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Original Article

Role of serum high density lipoprotein levels and functions in calcific aortic valve stenosis progression

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Abstract: Background: Clinical and epidemiological data well defines the role of atherosclerotic risk factors in pathogenesis of aortic stenosis. Especially dyslipidemia with elevated total and LDL cholesterol levels exerts certain histopathological changes on calcified valve tissue. Exact role of HDL in this process is not known. Objective: To evaluate the lipid profiles of patients with mild aortic valve stenosis with special focus on HDL; HDL subspecies, serum apoA1 levels, HDL related PON1 and PAF-AH enzyme activities and to correlate this with disease progression rates. Method: 42 patients (26 female; 16 male), with calcific aortic valve stenosis were enrolled in the study. Serum fasting lipid parameters, HDL subspecies (HDL2, HDL3), serum apoA1 levels and HDL related PON1 and PAF-AH enzyme activities were determined. All participants underwent detailed follow-up transthoracic echocardiography examination. Results: Among 42 study participants mean serum total cholesterol level was 195 ± 27.3 mg/dl, LDL-c level was 123 ± 19.1 mg/dl, HDL-c level was 44 ± 10.3 mg/dl and total cholesterol/HDL-c ratio was 4.64 ± 1.13. Basal peak aortic jet velocity (Vmax2) was 2.67 ± 0.39 m/sec, mean pressure gradient (Pmean2) was 15.6 ± 5.5 mmhg. Annual progression rate in peak aortic jet velocity (Vmax) was 0.23 ± 0.17 m/sec, in mean pressure gradient (Pmean) was 3 ± 2.1 mmhg. Annual progression rate in Pmean was most strongly correlated with serum HDL-c level and total/HDL-c ratio (r=-0.528 and 0.505; <0.001 and 0.001 respectively). Progression in Vmax values was positively correlated with serum LDL-c level and total/HDL-c ratio while negatively correlated with serum HDL-c levels (r=0.328, 0.499 and -0.464; P=0.034, 0.001 and 0.002 respectively). Among HDL subspecies HDL2 was the predominant type. HDL2 levels were found to be positively correlated with progression rates. There was no significant correlation between apolipoprotein A1 level and annual progression rate. Serum PON1 activity level was determined to be negatively correlated to doppler echocardiographic progression parameters while HDL related PAF-AH activity was independent of disease progression. Conclusion: Present study demonstrated a positive correlation between disease progression and serum total cholesterol/HDL-c ratio. Serum HDL-c level was inversely correlated with hemodynamic progression. The majority of HDL was HDL2 subtype. Among HDL related enzymes PON1 enzyme activity exhibited an inverse correlation with disease progression.

Keywords: Calcific aortic valve stenosis, high density lipoprotein, platelet activating factor acetyl hydrolase, Paraoxonase 1

Introduction

Calcific aortic valve stenosis (CAVS) is a disease continuum extending from mild valvular thickening to extensive valve calcification. It was once considered to be a passive degeneration as a consequence of wear and tear phenomenon during aging. However, today there are sufficient data pointing out an active process similar to atherosclerosis [1, 2]. Disease shares common cardiovascular risk factors with atherosclerosis including male gender, hypertension, smoking and dyslipidemia [3, 4]. Lipoprotein metabolism is of particular importance due to possible disease modification via 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors (statins). Although randomized controlled trials showed no benefit and raised doubt over efficacy of statin therapy for aortic stenosis (AS), their potential efficacy at early stages of aortic valve disease remains valid [5, 6].

