

## ESTABLISHING REFERENCE INTERVALS FOR LIPID PROFILE IN HEALTHY ADULT POPULATION OF AMBALAPPUZHA NORTH GRAMA PANCHAYATH, ALAPPUZHA, KERALA.

### Biochemistry

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| <b>Saleena Prameela CR</b>    | Associate Professor, Department of Biochemistry, Government Medical College Trivandrum.                       |
| <b>Bindu Gopinathan Nair*</b> | Professor, Department of Biochemistry, Government Medical College Trivandrum.<br>*Corresponding Author        |
| <b>Shamha Beegum M</b>        | Assistant Professor, Department of Biochemistry, Government Medical College Trivandrum.                       |
| <b>Kezia Blessinda R</b>      | Senior Resident, Department of Biochemistry, Sree Gokulam Medical College and Research Foundation Trivandrum. |

### ABSTRACT

**Introduction:** We established reference intervals for serum lipids in an apparently healthy adult population of Ambalappuzha North Grama panchayath in Alappuzha district of Kerala.

**Methods:** This cross-sectional study was done on 447 apparently healthy individuals of both sexes aged between 18-75 years who attended a health camp organized by the Department of Biochemistry, Government T.D. Medical College in association with the Ambalappuzha North Grama Panchayath. Samples were analyzed in a Beckman Clinical chemistry analyzer. Mean, standard deviation, median, 90% confidence limits for median and central 95<sup>th</sup> percentile were calculated. The 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles formed the lower and upper reference limits of population. Statistical analysis was done using SPSS ver. 16 software.

**Results:** Reference interval for Total cholesterol was 132.2-262 mg/dl, for HDL-cholesterol 28.2-62 mg/dl, for LDL-cholesterol 66.2-175 mg/dl, for triglyceride 65-218.8 mg/dl, for VLDL 13-43.8 mg/dl and Total cholesterol/HDL-cholesterol ratio 2.67 – 6.4. Reference intervals for Total cholesterol, LDL-cholesterol and Triglyceride were higher than the desirable limits suggested by NCEP ATP III guidelines and the reference intervals established by many studies. There was a gradual increase in these parameters with age up to 60 years. The reference intervals for HDL-cholesterol were lower in both males and females. Total cholesterol and LDL-cholesterol were higher in females compared to males.

**Conclusion:** There exists difference in reference intervals for various populations and hence there is a need for more population studies so that reference ranges for lipid parameters can be established which will help in better health care.

### KEYWORDS

Reference intervals (RI), Lipid profile, Total cholesterol/HDL-cholesterol ratio.

### INTRODUCTION

Health is a relative concept and can be assessed in terms of how different is the observed value of an individual from a relevant collection of reference values. Reference intervals are very essential for clinical laboratory test interpretation and patient care. The concept of reference interval was introduced by the International Federation for Clinical Chemistry (IFCC)<sup>1</sup>. According to the definition given by Ceriotti, Reference Interval (RI) is an interval that, when applied to the population serviced by the laboratory, correctly includes most of the subjects with characteristics similar to the reference group and excludes others<sup>2</sup>. Appropriate and reliable reference intervals when combined with data collected during medical interview and clinical examination help the physician to accurately interpret the laboratory test results. Majority of clinical laboratories are not able to implement their RI's. This may be due to the difficulty in obtaining sufficient numbers of references in individuals from a required population and the high cost of numerous tests to be performed. Thus, in practice, a great majority of the clinical laboratories use reference intervals reported in literature or in the manufacturers package inserts. The International Federation of Clinical Chemistry (IFCC) has recommended that each laboratory should have its own reference values<sup>1</sup>.

The National Committee for Clinical Laboratory Standards (NCCLS) now called Clinical and Laboratory Standards Institute (CLSI) has developed a scheme for the determination of Reference Interval. Reference individuals comprise a reference population from which is selected a reference sample group on which are determined reference values, upon which is observed a reference distribution and from this is calculated the reference limits which may define the RI's. Several methods are used to establish the RI's. However, most methods need healthy individuals as reference individuals. It is recommended to establish a RI by selecting a minimum of 120 healthy reference subjects which is considered to be statistically significant. Stratification by age and gender is also recommended. The values obtained from the sample group are then analysed to determine the 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles that form the 95% RI. The majority of RI's in use today refers to the central 95% of the reference population of subjects.

