Age and Sex: Important Determinants In Affecting The Levels Of Serum Apolipoprotein B And A1 In Indian Population

R Sharma, B Singh, M Mahajan, R Kant

Citation

Abstract

For the past many years, serum lipids and lipoprotein cholesterol levels have been used to assess the risk of coronary artery disease. Recently apolipoprotein A1 and apoB have been proposed as relatively better parameters to assess the risk of this disease. Age and sex are two non modifiable risk factors of CAD which may affect the levels of apoA1 and apoB and for investigating their role in CAD, age and sex related variations in these apolipoproteins must have to be considered. The main objective was to study the effect of age and sex on the levels of serum apoA1 and apoB in normal individuals (males and females) and secondly to investigate the role of these apolipoproteins as CAD risk indicators in Indian (Punjabi) population. Normal individuals (n=270) and coronary artery disease patients (n=290) were divided into three age groups: group1 (35-44yrs), Group11 (45-54yrs) and group111 (55-64yrs). Serum apolipoprotein A1 and B levels were estimated with immunoturbidemetric method in blood samples taken after12-hour overnight fast. Lipid and lipoprotein cholesterol levels were estimated with standard procedures. Serum apoB levels showed continuous increase with age in both normal males and females with males having significantly raised (p<0.01) apoB levels in each age group compared to females. Serum apoA1 levels did not show continuous increase, rather levels declined in the older age group (Group111) in normal males and females and this decline was relatively earlier in males. Within each age group, females were having relatively raised apoA1 levels than males. A significant (p<0.01) positive coefficient of correlation was observed between serum apoA1 and HDL cholesterol levels and between serum apoB and LDL cholesterol levels. Patients suffering from coronary artery disease were having significantly raised (p<0.01) serum apoB, LDL cholesterol and apoB/A1 ratio and significantly low (p<0.01) serum apoA1 and HDL cholesterol levels than age and sex matched normal individuals. Serum apoA1 and apoB levels were significantly affected by sex and age of a person. Males are at higher risk owing to relatively raised serum apoB levels and low serum apoA1 levels compared to females. ApoA1 and apoB could act as good candidates to act as CAD risk factors in our sample population.

INTRODUCTION

Cardiovascular disease is the most common cause of death worldwide. For the past many years total lipid profile i.e. total cholesterol, triglycerides, VLDL cholesterol and LDL cholesterol have been positively associated with coronary artery disease (CAD) while HDL cholesterol has inverse relation [1,2] But lipid and lipoprotein cholesterol levels have failed to explain the increased prevalence of CAD in normolipidemic patients[3]. Recently serum apoA1 and apo B have been proposed as relatively better markers for assessing the risk of CAD and its treatment regimen. ApoA1 is the constituent component of HDL and apoB is of LDL. It was shown in the AMORIS study that apoA1 and apoB might be of greatest value in predicting the risk of myocardial infarction especially in patients having low or normal LDL cholesterol level [4]. A few countries like Canada have incorporated apoB in their clinical management guidelines [5]. Age and sex are the two important factors seemed to affect lipid levels. Studies regarding age and sex related variations in serum apoA1 and apoB levels are few and are only confined to European, North American and few Australian and African populations [6,7,8]. Such data is very limited in Indian population, which has a very high incidence of coronary artery disease. Global burden of CAD is mainly concentrated in developing countries [9]. These apolipoproteins may act as important CAD risk indicators in our population. Hence the present study was aimed to assess age and sex related variations in serum apoA1 and B levels in normal individuals and to investigate the role of these apolipoproteins as CAD risk indicators in Indian (Punjabi) population.

