Clinical biomarkers of high-density lipoprotein dysfunction among middle-aged Turks

Orta yaşlı Türklerde HDL disfonksiyonunun klinik markörleri

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ABSTRACT

Objective: Impaired function of high-density lipoprotein (HDL) particles generates cardiometabolic disorders in people prone to impaired glucose tolerance for which clinical biomarkers need delineation.
Study design: Prospective population-based study.
Methods: Totally, data of 2725 adults followed-up over 7.3±3.0 years were analyzed by Cox regression analysis. C-reactive protein (CRP), complement C3 (C3), triglycerides (Trg) and HDL-cholesterol were tested to predict risk for incident diabetes or coronary heart disease (CHD).
Results: Beyond atherogenic dyslipidemia, high-Trg/normal HDL-cholesterol category was associated with elevated CRP and diabetes risk in women. Normotriglyceridemic men with normal HDL-cholesterol showed higher apolipoprotein A-I levels and higher diabetes risk than men having low HDL-cholesterol. Diabetes risk doubled in hypertriglyceridemic women regardless of HDL-cholesterol. Trg/HDL-C>2 in men and Trg>1.7 mmol/L in women were best predictors of diabetes. C3>1.3 g/L served additively in women alone. Regarding CHD risk, not CRP, but C3 contributed independently to Trg/HDL>2 in men [RR 2.46 (95% CI 1.33; 4.53)]; a high ratio was merely additive to elevated CRP in women. Among five cut-off values, predictive values for diabetes were highest for CRP >2.5 mg/L in men, Trg>1.7 mmol/L and C3>1.3 g/dL in women.
Conclusion: Trg/HDL-C ratio >2 and/or CRP >2.5 mg/L in men and Trg>1.7 mmol/L+C3>1.3 g/dL in women are most appropriate markers regarding impaired antiinflammatory or atheroprotective HDL function. In normotriglyceridemic men with normal HDL-cholesterol levels, diabetes risk may be elevated due to presumably dysfunctional apolipoprotein A-I. *(Anadolu Kardiyol Derg 2012; 12: 628-36)*
Key words: Coronary heart disease, Cox proportional regression analysis, C-reactive protein, diabetes type-2, HDL dysfunction, triglycerides, sensitivity, specificity

ÖZET

Amaç: Yüksek yoğunluklu lipoprotein (HDL) parçacıklarının fonksiyon bozukluğu, glikoz tolerans bozukluğuna yatkın kişilerde kardiyometabolik hastalık üretebildiğinden, klinik belirteçlerinin belirlenmesi gerekir.
Çalışma dizaynı: Öne dönük popülasyona dayalı.
Yöntem: Toplam 2725 yetişkin 7.3±3.0 yıllık takipte Cox regresyon analiziyle değerlendirildi. Başlangıçta ilgili olguların dışlanmasından sonra, ileride diyabet ve koroner kalp hastalığının gelişmesi bakımından, C-reaktif protein (CRP), kompleman C3 (C3), trigliseridler (Trg) ve HDL-kolesterolün öngörü değerleri incelendi.
Bulgular: Diyabet riski açısından kadınlarda, aterojen dislipidemi dışında, artmış CRP ile bağıntılı yüksek-Trg/normal HDL-kolesterol kategorisi ilişki gösterdi. Normal trigliserid ve HDL-kolesterol seviyesine sahip erkekler, düşük HDL-kolesterollü erkeklere kıyasla, daha yüksek apolipoprotein A-I düzeyiyle birlikte yüksek diyabet riski sergiledi. Hipertrigliseridemili kadınlarda diyabet riski, HDL-kolesterol düzeylerine bakılmaksızın, ikiye katlandı. CRP >2.5 mg/L ölçütü her iki cinsiyette diyabeti anlamlı öngördü; ama erkekte en iyi öngörücü Trg/HDL-C >2, kadında Trg>1.7 mmol/L ölçütleriydi. Ayrıca C3>1.3 g/L yalnızca kadında katkı sağladı. Koroner hastalık riski açısından erkekte Trg/HDL >2 oranına (RR 2.46 [%95 GA 1.33; 4.53]), C3 değil, CRP bağımsız öngörü ekledi; yüksek oran kadınlarda artmış CRP'ye katkı sağladı. Diyabet için en yüksek öngörü değerine, beş ölçütten, erkekte CRP >2.5 mg/L, kadında Trg >1.7 mmol/L ile C3 >1.3 g/dL sahipti.