For most analytes the lower and upper reference limits are assumed to determine 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles of the underlying distribution of values respectively<sup>3,4</sup>.

The important causes of mortality and morbidity throughout the world are ischemic heart disease and cerebrovascular disorders<sup>5,6</sup>. Many studies have clearly established that there is a relationship between lipids and other risk factors with cardiovascular and cerebrovascular events<sup>7,8,9</sup>. The widely accepted risk factors for atherosclerotic diseases include elevated total cholesterol, LDL cholesterol<sup>10</sup>, triglycerides<sup>11,12</sup> and reduced HDL cholesterol<sup>13,14</sup>. In clinical practice, the levels of lipids and lipoproteins are measured to diagnose dyslipidemia, to assess the risk of cardiovascular disease (CVD) and to decide subsequent treatments. The burden of atherosclerotic cardiovascular disease is a cause of concern in India.

During the past two decades the National Cholesterol Education Programme (NCEP) of US<sup>15</sup> and expert panels from certain Western and Eastern countries<sup>16,17</sup> have released clear cut guidelines for preventing mortality from coronary artery disease. NCEP expert committee has clearly defined the appropriate medical decision cut off points for serum total cholesterol, HDL cholesterol, LDL cholesterol and Triglycerides for their population. Most of the laboratories and clinicians use reference intervals established in western population which do not match with Indian population. Since serum lipids and lipoproteins levels are very much dependent upon genetic background, ethnicity and dietary pattern of a particular population<sup>18,19</sup>, it becomes mandatory to establish reference intervals for serum lipids and lipoproteins for healthy men and women in Indian population.

In India a number of studies are done to establish reference intervals for lipid parameters but most of these are done in North Indian populations. Only a few reference data is available among the South Indian population. To our knowledge there are no published reference intervals for lipid parameters in Kerala. Kerala has a very high prevalence of risk factors for coronary heart disease such as Obesity, Hypertension and Diabetes. Hence this study was done to provide a reference database for our population.

The aim of our study was to determine the serum level of lipids and lipoproteins like total cholesterol, triglycerides, high density lipoproteins cholesterol (HDL-C) and low-density lipoproteins cholesterol (LDL-C), to calculate the ratio of total cholesterol (TC) to HDL-C and to establish reference intervals for the above lipids and lipoproteins in the study population.

## MATERIALS AND METHODS

The study was approved by Institutional Ethics Committee of Govt TD Medical College, Alappuzha. A total of 447 apparently healthy subjects of either sex in the age group 18-75 years were included in the study. The subjects were selected by organizing free blood testing camps in Ambalapuzha North Grama Panchayath. A questionnaire comprising general health and lifestyle questions was distributed in order to include proper subjects and exclude irrelevant subjects to avoid confounding results.

As per IFCC guidelines<sup>1</sup> individuals with conditions like renal failure, congestive heart disease, liver disease, nutritional anaemias, infections, hypertension, Diabetes mellitus, hyperthyroidism, hypothyroidism, obesity (BMI > 30kg/m<sup>2</sup>) intake of pharmacologically active agents like alcohol, tobacco, OCP, drugs like antipsychotics, steroids and other agents causing hyperglycemia and pregnant women were excluded.

