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Could circulating fetuin A be a biomarker of aortic valve stenosis?

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ABSTRACT

Background: Aortic valve stenosis (AVS) is a multifactorial-progressive pathological process. In the past decades, many studies have focus their attention on circulating biomarkers able to identify AVS and/or to predict its progression. One of the many biomarkers studied is the fetuin A.
The aim of the present meta-analysis was to evaluate the correlation between fetuin A levels and end-stage AVS.
Methods and results: A systematic search was performed in three electronic databases (PubMed, Web of Science and Scopus), looking for studies that compared control subjects with AVS patients and that have measured fetuin A circulating levels in both groups. The main outcome was to evaluate the difference in circulating fetuin A concentration in the two groups. Seven studies, enrolling 2283 AVS patients and 1549 controls, were included. Differences between control subjects and AVS patients were expressed as standardized mean difference (SMD) with pertinent 95% confidence intervals (95%CI) and standard deviation (SD), analysing the data using a random effect model.
We found significantly lower circulating levels of fetuin A in AVS patients compared to healthy subjects (SMD: −0.96 μg/mL, 95% CI: −1.62, −0.30; $p = 0.004$). In addition, meta-regression analyses showed that several cardiovascular risk factors were significantly associated with circulating levels of fetuin A between patients affected by AVS and healthy controls.
Conclusion: In conclusion, our meta-analysis shows that AVS patients have significant lower circulating levels of fetuin A compared to control subjects. However, dedicated studies with large and matched cohorts are needed to validate these findings, evaluating if there is a real link or just a mere association.

1. Introduction

Aortic valve stenosis (AVS) is a multifactorial-progressive pathological process, associated with lipoprotein deposition, chronic inflammation and active calcification of aortic valve leaflets [1,2]. Therefore, AVS represents a disease continuum that encompasses mild valve thickening with normal functions and severe calcification with impaired leaflet [3]. AVS is one of most common heart valve disease in western countries after coronary heart disease and hypertension, with the prevalence up to 8% in elderly [4]. Despite its high prevalence, relatively little is known about its pathogenic mechanism [5] and no pharmacological treatment has been approved yet, leaving the aortic valve replacement as only available option to overcome the very poor prognosis once AVS symptom occur [6].

In the past decades, many studies have focus their attention on circulating biomarkers able to identify AVS and/or to predict its progression [7–10]. One of the many biomarkers is the fetuin A that is a glycoprotein synthesized by the liver and released in the circulation, functioning as potent inhibitor of soft tissue calcification [4]. Previously it has been shown that fetuin A forms a complex with calcium and phosphorus, increasing their solubility [11] and perturbing hydroxyapatite formation [12]. In addition, intracellular fetuin A inhibits apoptosis of vascular smooth muscle cells [11] and inhibits the calcification induced by transforming growth factor-β and bone morphogenetic proteins [13–15]. Since this protein has been strongly linked to vascular and valvular calcification, the aim of the present meta-analysis was to evaluate the correlation between fetuin A circulating levels and end-stage AVS.

2. Methods

A protocol for this review was prospectively developed, detailing the specific objectives, the criteria for study selection, the outcomes, and the statistical methods.

To identify all available studies, a detailed search pertaining to fetuin A circulating levels and AVS was conducted according to PRISMA (Preferred Reporting Items for Systematic reviews and Meta-Analyses) guidelines [16]. A systematic search was performed

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in the electronic databases (PubMed, Web of Science and Scopus), using the following search terms in all possible combinations: fetuin A, alpha-2-HS-glycoprotein, ba-alpha-2-glycoprotein, A2HS, HSGA, AHS and aortic valve stenosis. The last search was performed on 21st February 2017. Data were expressed as standardized mean difference (SMD) and standard deviation (SD); analysis was performed with random effect.

2.1. Data extraction and quality assessment

According to the pre-specified protocol, all studies evaluating the circulating levels of fetuin A in AVS patients compared with control subjects were included. Case-reports, case-series without a control group, reviews and animal studies were excluded. In addition, we excluded studies including patients with chronic kidney disease, since it has been previously show that this condition is highly associated with ectopic calcification and alter the circulating levels of fetuin A [17], and patients affected by rheumatic valve diseases. In particular, to be included in the analysis, a study had to provide the mean and SD of circulating fetuin A and reporting the definition of AVS following American or European guidelines [18,19]. Principal characteristic of the seven included studies are shown in Table 1. In each study, data regarding sample size, major clinical and demographic variables, and concentration of fetuin A in patients with AVS and control subjects were extracted.

To assess the quality each included study, we used the Newcastle-Ottawa Scale (NOS) that is specifically developed to evaluate the quality of nonrandomized observational studies [20]. NOS quality assessment results are reported in Supplemental Table S1.

2.2. Statistical analysis and risk of bias assessment

Statistical analysis was carried out using Comprehensive Meta-analysis (Version 2, Biostat, Englewood NJ-2005). Differences between control subjects and AVS patients were expressed as SMD with pertinent 95% confidence intervals (95% CI) and SD.

The overall effect was tested using Z scores and significance was set at $p < 0.05$. Statistical heterogeneity among studies was assessed with chi square Cochran's Q test and with I^2 statistic [21]. We defined heterogeneity as $I^2 > 25\%$.

Publication bias was assessed by the Egger's test and represented graphically by funnel plots of the standard difference in means versus the standard error. Visual inspection of funnel plot asymmetry was performed to address for possible small-study effect, and Egger's test was used to assess publication bias, over and above any subjective evaluation [22]. A $p < 0.10$ was considered statistically significant. In order to be as conservative as possible, the random-effect method was used for all analyses to take into account the variability among included studies [22]. In case of a significant publication bias, the Duval and Tweedie's trim and fill method was used to allow for the estimation of an adjusted effect size [22].