MATERIAL AND METHODS

In the present study two hundred and ninety patients (290) suffering from coronary artery disease (CAD) and 270 age and sex matched normal individuals were included. Patients were taken from the wards and OPDs of Guru Nanak Dev and ESI hospitals, Amritsar. Diagnosis of CAD was done by the treating experts on the basis of clinical symptoms, ECG changes and stress test. Patients were also subjected to angiography if required. CAD patients were also having some of the associated risk factors such as diabetes mellitus; hypertension etc and they were on the requisite treatment. Exclusion criteria included patients on lipid lowering drugs, having renal or thyroid disease and women having hysterectomy or on oral contraceptives or taking HRT. Normal individuals free from any evident symptom of the disease were taken as controls. They were subjected to ECG and stress test wherever possible to confirm their clinical state. Mean BMI of normal subjects was 26 ± 3 Kg/m^2 and of CAD patients was 27 ± 4 Kg/m^2. The control group represents the free living sample population and no selective criteria was included such as dietary restriction, increased/decreased physical activity or alcohol consumption or smoking status. Informed consent was taken from all the subjects included in the study. Both normal individuals and CAD patients were divided into 3 age groups: Group I (35-44 yrs), Group II (45-54 yrs) and Group III (55-64 yrs). Blood samples were taken after 12 hour overnight fast and serum was used for various investigations. Apolipoprotein A1 and B levels were estimated by immunoturbidemetric method [7] using kits from Diasys India Pvt Ltd. Reagents and calibrators were in accordance with WHO International reference material for these apolipoproteins. It has been reported that fasting/no fasting conditions do not affect apolipoprotein levels. [7] but fasting blood samples were preferred to avoid turbidity due to chylomicrons which could affect lipid levels. Total cholesterol levels were estimated by Zlatkis method as modified by Zak [10]. Triglycerides were estimated with enzymatic method from commercially available kits. HDL cholesterol was estimated after precipitating VLDL and LDL by using magnesium chloride and sodium phosphotungstate [11]. LDL cholesterol levels were estimated using the Friedwald's formula. [12]

STATISTICAL ANALYSIS

Standard deviation was calculated. Student's t test was applied to assess the significance

RESULTS

In normal males serum apoB levels tend to increase continuously from age group I to group III (Fig I). No in between decline in serum apoB levels was observed. Females also showed continuous increase in serum apoB levels with advancing age. But increase in serum apoB levels in males in each age group was relatively more as compared to females leading to significant sex related differences in apoB levels. Similar age and sex related variations were observed in serum LDL cholesterol levels in males and females (Fig II). A significant positive coefficient of correlation (r 0.68; p<0.01) was observed between serum apoB levels and LDL cholesterol levels. Age related variations in serum apoA1 levels were not similar to that of serum apoB levels. Serum apoA1 levels showed slight decrease in serum apoA1 levels after the age of 44 years in normal males and the levels continued to decrease till the age of 64 years (Fig I). No in between increase in serum apoA1 levels was observed in normal males. Females showed significant increase (p<0.01) in serum apoA1 levels after the age of 44 years. Then the levels declined after the age of 54 years but this decline was relatively earlier in males. No plateau in serum apoA1 levels was observed. In every age group females were having significantly raised (p<0.01) serum apoA1 levels as compared to males. Similar age and sex related variations were also observed in HDL cholesterol levels in normal males and females (Fig II). A significant positive coefficient of correlation (r 0.71; p<0.01) was observed between serum apoA1 and HDL cholesterol levels. The comparison between control group (males and females) and CAD patients (males and females) revealed that all parameters showed significant difference between the two populations (Table I). CAD patients were having raised apoB, apoB/apoA1 ratio, LDL cholesterol and triglycerides and low apoA1 and HDL cholesterol levels compared to control group.

Figure 1

Figure 1: Effect of age and sex on serum apoA1 and apoB levels in normal males and females.
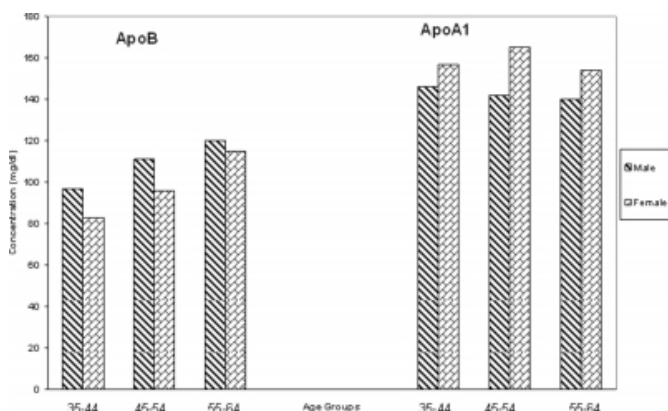

Figure 2

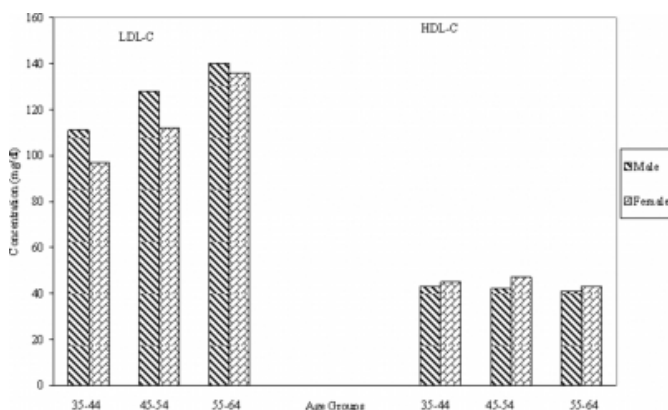Figure 2: Effect of age and sex on serum LDL and HDL cholesterol levels in normal males and females.