Blood samples were collected between 8 am and 10 am after overnight fasting for 12 hours. Under aseptic precautions 5ml of blood sample was collected in plain blood collection tubes. Cold chain was maintained throughout sample collection and transport to the lab. Serum was separated and samples were analysed within 6 hours in Central Biochemistry lab at Government TD Medical college Alappuzha. Analysis was carried out in a Beckman Coulter AU680 chemistry analyser using reagents procured from Beckman Coulter. Cholesterol was quantitated enzymatically by Cholesterol esterase, Cholesterol Oxidase and peroxidase method. Triglyceride estimation is based on a series of coupled enzymatic reactions using a lipase, glycerol kinase, glycerol phosphate oxidase and a peroxidase. For HDL determination first a human  $\beta$  lipoprotein antibody binds to lipoproteins other than HDL. HDL was then quantified by an enzyme chromogen system containing cholesterol esterase, cholesterol

oxidase and peroxidase. Low density lipoprotein cholesterol (LDL-C) was estimated using Friedwald's formula<sup>20</sup>. Control serum from Biorad and calibrators were run with each batch of analysis. External quality control was performed using EQAS, CMC Vellore.

## STATISTICAL ANALYSIS

Results were entered in Microsoft excel windows 10. Continuous variables were expressed as mean, standard deviation, percentiles and 90% CI of mean and 90% CI of median. The normality of the data was assessed by Kolmogorov-Smirnov (K-S) test. As the distribution of data was not normal, comparison of quantitative data between the two gender groups was done by Mann-Whitney U test and comparison of quantitative data among different age groups was done by Kruskal Wallis test. Data analysis was performed using SPSS ver16.0. The reference intervals were calculated non-parametrically from the central 95<sup>th</sup> percentile. The reference data was arranged in ascending order and ranks were assigned such that the lowest value is ranked 1 and the largest n. The limits that enclose 95% of the reference population were estimated from the rank numbers corresponding to the 2.5<sup>th</sup> percentile and 97.5<sup>th</sup> percentile.

**Table 1: Demographics**

|                          | Male (n=179)    | Female (n=268)  | Total (n=447)   |
|--------------------------|-----------------|-----------------|-----------------|
|                          | Mean $\pm$ SD   | Mean $\pm$ SD   | Mean $\pm$ SD   |
| Age(years)               | 37.7 $\pm$ 15.6 | 43.6 $\pm$ 13.4 | 41.1 $\pm$ 14.6 |
| BMI (kg/m <sup>2</sup> ) | 26.1 $\pm$ 1.7  | 25.3 $\pm$ 2.4  | 25.6 $\pm$ 2.2  |
| SBP (mm Hg)              | 120.7 $\pm$ 7.7 | 121.4 $\pm$ 7.2 | 121.1 $\pm$ 7.4 |
| DBP (mm Hg)              | 76.5 $\pm$ 6.2  | 78.1 $\pm$ 6.7  | 77.4 $\pm$ 6.5  |

## RESULTS

A total of 447 apparently healthy individuals enrolled for the medical camp were included in the study. Out of the 447 individuals, 179 were males and 268 were females aged between 18 and 75 years. Demographic details of reference individuals (Table 1) showed the mean age of 37.7 in males and 43.6 in females with an overall mean age of 41.1. Mean BMI of males was 26.1 Kg/m<sup>2</sup> and that of females was 25.3 Kg/m<sup>2</sup> with an overall mean BMI of 25.6 Kg/m<sup>2</sup>. Mean systolic and diastolic blood pressure were 121.1 and 77.4 mm of Hg respectively.

**Table 2: Lipids and lipoproteins expressed as mean, median, 90%CI, 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles in males and females.**

