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Importance of HDL Cholesterol as Predictor of Coronary Heart Disease in Jordan Population: The Role of HDL-Subfractions in Reverse Cholesterol Transport

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Abstract: The incidence of coronary heart disease (CHD) was assessed through cross-sectional study including 400 volunteer subjects aged between 16 and 65 years. An adequate incidence of atherosclerotic CHD was only found in male subjects greater than 40 years of age. The analysis and subsequent one year follow – up period was therefore confined to 200 male participants aged 40–65 years. In the follow–up period 65 participants developed atherosclerotic CHD only 20 definite non- fatal myocardial infraction (MI). Invariant analysis revealed a significant association between the incidence of atherosclerotic CHD and a high density lipoprotein cholesterol ($P \leq 0.001$) which remained after adjustment for other risk factors.

Key words: Cholesterol, HDL cholesterol, coronary heart disease, lipidemia, Jordan population, HDL–subfractions

Introduction

Over the past three decades, great progress has been made in identifying and correcting the risk factors for cardiovascular disease (CVD), such as smoking, high blood pressure and elevated plasma levels of total cholesterol and low-density lipoprotein (LDL) cholesterol. Despite this encouraging result, however, CVD is still the leading cause of death in many countries (Vincelj *et al*., 1997). Reduced level of high-density lipoprotein (HDL) cholesterol often associated with elevated plasma triglycerides may play a significant role in the risk of developing CVD (Cullen, 2000; Mediene *et al*., 2001). At the National Institute of Health (NIH) conference on HDL cholesterol and triglycerides held in Washington DC in February 1992, the panel concluded that level of HDL cholesterol below 35 mg dl^{-1} constituted a high risk for developing coronary heart disease (CHD). The group noted that in order to assess the risk of developing CHD, it was important to measure HDL cholesterol whenever cholesterol was measured. Paragh and Harangi (2001) reported that hypercholesterolemia patients with coronary heart disease showed significantly lower values of HDL cholesterol and higher total/HDL cholesterol ratio as compared with hypercholesterolemic subjects free of CHD. To further investigate the relation between HDL cholesterol and the risk of CHD, this paper presents results from the prospective study carried out on a selected sample from Jordan population.

Materials and Methods

Study population: Four hundred subjects were selected from three public hospitals (Islamic Hospital, Specialty Hospital and Al-Bashir Hospital) located in different parts of Jordan. Each participant was examined for CVD risk factors including smoking, high blood pressure and high level of both total cholesterol and LDL cholesterol and then subsequently monitored in order to record cardiovascular events including Myocardial infection and stroke. The study began in 2001 and recruitment phase was completed at the beginning of 2002. Full data records are held on a total of 400 participants, age range 16 to 65 years. The range age of the 282 men who participated in the study was 41.4± 11.2 years and that of the 118 women was 36.6 ± 12.5 years ($P \leq 0.001$).

Study design: On entry into the study, the case history of each participant was recorded using standardized questionnaires and their blood pressure was also measured. Blood sample was collected from each participant after 12 h fast, used for the determination of more than 7 laboratory parameters. The participants neither conducted nor arranged for any treatment intervention. Total cholesterol, triglycerides and HDL cholesterol were measured using enzymatic assays and for HDL cholesterol, a precipitation method commercially available from Boehringer Mannheim, Germany, was used in conjunction with Hitachi 737 autoanalyzer (Friedewald *et al*., 1972). LDL cholesterol was calculated by the Friedwald formula, if trighlycerides were less than 400 mg dl^{-1} (Friedewald *et al*., 1972). Other methods, such as systolic blood pressure, diastolic blood pressure and fasting blood sugar, used in the examinations are described in details elsewhere.

Follow-up study design: At study entry, participants were informed that they would be sent a questionnaire every six months to determine the occurrence of MI or stroke. The response rate to these questionnaires was 95%. For the following analyses two end points were considered: definite, non-fatal MI and atherosclerotic CHD. An explorative analysis was performed using statistical package for social sciences (SPSS-X) (Nie, 1983). The relationship between a variable and the risk of atherosclerotic CHD was described by dividing the patient series into tertiles of the studied variable and then calculating the incidence rate for each tertile. As is usual in prospective studies of CHD, the simultaneous contributions of several factors to the risk of major ischaemic heart disease were analyzed using the multiple logistic model (Nie, 1983).

