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Reference Values for Serum Lipid Profiles in Iranian Adults: A Spline-Based Quantile Regression Method

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ABSTRACT

Background: Reference measurements are used to screen for abnormal blood lipids. The problem is that these reference values obtained in one population cannot be effective for another population. This study aimed to determine the reference values for blood lipids profiles in the population aged 25-64 years in Yazd. **Methods:** This descriptive study was based on the data of Yazd Health Study (YaHS) on 3800 adults by cluster sampling. The data set included gender, age, total cholesterol (TC), triglyceride (TG), low-density lipoprotein-cholesterol (LDL-C), and high-density lipoprotein-cholesterol (HDL-C). The linear percentile regression model and the generalized additive model for location, scale, and shape (GAMLSS) were fitted to the data and the reference values were predicted according to the regression coefficients. R-3.0.1 software was used for data analysis. **Results:** Refrence values for TC, LDL-C, and HDL-C were 109.43-275.72, 45.58-177.70, and 29.95-62.22 mg/dl. The trend of TC, TG, and LDL-C levels increased with age in both genders, but the trend of HDL-C in men decreased with age and remained almost constant in women. **Conclusion:** In this study, for the population of Yazd, reference values for blood lipids were different in both genders and age groups. Reference values for lipid profile increased in men and women with age.These findings can be used in both prevention and clinical decisions.

Keywords: *Cholesterol; Blood lipids; Reference values*

Introduction

Over the last two centuries, industrial revolutions, technology, and economic and social developments have made main changes in the type of disease leading to death. Cardiovascular diseases have emerged as the predominant chronic diseases in most parts of the world, so it is predicted that cardiovascular disease is the leading cause of death and disability in the world at the beginning of the 21st century (Kelly *et al.*, 2012). Yazd city is located in Yazd province in the center of Iran and has a dry climate . The population of Yazd in 2017 was 656474 people. According to a study conducted on of people's health in Yazd in 2015, 27.9% of adults had obesity, 43.7% had abnormal blood

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pressure, and 13.6% had type 2 diabetes (Mozaffari-khosravi *et al.*, 2020).

According to the World Health Organization (WHO), at the beginning of the 20st century, cardiovascular diseases accounted for less than 10% of deaths in the world, but at the beginning of the 21st century, this rate increased to more than 50% in developed countries and 25% in developing countries. According to the WHO, developing countries will face an epidemic of non-communicable diseases over the next two decades. It is predicted that mortality from these diseases increased by 77% from 1990 to 2020, and these diseases will be the first leading cause of death in the world (World Health Organization, 1997). One of these diseases is cardiovascular disease. One of the important indicators for diagnosing cardiovascular diseases is determining the amount of blood lipids (lipid profile) and comparing it with the reference values in that community. Lipid profile is a strong risk factor for cardiovascular disease in various populations and is affected by factors, such as age, race, living environment, geographical conditions, gender, lifestyle, socioeconomic status, heredity, and eating habits. Moreover, risk factors, such as hypertension, diabetes mellitus, increased total cholesterol (TC), triglycerides (TG), low-density lipoprotein-cholesterol (LDL-C), and decreased high-density lipoprotein-cholesterol (HDL-C) in healthy people increase the risk of cardiovascular disease (Mainous *et al.*, 2004).

Due to racial and geographical differences, the average values of these indicators are different in different societies. Therefore, it is not easy to generalize the average of these indicators as a comparative reference in other societies. Therefore, it is necessary to separately measure the reference values of this biological index in different communities.

There are several ways to achieve normal values. One of these methods is experimental quantifiers. In this method, normal values are obtained based on percentiles obtained from age groups and it is not stable for the outlier. So that, to estimate the percentiles with appropriate accuracy in each age group, a large sample size is needed. On the other hand, by categorizing, the information of close groups may be lost (Luo, 2007, Neter *et al.*, 1996). Thus, many governments and the United Nations use percentile curves to measure the general well-being of populations, formulate health policies, and implement planned interventions and track their impact (Kutner *et al.*, 2005). Quantile regression is defined first, which is a powerful statistical method with the ability to calculate and draw 100 different regression curves corresponding to different percentile points, expressing a more comprehensive and complete picture of the data. The general shape of a percentile diagram includes a series of smoothed percentile curves that show how to change selected percentiles (usually 7th, 10th, 25th, 50th, 75th, 90th, and 95th percentiles) for the criterion measured in opposition to some independent variables (Cole and Green, 1992). Quantile regression is a widely used method in determining reference values as well as reference curves in many cases. This method is widely used in various fields of medical sciences (Yu *et al.*, 2003).