Table 1. Patient demographics

(n=42)	mean ± SD and (%)
Age	76.3 ± 8.76 (year)
Gender; Male/female	26/16 (61.9%/38.1%) n (%)
Systolic Blood Pressure	138 ± 11.7 (mmHg)
Diastolic Blood Pressure	85.2 ± 10.6 (mmHg)
Heart rate	79.2 ± 6.25 (bpm)
Coronary Artery Disease	19 (45.2%) n (%)
Diabetes Mellitus	12 (29%) n (%)
Hypertension	36 (85.7%) n (%)
Smoking	13 (31%) n (%)

Table 2. Serum lipid profiles

(n=42)	mean ± SD
Total Cholesterol	195 ± 27.3 (mg/dl)
LDL-c	123 ± 19.1 (mg/dl)
HDL-c	44.0 ± 10.3 (mg/dl)
TG	136 ± 54.2 (mg/dl)
Total Cholesterol/HDL Ratio	4.64 ± 1.13

Owing to its antiinflammatory and antioxidative properties high density lipoprotein (HDL) is vitally important in atheroprotection. But its role in CAVS pathogenesis has not fully understood yet. There are few reports linking low serum HDL level to accelerated disease progression [7]. Some animal studies demonstrated regression in the severity of valvular stenosis secondary to HDL related enzyme infusion, formerly called HDL therapeutics but data is scarce and contradictory [8].

We designed a study to evaluate the role of serum lipoproteins in CAVS progression with special emphasis on HDL function and structure.

Material and methods

Study population

We retrospectively evaluated the echocardiography records of patients diagnosed with isolated mild to moderate calcific AS in Gazi University Hospital between September 2009 and March 2011. 42 patients (26 female; 16 male) were enrolled in the study. All data about clinical and blood analysis was obtained from files. Patients with rheumatic valvular stenosis, bicuspid aortic valve, coexisting moderate to severe other valve pathology, moderate to severe aortic insufficiency, ejection fraction less than 50% and chronic kidney disorders (creatinine level >1.4 mg/dl) were excluded.

Study protocol was approved by local ethic committee and all participants gave written informed consent.

Echocardiographic examination

All participants underwent control echocardiographic examination via GE-Vingmed Vivid 7 system (GE-Vingmed Ultrasound AS, Horten, Norway). Standard echocardiographic images were acquired and M mode/2 dimensional/color and pulse doppler analyses were done by same investigator. All measurements were repeated three times and a mean value was calculated for each. Peak aortic jet velocity was calculated with a continuous wave doppler ultrasound scanning along the aortic valve in apical five chamber view, and peak pressure gradient was calculated from Modified Bernoulli Equation. Mean pressure gradient and mean aortic jet velocity were calculated from time-velocity integrals.

A minimum of 12 months and a maximum of 24 months were set apart between first and control echocardiographic imaging of each patient. Difference between basal and control echocardiographic recording for each parameter was calculated and then the average (annual rate of progression) was obtained by dividing the calculated differences to follow-up months times 12.

Biochemical measurements

Blood samples were drawn after 12 hour fasting period. Serum concentrations of fasting glucose, total cholesterol (TC), triglyceride (TG) and HDL-c were determined enzymatically. Low density lipoprotein cholesterol (LDL-c) was calculated using the Friedewald formula (provided that TG were <400 mg/dL; 4.5 mmol/L). Serum apolipoprotein (apo) AI level measurement and analysis of the apo AI-containing lipoprotein subclasses (HDL2 & HDL3) were performed enzymatically using commercially available elisa kits.

Serum PON1 (Paraoxonase 1) activity was determined via paraoxonase assay kit using paraoxon as substrate. P nitrophenol (formed

Table 3. Basal and control echocardiographic aortic valve doppler measurements

	Basal (n=42) mean ± SD	Control (n=42) mean ± SD	P*
V max (m/sec)	2.67 ± 0.396	2.99 ± 0.379	<0.001
V mean (m/sec)	1.94 ± 0.346	2.2 ± 0.339	<0.001
P max (mmHg)	29.1 ± 8.67	36.4 ± 9.24	<0.001
P mean (mmHg)	15.6 ± 5.55	19.8 ± 6.10	<0.001

*Paired Student's t-test.

Table 4. Annual progression rates of doppler echocardiographic measurements

Doppler Measurement	(mean ± SD)
P max	5.12 ± 4.22 (mmHg/year)
P mean	3.00 ± 2.11 (mmHg/year)
V max	0.228 ± 0.175 (m/sec/year)
V mean	0.182 ± 0.123 (m/sec/year)

from paraoxon under PON1 enzymatic activity) spectophotometric absorbance was determined and results were expressed as U/L.