Results

In contrast to other risk factors investigated, HDL cholesterol and the prevalence of low HDL cholesterol level were found to be almost independent of age in adults (Table 1, Fig. 1 a, b). However, on average, HDL cholesterol levels were 12 mg dl^{-1} lower in men than in women. Levels of plasma HDL cholesterol were lowered by the presence of hypertriglyceridemia or by diabetes mellitus, cigarette smoking, obesity and physical inactivity in men, while by contrast oral contraceptives, physical activity were found to raise plasma HDL cholesterol level (Paragh and Harangi, 2001). The analysis and 1 year follow–up period were confined only to 200 male participants aged between 40 and 65 years (Table 2) who had

Table 1: Distribution parameter of HDL cholesterol according to age

Age	N	Mean± SD (mg dl^{-1})	Median (mg dl^{-1})	5th percentile (mg dl^{-1})	95th percentile (mg dl^{-1})
Males					
16-24	35	45.4± 10.7	44	30	64
25-34	30	45.6± 11.5	44	29	65
35-44	50	45.8± 12.6	44	29	67
45-54	86	46.1± 11.9	45	29	67
55-64	81	46.4± 12.6	45	29	68
Females					
6-24	12	56.9± 14.3	56	36	82
25-34	20	58.1± 14.8	57	36	84
35-44	31	57.4± 14.6	56	35	83
45-54	38	58.0± 14.9	57	36	84
55-64	17	58.4± 15.0	57	36	85

Table 2: Mean value of age standardized factors for male participants, aged 40 to 65 Years

Variables	CHD- (n = 135)	CHD+ (n= 65)	P-value*
Total cholesterol (mg dl^{-1})	222.9± 41.0	251.8± 47.3	< 0.001
HDL cholesterol (mg dl^{-1})	45.2± 11.8	39.5± 10.6	< 0.001
LDL cholesterol (mg dl^{-1})	147.1± 35.9	176.2± 39.5	< 0.001
Triglycerides (mg dl^{-1})	135.2± 12.6	163.0± 10.9	< 0.001
Systolic blood pressure (mmHg)	132.7± 18.9	139.4± 21.2	< 0.001
Diastolic blood pressure (mmHg)	86.3± 11.1	89.5± 12.7	< 0.001
Fasting blood glucose (mg dl^{-1})	102.0± 21.1	108.2± 33.7	< 0.02

-CHD + and CHD - are the groups with and without development of atherosclerotic CHD within 1 year. Values are the means± SD. Unless otherwise indicate, Statistical analysis is significant when P ≤ 0.001,
n= number of subjects.

Table 3: Incidence of CHD per 100 subjects over 1 year period according to total cholesterol and HDL cholesterol levels

No. of subject	HDL cholesterol (mg dl^{-1})	Total cholesterol (mg dl^{-1})	Incidence (%)
1	> 55	< 200	0.5
1	35-55	< 200	2.0
2	< 35	< 200	3.5
2	> 55	200-249	1.5
3	35-55	200-249	3.0
5	< 35	200-249	11.0
3	> 55	250-300	3.3
4	35-55	250-300	3.5
6	< 35	250-300	20.0
4	> 55	> 300	10.0
10	35-55	> 300	8.0
24	< 35	> 300	28.0

*Normal HDL cholesterol level: 40 – 69 mg dl^{-1} (Mediene *et al.*, 2001). Normal total cholesterol level: 168 – 250 mg dl^{-1} (Friedewald *et al.*, 1972)

no prior history of MI or stroke. From the data (Table 2), coronary heart disease was developed in 65 participants out of the total samples (n= 200) and only 20 participants with definite non-fatal MI. The 135 subjects in non-CHD group had an age standardized mean HDL cholesterol level of 45.2± 11.8 mg dl^{-1}, whereas, in CHD (+) group the mean HDL cholesterol level was 39.5± 10.6 mg dl^{-1} (P ≤ 0.001). Also LDL cholesterol, systolic blood pressure and diastolic blood pressure were higher in CHD (+) group than in CHD (-) (Table 2). Thirty male participants (46.1%) of CHD (+) group (n= 65) showed HDL cholesterol level less than 35 mg dl^{-1}, while only 21 male participants (15.5%) of CHD (-) (n= 135) had HDL cholesterol below 35 mg dl^{-1}. When comparison was made between the effect of HDL cholesterol and the total cholesterol level on the incidence (%) of CHD (Table 3), individuals with HDL cholesterol below 35 mg dl^{-1} had an approximate 7 folds increased CHD within 1 year as compared with men who have HDL cholesterol level of > 55 mg dl^{-1}, while the incidence (%) of CHD among men with a plasma CHD cholesterol concentration of 35–55 mg dl^{-1} was increased 4 times (Table 3). An analysis of the incidence of CHD in terms of plasma concentration of HDL cholesterol and total cholesterol level showed a clear relation between the increasing incidence of CHD and the level of both HDL cholesterol and total cholesterol. Men participants with low HDL cholesterol (< 35 mg dl^{-1}) and high cholesterol level (> 300 mg dl^{-1}) showed an increase in the incidence of CHD to 28%, but in group with HDL cholesterol level (> 55 mg dl^{-1}) the incidence (%) of CHD was decreased to 10% despite the high cholesterol level (> 300 mg dl^{-1}). The incidence (%) of CHD in men participants over 1 year period is shown according to HDL cholesterol level when cholesterol levels were within the normal range (200 – 249 mg dl^{-1}) (Fig. 2). Individuals with HDL cholesterol level < 35 mg dl^{-1} had an approximate 8 folds increased risk of CHD as compared to men with HDL cholesterol level of > 55 mg dl^{-1}. The difference between men with HDL cholesterol level of 35-55 and > 55 mg dl^{-1} was 50% only (Fig. 2).