We also took into account that the differences in fetuin A circulating levels may be affected by differences in clinical and demographic characteristics of patients included in different studies (mean age, sex, diabetes, hypertension, smoking habit, coronary artery disease presence, low density lipoprotein levels, high density lipoprotein levels, triglyceride levels). To assess the possible effect of such variables in explaining the different results observed across studies, we planned to perform meta-regression analyses after implementing a regression model with the standardized mean difference of fetuin A levels as dependent variables (y) and the variables mentioned above as independent variables (x) [23].

In the frame of a sensitivity analysis, we have repeated analyses including only the studies judged as "high quality" according to NOS (i.e. NOS ≥5, indicating the median value found among included studies). In addition, we have repeated the analysis excluding one study at a time.

3. Results

After excluding duplicate results, the search retrieved 14 articles. Of these studies, no one was excluded because being off topic after scanning the title and/or the abstract. A total of 7 studies were excluded after full-length paper evaluation because they lacked of data of interest. Thus, seven studies [1,2,9,24–27], enrolling 2283 AVS patients and 1549 controls, were included in the qualitative and quantitative synthesis (Supplemental Fig. S1).

3.1. Study characteristics

Principal characteristic of the seven included studies are shown in Table 1.

The number of subjects varied from 30 to 3426, the mean age from 64 to 72 years, the prevalence of male gender from 37% to 59% and coronary artery disease was present in 17% to 61% of cases. Among the cardiovascular risk factors, hypertension was found in 45% to 85% of cases, diabetes was reported in 13% to 34% of cases, and smoking habit in 6% to 39% of cases. In addition, three studies reported low-density lipoprotein levels ranging from 109 to 206 mg/dL, high-density lipoprotein ranging from 47 to 56 mg/dL, and triglycerides from 128 to 143 mg/dL.

3.2. Circulating fetuin A levels in aortic valve stenosis

In the seven included studies [1,2,9,24–27], we found significantly lower circulating levels of fetuin A in AVS patients compared to healthy subjects. In particular, we found a SMD of −0.96 μg/mL (95%CI: −1.62, −0.30, $p = 0.004$; Fig. 1), with a significant heterogeneity among the studies ($I^2 = 94\%$, $p < 0.0001$).

3.3. Sensitivity analysis

Since the heterogeneity among studies was significant, we perform a sensitivity analysis. First, we excluded the two studies [2,26] that were considered of "low quality" after the NOS analysis (NOS ≤ 5; Supplemental Table S1). The circulating fetuin A levels remained significantly lower in AVS patients compared to control subjects (SMD: −0.64 μg/mL, 95% CI: −1.11, −0.17, $p = 0.007$; $I^2 = 71\%$, $p = 0.008$; Supplemental Fig. S2). Finally, we excluded one study at a time and all the results were confirmed. In particular, after excluding the study of Kapelouzou et al. [26], that reported the highest SMD between the two groups, the fetuin A levels remained significantly lower in AVS patients than controls (SMD: −0.52 μg/mL, 95% CI: −0.92, −0.12, $p = 0.01$; $I^2 = 82\%$, $p < 0.0001$).

Table 1
Demographic and clinical data of patients with aortic valve stenosis and controls included.

1st author and year		Patients (n)	Males (%)	Age (years)	HT (%)	Smoking (%)	Diabetes (%)	CAD (%)	BMI (kg/m^2)	TC (mg/dL)	LDL (mg/dL)	HDL (mg/dL)	TGs (mg/dL)
Adamcyk 2012 [24]	AVS	68	57	68.6 ± 7.7	85	6	24	56	28.7 ± 4.2	187 ± 52	111 ± 46	49 ± 16	135 ± 57
	Controls	20	12	66.1 ± 7.7	85	25	30	80	27.0 ± 3.3	178 ± 53	102 ± 47	40 ± 9	169 ± 155
Bortnick 2016 [2]	AVS	2027	41	72 ± 5	48	10	14	19	26.7 ± 4.5	–	133 ± 35	55 ± 16	135 ± 61
	Controls	1399	32	71 ± 4	41	11	11	14	26.8 ± 4.5	–	129 ± 36	57 ± 16	132 ± 56
Ferrari 2010 [1]	AVS	33	46	75.9 ± 7.2	82	33	36	24	–	–	–	–	–
	Controls	11	73	55.4 ± 24.2	64	9	27	9	–	–	–	–	–
*Kaden 2007 [25]	AVS	15	–	–	–	–	–	–	–	–	–	–	–
	Controls	15	–	–	–	–	–	–	–	–	–	–	–
*Kapelouzou 2015 [26]	AVS	60	–	–	–	–	–	–	–	–	–	–	–
	Controls	20	–	–	–	–	–	–	–	–	–	–	–
Sainger 2013 [9]	AVS	54	56	76.2 ± 1.1	74	41	28	33	–	–	–	–	–
	Controls	59	49	62.9 ± 2.2	73	37	20	36	–	–	–	–	–
Tutuncu 2016 [27]	AVS	26	50	67.2 ± 5.4	81	8	–	23	–	96.2 ± 8.8	190 ± 36	48 ± 3.1	120 ± 33
	Controls	25	68	60.5 ± 6.8	64	4	–	16	–	94.1 ± 9.8	222 ± 41	52 ± 4.1	137 ± 29

AVS: aortic valve stenosis; HT: hypertension; CAD: coronary artery disease; BMI: body mass index; TC: total cholesterol; LDL: low-density lipoptotein; HDL: high-density lipoprotein; TGs: triglycerides. * Data not available.
Age, BMI, TC, LDLc, HDLc, and TGs are expressed as mean values ± standard deviation.

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