Original Article

Reference Values for Serum Total Cholesterol Concentrations Using Percentile Regression Model: A Population Study in Mashhad

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Abstract

Background and purpose: Serum total cholesterol (TC) concentrations are affected by several factors including ethnicity, diet, geographic, and environmental determinants, and are related to another disease, including hypothyroidism, and renal and liver disease. It is associated with an increased risk of cardiovascular disease, particularly if associated with high levels of serum low-density lipoprotein (LDL). The distribution of TC levels within populations may be useful, and the current study aimed to determine the reference values and specific cut points in a population sample from Mashhad, Iran.

Materials and Methods: A cross-sectional study was conducted, and data was collected from 6518 individuals (2483 men and 4035 women) aged 25–64 year-old living in Mashhad city using a stratified cluster random sampling design.

Reference values for borderline and high TC levels in three age groups were obtained using a percentile regression model. Data were analyzed using Quantreg Software Package and R Ver. 3.1.2 Software.

Results: Within the population sample, 38% of the subjects were male and 62% of them were female. The mean and standard deviation for age were found to be 47.07 ± 9 years and 45.28 ± 9 years for men and women, respectively. Percentile regression showed that borderline TC levels for men and women aged 25-64 years were 198-216 mg/dl and 176-243mg/dl, respectively. The values for defining high TC levels were also 226-239 mg/dl in men and 202 - 271 mg/dl in women.

Conclusion: Our study estimated reference values and cut points for borderline and high TC separately in both men and women, and age-related sub-groups for a population derived from Mashhad. These findings could be used in local policy plans to allocate health resources.

Keywords: Reference Value; Total Cholesterol; Percentile Regression; Population

1. Introduction

High total cholesterol (TC) levels are associated with an increased risk of coronary heart disease (CHD) and stroke (1). Worldwide, it has been estimated that approximately one third of CHD is attributable to a raised serum total cholesterol, specifically LDL-cholesterol. Since CHD is one of the most common diseases both in developed and developing countries, high total cholesterol is a major cause of disease burden and mortality. Globally, a high TC is estimated to cause 4.5% of total mortality. (2.6 million deaths), and 2.0% of total disability-adjusted life years (DALYs), which is equal to 29.7 million DALYS. Therefore, detecting and lowering high level of serum TC can be helpful in reducing CHD and total mortality (2). Understanding the distribution of TC levels in each country is a valuable index for use in health planning. Since serum TC is caused by several factors including race, genetic factors, geographic, and environment determinants, diet and distribution of serum TC in different regions may be important to plan public health programs (3). Long-term exposure to high levels of plasma cholesterol may lead to the occurrence of CHD (4). Different cut-off values have been reported for old and young people (5-7). However, according to the expert panel of the US National Cholesterol Education Program (NCEP), the total cholesterol <200mg/dl was defined as desirable, 200-239 mg/dl as borderline, and >=240 mg/dl as high (5, 6). The absence of a reference value for a health system may lead individuals at low risk to be treated inappropriately, and even young people may not be recognized as being at potential risk and deprived of treatment. Reference values for total serum cholesterol have previously been published for an Iranian population in 2007 (7), but it is possible that these values may have changed over the last 8 years. Reference values can be used as a screening tool for identifying high risk groups in the society (8). There are many methods for determining reference values (9), such as percentile regression, which provides a robust estimate in real datasets that are less consistent with normality assumption (10, 11). This method was first proposed by Koenker and Bassett (1978), and may be regarded as an expansion on the ordinary least squared (OLS) regression technique (11, 12). Percentile regression can also be used to explore and describe the conditional distribution of response variables given predictor variables. In other words, it is a technique for investigating how dependent variables are associated with independent variables in all segments of the response distribution.

Knowing the risk factors of CHD could help the planning, training, and interventions aimed at reducing disease in young adults and middle-aged people. The aim of the present study was to determine the cut-off points and reference values for serum TC of individuals aged 25-64 years old who lived in Mashhad, Iran.

