the downward trend for AST in both genders in children and adolescents (47). The variation between Brussler *et al.* study and other mentioned studies may be due to the method of analysis and also, differences between baseline characteristics of populations.

Strengths and limitations

The greatest strength of this study was the use of a large database to obtain indirect RIs, using a sample populations from primary healthcare center. The limitation of the indirect method is consideration of unhealthy people, whilst those who come to primary healthcare centers do not usually have severe disease, and also, may come to these centers only to consult the general practitioner for routinely observations (35). Another strength is the consideration of a wide age range for each parameter; including children, adolescents, young and old adults. Although, the age range was limited for some of the variables, due to the available data. Also, this is the first study that reported indirect RI for BMI in a large database evaluation.

Conclusion

In the current study, we obtained the specific RIs for two genders and various age ranges for routinely biochemical tests and BMI, by utilizing the indirect method, which considered large databases for recommendation RIs. In this study, the RIs of uric acid, AST and ALT were higher in males. Although, the other variables had not different RIs among men and women. Also, age partitioning was not applied for none of the variables.

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Table 1. Characteristics of the population based on a large database from primary healthcare centers in Mashhad, Iran.

Variable	mean	SD	median	Q3-Q1
Age (y)	20.31	6.06	21	26-15
TC (mg/dl)	161.20	34.64	158	180-139
FBG (mg/dl)	83.10	16.10	82	89-76
HDL (mg/dl)	46.97	14.12	45	52-39
LDL (mg/dl)	96.26	28.49	93	112-77
TG (mg/dl)	109.68	69.04	96	132-70
Uric acid (mg/dl)	4.73	1.57	4.41	5.50-3.70
ALT (IU/L)	25.05	55.91	17	26-13
AST (IU/L)	23.78	40.10	20	25-16
BMI (Kg/m ²)	21.80	4.83	21.48	24.50-18.30

Abbreviations: TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglyceride; FBG: fasting blood glucose; BMI: body mass index; AST: aspartate aminotranferase; ALT: alanine aminotransferase.

Table 2. Reference Intervals for routine biochemical tests and body mass index based on a large database from primary healthcare centers in Mashhad, Iran.

Variable	Age (y)					
		Sample size (n)	Lower limit	Upper limit	Lower confidence	Upper confidence
FBG (mg/dl)	0-30	162572	63.4	104.9	60.4-66.4	100.4-109.4
TC (mg/dl)	18-120	708872	121.8	242.7	114.8-128.8	230.5-254.9
HDL (mg/dl)	18-120	283109	30.1	67	28.2-32	63.4-70.6
LDL (mg/dl)	18-120	274676	55.6	171.1	21.2-60	160.4-181
TG (mg/dl)	18-120	308516	26.7	225.6	23.1-30.3	206.5-244.7
Uric acid (mg/dl)	1-96	3975	2.7	10.1	2.5-2.9	9.4-10.8
		M 1484	2.8	8	2.6-3	7.5-8.5
		F 2491	2.2	6.2	2-2.4	5.8-6.6
ALT (IU/L)	10-30	4049	7.1	35.2	6.4-7.8	32.6-37.8
		M 1489	7.5	60.7	6.5-8.5	55.6-65.8
		F 2560	6.7	31.2	6-7.4	28.9-33.5
AST (IU/L)	10-30	3845	10.7	32.8	9.9-11.5	30.7-34.9
		M 1351	11.9	34.9	11-12.8	32.8-37
		F 2494	10.5	30.2	9.7-11.3	28.4-32
BMI (Kg/m ²)	0-30	346232	20.2	26.2	19.5-20.9	25.4-27

Abbreviations: TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglyceride; FBG: fasting blood glucose; BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase.

Table 3. Comparison of RIs from patients attending primary healthcare centers in Mashhad to previously published studies.