Quantile regression methods are applied to all clinical variables (or generally all biological variables) with a continuous quantitative scale. The resulting reference values are also called reference limits or norms. After reaching the reference values, if the amount of the variable for a person is less than the lower limit or more than the upper limit, the person may have a disorder. Hence, quantile regression is gradually emerging as a statistical method for estimating conditional quantile function models (World Health Organization, 2006).

Due to the importance of determining the native reference index for blood lipid measurements for each region and considering that in Yazd city no study has been conducted on reference values in age and gender groups for lipid profiles. This study was conducted to determine the reference values of blood lipid measurements based on age and gender using the GAMLSS statistical method, for res

Ethcal considerations: This study was approved by the research ethics committees of Shahid

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idents of Yazd city.

Materials and Methods

Study design and participants: The present study was a cross-sectional study that was performed using data collected in Yazd Health Study (YaHS) in Yazd city. The method of collecting the required data in the research was as follows: the questionnaires were completed in person by trained individuals by visiting the homes of people who were selected by multi-stage cluster sampling. Selection of clusters and sub-clusters was completely random and using a table of random numbers. In YaHS, 10,000 people were participated and about 4,000 people went to the laboratory for blood sampling and their information was used in this study.

YaHS was performed on people aged 25-64 in 2014-2015. The participants entered the study after obtaining informed consent. In this study, all questionnaires were without personal information and their information was confidential. It was done to take blood samples with their conscious desire and consent. All examination and blood sampling costs of the participants were free.

Measurements: for all participants, including information such as height, weight, age, gender, marital status, history of acute and chronic diseases, and blood lipids (TG, TC, LDL-C, HDL-C) were measured. Profile lipid values were obtained by fasting blood sampling and using chemical techniques and BT3000 autoanalyzer up to 6 hours after blood sampling. Details of the study have already been published (Mirzaei *et al.*, 2018).

Reference values should be based on a healthy community. Given that reference values were extracted in this study, biological differences should be obtained as much as possible in a disease-free community. This is a logical method; since the goal is to determine the reference values for healthy people in the community. Accordingly, to determine reference values, any individual who had at least one risk factor for hyperlipidemia (hypertension, obesity, diabetes) was excluded. The risk factors were consistent with criteria of NCEP ATP III (2005 revision) (Grundy *et al.*, 2005).

Ethcal considerations: This study was approved by the research ethics committees of Shahid Sadoughi University of Medical Sciences, code IR.SSU.SPH.REC.1395.74.

Data analysis: The data were entered into SPSS v.25.0 and analyzed using descriptive analysis (frequency, mean, and standard deviatio n), graphs, and statistical tables. Kruskal-Wallis and Mann-Whitney tests were used to compare lipid profile values in age and gender groups. P-value less than 5% was considered significant. GAMLLES package in R-4.0.5 software was used to calculate the values of quantile regression coefficients and draw reference graphs. The significance of quantile regression coefficients was calculated by the Wald test. Analysis of variance test for quantile regression was used to check the parallelism of the lines (Konker, 2017).

Results

According to the exclusion criteria to determine the reference population, the study population consisted of 3441 adults aged 25-64 years with a mean and standard deviation of 46.88 ± 13.8 years, including 1583 (46%) males and 1858 (54%) females.

Table 1 shows the mean and standard deviation with a confidence interval for lipid indices in men and women. The results showed that the mean TC (P = 0.0001) and LDL-C (P = 0.001) were higher in women than men, and this difference was statistically significant. In addition, the mean HDL-C was 52.22 mg/dl in women and 45.32 mg/dl in men and this difference was statistically significant (P = 0.0001). In the GAMLSS model, among selected statistical distributions for TC, Box-Cox Cole and Green (BCCG) distribution had the lowest value of the Akaike information criterion (AIC), and for TG, LDL-C and HDL-C, Box-Cox power exponential (BCPE) distribution was selected.

Table 2 shows the mean and standard deviation with a confidence interval for lipid indices in age groups in men. The results showed that the mean TC in the age group of 54-50 years was higher than the others, and the difference in the age groups was statistically significant (P = 0.0001). The mean LDL-C was different in age groups and this difference was statistically significant (*P* = 0.004). Meanwhile, the mean HDL-C in men in age groups was not statistically significant (P = 0.75). The reference values for lipid profile in age groups for men in the form of 2.5th and 97.5th percentiles are presented in **Table 2**.