2. Materials and Methods

This study was based on a cohort survey approved by Mashhad University of Medical Science with the code of 85134 (Mashhad Study) (13). The dataset consisted of three variables including age, gender, and total cholesterol on 6518 individuals aged 25–64 years who were selected randomly from Mashhad

urban population and gathered through stratified-cluster sampling method. For sampling purpose, in the original study, the whole city was, according to geographical features, divided into three districts. In each district, the population within the catchment area of each health center was regarded as a cluster. The attempt had been to select a number of clusters proportional to the population of each district. The information from the studied people was collected by going door-to-door. These people were justified in person about the plan by the statistical agent of the study, and in the case of desiring to participate in this plan, a written consent was obtained. In this stage, the family list including residence address, cluster number, telephone number, age, and the number of people living in that house were recorded, and then a complete map of selected cluster was provided. Then, an invitation was submitted to them to participate in the study and spend the time on visiting and filling questionnaire, then the implementation of required examination and experiments was specified. The subjects were, then, entered into the study after obtaining a written informed consent. The fasted lipid profile was also obtained using routine methods on an auto-analyzer BT3000 for individuals who were fasted for at least 12 h. To simplify the calculation, the values for serum TC were rounded. It should be noted that the study had received ethics approval of the Deputy of Research of Mashhad University of Medical Sciences (MUMS) before being conducted. The individuals aged 25–64 years old, with total cholesterol, and Gender were included in the study. To control for errors in measurement and recording data, the extreme outliers were also computed. Extreme outliers were

defined as values 2 times the interquartile range (25th to 75th percentiles) below the first quartile and above the third quartile for each total cholesterol (7,14). Consequently, the individuals with total cholesterol measurements ("extreme outliers") were excluded from the gender-specific samples. Quantile regression is a statistical technique intended to estimate and derive inferences about conditional quantile functions. Just as classical linear regression methods based on minimizing sums of squared residuals enable one to estimate models for conditional mean functions, quantile regression methods offer a method for estimating models for the conditional median function, and the full range of other conditional quantile functions (10, 11). For pth percentile regression line, p% of the data are below, and (100-p) % of the data are above the fitted line. In comparison to the OLS methods, percentile regression approaches are more efficient especially when the distribution of residuals does not comply with normality assumptions. The percentile regression model was used to determine the reference values for borderline and high TC levels in three age groups. The parameter estimation of the regression model is derived using the Least Absolute Deviation (LAD) (11). The cutoff points of serum TC of 7 selected smoothed percentiles (5th, 10th, 25th, 50th, 75th, 90th, and 95th) were presented for three age groups. NCEP has previously reported the seventy-five population percentile of serum TC level as a cutoff point of high 'risk percentile' for CHD (6). On this basis, serum TC levels were divided into three categories, so that serum TC below 75th percentile was defined as the desired level, while the amount between 75th and 90th percentile was

considered as borderline, and the quantity greater than 90th percentile was defined as high (7, 15). The subjects were then categorized according to age (25-34, 35-44, and 45-64 years old). The collected data were analyzed using the Quantreg Software Package and R Ver. 3.1.2, while the significance level was set at $p < 0.05$. The study was approved by the Ethic Committee of Mashhad University of Medical Sciences with the code 931326.

3. Results

Data was collected from 6518 individuals (2483 men and 4035 women) aged 25–64 year-old in Mashhad city, while 38% of the subjects were men and 62% of them were women. Mean and standard deviation of age were 47.07±9 years and 45.28±9 years