|                         | Gender | N   | Mean $\pm$ SD    | 90% CI for mean | median (90% CI for median) | 2.5 <sup>th</sup> -97.5 <sup>th</sup> Percentile | p value |
|-------------------------|--------|-----|------------------|-----------------|----------------------------|--------------------------------------------------|---------|
| Total Cholesterol (mg%) | Male   | 179 | 192.2 $\pm$ 33.6 | 188-196.3       | 192(184-199)               | 124-254.5                                        | 0.002   |
|                         | Female | 268 | 202.6 $\pm$ 35.7 | 199-206.2       | 207.5(199-212)             | 134.2-265.1                                      |         |
|                         | Total  | 447 | 198.4 $\pm$ 35.2 | 195.7-201.2     | 200(195-205)               | 132.2-262                                        |         |
| Triglyceride (mg%)      | Male   | 179 | 144.8 $\pm$ 38.6 | 140-149.6       | 150(143-155)               | 67-199.5                                         | <0.001  |
|                         | Female | 268 | 125.3 $\pm$ 41.2 | 121.1-129.4     | 118(113-124)               | 65-219.3                                         |         |
|                         | Total  | 447 | 133.1 $\pm$ 41.2 | 129.9-136.3     | 130(125-136)               | 65-218.8                                         |         |
| VLDL (mg%)              | Male   | 179 | 29 $\pm$ 7.7     | 28-29.9         | 30(28.6-31)                | 13.4-39.9                                        | <0.001  |
|                         | Female | 268 | 25 $\pm$ 8.2     | 24.2-25.9       | 23.6(22.6-24.8)            | 13-43.9                                          |         |
|                         | Total  | 447 | 26.6 $\pm$ 8.3   | 26-27.3         | 26(25-27.2)                | 13-43.8                                          |         |
| HDL (mg%)               | Male   | 179 | 41.8 $\pm$ 7.6   | 40.9-42.7       | 42(41-43)                  | 25-57.5                                          | <0.001  |
|                         | Female | 268 | 49.6 $\pm$ 6.9   | 48.9-50.3       | 49(49-50)                  | 36.7-63.3                                        |         |
|                         | Total  | 447 | 46.5 $\pm$ 8.1   | 45.9-47.1       | 48(46-48)                  | 28.2-62                                          |         |
| LDL (mg%)               | Male   | 179 | 121.4 $\pm$ 27.9 | 118-124.9       | 122(118-129)               | 66-173                                           | 0.009   |
|                         | Female | 268 | 128.3 $\pm$ 31   | 125.2-131.5     | 133(127-136)               | 65.2-178                                         |         |
|                         | Total  | 447 | 125.6 $\pm$ 30   | 123.2-127.9     | 129(123-132)               | 66.2-175                                         |         |
| Total Cholesterol/HDL   | Male   | 179 | 4.70 $\pm$ 0.94  | 4.59-4.82       | 4.72(4.55-4.84)            | 2.95-6.79                                        | <0.001  |
|                         | Female | 268 | 4.15 $\pm$ 0.87  | 4.06-4.24       | 4.07(3.98-4.20)            | 2.59-5.84                                        |         |
|                         | Total  | 447 | 4.37 $\pm$ 0.94  | 4.30-4.44       | 4.33(4.22-4.45)            | 2.67-6.4                                         |         |

**Table 3: Lipids, lipoproteins expressed as mean, median, 90%CI, 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles in different age groups.**

|                         | Age   | N   | Mean $\pm$ SD    | 90% CI for mean | Median (90% CI for median) | 2.5 <sup>th</sup> -97.5 <sup>th</sup> percentile | p value |
|-------------------------|-------|-----|------------------|-----------------|----------------------------|--------------------------------------------------|---------|
| Total Cholesterol (mg%) | <30   | 104 | 176.4 $\pm$ 30.7 | 171.4-181.4     | 176(170-184)               | 110.8-223.1                                      | <0.001  |
|                         | 30-44 | 158 | 198.2 $\pm$ 30.7 | 194.1-202.2     | 198(192-205)               | 131.7-258                                        |         |
|                         | 45-59 | 149 | 212.6 $\pm$ 36.1 | 207.7-217.5     | 217(209-225)               | 146.3-272.3                                      |         |
|                         | >60   | 36  | 204.3 $\pm$ 30.5 | 195.7-212.9     | 211.5(189-218)             | 141-260                                          |         |
|                         | Total | 447 | 198.4 $\pm$ 35.2 | 195.7-201.2     | 200(195-205)               | 132.2-262                                        |         |
|                         |       |     |                  |                 |                            |                                                  |         |
| Triglyceride (mg%)      | <30   | 104 | 124.7 $\pm$ 39.2 | 118.3-131.1     | 119(112-128)               | 61.9-193                                         | 0.057   |
|                         | 30-44 | 158 | 131.7 $\pm$ 42.1 | 126.1-137.2     | 130.5(120-140)             | 64-219.4                                         |         |
|                         | 45-59 | 149 | 140.2 $\pm$ 41.9 | 134.5-145.9     | 136(129-141)               | 69.8-221                                         |         |
|                         | >60   | 36  | 133.9 $\pm$ 36.3 | 123.7-144.2     | 140(116-150)               | 73-0.191                                         |         |
|                         | Total | 447 | 133.1 $\pm$ 41.2 | 129.9-136.3     | 130(125-136)               | 65-218.8                                         |         |
|                         |       |     |                  |                 |                            |                                                  |         |

