Anti-müllerian hormone levels, subclinical atherosclerosis and cardiovascular risk factors in healthy premenopausal women

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Introduction

Anti-Müllerian hormone (AMH) is a member of the transforming growth factor b superfamily peptides, which plays a key role in human reproduction in both genders. In women, AMH is secreted by granulosa cells of the late preantral and small antral follicles and is considered as a reliable marker of ovarian reserve. Cardiovascular (CV) disease (CVD) is the leading cause of mortality in women. In general, the prevalence of CVD in premenopausal women is suboptimally studied. The presence of subclinical CVD can be estimated by using surrogate markers of atherosclerosis and vascular structure and function, such as intima–media thickness (IMT), flow-mediated dilation (FMD) and carotid–femoral pulse-wave velocity (PWV). We recently demonstrated that the C242T polymorphism of the CYBA gene and the G894T polymorphism of the NOS3 gene are associated with subclinical atherosclerosis in premenopausal women.

Objectives

The aim of this study was to investigate the association between AMH concentrations and subclinical CVD indices in healthy premenopausal, normally menstruating women.

Materials & Methods

In this cross-sectional study 70 healthy, normally ovulating, reproductively active women were recruited. Fasting venous blood samples were obtained for hormonal and biochemical assessment. Genotyping was performed, using real-time PCR. Indices of vascular structure and function were sonographically assessed and included carotid and femoral intima-media thickness (IMT), flow-mediated dilation (FMD), carotid-femoral pulse-wave velocity (PWV), and augmentation index.

Results

The descriptive characteristics of the participants are presented in Table 1, stratified according to levels of AMH higher vs lower than the median value of 2.55 ng/mL (Tables 1a and 1b). The prevalence of the reported abortions in the general sample was low (4.3%). Moreover, almost half of the women were nulliparous and more than a third were current smokers. Mean AMH levels were lower in smokers than in non-smokers (2.55±2.54 vs 3.88±2.75 ng/mL, respectively; p=0.048). In contrast, there was a trend for higher sICAM-1 concentrations in smokers compared with non-smokers (251.3±111.8 vs 193.5±69.3 ng/mL; p=0.052).

An inverse association between IMT in all segments and mean lnAMH concentrations was observed [r-coefficient for combined carotid IMT, CCA-IMT, CB-IMT, ICA-IMT and FA-IMT: -0.428 (p<0.001), -0.317 (p=0.009), -0.455 (p<0.001), -0.304 (p=0.012) and -0.312 (p=0.010), respectively]. lnAMH was negatively associated with TC levels (r-coefficient: -0.273, p=0.029), LDL-C levels (r-coefficient=-0.262, p=0.037) and age (r-coefficient=-0.435, p<0.001).

Table 1a. Descriptive characteristics for demographic – anthropometric indices as well as for biochemical/hormonal parameters for the 70 women of our study

	AMH <2.55 ng/mL Mean±SD or frequency (%)	AMH ≥2.55 ng/mL Mean±SD or frequency (%)	ANOVA p-value
Demographic/anthropometric parameters			
Age (years)	**35.3±5.0**	**29.6±6.4**	**<0.001**
Age at menarche (years)	12.9±1.3	13.1±1.2	0.999
Body mass index (kg/m^2)	24.7±4.8	23.9±3.9	0.472
Waist (cm)	84.5±11.9	82.8±10.8	0.565
Waist to hip ratio	0.8±0.1	0.7±0.1	0.672
Duration of menses (days)	3.8±1.0	4.4±0.9	0.131
Duration of cycle (days)	**27.2±1.6**	**28.7±1.8**	**0.001**
Parity			
• No children	**65.6**	**93.8**	**0.017**
• 1 child	**9.4**	**0**	
• 2 children	**25**	**6.2**	
Abortions (number)	6.2	3.1	0.601
Smoking	**53.1**	**25**	**0.021**
Biochemical parameters			
Glucose (mg/dL)	86.1±8.9	81.7±9.2	0.056
Insulin (μIU/mL)	7.3±2.9	6.7±2.4	0.373
HOMA-IR	1.6±0.7	1.4±0.6	0.235
s-ICAM-1 (ng/mL)	228.9±109.1	195.2±53.9	0.251
Haptoglobin (mg/mL)	7.1±2.0	7.5±1.6	0.583
hsCRP (ng/mL)	1.1±1.6	2.1±6.2	0.353
Total cholesterol (mg/dL)	**189.3±41.1**	**165.9±20.7**	**0.006**
Triglycerides (mg/dL)	67.7±26.9	55.7±23.8	0.065
HDL-C (mg/dL)	59.4±11.7	62.1±10.7	0.347
LDL-C (mg/dL)	**116.2±45.7**	**95.2±22.1**	**0.022**