in men and women, respectively. In order to assess if cholesterol changes in a linear fashion with age, locally weighted scatter plot smoothing (LOWESS) and smooth spline methods were fitted to the scatter plot of cholesterol against age. LOWESS and smooth spline methods in Figure1 show the Serum TC among younger men (under 45) which is increased by age, but man's total cholesterol (above 45) has been decreasing gradually with an increase in age, but in women trend Serum TC among aged 25–64 years increases with age. Figure 1 shows that the fitted line using either methods follows a smooth line, so the application of percentile linear regression models to the present data could be justified.
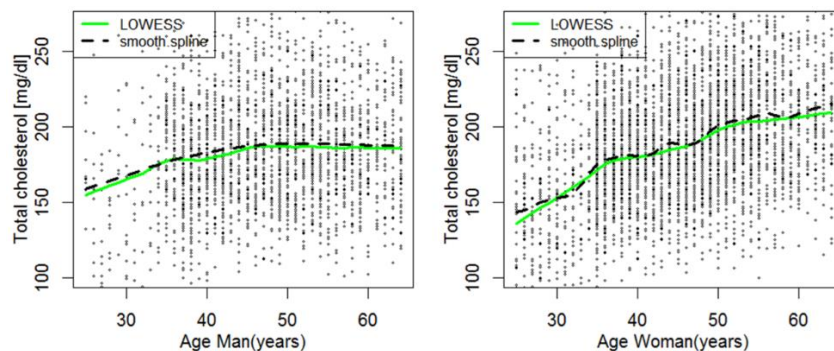

Figure1. Nonparametric regression for men and women aged 25-64 years old.

Table 1 shows the smoothed percentiles for men and women. It is illustrated that Serum TC increased gradually by age both for men and women. The median (50th percentile) of serum TC in men and women aged 25 years was 169 and 153 mg/ dl, respectively, while in men and women aged 64 years, the corresponding values were 190 and 219 mg/dl, respectively. In women, the increase of cut-off values for borderline and high TC levels ranged from 176 mg/dl to 243 mg/dl, and the corresponding values were from 198 mg/dl to 216 mg/dl in men. A high serum TC level was also defined by a cut-

off of 226 mg/dl and 202 mg/dl, respectively, in men and women at the age of 25 years. However, the corresponding values were 239 mg/dl and 271 mg/dl, respectively, in men and women at 64 years of age. A high serum TC level in men aged 25 years and women aged 39 years was also approximate (226mg/dl). On average, the serum TC levels were higher for men than for women until the age of 42 years, and at this age, the mean value was equivalent (231 mg/dl) in both sexes. Beyond 42 years, the levels of TC were higher in women, though.

Table1. Total cholesterol (mg/dl) levels for men and women
(percentiles: 5 10 25 50 75 90 95) according to age

Age	Percentile for men							Percentile for women						
	5th	10th	25th	50th	75th	90th	95th	5th	10th	25th	50th	75th	90th	95th
25	104	119	146	169	198	226	258	90	107	129	153	176	202	214
26	105	120	147	169	199	226	257	92	108	131	155	178	204	216
27	106	121	147	170	199	226	257	94	11	132	157	179	206	218
28	107	122	148	170	200	227	257	95	112	134	159	182	208	220
29	108	123	148	171	200	227	257	97	114	135	160th	183	209	222
30	109	124	149	171	200	227	257	99	115	137	162	185	211	224
31	110	124	150	172	201	228	257	101	117	139	164	187	213	226
32	110	125	150	173	201	228	257	103	119	140	165	188	214	228
33	111	126	151	173	202	229	256	104	121	142	167	190	216	230
34	112	127	151	174	203	229	256	106	123	143	169	192	218	232
35	113	128	152	174	203	229	256	108	124	145	170	194	220	234
36	114	129	153	175	203	230	256	110	126	147	172	195	221	236
37	115	129	153	175	204	230	256	112	128	148	174	197	223	238
38	116	130	154	176	204	230	256	113	129	149	176	199	225	240
39	117	131	154	175	205	231	256	115	131	151	177	200	226	242
40	118	132	155	177	205	231	255	117	133	153	179	202	228	244
41	119	133	156	178	205	231	255	119	135	155	181	204	230	246
42	120	133	156	178	206	232	255	121	136	156	182	205	231	248
43	121	134	157	179	206	232	255	122	138	158	184	207	233	250
44	121	135	157	179	207	232	255	124	140	159	186	209	235	252
45	122	136	158	180	207	233	255	126	142	161	188	211	237	254
46	123	137	159	180	208	233	255	128	144	163	189	212	238	256
47	124	138	159	181	208	233	254	130	145	164	191	214	240	258
48	125	138	160	182	209	234	254	131	147	166	193	215	242	260
49	126	139	160	182	209	234	254	133	149	167	194	217	244	262
50	127	140	161	183	210	235	254	135	150	169	196	219	246	264
51	128	141	162	183	210	235	254	137	152	171	198	221	248	266
52	129	142	162	184	210	235	254	139	154	172	199	223	250	268
53	130	142	163	184	211	236	254	140	155	174	201	224	252	270
54	131	143	163	184	211	236	253	142	157	175	203	226	254	272
55	122	144	164	185	212	236	253	144	159	177	204	227	256	274
56	123	145	165	186	212	237	253	146	161	179	206	229	257	276
57	124	146	165	187	213	237	253	148	163	180	208	231	259	278
58	125	147	166	187	213	237	253	149	164	182	210	233	260	280
59	126	147	166	187	214	238	253	151	166	183	211	234	262	282
60	127	148	167	188	214	238	253	153	168	185	213	236	264	284
61	128	149	168	189	214	238	252	155	170	187	215	238	266	286
62	129	150	168	189	215	239	252	157	171	188	216	239	268	288
63	130	151	169	190	215	239	252	158	173	190	218	241	270	290
64	131	151	169	190	216	239	252	160	175	191	219	243	271	292