Table 3 shows the mean and standard deviation with a confidence interval for lipid indices in age groups in women. The results showed that the mean TC in the age group of 40-44 years was higher than the others, and the difference in the age groups was statistically significant (P = 0.0001). The mean LDL-C was different in age groups and this difference was statistically significant (*P* = 0.004). Meanwhile, the mean HDL-C in men in age groups was not statistically significant (*P* = 0.75). The reference values for lipid profile in age groups for women in the form of 2.5th and 97.5th percentiles are presented in **Table 3**.

Table 1. Descriptive information of blood lipids levels in men and women.

Blood lipids	sex	N	Mean	SD	95% confidence interval Lower bound	Upper bound	P-value[a]
TC (mg/dl)	Men	1583	191.19	42.21	189.11	193.27	0.0001
	Women	1858	197.44	42.93	195.49	199.40	
	Total	3441	194.56	42.71	193.14	195.99	
LDL-C (mg/dl)	Men	1583	111.75	36.18	109.97	113.54	0.004
	Women	1858	115.34	36.72	113.67	117.01	
	Total	3441	113.69	36.51	112.47	114.91	
HDL-C (mg/dl)	Men	1583	45.34	8.94	44.90	45.78	0.0001
	Women	1858	52.22	11.17	51.71	52.73	
	Total	3441	49.05	10.77	48.69	49.41	

TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; [a]: Student t-*test*.

Table 2. Descriptive information of blood lipids levels in age groups in men.

Blood lipids	Age groups (y)	N	Mean	SD	Percentiles 2.5th	97.5th	95% confidence interval Lower bound	Upper bound	P-value[a]
TC (mg/dl)	20-24	59	191.07	42.79	111.46	291.57	179.92	202.22	0.0001
	25-29	157	195.49	45.37	111.43	290.39	188.34	202.64	
	30-34	175	190.61	43.02	112.58	288.57	184.19	197.03	
	35-39	192	195.97	42.29	115.72	287.62	189.95	201.99	
	40-44	178	198.54	46.30	116.92	286.71	191.70	205.39	
	45-49	184	197.51	39.70	117.75	284.86	191.74	203.29	
	50-54	158	198.51	40.63	121.93	281.81	192.13	204.90	
	55-59	119	192.84	35.27	124.58	276.61	186.44	199.24	
	60-64	151	183.74	37.43	122.54	270.84	177.72	189.76	
	65-69	128	177.08	41.17	116.95	265.94	169.88	184.28	
	>70	82	162.11	33.76	110.42	260.52	154.69	169.53	
	All	1583	191.19	42.21	109.43	275.72	189.11	193.27	
LDL-C (mg/dl)	20-24	59	115.74	35.88	49.76	192.17	106.38	125.09	0.0001
	25-29	157	117.47	37.36	48.01	186.10	111.58	123.36	
	30-34	175	107.37	35.33	46.79	180.85	102.10	112.65	
	35-39	192	116.75	36.60	46.39	178.23	111.54	121.96	

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	40-44	178	117.44	37.14	45.78	178.12	111.94	122.93	
	45-49	184	114.39	36.82	45.38	177.74	109.03	119.74	
	50-54	158	114.52	38.07	45.99	177.36	108.54	120.50	
	55-59	119	109.91	33.59	46.63	177.10	103.81	116.00	
	60-64	151	106.66	33.67	47.58	176.24	101.24	112.07	
	65-69	128	104.73	35.76	48.85	174.25	98.47	110.98	
	>70	82	95.00	27.97	49.85	170.52	88.86	101.15	
	All	1583	111.75	36.18	45.58	177.70	109.97	113.54	
HDL-C (mg/dl)	20-24	59	45.15	9.12	27.63	63.39	42.78	47.53	0.75
	25-29	157	45.43	9.51	28.18	63.82	43.93	46.93	
	30-34	175	44.79	8.94	28.78	64.43	43.46	46.13	
	35-39	192	45.76	8.79	29.50	64.83	44.51	47.01	
	40-44	178	45.90	8.82	29.93	64.54	44.59	47.20	
	45-49	184	44.63	9.26	30.08	64.01	43.28	45.97	
	50-54	158	45.03	8.80	30.52	63.71	43.65	46.41	
	55-59	119	44.26	7.66	31.05	63.88	42.87	45.65	
	60-64	151	45.81	9.03	31.59	64.65	44.36	47.27	
	65-69	128	45.90	9.04	31.85	65.17	44.32	47.48	
	>70	82	46.27	9.47	31.56	64.88	44.19	48.35	
	Total	1583	45.34	8.94	29.95	62.22	44.90	45.78	

TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; [a]: ANOVA *test*.