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Sonuç: HDL'nin anti-enflamatuvar veya ateroprotektif işlevi bozukluğunun en uygun belirteçleri, erkekte Trg/HDL-K oranı >2 ve/veya CRP >2.5 mg/L, kadında Trg>1.7 mmol/L +C3>1.3 g/L ölçütleridir. Trigliserid ve HDL-kolesterolü normal düzeyli erkekte diyabet riski muhtemelen disfonksiyonlu apolipoprotein A-I aracılığıyla artmaktadır. *(Anadolu Kardiyol Derg 2012; 12: 628-36)*
Anahtar kelimeler: Koroner kalp hastalığı, Cox oransal regresyon analizi, C-reaktif protein, diyabet tip-2, HDL disfonksiyonu, trigliseridler, duyarlılık, özgüllük

Introduction

Interest in high-density lipoproteins (HDL) is growing due to findings linking diabetes and atherosclerosis to the formation of dysfunctional HDL. In type-2 diabetes, observed deficient anti-inflammatory properties of HDL have been ascribed to mechanisms including HDL enrichment with conformational alterations of apolipoprotein (apo) A-I, glycation of apolipoproteins and oxidative modification of HDL lipids (1). On the other hand, as the protective properties of HDL are impaired, stress-induced β-cell apoptosis and islet inflammation may prevail and contribute to the pathogenesis of diabetes (2). The role of oxidized phospholipids and HDL has been hypothesized in atherogenesis (3). Of the phospholipids carried in HDL, ceramide has been implicated in the pathogenesis of insulin resistance and has many pro-inflammatory properties (4). One potential mechanism implicating dysfunctional HDL in the pathogenesis of cardiovascular disease was suggested as malondialdehyde impairing the cholesterol transporter ABCA1 activity of apo A-I (5).

From an epidemiologic viewpoint, normal or high HDL-cholesterol levels have been noted to fail to protect against vascular disease by an absent inverse relation between HDL-cholesterol and coronary heart disease (CHD) incidence in subjects at high risk (6), and a systematic review disclosed that no association existed between treatment-induced change in HDL-cholesterol and risk ratios for cardiovascular disease morbidity and mortality, when changes in low-density lipoprotein (LDL)-cholesterol were adjusted for (7). In dyslipidemic patients, normal levels of HDL-cholesterol were not associated inversely, but rather positively (albeit insignificantly) with elevated gamma-glutamyl transferase (GGT) activity (8), recognized to reflect generation of reactive oxygen species (9). HDL-cholesterol levels did not significantly predict newly developing cardiovascular disease in diabetic Iranian men and women, nor in non-diabetic females (10).

HDL-cholesterol levels in Scandinavian subjects with impaired glucose tolerance did not protect against type-2 diabetes (11). Middle-aged and elderly Turkish adults were the first general population in whom HDL dysfunction has been documented in prospective studies to fail to protect against diabetes and/or CHD (12, 13). Mechanisms related to a pro-inflammatory status/oxidative stress are of utmost importance in cardiometabolic risk, implicate huge public health and are more pronounced in women (14).

Managing HDL dysfunction and its consequences obviously requires the clinical identification of individuals with such impairment, an uninvestigated area.

We, therefore, aimed in this study to delineate which parameters may serve as an indicator of impaired function of HDL particles in Turkish women or men searching for cut-off points that may be utilized clinically. Based on our previous experience (14), we focused on serum fasting triglycerides (Trg), its ratio to HDL-cholesterol, C-reactive protein (CRP) and complement C3 (C3).

Methods

Study design

A prospective population-based study.

Population sample

The Turkish Adult Risk Factor (TARF) is a longitudinal population-based cohort study on cardiac disease and its risk factors in Turkey carried out biennially in all geographical regions (15). It involves a random sample of the Turkish adult population, representatively stratified for sex, age, geographical regions and rural-urban distribution (15). Inclusion of participants was based on availability of combined measurements of fasting Trg and HDL-cholesterol at baseline (n=2725). Participants, 28 years of age or older at baseline, were examined periodically up to the survey 2008/09. Subjects with no follow-up (n=196) were further excluded from prospective analyses. The survey conformed to the principles embodied in the Declaration of Helsinki and was approved by the Istanbul University Ethics Committee. Individuals of the cohort gave written consent for participation. Data were obtained by history of the past years via a questionnaire, physical examination of the cardiovascular system, sampling of blood and recording of a resting 12-lead electrocardiogram.

Measurements of risk variables

Blood pressure was measured on the right arm using a sphygmomanometer (Erka, Bad Tölz, Germany) after 10 minutes of rest while seated, and the mean of two recordings at least 3 min apart was recorded. Waist circumference was measured with a tape [Roche LI95 63B 00, (Roche Diagnostics, Mannheim, Germany.)], the subject standing and wearing only underwear, at the level midway between the lower rib margin and the iliac crest. Self-reported cigarette smoking was categorized into never, former and current smokers.