|                         |       |     |            |             |                 |            |        |
|-------------------------|-------|-----|------------|-------------|-----------------|------------|--------|
| VLDL-C (mg%)            | <30   | 104 | 24.9±7.8   | 23.7-26.2   | 23.8(22.4-25.6) | 12.4-38.6  | 0.057  |
|                         | 30-44 | 158 | 26.3±8.4   | 25.2-27.4   | 26.1(24-28)     | 12.8-43.9  |        |
|                         | 45-59 | 149 | 28±8.4     | 26.9-29.2   | 27.2(25.8-28.2) | 14-44.2    |        |
|                         | >60   | 36  | 26.8±7.3   | 24.7-28.8   | 28(23.2-30)     | 14.6-38.2  |        |
|                         | Total | 447 | 26.6±8.3   | 26-27.3     | 26(25-27.2)     | 13-43.8    |        |
| HDL- C (mg%)            | <30   | 104 | 45.1±10.4  | 43.4-46.8   | 44(42-47)       | 25-62.4    | 0.042  |
|                         | 30-44 | 158 | 46.2±7.7   | 45.2-47.2   | 48(46-49)       | 28-59      |        |
|                         | 45-59 | 149 | 47.9±6.8   | 47-48.8     | 48(47-49)       | 36-64.3    |        |
|                         | >60   | 36  | 45.7±7     | 43.8-47.7   | 45.5(42-48)     | 32-59      |        |
|                         | Total | 447 | 46.5±8.1   | 45.9-47.1   | 48(46-48)       | 28.2-62    |        |
| LDLC (mg%)              | <30   | 104 | 107.4±25.5 | 103.2-111.5 | 108.5(100-114)  | 56.5-145.1 | <0.001 |
|                         | 30-44 | 158 | 125.3±26.3 | 121.8-128.7 | 127.5(122-133)  | 69.9-169   |        |
|                         | 45-59 | 149 | 137.1±31.1 | 132.9-141.3 | 143(134-149)    | 79.3-179   |        |
|                         | >60   | 36  | 131.9±27.5 | 124.2-139.7 | 136.5(123-147)  | 63-178     |        |
|                         | Total | 447 | 125.6±30   | 123.2-127.9 | 129(123-132)    | 66.2-175   |        |
| Total Cholesterol/HDL-C | <30   | 104 | 4.08±0.98  | 3.92-4.24   | 4.08(3.83-4.25) | 2.41-6.13  | 0.003  |
|                         | 30-44 | 158 | 4.4±0.93   | 4.27-4.52   | 4.32(4.14-4.48) | 2.85-6.67  |        |
|                         | 45-59 | 149 | 4.51±0.9   | 4.38-4.63   | 4.48(4.24-4.68) | 3-6.38     |        |
|                         | >60   | 36  | 4.55±0.82  | 4.31-4.78   | 4.49(4.41-4.84) | 2.87-5.97  |        |
|                         | Total | 447 | 4.37±0.94  | 4.30-4.44   | 4.33(4.22-4.45) | 2.67-6.4   |        |

Mean ± SD, 90% CI of mean, 90 % confidence intervals of median, *p* value for mean and 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles for various parameters of both sexes (Table 2) and different age groups (Table 3) of target population were tabulated. The frequency histograms of various parameters of target population are depicted in figure 1.