Table 3. Descriptive information of LDL-C, HDL-C, and TC levels in age groups in women

Blood lipids	Age groups (y)	N	Mean	SD	Percentiles		95% confidence interval		P-value[a]
					2.5^{th}	97.5^{th}	Lower bound	Upper bound	
TC (mg/dl)	20-24	40	201.50	42.03	123.26	300.78	188.06	214.94	0.0001
	25-29	143	209.48	47.57	125.21	304.97	201.61	217.34	
	30-34	239	210.08	52.59	127.21	306.53	203.38	216.78	
	35-39	192	209.28	44.14	129.49	303.29	203.00	215.56	
	40-44	246	210.76	40.16	132.70	296.39	205.72	215.80	
	45-49	190	199.09	37.37	134.56	285.86	193.75	204.44	
	50-54	209	196.32	35.19	132.20	273.57	191.52	201.12	
	55-59	157	187.38	33.10	127.04	263.61	182.16	192.59	
	60-64	180	183.06	39.23	121.85	258.57	177.29	188.83	
	65-69	162	176.64	32.16	119.30	256.50	171.65	181.63	
	>70	100	167.53	36.11	117.21	257.38	160.36	174.70	
	All	1858	197.44	42.93	29.95	62.22	195.49	199.40	
LDL-C (mg/dl)	20-24	40	112.84	37.03	49.49	212.17	100.99	124.68	0.0001
	25-29	143	124.98	41.33	51.34	208.99	118.15	131.81	
	30-34	239	122.47	47.35	52.64	205.20	116.44	128.51	
	35-39	192	121.14	36.91	52.98	200.54	115.89	126.40	
	40-44	246	124.74	37.56	54.27	195.68	120.03	129.46	
	45-49	190	116.05	33.09	56.50	190.38	111.31	120.78	
	50-54	209	116.54	30.11	57.92	182.87	112.44	120.65	
	55-59	157	108.83	30.11	56.85	175.97	104.09	113.58	
	60-64	180	106.94	32.50	54.57	172.23	102.16	111.72	
	65-69	162	101.37	26.84	52.77	168.94	97.21	105.54	
	>70	100	95.34	28.59	49.05	166.92	89.67	101.01	
	All	1858	115.34	36.72	52.80	110.20	113.67	117.01	
HDL-C (mg/dl)	20-24	40	53.80	11.85	27.63	63.39	50.01	57.59	0.55
	25-29	143	51.48	11.24	28.18	63.82	49.62	53.33	
	30-34	239	52.29	11.32	28.78	64.43	50.85	53.74	

Table 3. Descriptive information of LDL-C, HDL-C, and TC levels in age groups in women

Blood lipids	Age groups (y)	N	Mean	SD	Percentiles 2.5th	Percentiles 97.5th	95% confidence interval Lower bound	95% confidence interval Upper bound	P-value[a]
	35-39	192	52.29	11.54	29.50	64.83	50.64	53.93	
	40-44	246	53.08	12.02	29.93	64.54	51.57	54.59	
	45-49	190	51.40	11.17	30.08	64.01	49.80	53.00	
	50-54	209	51.07	9.43	30.52	63.71	49.79	52.36	
	55-59	157	52.16	10.02	31.05	63.88	50.58	53.74	
	60-64	180	52.11	11.29	31.59	64.65	50.45	53.77	
	65-69	162	53.62	12.33	31.85	65.17	51.71	55.54	
	>70	100	52.20	10.48	31.56	64.88	50.12	54.28	
	All	1858	52.22	11.17	34.20	50.93	51.71	52.73	

TC: Total cholesterol; LDL-C: Low density lipoprotein cholesterol; HDL-C: High density lipoprotein cholesterol; [a]: ANOVA *test*.

Discussion

TC, HDL-C, LDL-C, and TG are checked periodically by physicians to assess risk factors for heart disease (Detection and Adults, 1993, Solberg and Stamm, 1991). According to the recommendations of the International Federation of Clinical Chemistry **(**IFCC) and national cholesterol education programme (NCEP), sufficient samples should be selected from the population in each country or region and lipid indices in individuals should be evaluated. There are usually large variations in lipid levels according to different populations, age, gender, eating habits, lifestyle, socioeconomic status, and race (D'agostino *et al.*, 2008). Given that Yazd city has a dry and desert climate and has a lifestyle and eating habits adapted to this weather conditions, determining the normal range of lipid indicators can help doctors to diagnose people at high risk of cardiovascular disease.