Abbreviations: HDL-C: high-density lipoprotein cholesterol; HOMA-IR: homeostasis model assessment of insulin resistance; hsCRP: high-sensitivity C-reactive protein; LDL-C: low-density lipoprotein cholesterol; AMH: anti- Müllerian Hormone; s-ICAM-1: soluble cell adhesion molecule 1

Table 1b. Mean values of vascular structure and function indices for the 70 women of our study

	AMH <2.55 ng/mL Mean±SD or frequency (%)	AMH ≥2.55ng/mL Mean±SD or frequency (%)	ANOVA p-value
Intima-media thickness			
Common carotid artery (mm)	**6.3±0.6**	**5.8±0.1**	**0.004**
Carotid bulb (mm)	**7.1±1.7**	**5.6±1.0**	**<0.001**
Internal carotid artery (mm)	**6.6±1.9**	**5.5±1.4**	**0.012**
Combined (mm)	**6.7±1.3**	**5.7±0.9**	**<0.001**
Femoral artery (mm)	**7.8±1.3**	**6.8±1.2**	**0.005**
Atherosclerotic plaques			
Combined	12.5	3.1	0.162
Femoral artery	—	—	
Vascular function indices			
FMD (%)	4.2±3.7	4.4±2.8	0.799
Absolute Brachial Diameter change (mm)	39.5±12.8	37.9±17.3	0.679
PWV (m/s)	6.8±0.5	6.9±1.1	0.717
Aix (%)	19.1±9.6	15.3±9.9	0.120
SBP (mmHg)	101.7±7.3	103.3±10.4	0.558
DBP (mmHg)	69.4±7.3	68.3±6.6	0.515

Abbreviations: Aix: heart rate adjusted augmentation index; DBP: diastolic blood pressure; FMD: flow mediated dilation; PWV: pulse wave velocity; SBP: systolic blood pressure

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Results (continued)

We evaluated the independent association between age and combined carotid IMT, for women with AMH levels higher or lower than the median of 2.55 ng/mL. For women with AMH levels <2.55 ng/mL, age correlated significantly with IMT (r-coefficient=0.572, p=0.001). In contrast, in women with AMH levels >2.55 ng/mL, age was not significantly correlated with combined carotid IMT values (r-coefficient=0.347, p=0.052).

Table 2. Association between ln-AMH levels and subclinical atherosclerosis indices, as well as cardiovascular risk factors, in our cohort of premenopausal women

	r-coefficient	p-value
Combined-IMT	**-0.428**	**<0.001**
CCA-IMT	**-0.317**	**0.009**
CB-IMT	**-0.455**	**<0.001**
ICA-IMT	**-0.304**	**0.012**
FA-IMT	**-0.312**	**0.010**
FMD (%)	-0.125	0.347
Absolute Brachial artery diameter change	0.022	0.867
PWV (m/s)	-0.022	0.860
AIx (%)	-0.238	0.062
Total cholesterol (mg/dL)	**-0.273**	**0.029**
lnTriglycerides (mg/dL)	-0.137	0.279
HDL-C (mg/dL)	0.120	0.345
LDL-C (mg/dL)	**-0.262**	**0.037**
Glucose (mg/dL)	-0.207	0.101
Insulin (μIU/mL)	-0.013	0.917
HOMA-IR	-0.052	0.681
lnCRP	-0.007	0.953
SBP (mmHg)	0.057	0.652
DBP (mmHg)	-0.101	0.427
ICAM (ng/mL)	-0.111	0.501
BMI (kg/m²)	-0.131	0.303
Haptoglobin (mg/mL)	0.171	0.299
Age (years)	**-0.435**	**<0.001**

Abbreviations: AIx: heart rate adjusted augmentation index; AMH: anti- Müllerian hormone; CB: carotid bulb; CCA: common carotid artery; DBP: diastolic blood pressure FA: femoral artery; FMD: flow mediated dilation; HDL-C: high density lipoprotein; HOMA-IR: homeostasis model assessment of insulin resistance; ICA: internal carotid artery; IMT: intima media thickness; LDL-C: low density lipoprotein; lnCRP: log transformed levels of C-reactive protein; PWV: pulse wave velocity; SBP: systolic blood pressure lnTriglycerides: log transformed levels of triglycerides

Notes: "Bold" indicates statistical significance, which was set at p-value <0.05.

There was no correlation with other markers of subclinical atherosclerosis or CVD risk factors (Table 2). After adjustment for the presence of traditional CVD risk factors (such as age, BMI, TC, smoking), the association between AMH concentrations (higher vs lower than the median value of 2.55 ng/mL) and combined carotid IMT (b-coefficient: -0.339; p=0.002), as well as CB-IMT (b-coefficient: -0.336; p=0.004), remained significant. These data are presented in detail in Table 3. The results remained significant even after Bonferroni correction for 6 independent tests (0.05/6 = 0.0083).