Gender related differences were observed for all analytes (*p*<0.05) (Table 2). The mean total cholesterol in men was 192.2 mg/dl and that of women 202.6 mg/dl. Mean Triglyceride levels were 144.8 mg/dl in males compared to 125 mg/dl in females. Mean VLDL cholesterol was 29 mg/dl in males and 25 mg/dl in females. Mean HDL was 41.8 mg/dl in males and 49.6 mg/dl in females.

Mean LDL cholesterol was 121.4 mg/dl in males and 128.3 mg/dl in females. TC/HDL cholesterol ratio was 4.70 in males and 4.15 in females. Total cholesterol and LDL cholesterol were found to be higher in females compared to males whereas Triglycerides, VLDL, TC/HDL ratio were higher in males compared to females. HDL cholesterol was found to be higher in females compared to males. Significant age related differences were also observed (Table 3) for total cholesterol, HDL-cholesterol and LDL-cholesterol. However Triglyceride levels did not vary significantly across various age groups.

Total cholesterol and LDL-cholesterol levels were higher up to 60 years in this study and above 60 year age group, they were decreased. There was insignificant increase in triglycerides and VLDL cholesterol levels upto 60 years and above 60 years the levels decreased. Total cholesterol/HDL-cholesterol ratio also showed similar changes. 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles for each parameter, gender wise and age wise, are also represented in Table 2 and Table 3.

In this study the 95% reference range for total cholesterol was 124-254.5 mg/dl for males, 134.2-265.1 mg/dl for females and 132.2 - 262 mg/dl for the whole population. As per NCEP ATP III guidelines<sup>16</sup> a total cholesterol concentration of 200-239 mg/dl is classified as borderline high and a level of >240 mg/dl as high. In the present study the upper limits of total cholesterol for both men and women are above these limits. For Triglycerides the 95% reference intervals were 67-199.5 mg/dl for men and 65-219.3 mg/dl for women and 65- 218.8 mg/dl for the whole population. The NCEP decisional concentration for triglyceride is <150 mg/dl: desirable, 200-339 mg/dl: borderline high and >339 mg/dl: high. The values for Triglycerides in the present study were above the desirable range for both genders.

In the present study the 95% reference intervals for HDL-cholesterol were 25-57.5 mg/dl in males, 36.7-63.3 mg/dl in females and 28.2-62 mg/dl in the total population. The NCEP decisional concentration for HDL-cholesterol is <30 mg/dl: low and >60 mg/dl: desirable. In the present study the values for HDL-cholesterol were low for men.

For LDL-cholesterol the 95 % reference intervals represented by 2.5<sup>th</sup> and 97.5<sup>th</sup> percentiles were 66-173 mg/dl for men, 65.2-178 mg/dl for women and 66.2-175 mg/dl for the whole population. The NCEP decisional concentration for LDL-cholesterol is <130 mg/dl: desirable, 130-159 mg/dl: borderline high and >160 mg/dl: high. In our study the upper limits of LDL-cholesterol are also above these limits.

The 95% reference range for Total cholesterol/HDL-cholesterol ratio in our study was 2.95-6.79 in males, 2.59-5.84 in females and 2.67-6.4 for the whole population. The upper limits are higher in males compared to females.

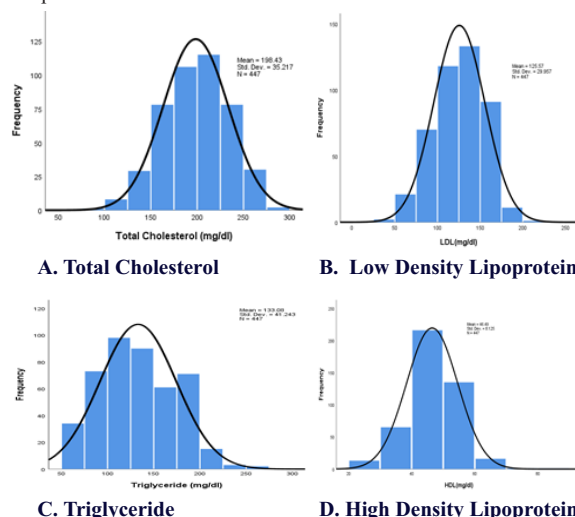


Figure 1. Frequency histogram of lipid parameters

## DISCUSSION

Serum lipids are important determinants of cardiovascular risk. Being very appropriate drug targets, lipid parameters like Cholesterol, LDL-cholesterol, HDL-cholesterol are monitored routinely in all diagnostic laboratories. Most of the clinical laboratories refer to clinical data or manufacturer insert sheets for the interpretation of observed values or follow the NCEP guideline. The concentration of these analytes are dependent on ethnicity, dietary habits and genetic makeup, age, gender, lifestyle and environmental factors.