Discussion

The level of HDL cholesterol has been documented to be inversely related to the incidence of CHD in several prospective, epidemiological studies (Mediene *et al.*, 2001; Cullen, 2000; Mikhailidis and Wierzbicki, 2000; Millo, 1999, Vincelj *et al.*, 1997; Koren *et al.*, 1996). In accordance with our findings, six studies (Paragh and Harangi, 2001; Mediene *et al.*, 2001; Cullen, 2000;

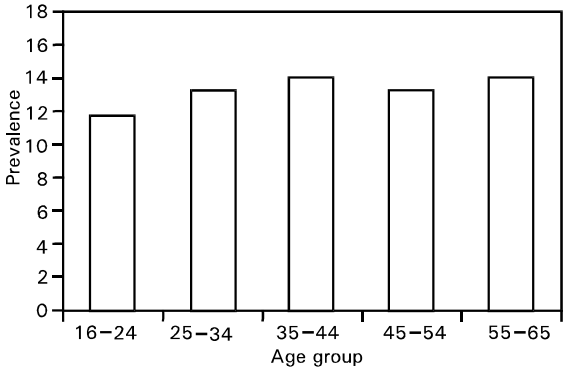

Fig. 1a: Prevalence of low HDL-cholesterol concentration (< 35 mg dl^{-1}) in relation to age in men

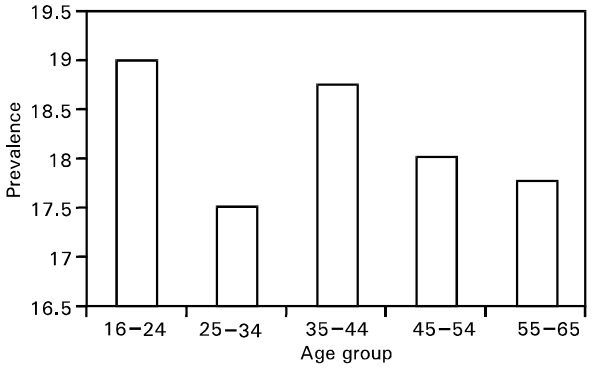

Fig. 1b: Prevalence of low HDL-cholesterol concentration (< 45 mg dl^{-1}) in relation to age in women

Vincelj *et al.*, 1997) showed that the inverse association was statistically significant after adjustment for other risk factors. The results are corroborated further by the data of the Helsinki Heart Study (Frick *et al.*, 1987). Another study (Farmer and Gotto, 1995) found that within each LDL cholesterol tertile of subjects treated with either placebo or gemfibrosil, as anti-hyperlipidemic drugs, the risk of CHD increased with decreasing plasma concentration of HDL cholesterol. This risk pattern remained essentially similar after adjustment for age, smoking and systolic blood pressure. The anti-atherogenicity of HDL cholesterol is usually explained by its ability to remove excess cholesterol from non-hepatic cells to the liver (Eisenberg, 1984; Tall, 1990). The increase in the concentration of serum HDL cholesterol and the fall in that of LDL cholesterol were both associated with reduced risk CHD, where as the changes in the amount of total cholesterol and triglycerides in serum were not correlated well with the risk of CHD (Paragh and Harangi, 2001; Cullen, 2000). Some studies (Von *et al.*, 1994a; Von *et al.*, 1994b; Assmann *et al.*, 1994) questioned the anti-atherogenic role of HDL cholesterol. They reported that various subfractions of HDL play different roles in reverse cholesterol transport and some of these minor subfractions are present even in individuals who have HDL cholesterol deficiency.

In order to explain the proper role of HDL cholesterol in decreasing the CHD incidence, several studies gave the basis of the HDL subfractions role in lowering the cholesterol transport by LDL (Von *et al.*, 1994a, b). In Von *et al.* (1994b) study, three subclasses of HDL were separated namely; pre B1 – LPA-I (lipoprotein A-I), pre B2-LPA-I and pre B3- LPA-I. The remaining majority of HDL has β–mobility which is differentiated into β-LPA-12 and β-LPA-13 as presented by Castro and Fielding (1988).