Plasma concentrations of total and HDL-cholesterol, fasting Trg and glucose were determined at baseline examination by enzymatic dry chemistry using a Reflotron apparatus (Roche Diagnostics, Mannheim, Germany). In the final four surveys, the stated parameters, insulin, CRP and C3 values were assayed in a single central laboratory. Blood samples were shipped to

Istanbul to be stored in deep-freeze at -75°C, until analyzed. Concentrations of insulin were determined by the electrochemiluminescence immunoassay ECLIA on Roche Elecsys 2010 (Roche Diagnostics, Mannheim, Germany). Serum concentrations of apo A-I, apo B and CRP were measured by the Behring nephelometry (Behring Diagnostics, Marburg, Germany). External quality control was performed with a reference laboratory in a random selection of 5-6% of participants. Data on CRP and C3 were available in 86% and 46% of tracked participants, respectively.

Definitions and outcomes

Individuals with type-2 *diabetes* were diagnosed with criteria of the American Diabetes Association (16), namely when plasma fasting glucose was ≥7 mmol/L (or 2-h postprandial glucose ≥11 mmol/L) and/or the current use of diabetes medication. Individuals with abdominal obesity were identified using cut-points of ≥95 cm in men (17) and ≥88 cm in women. Atherogenic dyslipidemia was defined as a lipid disorder consisting of high Trg (≥1.7 mmol/L) combined with low HDL-cholesterol (<1.03/<1.3 mmol/L) (18).

Diagnosis of nonfatal CHD was based on the presence of angina pectoris, a history of myocardial infarction with or without accompanying Minnesota codes of the electrocardiograms (19) or on a history of myocardial revascularization. Typical angina and, in women, age >45 years were prerequisite for a diagnosis when angina was isolated. ECG changes of "ischemic type" of greater than minor degree (Codes 1.1-2, 4.1-2, 5.1-2, 7.1) were considered as myocardial infarct sequel or myocardial ischemia, respectively. Cause of death was assigned in accordance with the information on the mode of death obtained from first-degree relatives and/or local health personnel, considering also pre-existing clinical and laboratory findings elicited during biennial surveys.

Statistical analysis

Statistical analyses were performed using SPSS-10 for Windows (SPSS Inc., Chicago, Ill., Nr. 9026510, USA). Descriptive parameters were shown as mean±standard deviation (SD) or in percentages. Due to skewed distribution, log-transformed values and geometric means were used for serum CRP and insulin. Two-sided t-tests and Pearson's Chi-square tests were used to analyze the differences between means and proportions of two groups. Participants were analyzed in each sex in 4 groups of normal or elevated Trg (≥1.7 mmol/L) and normal or low HDL-cholesterol (<1.03/<1.3 mmol/L) since dysfunction of HDL particles depends largely to elevation in Trg and to the presence of enhanced low-grade inflammation. In predicting outcomes at baseline examination in multivariate analyses, estimates (and 95% confidence intervals) for relative risk (RR) were obtained by use of Cox proportional hazards regression analyses in models that controlled for potential confounders. Cases with prevalent diabetes (n=196) or CHD (n=185) were excluded in the respective prospective analyses. RRs for 1 SD of the log-transformed CRP were expressed in terms of a 3-fold increment. Cut points of few parameters relative to atherogenic dyslipidemia and low-grade inflammation were explored to potentially identify individuals at high likelihood of HDL dysfunction. Areas under ROC curve for each cut-off were evaluated for specificity, sensitivity and positive and negative predictive values yielded. A value of $p<0.05$ on the two-sided test was considered statistically significant.

Results

Mean follow-up of the study population was 7.3±3.0 (range 1 to 11) years. Baseline characteristics of the study sample (n=2725) are provided in Table 1. Wide waist girths, high fasting triglyceride and low HDL-cholesterol levels were main distinctive features; prevalence of current smoking and alcohol usage was low in females.

Figure 1 shows both the distribution of mean CRP levels and incidence of type-2 diabetes in 4 categories of Trg and HDL-C in each sex. The graph reflects that two-thirds of men or women have low HDL-cholesterol levels. Concentrations of CRP were not significantly different among men in any lipid category, among which relatively few men had normal HDL-cholesterol in the hypertriglyceridemic category. CRP was increased by 11% in categories with elevated triglyceride levels. In women, in contrast, CRP values were by 70% higher with elevated than with normal triglyceride levels ($p<0.001$). In the hypertriglyceridemic categories the number of women with normal HDL-cholesterol was still fewer. The data in Fig. 1 thus indicate that any variation in CRP concentrations was independent of variation in dyslipidemic categories in men whereas elevated CRP was linked to hypertriglyceridemia among women.