The present study showed higher levels of total cholesterol, LDL-cholesterol, triglyceride and lower levels of HDL-cholesterol in both genders and different age groups. This is comparable with the study conducted by Ashavaid et al (2005)<sup>21</sup>, where the levels of different lipids were elevated. But in their study the reference ranges were wider with higher upper limits when compared to others. They reported total cholesterol in the range of 129.8 to 284 mg/dl, HDL-cholesterol in the range of 29-73 mg/dl, LDL-cholesterol 67-185.2 mg/dl, Triglyceride 44.8-258.6 mg/dl and Total cholesterol/HDL-cholesterol ratio 2.66-7.06. The upper limits of most parameters were lower in females when compared to males, whereas in the present study the mean total cholesterol and LDL-cholesterol levels were higher in females.

In the study by Malati et al (2009)<sup>22</sup> the mean total cholesterol levels were 175.1 mg/dl in males and 175.4 mg/dl in females. Mean LDL-cholesterol levels were 102.4 mg/dl in males and 99.2 mg/dl in females; mean HDL-cholesterol levels were 41.9 mg/dl in males and

47.2 mg/dl in females, mean triglyceride levels were 139.9 mg/dl in males and 120 mg/dl in females. Mean Total cholesterol/HDL-cholesterol ratio was 3.85 in males and 4.15 in females. Mean Total cholesterol and LDL-cholesterol levels were lower than that of present study. Mean HDL-cholesterol, mean triglyceride and Total cholesterol/HDL-cholesterol ratio were comparable to our study. A steady increase of total cholesterol, LDL-cholesterol and triglyceride levels until the 6<sup>th</sup> decade were found in their study which is comparable to the results of the present study.

In another south Indian study done in Mangaluru, Karnataka by Ireshanavar et al (2019)<sup>23</sup>, the reference range for total cholesterol in males and females were 116-240 mg/dl and 106-240.8 mg/dl; reference range for triglyceride in males and females were 38-199 mg/dl; reference range for VLDL-cholesterol in males and females were 7.7-42 mg/dl and 7.8-39.8 mg/dl; reference range for LDL-cholesterol in males and females were 59.5-177 mg/dl and 59.8-166.9 mg/dl respectively and reference range for HDL-cholesterol in males and females were 32.7-68 mg/dl.

The total cholesterol and LDL-cholesterol levels were much higher in our population compared to that of Karnataka. The Karnataka population also had higher HDL-cholesterol levels attributed to high intake of fish in the diet.

According to the study by Sivaranjini et al (2003) done on a healthy population of middle district of Southern Tamilnadu<sup>24</sup> mean values of total cholesterol were 172.7 mg/dl in males and 164.5 mg/dl in females, mean Triglyceride 100.1mg/dl in males and 92.9 mg/dl in females, mean LDL -cholesterol 113.1 mg/dl in males and 102.9 mg/dl in females and mean VLDL-cholesterol levels were 19.8 mg/dl in males and 18.5 mg/dl in females. All these values were lower when compared to the present study group. The mean HDL-cholesterol levels were 39.6 mg/dl in males and 42.8 mg/dl in females which were slightly lower than the present study. According to the study by Vaneet Kaur et al<sup>25</sup> to establish reference intervals of lipid profile in Punjab, a mean total cholesterol level of  $184.4 \pm 32.3$  mg/dl was observed in females which was significantly higher compared to males ( $179.9 \pm 35.3$ mg/dl). Also an elevated reference interval of 98-270 mg/dl and 100-305 mg/dl for males and females respectively were observed. The mean levels were higher in our study but the upper limits of reference intervals of total cholesterol in study conducted in Punjab was higher. The mean HDL-cholesterol level was also significantly higher in females than males in the study population of Punjab as with the present study. The overall mean LDL-cholesterol was higher in females ( $115.2 \pm 31.2$  mg/dl) than males ( $112 \pm 32.9$ ) as in our study.