All Apo B containing lipoprotein subclasses were precipitated via dextrane sulphate-Mg chloride solution. HDL related platelet activating factor acetyl hydrolase (PAF-AH) activity was measured from the supernatant by PAF acetylhydrolase assay kit and the results were expressed as mmol/min/ml.

Statistical analysis

Statistical analyses were performed using the SPSS software version 17.0 (SPSS Inc., Chicago, IL., USA). The variables were investigated using visual (histograms, probability plots) and analytical methods (Shapiro-Wilk test). Continuous variables were expressed as mean ± 1 standard deviation; categorical variables were expressed as numbers and percentages. Paired Student's t-test was used to compare the measurements at two time points. While investigating the associations between variables, correlation coefficients and their significance were calculated using the Spearman test. A correlation coefficient greater than 0.8 is accepted as strong, whereas a correlation coefficient less than 0.5 is described as weak. A 5% type-I error level was used to infer statistical significance.

Results

42 patients (16 male, 26 female; age 76 ± 8.7 years) with mild to moderate isolated CAVS were enrolled in the study. Patients' demographics were summarized in **Table 1**.

Mean serum total cholesterol level was 195 ± 27.3 mg/dl, LDL-c level was 123 ± 19.1 mg/dl, HDL-c level was 44 ± 10.3 mg/dl. and total cholesterol/HDL-c ratio was 4.64 ± 1.13 (**Table 2**).

Basal peak aortic jet velocity (V_{max2}) was 2.67 ± 0.39 m/sec, peak pressure gradient was (P_{max2}) 29.1 ± 8.67 mmHg, mean pressure gradient (P_{mean2}) was 15.6 ± 5.5 mmHg and mean aortic jet velocity (V_{mean2}) was 1.94 ± 0.34 m/sec (**Table 3**).

Follow-up echocardiographic measurements were as follows; peak aortic jet velocity (V_{max2}) was 2.99 ± 0.38 m/sec, peak pressure gradient was (P_{max2}) 36.4 ± 9.24 mmHg, mean pressure gradient (P_{mean2}) was 19.8 ± 6.10 mmHg and mean aortic jet velocity (V_{mean2}) was 2.20 ± 0.34 m/sec (**Table 3**).

Mean duration between first and control echocardiographic examination was 17.9 ± 3.3 months. Annual progression rate in peak aortic jet velocity (V_{max}) was 0.23 ± 0.17 m/sec, in peak pressure gradient was (P_{max}) 5.1 ± 4.2 mmHg, in mean pressure gradient (P_{mean}) was 3 ± 2.1 mmHg and in mean aortic jet velocity (V_{mean}) was 0.18 ± 0.12 m/sec (**Table 4**).

Annual progression rate in P_{mean} was most strongly correlated with serum HDL-c level and total/HDL-c ratio (r=-0.528 and +0.505; P=0.001 and 0.001 respectively). There was a weak positive correlation with serum LDL-c level (r=0,325; P=0,036). Progression at V_{max} values was positively correlated with serum LDL-c level and total/HDL-c ratio while weak negative correlated with serum HDL-c levels (r=0.328, 0.499 and -0.464; P=0.034, 0.001 and 0.002 respectively) (**Table 5**).

Among HDL subspecies HDL2 was the predominant type (mean HDL2 59.0 ± 15.1 mg/dl; mean HDL3 12.0 ± 3.57 mg/dl; P<0.001) (**Table 6**). HDL2 levels were found to be weak positive correlated with P_{max}, V_{max} and V_{mean}

Table 5. Aortic valve doppler echocardiographic progression rates and serum lipid levels correlations

		Pmax (mmHg/year)	Pmean (mmHg/year)	Vmax (m/sec/year)	Vmean (m/sec/year)
LDL-c (mg/dl)	r	0.385	0.325	0.328	0.253
	P	0.012	0.036	0.034	0.106
HDL-c (mg/dl)	r	-0.490	-0.528	-0.464	-0.524
	P	0.001	<0.001	0.002	<0.001
Total Cholesterol (mg/dl)	r	-0.029	-0.060	-0.024	-0.047
	P	0.853	0.705	0.879	0.770
Total Cholesterol/HDL-c	r	0.536	0.505	0.499	0.490
	P	<0.001	0.001	0.001	0.001