Figure 3

Table 1: Lipid, lipoprotein cholesterol and apolipoprotein levels in control group and CAD patients.

Subjects	Control Males			Male patients			Control Females			Female patients		
Age groups (yrs)	35-44 (n=40)	45-54 (n=50)	55-64 (n=50)	35-44 (n=40)	45-54 (n=50)	55-64 (n=60)	35-44 (n=35)	45-54 (n=45)	55-64 (n=60)	35-44 (n=35)	45-54 (n=40)	55-64 (n=65)
TC	180±12.4	195±13.1	215±12.8	210±12.1	230±13.8	240±13.2	170±12.4	189±11.6	210±13.1	200±11.8	220±12.1	233±13.4
TG	130±11.6	141±12.4	156±13.4	140±12.6	151±12.8	160±11.4	140±11.9	150±12.2	167±11.5	150±12.1	161±13.1	168±12.8
VLDL-C	26±8.4	28±9.1	31±10.1	28±9.4	30±10.1	32±9.6	28±8.6	30±8.1	31±8.0	30±9.1	32±9.5	33±9.6
LDL-C	111±10.1	128±11.5	140±11.7	144±11.1	159±12.7	168±12.4	97±11.1	112±12.4	136±12.6	130±12.4	146±13.1	158±12.8
HDL-C	43±9.1	42±10.2	41±10.3	38±10.1	41±9.3	40±10.5	45±9.4	47±10.2	43±9.5	40±10.2	42±9.9	41±10.0
ApoB	97±11.9	111±12.6	120±13.2	129±12.9	140±12.2	155±13.1	83±13.1	96±12.4	115±14.1	122±11.4	135±12.5	163±13.1
ApoA1	146±13.2	142±13.0	140±12.4	100±12.2	110±11.5	103±12.4	157±12.6	165±13.2	154±12.1	125±12.2	131±13.6	119±11.9
ApoB:A1 ratio	0.66±0.14	0.72±0.18	0.85±0.21	1.2±0.41	1.3±0.51	1.5±0.58	0.53±0.10	0.58±0.09	0.74±0.12	0.97±0.11	1.0±.20	1.3±0.34

P<0.01 (comparison of lipid levels between patients and controls in the respective age groups).
P<0.001 (comparison of apolipoprotein levels and ApoB/A1 ratio between patients and controls in the respective age groups).
Results were expressed as Mean± S.D. mg/dl
TC= Total cholesterol ; TG= triglycerides; n= number of cases.

DISCUSSION

Apolipoprotein B is a constituent component of LDL and apoA1 of HDL. Recently they have been proposed as better markers to assess the risk of coronary artery disease. Some workers have assessed the role of these apolipoproteins in coronary artery disease but the conclusions drawn are variable [13,14]. Age and sex are two non modifiable risk factors of CAD which may affect serum apolipoprotein levels. Data regarding the effect of age and sex on serum apoA1 and apoB levels is very limited in Indian population. These uncertainties are limiting the use of these apolipoproteins in routine diagnosis, which may prove beneficial in our population having high incidence of coronary artery disease. To investigate their role in CAD, age and sex related variations in serum apoA1 and apoB must have to be considered. In the present study serum apoA1 levels showed minor variations with age in normal males and females. In males apoA1 levels slightly declined after the age of 44 yrs. But in females a relative increase in serum apoA1 levels were observed after the age of 44 yrs and then the levels declined after the age of 54 yrs. Sex related differences in serum apoA1 levels were clearly significant. In every age group females were having significantly raised serum apoA1 levels than males. Even in the older age group significant difference in serum apoA1 levels were observed between normal males and females. Similar age and sex related variations were also observed in serum HDL cholesterol levels. ApoA1 is a constituent apolipoprotein of HDL and any change in apoA1 levels may affect the cholesterol uptake property of HDL. ApoA1 plays a key role in reverse cholesterol transport. It also activates LCAT which is essential for cholesterol esterification [15]. Relatively raised serum apoA1 levels in females could be responsible for the raised HDL cholesterol levels in them.