In plasma obtained from patients with apo A-I deficiency, Tangier disease (TD) and Lecithin cholesterol acyl transferase deficiency

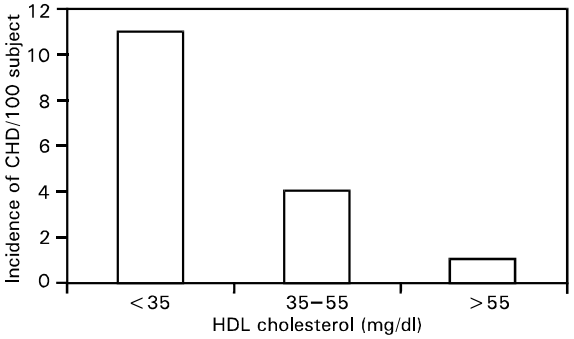

Fig. 2: Incidence of CHD per 100 men over 1 year period according to HDL cholesterol level
Note: The data in Fig. 2 were selected at normal cholesterol level (200–249 mg dl^{-1})

(LCAT-deficiency), the distribution of LpA-I subfractions differs from that found in plasma taken from normal subjects (Von *et al.*, 1994a). LPA-I is absent from plasma with apo A-I deficiency. Only pre β2 and pre β3 of HDL are present in patient plasma with LCAt deficiency and TD. Several reports (Tall, 1990; Huang *et al.*, 1994), using 1 min. pulse incubation of plasma with [^{3}H] cholesterol loaded fibroblasts, have identified pre β1 LPA-I and β-lipoprotein E (β-LPE) as initial acceptors cell-derived cholesterol. Also in plasma derived from normal subjects, the amount of [^{3}H] cholesterol accumulating in β-LPE equals the amount in pre β1-LPA-I. In apo A-I deficiency where LPA-I is absent, only β-LPE can absorb cell-derived [^{3}H] cholesterol which is accompanied by a 50% decrease in the uptake of cellular [^{3}H] cholesterol after 1 min pulse incubation (Huang *et al.*, 1994). The same reduction in efflux of cellular cholesterol has been observed in plasma derived from normal subjects which has been depleted of apo A-I by anti apo-A immuno-affinity chromatography (Kawano *et al.*, 1993). This suggests that lipoproteins which do not contain apo A-I, especially β-LPE, play an important role in the uptake of cellular cholesterol into plasma. It has been found by Ordavas and Schaefer (1994) that β-LPE is immunodetectable only in the plasma of individuals who carry at least one apo E_3 allele. Plasma which contains apo E_3 releases [^{3}H] cholesterol from fibroblasts into both β-LPE and pre-β1-LPA-I. β-LPE is an important contributor to initial cholesterol efflux into normal plasma and is a major component of the residual activity, resulting in cholesterol release from cells in apo A-I deficient plasma (Davignon *et al.*, 1988). The failure of apo E_4 to form β-LPE may account for the increased risk of MI which is associated with this isoform (Ordavas and Schaefer, 1994). In TD and genetic LCAT-deficiency, cell derived [^{3}H] cholesterol is taken up by both pre β1-LPA-I and β-LPE. However, in comparison with normal plasma, the uptake of cellular [^{3}H] cholesterol in these subjects after 1 min pulse, was reduced by 35% (TD) and 20% (LCAT-deficiency) (Von *et al.*, 1994a). In normal plasma, cholesterol taken up by pre β1-LPA-I is transferred to LDL via pre β2-LPA-I, pre β3-LPA-I and β-LPA-I (Huang *et al.*, 1993). The minority of cholesterol is esterified in pre β3-LPA-I which contains cholesterol ester transfer protein (CETP), LCAT and apo D, while the majority of the cholesterol is esterified in β-LPA-I after re-transfer for LDL-cholesterol. In plasma which is deficient in HDL cholesterol, cell-derived [^{3}H] cholesterol is transferred more rapidly to LDL than in plasma derived from normal subject, while in plasma which is deficient in LCAT, [^{3}H] cholesterol remains non-esterfied (Huang *et al.*, 1993). In plasma derived from subjects with apo A-I deficiency or TD, the transfer of [^{3}H] cholesterol enters to LDL is enhanced compared with plasma derived from normal subjects.

In conclusion, deficiency of HDL-cholesterol enhances the transport of cholesterol more rapidly to LDL, also genetic deficiency of LCAT and genetic variations in apo E influence the formation of β-LPE and the initial uptake of cell-derived cholesterol into plasma, which may influence the risk of developing CHD.

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