In the present study, the normal range for TC in men and women was presented based on age group. However, in similar studies, such as the Agrawal et al. in India (Agrawal *et al.*, 2014) and Kaur et al.in Punjab (Kaur *et al.*, 2012), the normal range for TC was presented only for the whole community and did not provide age and gender. In the current study, the normal range based on percentiles for cholesterol was 193.1-195.9 mg/dl for the whole population, for HDL-C was 112-114.9 mg/dl, and for LDL-C was 48.7-49.4 mg/dl. In the study by Agrawal *et al.*, the normal range based on percentiles for cholesterol was 85-211 mg/dl for the whole population, for HDL-C was 20-63 mg/dl, and for LDL-C was 50-147 mg/dl (Agrawal *et al.*, 2014). The upper limit values of the normal range in the present study were lower than the normal range of the global standards (200 mg/dl for TC, 160 for LDL-C, and 60 for HDL-C). According to the data of the present study, the upper limit for the normal range obtained in most age and gender groups was higher than the world standard for cholesterol and lower than the standard for HDL-C and LDL-C (Detection and Adults, 1993).

The difference between normal ranges obtained in the present study and global standards may be due to differences in lifestyle, diet, and low physical activity in urban areas. Comparison of the results obtained in the present study with the study of Goswami *et al.* (Goswami and Bandyopadhyay, 2003), Das *et al.* (Das and Saikia, 2009), and Agrawal *et al.* (Agrawal *et al.*, 2014) showed that the mean TC, LDL-C, and HDL-C in the present study were higher than similar studies.

In studies of other countries, reference values are shown in the form of 2.5, 97.5 percentiles, 5^{th} and 95^{th} percentiles, and mean ± 2SD. The results of the study in Tehran showed that the reference values for TC in men were 126-126 mg/dl and in women 117-235.9 mg/dl and were upper bonds smaller than the present study. Similarly, the reference values for

LDL-C and HDL-C were somewhat different from the current study (Rahmani *et al.*, 2019). Another similar study was conducted in Ahvaz in southern Iran and the results were upper bonds lower than the present study. This difference could be due to dietary patterns and lifestyle (Jalali *et al.*, 2013).

Studies in other countries, such as China, Japan, and the United States have reported higher HDL-C values in women which are consistent with the results of the present study. (Ashavaid *et al.*, 2005, Carroll, 1993, Das and Saikia, 2009, Li *et al.*, 2004, Li *et al.*, 2005, Noma *et al.*, 1991, Reddy *et al.*, 2006). Therefore, for better evaluation, it is recommended to conduct the same study in the whole Yazd province, especially in rural areas, to have better generalizability for the results. As a result, people at high risk for cardiovascular disease can be accurately identified. Fat-tailed distribution usually produces outlier and such points have a profound effect on the estimation of ordinary least squares. So that they estimate the least squares and consequently make the prediction difficult; since the analysis is the least squares method.

To solve this problem, the researchers used other regression methods that are less sensitive to the outlier. These methods produce estimators that reduce the effect of outliers. Therefore, the most important reason for using quantile regression in this study was its inherent strength against outlier in the response variable, while normal regression is sensitive to the outlier. The effect of the outlier on the slope of quantities of quantile regression is limited. Moving the observations away from the fitted regression quadrant has little effect on the model fit. Finally, if the data is skewed and the goal is to obtain the relationship between the explanatory variables and all aspects of the distribution, especially the terminal quantifiers, the quantile regression model or the GAMLSS method can be used.

Modern statistical methods, such as the generalized collective model for location, scale, and shape may not be widely used by researchers. One reason is that researchers are not familiar with these methods. Another reason is the need for relatively high sample size for each of the classes in the covariates or independent variables. Overall, a better understanding can extend the use of this method and improve the understanding of relationships in health research.

Conclusion

In this study, for the population of Yazd, reference values for blood lipids were different in both sexes and age groups. These findings can be used in clinical decisions. Therefore, it is suggested that future studies examine the reference range for other blood parameters that were not examined in this study, such as triglycerides, lipoproteins.

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Authors' contributions

Momayyezi M, and Fallahzadeh H, conceived and developed the idea for the article; Mirzaei M, collected data; Momayyezi M, and Fallahzadeh H, prepared numerous drafts; Fallahzadeh H, contributed to the statistical analysis; Fallahzadeh H, Momayyezi M, and Mirzaei M revised the manuscript. All the authors read and approved the final manuscript.

Conflict of interest

The authors declare that there is no conflict of interest.

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