Variable	RIs of other studies			RIs of primary healthcare center of Mashhad
FBG (mg/dl)	Turkey 2006 (33) Hospital-based data N= 73,883 18 – 45 YO M: 74.94 – 109.89 F: 69.90 – 106.83			Primary healthcare center N= 162,572 0 – 30 YO M & F: 63.4 – 104.9
TC (mg/dl)	Turkey 2006 (33) Hospital-based data N= 73,883 18 – 45 YO M: 81.96 – 277.22 F: 112.90 – 256.34	Turkey 2016 (36) Hospital-based data N= 1,366,948 18 – 65 YO M: 116.76 – 258.28 F: 112.12 – 252.48	Canada 2014 (34) Hospital-based data 0 – 18 YO <1 YO, M & F: 28.22 – 249.77 1 – 18 YO, M & F: 90.47 – 218.84	Primary healthcare center N= 708,872 18 – 120 YO M & F: 121.8 – 242.7
HDL (mg/dl)	Turkey 2006 (33) Hospital-based data N= 73,883 18 – 45 YO M: 28.99 – 59.93 F: 31.70 – 73.07	Turkey 2016 (36) Hospital-based data N= 1,366,948 18 – 65 YO M: 20.87 – 50.26 F: 27.83 – 78.10	Canada 2014 (34) Hospital-based data 0 – 18 YO <1 YO, M & F: 0.38 – 73.85 1 – 4 YO, M & F: 11.98 – 74.24 4 – 13 YO, M & F: 22.42 – 80.42 13 – 18 YO, M: 20.10 – 72.30; F: 15.46 – 80.42	Primary healthcare center N= 283,109 18 – 120 YO M & F: 30.1 – 67
LDL (mg/dl)	Turkey 2016 (36) Hospital-based data N= 1,366,948 18 – 65 YO M: 57.61 – 192.55 F: 56.06 – 173.21			Primary healthcare center N= 274,676 18 – 120 YO M & F: 55.6 – 171.1
TG (mg/dl)	Turkey 2006 (33) Hospital-based data N= 73,883 18 – 45 YO M: 37.17 – 198.24 F: 33.63 – 195.58	Turkey 2016 (36) Hospital-based data N= 1,366,948 18 – 65 YO M: 52.21 – 276.12 F: 55.75 – 239.83		Primary healthcare center N= 308,516 18 – 120 YO M & F: 26.7 – 225.6

Uric acid (mg/dl)	Turkey 2006 (33) Hospital-based data N= 73,883 18 – 45 YO M: 1.84 – 5.54 F: 2.68 – 7.56				Primary healthcare center N= 3975 1 – 96 YO M: 2.8 – 8 F: 2.2 – 6.2
ALT (IU/L)	Turkey 2006 (33) Hospital-based data N= 73,883 18 – 45 YO M: 6 – 40.80 F: 3 – 32.40	Turkey 2016 (36) Hospital-based data N= 1,366,948 18 – 65 YO M: 10 – 55 F: 6 – 30	Netherlands 2019 (35) Primary care center N= 7,574,327 1 – 5 YO= M: 9 – 35, F: 10 – 33 6 – 12 YO= M: 10 – 35, F: 9 – 33 13 – 18 YO= M: 9 – 42, F: 8 – 35 19 – 50 YO= M: 13 – 71, F: 9 – 44 51 – 65 YO= M: 13 – 64, F: 11 – 52 66 – 80 YO= M: 11 – 54, F: 10 – 46 >81 YO= M: 9 – 42, F: 8 – 37	Canada 2014 (34) Hospital-based data 0 – 18 YO <1 YO, M & F: -1 – 65 1 – 13 YO, M & F: 3 – 61 13 – 18 YO, M: 3 – 60, F: 7 – 45	Primary healthcare center N= 4049 10 – 30 YO M: 7.5 – 60.7 F: 6.7 – 31.2
AST (IU/L)	Turkey 2006 (33) Hospital-based data N= 73,883 18 – 45 YO M: 10.8 – 37.2 F: 6 – 36	Turkey 2016 (36) Hospital-based data N= 1,366,948 18 – 65 YO M: 9 – 28 F: 9 – 24	Netherlands 2019 (35) Primary care center N= 7,574,327 1 – 5 YO= M: 24 – 53, F: 23 – 49 6 – 12 YO= M: 19 – 42, F: 15 – 46 13 – 18 YO= M: 14 – 39, F: 12 – 33 >19 YO= M: 14 – 43, F: 13 – 38	Canada 2014 (34) Hospital-based data 0 – 18 YO <1 YO, M & F: -50 – 139 1 – 7 YO, M & F: 17 – 64 7 – 12 YO, M & F: 14 – 53 12 – 18 YO, M: 6 – 54, F: 7 – 44	Primary healthcare center N= 3845 10 – 30 YO M: 11.9 – 34.9 F: 10.5 – 30.2
BMI (Kg/m²)	No other studies recommended RI for BMI by using indirect method.				Primary healthcare center N= 346232

		0 – 30 YO M & F: 20.2 – 26.2
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Abbreviations: TC: total cholesterol; HDL-C: high-density lipoprotein cholesterol; LDL-C: low-density lipoprotein cholesterol; TG: triglyceride; FBG: fasting blood glucose; BMI: body mass index; AST: aspartate aminotransferase; ALT: alanine aminotransferase; M: male; F: female.