T786C polymorphism of eNOS gene and risk of developing atherosclerosis in women with endopelvic endometriosis

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INTRODUCTION

- Endometriosis affects younger women, while atherosclerosis is a disease that occurs in elder women. These two diseases, with no apparent connection between them, represent situations where activated macrophages and lipoproteins come together. Both have tissue macrophages that expressing specific receptors and these receptors are exposed to lipoproteins. In both diseases, the common features include: chemotaxis, conservation of monocyte/macrophage differentiation, development of monocytes and smooth muscle cells (or endometrial cells), activation of the inflammatory process, and cytotoxicity.
- Endothelial nitric oxide synthase (eNOS) plays an important role in the regulation of cardiovascular function. There are some studies that relating the impact of T786C polymorphism of eNOS gene in the development of premature myocardial infraction (MI) in individuals whose coronary arteries are characterized by atheromatic burden (1,2,3). In particular, homozygosity for T786C has been shown to lead to elevated eNOS production (4).

OBJECTIVE

In our study we examined whether patients with endometriosis express the T786C polymorphism of eNOS gene and so if there is a strong correlation between endometriosis and the development of atherosclerosis in the same patients.

PATIENTS (STUDY PROTOCOL)

In our study 17 patients with laparoscopically confirmed endometriosis were studied. The staging of the disease was based on the updated criteria of the American Fertility Society (American Fertility Society). At the same time, 103 women of reproductive age and disease free were used as controls. The study lasted 18 months.

MATERIALS AND METHODS

PATIENTS: STUDY INCLUSION CRITERIA

1. Laparoscopically confirmed endometriosis
2. Lack of medical history of gynecological cancer
3. Lack of medical history of ischemic heart disease or thromboembolic disease
4. Abstain from any treatment with antioxidants, anti-inflammatory medication or hormone therapy preparations for at least 6 months prior to laparoscopy
5. Absence of inflammatory markers (C reactive protein, white blood cells) or febrile illness.

DNA ANALYSIS

- **ISOLATION OF GENOMIC DNA**

The isolation of total genomic DNA was performed on peripheral blood samples using standard methods (CVD strip assay. A. Viennalab, Austria)

- **T786C POLYMORPHISM: ENOS (-786 T / C)**

❖ The presence of the genetic polymorphism T786C of eNOS (-786 T / C) was studied.
❖ Procedures and PCR amplification of the target sequence used biotinylated primers:
5 'CACCTGCATTCTGGAACTGTA 3'
& 5 'GCCGCAGTAGCAGAGAGAGAC 3'.
❖ The cycles of PCR stantaristikan as follows:
2 min of initial denaturation at 94oC, then 35 cycles of amplification (15 sec denaturation at 94oC, 30 sec primers binding at 58oC, 30 sec elongation at 72oC) and final elongation 3 min at 72oC.
❖ In the elongation PCR products were hybridized with a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and specific color substrate (CVD strip assay A, Viennalab, Austria).

- **ELECTROPHORESIS OF DNA ON AGAROSE GEL**

STATISTICAL ANALYSIS

The statistical analysis was performed with the statistical program SPSS (Statistical Package for the Social Sciences-version 10.1). The level of statistical significance was set at $p<0.05$.

RESULTS

Patients (n=17)				P
eNOS (-786 T/C)		Total	Controls (n=103)	P
	TT	5 (29%)	70 (68%)	<0,001
	TC	9 (53%)	27 (26%)	<0,001
	CC	3 (18%)	6 (6%)	<0,001
Allele frequency	T	19(56%)	167(81%)	0,03
	C	15(44%)	39 (19%)	<0,001

P: patients vs controls, NS: Not Significant

- The prevalence of homozygosity for C allele was significantly higher in patients compared with controls (18% versus 6%, p <0,001) (Table 1). Furthermore, the incidence of the C allele in the patients were significantly higher compared with controls (44% versus 19%, p <0,001) (Table 1).

- Genotypes for eNOS (-786 T / C) are:
1) Genotype T / T Phenotype: Normal
2) Genotype T / C- Phenotype: heterozygote
3) Genotype C / C- Phenotype: homozygotes

DISCUSSION

- T786C genetic polymorphism is associated with the development of coronary heart disease (5,6,7,8,9). Regarding the risk of infraction in young people whose coronary arteries are characterized by a significant degree of atherosclerotic plaques, seems that T786C genetic polymorphism plays an important role.
- In our study, we studied 17 women with endometriosis and 103 healthy women without endometriosis or atherosclerosis and we found that the prevalence of homozygosity for allele C (CC) was significantly higher in patients than in healthy subjects (18% vs. 6%, p <0.001) while the frequency of the C allele in the patients were significantly higher than in healthy subjects (44% versus 19%, p <0,001).

CONCLUSIONS

Our data suggest that there is a significant higher frequency of homozygosity for the T786C allele of the eNOS gene (CC genotype) in patients with endopelvic endometriosis compared to the control group. So it seems that patients with endopelvic endometriosis have a significant risk of developing atherosclerosis sometime in their life. A larger number of participants and different populations are required in future studies.

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