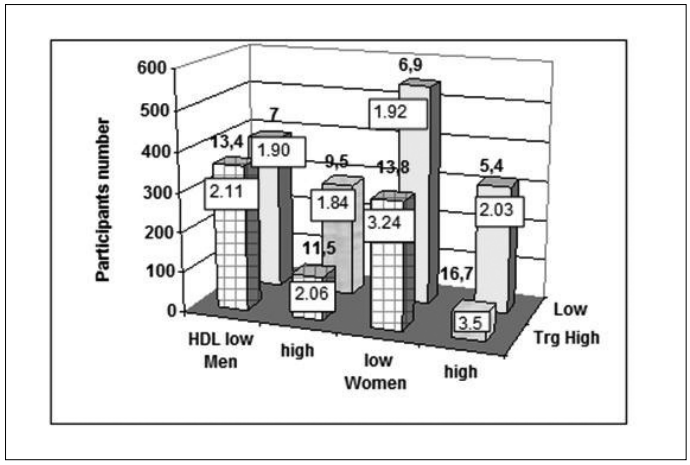

Figure 1. Number of participants (in bars) are illustrated in each of the 4 categories of high/low triglyceride and HDL-cholesterol concentrations, along with the respective geometric mean CRP values (in mg/L) and incidence of diabetes (in %) among men and women. Unanticipatedly high diabetes incidences in women with high triglyceride/normal HDL-C (16.7%) and men with low triglyceride/normal HDL-C (9.5%) are highlighted

CRP - C-reactive protein, HDL - high-density lipoprotein

Table 1. Selected characteristics in non-diabetic sample by gender (n=2725)

Variables	Men			Women			
	n	Mean	SD	n	Mean	SD	*p
Age, years	1308	48.5	12.2	1417	48.0	11.9	0.30
Waist circumference, cm	1289	94.3	10.9	1396	90.9	12.5	<0.001
Body mass index, kg/m^2	1249	26.8	5.4	1358	29.0	5.7	<0.001
Systolic BP, mmHg	1288	126.8	22.1	1398	132.4	25.6	<0.001
Diastolic BP, mmHg	1288	81.0	13.0	1398	82.8	13.9	0.001
Fasting glucose, mmol/L	1119	5.4	1.5	1234	5.5	1.5	0.068
Total cholesterol, mmol/L	1256	4.7	1.0	1361	4.88	1.04	<0.001
F. triglycerides¶, mmol/L	1308	1.54	1.73	1417	1.34	1.66	<0.001
HDL-cholesterol, mmol/L	1308	0.98	0.3	1417	1.17	0.3	<0.001
Trg/HDL-cholesterol¶	1308	3.74	1.99	1417	2.71	1.89	<0.001
Apolipoprotein A-I, g/L	851	1.26	0.34	932	1.39	0.33	<0.001
Apolipoprotein B, g/L	846	1.14	0.35	927	1.12	0.36	0.43
CRP¶ mg/L	1123	1.94	3.13	1225	2.25	3.17	0.002
Complement C3, g/L	569	1.29	0.27	691	1.34	0.28	0.001
Fasting insulin¶ mIU/L	753	7.45	2.08	908	7.72	1.93	0.29
	Men, n	n	%	Women, n	n	%	
Current smoking,	1288	679	52.7	1397	254	18.2	<0.001
Alcohol usage	1285	243	18.9	1394	11	0.8	<0.001

Data are presented as mean and SD, number and percentage

*Two-sided unpaired t-test and Pearson's Chi-square tests were used

BP - blood pressure, CRP - C-reactive protein, F. - fasting, HDL - high-density lipoprotein

Higher diabetes risk in males with normal triglyceride and HDL-cholesterol levels

In men, in conjunction with normal triglyceride levels, the normal HDL-C category surprisingly revealed a higher risk of diabetes than the low HDL-C category. This could not be accounted for by waist circumference, fasting glucose, insulin, homeostatic model assessment (HOMA) and C3 each of which was significantly higher in men with low HDL-C category, by apo B and current smoking which were similar in the two groups (details not shown). A higher risk was associated in the normal than the low HDL-C category even after age adjustment (Table 2). Serum apo A-I (1.353 g/L) was highest in men with normal HDL-C category ($p<0.05$).

Trg/HDL-cholesterol categories in risk for diabetes

Cox regression analysis in predicting incident diabetes by Trg/HDL-cholesterol categories adjusted for age and CRP are given in Table 2, separately for genders. Low Trg-low HDL-C served as referent regarding diabetes risk in men. In the presence of hypertriglyceridemia, diabetes risk was over 2-fold regardless whether HDL-C levels were low or normal. In the group with normal HDL-C levels, men had (independently of CRP) an HR 2-fold as high as the referent and in women HR was 3.47 (95%CI 1.6-7.5) which attenuated only modestly after adjustment for CRP. Entering CRP in Model 2 raised HRs of the high-Trg categories in men (CRP independent of HDL dysfunction); in women, it lowered the HR (CRP presumably mediated).

Testing a threshold of 1.5 mmol/L for Trg did not provide an advantage in the prediction of diabetes by the studied groups of Trg/HDL.

In a third model, we tested the sex-specific predictive value for diabetes risk of the cut-off values of Trg/HDL-cholesterol ratio and CRP along with individual standard cut-off values of Trg and HDL- cholesterol. While in men the ratio and CRP were significant predictors alone, in women hypertriglyceridemia and CRP alone were significant. Dysfunction of normal HDL-C concentrations in women is suggested by an RR of HDL-cholesterol higher than 1 and an RR of the high ratio cut-off value lower than 1.