Table 3. Stepwise multivariate regression analysis including indices of vascular structure as dependent variables and AMH levels higher or lower than the median value of 2.55 ng/mL, as well as cardiovascular risk factors as independent variables.

	Model R^2	b-coefficient	p-value
Combined-IMT (mm)	46.7%		
Age (years)		**0.400**	**<0.001**
BMI (kg/m²)		**0.278**	**0.005**
SBP (mmHg)		-0.090	0.386
DBP (mmHg)		-0.036	0.711
Total cholesterol (mg/dL)		0.141	0.156
Smoking		**-0.215**	**0.033**
sICAM-1 (ng/mL)		0.030	0.762
AMH ≥2.55 ng/mL *		**-0.339**	**0.002**
lnCRP		-0.016	0.874
Haptoglobin (mg/mL)		0.079	0.416
CCA-IMT (mm)	34.3%		
Age (years)		**0.509**	**<0.001**
BMI (kg/m²)		0.023	0.827
SBP (mmHg)		-0.066	0.525
DBP (mmHg)		-0.066	0.534
Total cholesterol (mg/dL)		0.027	0.799
Smoking		0.082	0.457
sICAM-1 (ng/mL)		**0.218**	**0.043**
AMH ≥2.55 ng/mL *		-0.110	0.342
lnCRP		-0.095	0.369
Haptoglobin (mg/mL)		0.137	0.185

Table 3 (continued). Stepwise multivariate regression analysis including indices of vascular structure as dependent variables and amh levels higheor lower than the median value of 2.55 ng/ml, as well as cardiovascular risk factors as independent variables

CB-IMT (mm)	35.6%		
Age (years)		**0.237**	**0.044**
BMI (kg/m²)		**0.310**	**0.004**
SBP (mmHg)		-0.039	0.732
DBP (mmHg)		0.126	0.234
Total cholesterol (mg/dL)		0.198	0.068
Smoking		-0.176	0.105
sICAM-1 (ng/mL)		-0.007	0.947
AMH ≥2.55 ng/mL *		**-0.336**	**0.004**
lnCRP		0.038	0.726
Haptoglobin (mg/mL)		0.012	0.910
ICA-IMT (mm)	26.6%		
Age (years)		**0.330**	**0.004**
BMI (kg/m²)		**0.360**	**0.002**
SBP (mmHg)		-0.176	0.140
DBP (mmHg)		-0.155	0.167
Total cholesterol (mg/dL)		0.125	0.263
Smoking		-0.160	0.162
sICAM-1 (ng/mL)		-0.063	0.575
AMH ≥2.55ng/mL *		-0.166	0.169
lnCRP		-0.120	0.298
Haptoglobin (mg/mL)		0.009	0.938
FA-IMT (mm)	11.0%		
Age (years)		**0.352**	**0.004**
BMI (kg/m²)		-0.029	0.813
SBP (mmHg)		-0.049	0.682
DBP (mmHg)		0.071	0.560
Total cholesterol (mg/dL)		-0.060	0.626
Smoking		0.064	0.609
sICAM-1 (ng/mL)		-0.092	0.459
AMH ≥2.55 ng/mL *		-0.235	0.076
lnCRP		-0.195	0.107
Haptoglobin (mg/mL)		-0.010	0.934

Abbreviations: AMH: anti-Müllerian hormone; BMI: body mass index; CB: carotid bulb; CCA: common carotid artery; DBP: diastolic blood pressure; FA: femoral artery; ICA: internal carotid artery; IMT: intima media thickness; SBP: systolic blood pressure; sICAM-1: soluble cell adhesion molecule 1
* Reference group: AMH <2.55 ng/mL

Notes: "Bold" indicates statistical significance, which was set at the level of p=0.05.

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Discussion

The main finding of this study is the inverse association between AMH concentrations and IMT values in all carotid segments (CCA, CB, and ICA), as well as FA-IMT.

Most of these associations (combined carotid IMT and CB-IMT) remained significant after adjustment for traditional CVD factors, such as age and BMI. However, no correlation with indices of vascular function, such as FMW, PWV and AIx, was observed. Furthermore, AMH was positively associated with TC levels and was lower in smokers than in non-smokers.

Conclusion

In this cross-sectional study in premenopausal women, AMH concentrations were inversely associated with the presence of subclinical atherosclerosis, independently of traditional CVD risk factors. However, no association with indices of vascular function, such as FMW, PWV and AIx, was found. The key issue for future studies is to investigate whether AMH exerts a pathogenetic role in the atherosclerotic process or just reflects the status of ovarian reserve, as a parallelism with arterial aging.

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