Figure 2 is a graphical representation of TC values against age for the different percentiles. Those values of TC that fall below the lower dotted line are regarded as desirable, whereas values above the higher dotted line are considered high. Values that are located between the dotted lines are also classified as borderline. The estimated equation of cut-off values for men aged 25-64 (75th percentiles) was $187+0.45\times(age)$, while it was $134+1.73\times(age)$ for women. Hence, with each one year increase in age, the cut-off point for borderline TC increased to be 0.45 ml/dl, and 1.73 ml/dl in men and women, respectively. The equation for the cutoff values for high TC level (90th percentiles) was $217+0.35 \times (age)$ in men and $160+1.73\times(age)$ in women. With one year increase in age, the cut-off point for high

cholesterol also increased to be 0.45 ml/dl and 1.73 ml/dl in men and women, respectively. Serum TC in early adolescence in men was more than women of the same age, but this was reversed after the age of 42 years. This situation remained even in the older age, so that women had a higher level of serum TC compared with men (Table 1). To investigate if the quantile regression lines fitted for each percentile was parallel with the others, the ANOVA method of data analysis was used. As is clear in Figure2, the quantile regression lines were parallel in women (F=1/23, P-value= 0/28, Df=6) but not in men (F=3, P-value= 0.006, Df=6). Further analysis of the data in men revealed that if the fitted line for 95th percentile was omitted, the rest of lines would be parallel (F=1/77, P-value= 0/11, Df=5).
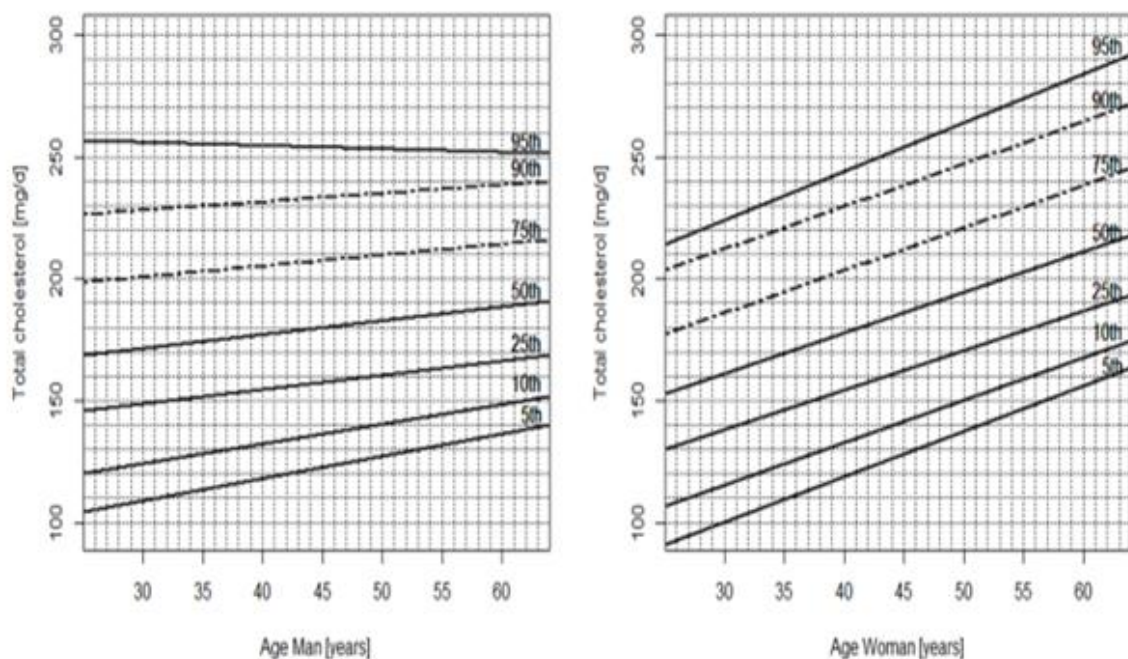