In a subset in whom C3 determinations had been available, these categories were further analyzed jointly with a cut-off value of serum C3 >1.3 g/L in predicting diabetes (Table 3). C3 contributed little in men independently, whereas in women it was predictive by a 2-fold HR independently of CRP levels and hypertriglyceridemia. The high Trg-normal HDL category exhibited also a 2-fold risk without attaining significance, likely due to few cases in this group.

Table 2. Cox regression analysis for prediction of incident type-2 diabetes by serum triglyceride (Trg) and HDL-cholesterol categories, adjusted for age and C-reactive protein[e]

	Men	n=124/1237[a]	Women	n=116/1336[a]
Independent variables	**RR**	**95% CI**	**RR**	**95% CI**
Model 1				
Age, 11 years	1.26	1.06; 1.48	1.03	0.85; 1.27
High Trg-normal HDL-C	2.00	1.06; 3.76	3.47	1.61; 7.48
Low Trg-normal/low HDL-C[c]	1.37	0.82; 2.28	1.26	0.73; 2.18
High Trg-low HDL-C[d]	2.31	1.47; 3.63	2.77	1.63; 4.74
	n=110/1066[a]		n=95/1157[a]	
Model 2[b]				
High Trg-normal HDL-C	2.25	1.15; 4.37	2.88	1.28; 6.49
Low Trg-normal/low HDL-C[c]	1.33	0.76; 2.32	1.20	0.57; 2.14
High Trg-low HDL-C[d]	2.42	1.50; 3.92	2.05	1.14; 3.68
C-reactive protein[e] 3-fold increment	1.23	1.11; 1.37	1.30	1.13; 1.48
Model 3 b				
Trg/HDL-C ratio (mmol) >2/>1.5	2.15	1.12; 4.13	0.71	0.36; 1.39
Triglycerides >1.7 mmol/L	1.25	0.68; 2.29	2.61	1.38; 4.94
HDL-cholesterol <1.03/1.3 mmol/L	0.73	0.47;1.14	1.09	0.66;1.79
C-reactive protein[e] 3-fold increment	1.28	1.15; 1.44	1.18	1.04; 1.354

[a]incident cases/ number at risk, [b]all models were age-adjusted; significant values are highlighted in bold, [c]referent : category with low Trg-normal HDL-C in women, but low Trg-low HDL-C in men, [d]defines atherogenic dyslipidemia, [e]log-transformed values
HDL - high-density lipoprotein

Table 3. Cox regression analysis for prediction of incident type-2 diabetes by serum triglycerides (Trg), HDL-cholesterol and their ratio, adjusted for age and elevated C3 and/or C-reactive protein levels

	Men	n=64/539[a]	Women	n= 61/651[a]
Independent variables	**RR**	**95% CI**	**RR**	**95% CI**
Model 1				
Age, 11 years	1.35	1.06; 1.73	1.12	0.85; 1.46
High Trg-normal HDL-C	0.91	0.35; 2.39	2.01	0.70; 5.75
Low Trg-low HDL-C	0.54	0.27; 1.09	0.90	0.45; 1.83
High Trg-low HDL-C[c]	1.20	0.62; 2.33	1.68	0.82; 3.44
Complement C3, >1.3 g/dL	1.48	0.87; 2.53	2.03	1.15; 3.57
Model 2[b]				
Trg/HDL-C >2/>1.5	1.46	0.64; 3.31	0.51	0.22; 1.21
C-reactive protein[d] 3-fold increment	1.28	1.09; 1.49	1.22	1.03; 1.45
Triglyceride >1.7 mmol/L	1.42	0.66; 3.07	2.64	1.20; 5.81
HDL-cholesterol <1.03/<1.3 mmol/L	0.80	0.44; 1.44	1.04	0.56; 1.94
Complement C3, >1.3 g/dL	1.26	0.74; 2.17	1.80	1.01; 3.23

[a]incident cases/ number at risk. [b]both models were age-adjusted; significant values are highlighted in bold, [c]defines atherogenic dyslipidemia, [d]log-transformed values
HDL - high-density lipoprotein

Sex-specific CHD risk determinants

Predictors for incident CHD risk were analyzed by Cox regression first in two age-adjusted models comprising the Trg/HDL-cholesterol categories, without and with CRP (Table 4). The category atherogenic dyslipidemia imparted roughly a 2-fold risk in each gender. In men, the potentially HDL-dysfunctional category additionally and significantly conferred an over 3-fold risk, whereas CRP proved not to have independent contribution. In women, CRP was the independent determinant while the moderately high HRs in high-triglyceride categories failed to reach significance.