The reference intervals for total cholesterol, triglycerides, VLDL-cholesterol and LDL-cholesterol were lower in the study conducted in a healthy population in Western Maharashtra by Durgawala et al<sup>26</sup>. A study conducted to establish the reference intervals for serum lipid profile in the healthy population of Anand district, Gujarat, by Modi et al (2014)<sup>27</sup> showed findings similar to the present study in both genders and different age groups except for the lower reference limits which were towards the lower side.

Madhumita Das and Mauchumi Saikia did a study<sup>28</sup> on the reference values of lipid profile in Assamese population in the age group of 20-80 years and concluded that the reference ranges of cholesterol, triglycerides and LDL-cholesterol were wider in all age groups. The HDL and LDL levels in their study were almost similar to those used in the laboratory. The reference intervals in the present study were higher compared to the study by Madhumita Das et al.

The values of the present study follow a similar pattern as in studies conducted in some foreign countries. Except for a higher HDL cholesterol level and lower LDL-cholesterol level the results of present study were comparable to Li et al (2005)<sup>29</sup>. Cohen et al<sup>30</sup> studied 30 year trends in serum lipids among United states adults from the National Health and Nutrition Examination survey II,III and 1996-2006. The lipid levels reported by NHANES II survey is comparable with our study.

Mahato et al (2018) studied the reference intervals of lipid parameters for Nepalese population<sup>31</sup> and determined the reference intervals for total cholesterol (97.83-245.17 mg/dl), triglyceride (37.2-294 mg/dl), HDL-cholesterol (10.8-56.45 mg/dl) and LDL-cholesterol (40.60-

154.68 mg/dl). The reference intervals for all parameters were wider with the lower reference limits towards the lower side.

In a study done on Iranian population<sup>32</sup> the mean total cholesterol reported was 175.3 mg/dl in females and 177.07 mg/dl in males, mean LDL-cholesterol concentration was 107.1 mg/dl in females and 111.48 in males, mean HDL-cholesterol of 46.37 in females and 38.13 in males, mean triglycerides of 108.95 in females and 127.58 in males. Mean values of all the lipid parameters were low compared to the present study.

Lipids, lipoproteins and their 95% reference intervals for the study population from Ambalappuzha North Grama panchayath in Alappuzha district, Kerala, were higher than levels suggested by NCEP ATP III guideline, even in the younger age groups. There was also considerable difference from the reference intervals obtained from studies conducted in different parts of India as well as other countries.

Kerala is the most advanced Indian state in terms of epidemiological transition. People consume large quantities of marketed products high in salt, sugar, saturated fat and trans fats. Energy needs declined steadily because of mechanization at the workplace and increasing sedentary lifestyle style<sup>33</sup>. Hence it is debatable whether a revision of reference values for lipids in our population would be wise. We need more studies involving larger populations in different centres of our state. When the data from the National Health And Nutrition Examination Survey (NHANES) II and III and NHANES 1996 to 2006 were examined, it was found that there was a decrease in the levels of Total cholesterol, LDL-cholesterol and a significant increase in mean HDL-cholesterol levels<sup>30</sup>. This was attributed to an increased awareness of risk of high cholesterol and greater use of lipid lowering drugs. Currently our aim should be to increase awareness among our people for modification of life style, changes in the diet and increase in physical activity. The people at risk for coronary artery disease could be put on medication.

## CONCLUSION

We could conclude from our study that there exist differences in reference intervals for different populations. Our results may be beneficial in formulating medical decision limits for serum lipids pertaining to the population of Kerala. The findings of our study can be supported with multiple large population studies. Lifestyle and diet modifications may be implemented to reduce coronary artery disease risk.

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