Table 6. Serum HDL2, HDL3 and apoA1 levels

	mean ± SD
HDL2	59.0 ± 15.1 (mg/dl)
HDL3	12.0 ± 3.57 (mg/dl)
ApoA1	273 ± 68.3 (mg/dl)

(r=0.316, 0.405, 0.395; P=0.042, 0.08, 0.01 respectively) (**Table 7**). Mean apolipoprotein A1 level was 273 ± 68.3 mg/dl and there was no correlation between annual progression rate (**Tables 6**, **7**). Apolipoprotein A1 was noted to be negatively correlated with HDL2 (r=-0.369; P=0.016).

Serum PON1 activity was 50 ± 26 U/L and HDL related PAF-AH activity was 10 ± 6.6 mmol/min/ml. PON1 activity level was determined to be negatively correlated to P_{max}, P_{mean}, V_{max} and V_{mean} (r=-0.359, -0.365, -0.322, -0.412; P=0.02, 0.018, 0.038, 0.007 respectively). While HDL related PAF-AH activity was independent of disease progression (**Table 8**).

Discussion

Present study demonstrated a positive correlation between disease progression and serum LDL-c level. Total cholesterol/HDL-c ratio exhibited even a stronger positive correlation. Serum HDL-c level was inversely correlated with hemodynamic progression whereas higher HDL2 subgroup level was associated with higher annual progression rates. Majority of HDL was HDL2 subtype. Serum apo A1 level and plasma HDL-related PAF-AH activity were found to have no effect on hemodynamic deterioration but serum PON1 activity exhibited an inverse correlation.

Calcific aortic stenosis is a progressive disease of the elderly; 2-4% of population over 65 years were affected [9]. Disease has a progressive nature with annual increase of 7 mmHg in mean pressure gradient, 0.3 m/sec in peak jet velocity and an annual decrease rate of 0.1 cm^2 in valve area. Hemodynamic progression rate accelerates as disease severity increases. Relatively slow progression rate (3 mmHg/year) in the present study is the result of lower basal pressure and velocity measurements. Simvastatin and Ezetimibe in Aortic Stenosis (SEAS) study, which also recruited patients with low peak jet velocity and pressure gradient (3.09 m/sec and 23 mmhg respectively), reported an annual progression rate of 2.7 mmhg/year [6].

Several studies investigating the association of statin use with slowed disease progression were published. Results from retrospective clinical trials support an association between statin use and slowed disease progression [10, 11]. On contrary randomized large studies fail to support this effect and raise doubts about the role of lipoprotein metabolism in CAVS pathophysiology [5, 6]. However, even after SEAS, data in support of lipid-lowering therapies continue to mount [12].

Interpersonal variation in disease progression is prominent and lipoprotein metabolism has operative effects. Although extensively studied in the pathogenesis of atherosclerosis, role of HDL in CAVS process has not fully illuminated yet. Low HDL-c level was defined as a risk factor for CAVS [13]. Lommi et al. demonstrated a decrease in valvular HDL-c levels of patients undergoing valve replacement and speculated that HDL-c decreases valvular calcification via osteoprotegerin stimulation and TNF-α expression inhibition [14]. Our study revealed a strong inverse correlation between serum HDL-c level and disease progression while LDL-c was found to have a moderate effect. It is known that LDL-progression relationship loosens with increasing age with a relatively weak association after age 65 [15]. Yılmaz et al. reported similar find-

Table 7. Aortic valve doppler echocardiographic progression rates and serum HDL2, HDL3 and apoA1 levels