In a second set of two regression models, we examined the combined cut-off values of sex-specific Trg/HDL-C ratio and CRP as predictors of incident CHD, adding C3 in one model (Table 4). Elevated C3 did not contribute additively to a high lipid ratio significantly predicting in men, but elevated CRP did independently contribute to a low lipid ratio by doubling the HR in each sex. In women, elevated CRP added to the prediction in the presence of a high Trg/HDL as well.

Sensitivity and specificity of the cut-off value for the cardiometabolic risks

Area under ROC curve of serum C3 for the prediction of *diabetes* was 0.60 (0.52-0.67, p=0.013) in men, 0.63 (0.57-0.70, p=0.001) in women. The triglyceride threshold had highest specificity and the C3 cut-off the highest sensitivity in women; so that the use of combined cut-offs was best for diabetes with a 95% negative and a 16% positive predictive value (Table 5). Trg/HDL-C ratio of >2 (p=0.003) and CRP >2.5 mg/L (p<0.001) were best indicators in men.

For incident *CHD*, a cut-off of Trg>1.7+C3 >1.3 had a positive predictive value of 20% and a negative predictive value of 92% in women (p=0.003). It was not significant in men. Hence, use of a cut-off of Trg>1.7+C3 >1.3 was most appropriate as a marker regarding impaired antiinflammatory and atheroprotective HDL function in women. The positive predictive value of the high Trg-normal HDL-C category in men was 19.2% and the negative predictive value 91.3% (p=0.013). These values were 17% and 91%, respectively, in women (p=0.002).

It is concluded that Turkish adults who have HDL-C ≥1.03/≥1.3 mmol/L concomitant with fasting Trg >1.7 mmol have a high likelihood of HDL dysfunction with respect to antiinflammatory and/or

Table 4. Cox regression analysis for prediction of incident coronary heart disease by serum Trg/HDL-cholesterol categories, adjusted for age and elevated C3 and/or C-reactive protein

Independent variables	Men		Women	
	RR	95% CI	RR	95% CI
Model 1	n=143/1234[a]		n=148/1350[a]	
Age, 11 years	1.71	1.49; 1.98	1.75	1.51; 2.02
High Trg-normal HDL-C	3.39	1.94; 5.90	1.77	0.89; 3.51
Low Trg-low HDL-C	1.35	0.83; 2.19	1.09	0.70; 1.70
High Trg-low HDL-C[c]	2.05	1.27; 3.31	1.85	1.20; 2.85
Model 2[b]	n=125/1057[a]		n=129/1163[a]	
High Trg-normal HDL-C	3.19	1.73; 5.86	1.62	0.81; 3.24
Low Trg-low HDL-C	1.42	0.85; 2.37	1.00	0.63; 1.60
High Trg-low HDL-C[c]	2.04	1.22; 3.41	1.49	0.93; 2.38
C-reactive protein[d] 3-fold increment	1.06	0.96; 1.18	1.20	1.07; 1.34
Model 3[b,e]	n=125/1057[a]		n=129/1163[a]	
Trg/HDL high; CRP <2.5	3.48	2.12; 5.72	1.60	0.88; 2.91
Trg/HDL low; CRP >2.5	2.24	1.35; 3.73	1.46	0.92; 2.32
Trg/HDL high; CRP >2.5	2.90	1.65; 5.10	2.22	1.39; 3.53
Model 4[b,e]	n=65/525[a]		n=74/625[a]	
Trg/HDL high; CRP <2.5	2.56	1.27; 5.18	1.39	0.56; 3.46
Trg/HDL low; CRP >2.5	2.13	1.06; 4.26	1.97	1.02; 3.77
Trg/HDL high; CRP >2.5	3.44	1.61; 7.33	3.28	1.66; 6.47
Complement C3, >1.3 g/dl	0.84	0.50; 1.41	1.07	0.65; 1.78

[a]incident cases/ number at risk, [b]all models were age-adjusted; significant values are highlighted in bold, [c]defines atherogenic dyslipidemia, [d]log-transformed values, [e]referent is the category of low Trg/HDL-C ratio with CRP <2.5 mg/L.
C3 - complement C3, CRP - C-reactive protein, HDL - high-density lipoprotein, Trg - triglycerides

Table 5. Sensitivity and specificity for prediction of incident diabetes by cut-off values of serum lipids, lipid ratio, elevated C-reactive protein and complement C3

Selected cut-off values	Men (n=1237) Sensitivity	Specificity	Women (n=1336) Sensitivity	Specificity
Triglycerides >1.7 mmol/L	52	61	48	72
C-reactive protein[a] >2.5 mg/L	59	63	63	54
Complement C3[b] >1.3 g/dL	56	56	71	48
Triglycerides>1.7+ C3>1.3	55	63	63	69
Trg/HDL-C ratio >2/>1.5	51	63	47	69
HDL-C >1.03/>1.3 mmol/L	65	35	75	31

[a]n=2223, [b]n=1190 Most appropriate cut-off values are highlighted in bold
HDL - high-density lipoprotein

atheroprotective properties. These cut-off values have a low sensitivity despite a high specificity with respect to CHD risk; the sensitivity regarding risk of diabetes can be substantially raised in women by using Trg >1.7 mmol, alone or in conjunction with complement C3 >1.3 g/L and in men by using Trg/HDL-C >2 and/or CRP >2.5 mg/L.