On the contrary serum apoB levels continuously increased with age in both normal males and females. No decline in its levels was observed in either sex. In every age group, males were having relatively raised serum apoB levels than females. A continuous increase in serum apoB levels in both the sexes may indicate a physiological increase in risk of CAD with advancing age .This risk seemed to be more pronounced in males having increased number of atherogenic particles (apoB) and decreased number of anti-atherogenic particles (apoA1). Very similar results were reported in Swedish population study. [7] It was reported that apoB levels increased with age in both the sexes while serum apoA1 showed minor age related variations. The same study reported similar sex related differences in apoA1 and apoB levels as observed in the present study. Age groups taken in the Swedish study were different from the present study but that did not affect the results. Another study in Mexican

population also reported similar results [16]. Although the results of the Swedish and Mexican studies were similar to the results of the present study, slight variations in the average levels of these apolipoproteins were observed. In the Mexican population study, relatively lower mean levels of serum apoA1 and apoB were reported as compared to the results of the present study. This strengthens the need to obtain more data regarding these apolipoproteins in population-based studies worldwide. LDL cholesterol levels also undergo similar age and sex related variations in normal males and females as were observed in serum apoB levels. A positive coefficient of correlation was observed between serum LDL cholesterol levels and apoB levels. ApoB plays an important role in lipid metabolism. It maintains the integrity of LDL and also facilitates delivery of cholesterol to the peripheral cells. Any variation in apoB levels could alter the cholesterol content in LDL leading on to the observed variations.

CAD Patients (males and females) had significantly low serum apoA1 and HDL cholesterol levels and significantly raised serum apoB and LDL cholesterol levels as compared to age and sex matched normal individuals (males and females). In every age group serum apoB/apoA1 ratio was significantly higher in CAD patients compared to normal individuals indicating increased atherogenicity. Raised apoB/A1 ratio has been associated with increased risk of CAD [17]. Significant difference in serum apoB levels of CAD patients and normal individuals has already been reported in our previous published data [18]. Low serum apoA1 levels have been reported in patients suffering from coronary artery disease [19]. This clearly shows that apolipoprotein A1 and B are good candidates to act as risk factors for CAD. Similar results were also reported in one study in Indian population. [20]. Though this study reported that apoA1 and B could as better predictors in evaluating the risk of coronary artery disease, the absolute values of these apolipoproteins reported were significantly lower from the present study. This may be due to other factors such as difference in geographical distribution or different dietary habits, and cultural and behavioral factors of the population studied which may affect the levels of these apolipoproteins. Moreover the study design was different from that of the present study.

CONCLUSIONS

However this study concluded that serum apoB and apoA1 levels differ significantly with age and sex of a person and could act as good candidates in evaluating the risk of CAD.

The number of cases in each age group may not be an indicative of CAD prevalence due to small sample size. This study emphasizes the need to have more data regarding the distribution of these apolipoproteins within different geographic regions and in other populations especially in developing countries due to high disease burden. Data regarding age and sex related variations in apoA1 and apoB in larger population could help to establish their reference levels in our country.

STUDY LIMITATIONS

This was a sample population study. No selective criteria were made to link any association of these apolipoproteins with other factors such as physical activity, smoking, alcoholism, nutritional factors. All these factors may overestimate or underestimate the levels of apoA1 and B. Despite these facts; apolipoprotein profile appeared to be favorable in our study participants and could act as important parameters in evaluating the risk of coronary artery disease in addition to lipid levels.