Figure 2. Total cholesterol level percentiles for men and women aged 25-64 year old.Dashed lines show the borderline of TC level.

The researchers have summarized 75th and 90th percentiles of serum TC in three age groups (25-34, 35-44, and 45-64). Then, 75th and 90th percentiles in each age group

were rounded to derive cut-points for borderline and high levels of serum TC specific to Mashhad population.

Table 2. Reference values of total cholesterol according to age groups

Age group (y)	Women			Men		
	N			N		
25-64	4035	**Borderline**	**High**	2483	**Borderline**	**High**
25-34	669	185	210	436	200	225
35-44	1485	200	225	695	205	230
45-64	1881	225	255	1352	210	235

As can be seen in Table 3, the regression coefficients for the selected percentile were found to be positive, indicating that an increase in the serum TC was expected with age increase. Moreover, the regression coefficients for age of 25–64 years old, showed that a one-year increase in age for percentiles (the 0.05, 0.10, 0.25, 0.50, 0.75, 0.90, and 0.95), would increase the serum TC level as follows: 1.43 1.32 1.17 1.18 1.29 1.33 1.40, while according to the results of OLS regression, this increase in the serum TC was about 1.26 unit. Figure 2 presents the values of regression coefficients with 95% confidence intervals which are given in Table 3. This chart shows that in the study of the population, the slope of least squares regression line and percentile regression was always positive. Percentile Regression Slope also reduced in percentiles before 25th percentile but it was still positive, and between the 25th and 50th percentiles, it was almost constant, while after the 50th percentile, it increased.

Table 3. Regression coefficients of total cholesterol and their %95 confidence intervals in the models of ordinary least squares regression (OLS), and percentile regression for the 0.05 ,0.10, 0.25, 0.50, 0.75, 0.90 and 0.95 percentiles (Q5, Q10, …)

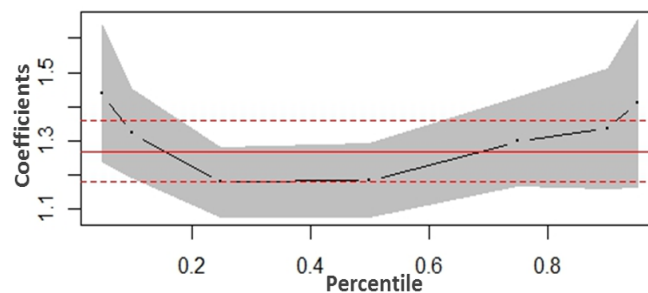|	Intercept	Regression coefficient	95% CI
OLS	129	1.26	1.19 - 1.37
Q5	60.62	1.43	1.25 – 1.63
Q10	78.71	1.32	1.21 - 1.45
Q25	106.88	1.17	1.08 – 1.29
Q50	130.45	1.18	1.08– 1.30
Q75	151.62	1.29	1.18 – 1.45
Q90	176.33	1.33	1.18 – 1.54
Q95	189.81	1.40	1.18 – 1.66

Figure 3. Percentile Regression Coefficients for smoothed percentiles with 95% confidence intervals (the red line shows the slope of the least squares method).