Discussion

Proinflammatory HDL as a biomarker for atherosclerosis has been described even in patients with systemic lupus erythematosus and rheumatoid arthritis (20). Given that HDL dysfunction is an important element in the development of cardiometabolic risk among middle-aged and elderly Turks (14, 21), we sought to delineate most suitable clinical biomarkers of impaired HDL function. The high Trg-normal HDL-cholesterol category revealed presence of HDL dysfunction independent of CRP by conferring significant risk of diabetes in both sexes and CHD in men. Cut-off points of Trg (>1.7 mmol/L), Trg/HDL-C ratios of >2/>1.5, CRP >2.5 mg/L, and C3 of >1.30 g/dL, markers of enhanced low-grade inflammation, and determined by areas under ROC curve, were tested sex-specifically in multivariably adjusted Cox regression models and for positive and negative predictive values. As markers regarding impaired antiinflammatory or atheroprotective HDL function, a Trg/HDL-C ratio of >2 and/or CRP >2.5 mg/L in men and a cut-off of Trg >1.7+C3 >1.3 in women were most appropriate. Moreover, in normotriglyceridemic men with normal HDL-cholesterol levels, the diabetes risk was unexpectedly elevated in the presence of raised and presumably dysfunctional apoA-I concentrations.

In the presence of hypertriglyceridemia, diabetes risk was doubled in each sex with little regard to HDL-cholesterol levels suggesting that normal HDL-cholesterol concentrations associated with hypertriglyceridemia were likely dysfunctional. A similar tendency to dysfunctionality of serum apoA-I associated with normal triglyceride and HDL-cholesterol levels likely existed in men. This is consistent with what we reported previously that high compared with low serum apoA-I levels in men nearly double the risk for incident diabetes, additively to age, body mass index, CRP, HDL-cholesterol among Turks (12).

Sex difference in HDL dysfunction

A fundamental sex difference was related to the association between CRP elevation and hypertriglyceridemia. In women, in contradistinction to men, CRP values were by 70% higher in company of elevated than with normal triglyceride levels indicating the linkage of elevated CRP to hypertriglyceridemia among women. Waist girth quartiles from quartile II on in women conferred excess risk of both atherogenic dyslipidemia and independent excess risk of incident CHD in this cohort (22). We found that, in addition to CRP, the ratio should be more appropriately used as a marker of HDL dysfunction relative to diabetes risk in men, at variance from using high Trg in women.

Hypertriglyceridemia appeared to be a major determinant of diabetes in both genders and of CHD in men, the CRP elevation associated with hypertriglyceridemia mediated it in women. These findings are parallel to those of Corsetti et al. (23) who identified a subgroup of non-diabetic postinfarction patients at high risk for recurrent coronary events. High HDL alone was an independent predictor of risk in this subgroup derived from inter-

action of hypercholesterolemia [representing presumably excess Lp(a), intermediate- density and very low-density lipoproteins (VLDL)] and high levels of CRP (and apoA-I). The relationship of elevated CRP and HDL dysfunction to risk of diabetes or CHD disclosed differences in the sexes. Though elevated CRP was an independent determinant of diabetes in both genders, it was so in regard to CHD risk only in women, requiring mediation by HDL dysfunction in men. Thus HDL dysfunction -presumably induced by hypertriglyceridemia and elevated CRP- directly conferred CHD risk in men but was not independent of the pro-inflammatory state in women (Fig. 2). We reported evidence in women that hypertriglyceridemia, dysfunctional HDL and other potential pro-inflammatory mediators not included in the model might be involved *in toto* in determining the CHD risk (24).

A high Trg/HDL ratio alone was predictive of CHD risk only in men, whereas combined presence of high Trg/HDL ratio and elevated CRP conferred CHD risk in women. The interplay across gender, hypertriglyceridemia, elevated CRP and HDL dysfunction in the development of cardiometabolic risk is illustrated in the Figure 2. Sex differences involve the joint role of the pro-inflammatory state, represented by elevated circulating Trg, CRP and HDL dysfunction, as well as a greater role of complement C3 in women (25, 26). The Other elements of enhanced low-grade inflammation/oxidative stress such as increased cellular adhesion molecules and elevated serum 8-isoprostanes (27), shown to exist in cardiometabolic disorders, may well be involved.

By showing in normotriglyceridemic men that, despite significantly higher abdominal obesity and insulin resistance in the category with low than normal HDL-cholesterol levels, the category with normal HDL-cholesterol was subject to an insignificantly higher (by 1.35-fold) diabetes risk; such men had high (and presumably dysfunctional) apoA-I concentrations.