		Pmax (mmHg/year)	Pmean (mmHg/year)	Vmax (m/sec/year)	Vmean (m/sec/year)
ApoA1 (mg/dl)	r	-0.085	-0.192	-0.088	-0.199
	P	0.592	0.223	0.581	0.207
HDL2 (mg/dl)	r	0.316	0.241	0.405	0.395
	P	0.042	0.124	0.008	0.01
HDL3 (mg/dl)	r	0.275	0.293	0.258	0.294
	P	0.086	0.066	0.108	0.066

Table 8. Aortic valve doppler echocardiographic progression rates and serum PON1 ve HDL related PAF-AH enzyme activities

		Pmax (mmHg/year)	Pmean (mmHg/year)	Vmax (m/sec/year)	Vmean (m/sec/year)
PON1 activity (U/l)	r	-0.359	-0.365	-0.322	-0.412
	P	0.02	0.018	0.038	0.007
PAF-AH activity (mmol/min/ml)	r	0.11	0.252	0.121	0.283
	P	0.487	0.108	0.446	0.069

ings; they found a stronger relationship between disease progression and HDL-c and/or total cholesterol/HDL-c ratio than LDL-c level [7]. However HDL shared the same fate with LDL; despite considerable increase in serum HDL-c level in Aortic Stenosis Progression Observation Measuring Effects of Rosuvastatin (ASTRONOMER) trial valvular destruction cannot be withheld [5].

High density lipoprotein is a heterogeneous molecule composed of subgroups with different density; diameter and electrophoretic properties. These subgroups had been extensively studied in atherosclerosis although results are highly conflicting. Kuopi Ischeamic Heart Disease Risk Factors and Quebec City Suburbs trials demontrated an inverse relationship between risk of atherosclerotic events and HDL2 subtype; Physician's Health Study defined HDL3 as a strong and independent antiatherosclerotic molecule whereas in Gofman's Livermore Cohort both HDL2 and HDL3 were defined as strong predictors of future atherosclerotic events [16-19]. HDL subgroups had not been studied in CAVS patients except the report by Arsenault et al. which found no difference among HDL size and pre-β HDL levels between aortic valve stenosis patients and controls [20]. Present study revealed HDL2 as the predominant HDL subtype and revealed its positive correlation with disease progression.

Apolipoprotein A1 is the main apolipoprotein in HDL structure participating in reverse cholesterol transport and antioxidative activity. Amount and conformation of ApoA1 in HDL structure is crucial and both are disturbed in chronic inflammatory conditions. As ApoA1 level in HDL structure decreseas ApoA1-cholesterol interaction declines resulting in less efficient reverse cholesterol transport and antioxidation. In CAVS patients present study demonstrated an inverse correlation between HDL2 and ApoA1 level. This ApoA1 depleted HDL2 fails to prevent LDL oxidation and resultant ox-LDL acumulates in valve structure and further worsens fibrocalcific remodeling [21].

PON1 is a HDL related plasma esterase with antioxidative activity. There are a number of studies in the literature reporting attenuated enzyme activity in conditions characterized with low grade inflammation (i.e DM, metabolic syndrome) [22, 23]. CAVS is another disease of inflammation and is also associated with low PON1 activity [24]. Similarly we observed negative correlation between disease progression rate and enzyme activity.

HDL-related PAF-AH is another enzyme with antioxidative and antiinflammatory activity. PAF-AH is a dual acting enzyme whose role is primarily determined by its plasma carrier. LDL related PAF-AH exhibits proatherogenic activity whereas HDL related PAF-AH is antiatherogenic. Patients with severe CAVS were shown to have elevated plasma lipoprotein associated PAF-AH level [25]. HDL related PAF-AH activity is however had not been studied until present study and we observed no correlation between disease progression and enzyme activity.

Conclusion

In our study we confirmed the association between aortic valve stenosis and serum HDL

level and total cholesterol/HDL ratio as reported by previous studies. Analysis of the molecule revealed HDL2 as the predominant subtype in an inverse relationship with apolipoprotein A1 and at least one of the HDL related enzymes which are crucial for its useful effects may play role in disease progression. Future studies regarding not simply HDL level but HDL functionality in CAVS progression are needed.

Disclosure of conflict of interest

None.

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