Figure 3 is a schematic presentation of Table 2 which demonstrates how the coefficients changed with the selected percentiles. Figure 3 indicates the coefficients of percentile regression for different values of percentiles 5th and 10th. Also, the coefficients followed a decreasing trend under 25th percentile but an increasing trend above the 50th percentile, while they remained unchanged between these two values.

4. Discussion

In the present study, the researchers made an attempt to estimate the reference values of total serum cholesterol levels using the percentile regression model. The results of this study could be applied as a basis for judgment on who should be regarded as having borderline or high serum TC in comparison to others in the same age and sex in people aged 25 to 64 years living in Mashhad. Evidences show that Most of the studies have previously been done on cholesterol in the USA (5, 6, 16). In recent years; some other countries determine reference values by using empirical percentiles, as well (17-18). Borderline TC level was reported to be 209-243 mg/dl and 205-261 mg/dl in men and women aged 25-64 years old, respectively, by NCEP. However, in the current study, it was found to be 176-243 mg/dl for women and 198-216 mg/dl for men. Hence, the serum TC concentration distribution in Mashhad (Iran) was found to be lower than in USA (5, 6). Because of the sampling method (Complex Sample Survey analysis) by Hosseini (7), TC measurements were transformed to the logarithmic scale in order to obtain approximately normal distributions. They also reported to be 200 mg/dl TC level for women aged 30 years and men aged 32 years, but in our study,

this value was observed to be 39 for men and 30 for women. The fact that high TC level was lower in men than women was consistent with the finding of some other studies (3). However, their estimation of these levels was almost 10 mg/dl lower than that of us. This difference could be attributed to differences in age distribution and lifestyle or methods of analysis. In another study, the borderline TC was 210-234 mg/dl in men and 199-221 mg/dl in women aged 35-44 years old in Korea (17), which was not in line with the findings of this study. Hence, in the present study, the borderline TC in men was less than Korean study, whereas in women, it was greater than their research. At the same time, the desirable TC level in Korea was less than 217 mg/dl and 231 mg/dl for males and females aged 45-64 years, respectively, while it was less than 210 mg/dl for men and 225 mg/dl for women in our study. It could further be concluded that in general the TC level of Mashhad people was less than Korean. In a different context, 5 percent of the Malaysian population (18) had TC greater than 240 mg/dl, while in the present study, it was 10%. TC level of 25% of the people who took part in this study were reported to be in borderline; however, it was only 15% in Malaysia. Also, 85% of Malaysian had lower cutoff point of 200 mg/dl, whereas in our study, this value was 65%. High TC reference values in Malaysia until 50 years of age was greater in men as compared to women, but this pattern reversed after 50 years of age. On the other hand, high TC reference values in our study until 42 years was greater in men as compared to women but this value changed in favor of women compared to men after 42 years of age. This fact indicated that the individuals who participated in the Mashhad study with respect to Malaysian

were exposed to increasing amount of TC in women as compared to men in lower ages (13). High serum TC level in men and women aged 45-64 years of age were 235 mg/dl and 255 mg/dl, respectively. This value was 10 units (mg/dl) less than high TC cut points in the study of Hosseini et al. in both sexes and same age groups (7). The main reason for this controversy could be attributable to non-identical distribution of individuals in various ages of 25-64 years in our study. Another reason may be due to lower total cholesterol of Mashhad people in comparison with Iranian population average TC.

5. *Conclusion*

We have found different reference values and cut points of borderline and high serum TC separately for sex and age group for the population living in Mashhad. Serum TC concentration changed with age and was different in men and women. Because of the important role of cholesterol level in increasing the risk of non-communicable disease including cardiovascular disease, it seems that these findings could be used in local preventive plans and health policies.

Limitations and suggestions

The great advantage of percentile regression compared to empirical quantile is that it uses all data in order to determine reference values. However, our sample was collected from a limited geographical area. Further studies are needed to identify people who are at risk of heart disease, and longitudinal studies are required to validate the risk categories.

Acknowledgments

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