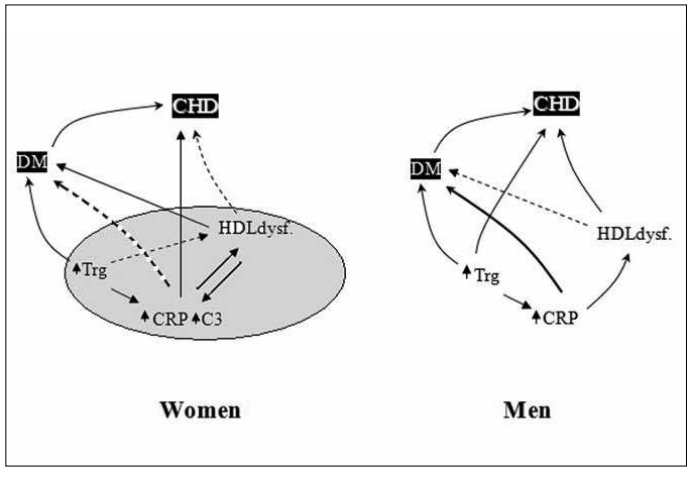

Figure 2. Depicts interaction between hypertriglyceridemia, elevated CRP and HDL dysfunction in the development of type-2 diabetes and coronary heart disease in men and women. Distinct sex differences are indicated which involve in females the joint role of the pro-inflammatory state including that of complement C3

C3 - complement C3, CRP - C-reactive protein, HDL - high-density lipoprotein

Evidence for HDL dysfunction in segments at risk in other populations

HDL dysfunction is particularly relevant in regard to increased cardiovascular risk in diabetic individuals, not fully explained by traditional risk factors. Oxidative stress may contribute to atherogenesis by mechanisms other than LDL oxidation. Data are emerging that atherogenic dyslipidemia can reduce endogenous antiinflammatory pathways mediated by HDL and amplify pro-inflammatory actions of VLDL (28). VLDL that bear apo C-III can induce expression of vascular cell adhesion molecule-1 in vascular endothelial cells (29). Serum apo C-III residing on HDL particles revealed to be a key diabetogenic factor among Turks (30). In the Hoorn Study (31), findings were provided suggesting that endothelial dysfunction and low-grade inflammation explained ~43% of the increase in cardiovascular mortality conferred by diabetes. An atherogenic risk factor profile was observed in the offspring of diabetic patients who had normal glucose tolerance and whose 2-h post-load glucose did not return to the fasting level (32).

Some evidence of HDL dysfunction has been observable in populations beyond Turkish adults, notably in those of the Middle East (10) and in Western subjects at high risk of diabetes or CHD (6, 8, 29, 33).

Finally, it would be appropriate to comment briefly on the controversy raised in regard to the relatively high prevalence of low HDL-cholesterol among Turkish adults documented by Mahley et al. (34) and the TARF study. We may compromise by stating that the previous statement is valid for adults in general, but the more individual studies on clinical patients are concerned with older, hypertensive, diabetic subjects and those with CHD, the higher is the prevalence of high (and dysfunctional) HDL, reflecting the heterogeneity in protective functions of high HDL concentrations.

Public health implications of HDL dysfunction

Half of incident cases of diabetes and about one-quarter of all new cases of CHD are estimated to be attributable to this impaired function of protective (apo) lipoproteins among Turks (14). Among Westerners, the large population segment consisting of impaired glucose tolerance and (atherogenic) dyslipidemia is, in our opinion, intimately linked to the risk of HDL dysfunction. Hence, clinical indices marking this risk should be valuable.

Study limitations

The prospective analysis of a relatively large population-based cohort having in a substantial proportion HDL dysfunction is the strength of the present study, as is the finding of appropriate simple biochemical variables as markers of such dysfunction. The relatively low positive predictive values of biomarkers should not underestimate their clinical utility in view of their very high negative predictive values. Cut points proposed here will help identify HDL dysfunction in middle-aged Turks, and these cut-offs may not be exactly applicable for other populations in

whom such criteria may similarly be developed. But the basic rationale and utility of seeking clinical biomarkers of impaired HDL function is not to be disputed. The lack of using oral glucose tolerance test in the identification of diabetes may have been a factor narrowing the discrimination between presence and absence of this metabolic disease, but this does not represent a systematic bias and influences in a limited way the clinical biomarkers of HDL dysfunction.

Conclusion

As most appropriate clinical biomarkers of impaired anti-inflammatory or atheroprotective HDL function, we found a Trg/HDL-C ratio of >2 and/or a CRP level >2.5 mg/L in men and a cut-off of fasting Trg >1.7 mmol/L, preferably combined with C3>1.3 g/L, in women. These indicators had acceptable positive predictive and high negative predictive values. Moreover, in normotriglyceridemic men with normal HDL-cholesterol levels, diabetes risk was elevated in the presence of elevated and presumably dysfunctional apo A-I concentrations.

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