Endometriosis vs *Fusobacterium spp.* – microbiological and molecular analysis of endometrial tissue samples of endometrium patients vs control. RES-289

[1]MAJA CZERNIACHOWSKA, [1]AGNIESZKA KIRYSZEWSKA-JESIONEK, [1]MAŁGORZATA BRAUNCAJS, [1]PIOTR MACHNICKI, [2]FILIP FRANCISZEK KARUGA, [2]DOROTA KOLASA-ZWIERZCHOWSKA, [2]PAULINA GŁOGOWIEC, [2]KRZYSZTOF SZYŁŁO, [1]DOROTA PASTUSZAK-LEWANDOSKA

[1]Department of Microbiology and Laboratory Medical Immunology [2]Klinika Ginekologii, Ginekologii Onkologicznej i Leczenia Endometriozy

INTRODUCTION

Endometriosis is a benign proliferative process, caused by the growth of extrauterine, endometrium-like tissue it is most commonly found in the abdominal cavity. Endometriosis proves to be a prevalent issue, affecting approximately 10-15% of women in a reproductive age. Despite the exact pathogenesis not fully understood et, recent studies suggest that it may be affected by the presence of bacteria in endometrial tissue and suggested a causal role.

AIM OF STUDY

The study aims to analyse the prevalence of *Fusobacterium spp.* in samples collected from women diagnosed with endometriosis compared to the healthy control - women without the disease. It is the first step in analyzing the potential role of *Fusobacterium spp.* in the pathogenesis of endometriosis and in the future, allowing for understaanding the causes of the disease,

METHODOLOGY

The current stage of study included 16 female patients: 7 patients with endometriosis and 9 non-endometriosis patients who underwent hysterectomy due to other reasons. From each of the patients, the following samples were collected prior to surgery: (i) oral swabs, (ii) cervical swab, and during surgery: (iii) peritoneal fluid sample, (iv) cyst wall sample. In both groups of patients, a 1 cm2 endometrial cyst wall sample was collected during surgery, after which it was put into sterile container. Cyst wall samples were also incubated in appropriate broth medium in anaerobic conditions. Additionally, oral and cervical swabs were taken from each patient: 1st collected into the tube with Amies transport medium, and 2nd collected into a sterile tube with no medium and frozen in -20°C. All swabs previously kept in Amies transport medium were cultured with appropriate broth and agar medium and incubated in anaerobic conditions. Moreover, peritoneal fluid samples were also collected into sterile container and remained frozen in -20C. Fluid samples were also incubated in broth medium in anaerobic conditions. Each of the samples then underwent both a DNA as well as microbiological analysis. DNA was isolated from all taken samples using „GeneMATRIX Tissue & Bacterial DNA purification Kit", by the EURX, and then amplified using PCR using the following starters: FUSO1 (5'-GAG AGA GCT TTG CGT CC-3') and FUSO2 (5'-TGG GCG CTG AGG TTC GAC -3'), which are known to be the universal starters in detecting clinically significant species of *Fusobacterium*.

RESULTS

PCR analysis revealed the presence of *Fusobacterium* DNA in 14 out of 16 oral swab samples: 8 out of 9 in the control group and 6 out of 7 in the endometriosis group. However, there was no statistically significant difference between the groups. DNA analysis of other samples - including cervical swabs, peritoneal fluid, and endometrial cyst walls all yielded negative results. In the microbiological analysis, no *Fusobacterium* colonies were cultured.

CONCLUSION

To draw final conclusions, further analysis based on a larger group of patients is necessary.

PROCEDURE

Diagnosed with endometriosisn (7)

Underwent hysterectomy for other reasons (9)

Sample collection

Prior to surgery:

1. Oral swabs
2. Cervical swab

During surgery:

3. Peritoneal fluid sample
4. Cyst wall sample

PCR DNA ANALYSIS

DNA isolation - „GeneMATRIX Tissue & Bacterial DNA purification Kit", by EURX
Starters - FUSO1 (5'-GAG AGA GCT TTG CGT CC-3'), FUSO2 (5'-TGG GCG CTG AGG TTC GAC -3')

MICROBIOLOGICAL ANALYSIS

Schaedler Anaerobe Broth and Schaedler Anaerobe Agar with Sheep Blood - Each of the distinct colonies -> isolated and cultured again using Schaedler Anaerobe Agar with Sheep Blood, identified using (MALDI-TOF) VITEK MS.