ACKNOWLEDGEMENT

References

1. Castelli WP, Garrison RJ, Wilson PW, Abott RD, Kalousdian S and Kannel WB. Incidence of coronary heart disease and lipoprotein cholesterol levels. The Framingham Study. JAMA. 1986; 256: 2835 - 8.
2. Gordon DJ and Rifkind BM. High-density lipoprotein - the clinical implications of recent studies. N Engl J Med. 1989; 321: 1311 - 6.
3. Hughes LO. Insulin, Indian origin and ischemic heart disease. Int J Cardiol. 1990; 26: 1 - 4.
4. Walldius G, Jungner I, Holme I, Aastveit AH, Kolar W and Steiner E. High apolipoprotein B, low apolipoprotein A-1, and improvement in the prediction of fatal myocardial infarction (AMORIS Study) : a prospective study. Lancet 2001; 358: 2026 - 33.
5. Connely PW, Poapst M, Davignon J, Lussier-Cacan S Reeder B,Lessard R, Hegele RA and Csima A. Reference levels of plasma apolipoproteins A1 and B , and association with nonlipid risk factors in the populations of two Canadian provinces: Quebec and Saskatchewan. Canadian Heart Health Surveys Research Group. Can J Cardiol 1999; 15:409-418.
6. Zunic G, Jelie - Ivanovic Z, Spasie S, Stojiljkovie A and Singh NM. Reference values for apolipoproteins A-1 and B in Healthy subjects by age. Clinical chemistry. 1992; 38: 566 - 9.
7. Jungner I, Marcovina SM, Walldius G, Holme I, Kolar W and Steiner E. Apolipoprotein B and A-1 value in 147576. Swedish males and females, standardized according to the world health organization - International Federation of Clinical First International Reference Materials. Clinical

Chemistry 1998; 44: 1641 - 49.

8. Avogaro P, Cazzolato G, Bittolo BonG, Belussi F and Quinci GB. Values of apoA1 and apoB in humans according to age and sex. Clin chim Acta 1979;16:311-315.

9. Yusuf S, Reddy s, Ounpuu S and Anand S. Global burden of cardiovascular disease, part1: general considerations, the epidemiologic transition, risk factors and impact of urbanization. Circulation 2001;104:2746-2753.

10. Zlatkis A, Zak B and Boyle A. A new method for the direct estimation of cholesterol. J Lab Clin Med 1953;41:486-492.

11. Burstein M, Scholnick HR and Morfin RJ. Estimation of High density lipoprotein cholesterol. J Lipid Res 1970;11:583-593.

12. Friedewald WT, Levy RL and Fredrickson DS. Estimation of concentration of low density lipoprotein cholesterol in plasma without the use of preparative ultracentrifuge. Clin chem. 1972;18:499-502.

13. Lamarche B, Moorjani S, Lupien PJ, Contin B, Bernard PM, Dagenais GR and Despres JP. Apoliprotein A1 and B levels and the risk of ischemic heart disease during a five year follow up of men in the Quebec Cardiovascular study. Circulation 1996; 94:273-278.

14. Sharrett AR, Ballantyne CM, Coady SA, Heiss G, Sorlie PD, Castellier D and Patch W. Coronary heart disease prediction from lipoprotein cholesterol levels, triglycerides, lipoprotein(a), apolipoproteins A1 and B and HDL density subfractions: the Atherosclerosis Risk in Communities (ARIC) study. Circulation 2001;104:1108-1113.

15. Acton S, Riggoti A, Landshulz KT, Zu S, Hobbs S and Krieger M. Identification of scavenger receptor SR-BI as a high density lipoprotein receptor. Science 1996;271:518-520.

16. Valles V, Aguilar- Salinas CA, Gomez- Perez FJ, Rojas R, Franco A and Olaiz G, Rull JA and Sepulveda J. Apolipoprotein B and A-1 distribution in Mexican Urban Adults: Results of a Nation wide survey. Metabolism 2002; 51: 560 - 68.

17. Gotto AM, Whitney T, Stein A, Shapiro DR, Clearfield M and Weis S et al. Relation between baseline and on treatment lipid parameters and the first acute major coronary events in the Air Force/texas coronary Atherosclerosis Prevention study (AFCAPS/TEXCAPS). Circulation 2000;101:477-484.

18. Sharma R, Mahajan M and Mahajan R. Comparative account of serum lipids, lipoproteins and apolipoprotein B in patients of coronary artery disease. Indian Journal of Clinical Biochemistry 2004; 19:10-13.

19. Francis MC and Frohlich JJ. Coronary artery disease in patients at low risk: apolipoprotein A1 as independent risk factors. Atherosclerosis 2001; 155:165-170.

20. Bahl VK, Vaswani M, Thatai D and Wasir HS. Plasma levels of apolipoproteins A1 and B in Indian patients with angiographically defined coronary artery disease. Int J Cardiol 1994;46:143-149.

Author Information

Ritu Sharma
Department of Molecular Biology and Biochemistry, Guru Nanak Dev University

Balwant Singh
Department of Molecular Biology and Biochemistry, Guru Nanak Dev University

Mridula Mahajan
Department of Biochemistry,, Govt. Medical College

Ravi Kant
Department of Medicine, ESI Hospital