Exosomes as biomarkers in endometriosis

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Study question:
Could exosomes be considered as biomarkers of endometriosis?

Summary answer:
Potentially – we found exosomes in peritoneal fluid of endometriosis and control samples. The differences observed in number and size likely translates into qualitative differences.

What is known already:
Endometriosis, the presence of endometrial lesions outside the uterus, is a common condition affecting up to 10% of women of reproductive age. Patients suffer from pain and infertility. The current diagnostic model relies on non-specific symptoms, the (inconsistent) application of endometriosis risk factors, and invasive diagnostic laparoscopy as the gold standard. There is no clinically relevant biomarker for endometriosis yet.Recently, nano-sized extracellular vesicles – exosomes – produced by virtually every cell, have been described in diseases such as cancer, diabetes and pre-eclampsia. Exosomes could similarly be important in endometriosis and serve as biomarkers of the disease.

Study design, size, duration:
Peritoneal fluid (PF) samples were obtained from participants in the ENDOX study at the Endometriosis CaRe Centre, Nuffield Department of Women's and Reproductive Health, University of Oxford (REC ref. 09/H0604/58) under WERF EPHect standards. Women between 18-49 years of age (n=28) who had undergone diagnostic laparoscopy for abdominal or pelvic pain and/or subfertility investigation were classified according to menstrual cycle phase (proliferative or secretory) and severity of endometriosis (ASRM stages I+II or stages III+IV).

Participants/materials, setting, methods:
Groups, control proliferative, n=4; control secretory, n=2; StI+II proliferative, n=9; StI+II secretory, n=7; StIII+IV proliferative, n=3; StIII+IV secretory, n=3. Exclusion criteria: Hormonal treatment, malignancy, pregnancy, breastfeeding and inability to understand the consent form. 1 mL PF was centrifuged to remove cells, debris and microvesicles. Exosomes were enriched by size-exclusion chromatography and confirmed by nanoparticle tracking analysis (NTA), immunoblotting and transmission electron microscopy (TEM). The study of exosome cargo by mass spectroscopy and RNA-sequencing is ongoing.

Main results and the role of chance:
We confirmed the presence of exosomes in PF from women at different stages of endometriosis and from disease-free patients at different menstrual cycle phases by TEM (n=6 pooled group samples) and NTA (n=6 pooled group samples). Enriched exosomes were positive for CD9 and Syntenin. The mean size of PF particles from women with endometriosis was 217 ± 11.2 nm (n=4 pooled group samples), whereas in non-endometriotic women it was 182.9 ± 9.11 nm (n=2 pooled group samples). We observed a higher concentration of PF particles in stage I-II endometriosis compared to controls in both proliferative and secretory phases ($p<0.0001$) (n=2 pooled group samples). In stage III-IV endometriosis, the opposite was seen in the proliferative phase ($p<0.0001$) (n=2 pooled group samples).

Limitations, reasons for caution:
Due to the very limited material obtained, PF samples had to be pooled according to menstrual cycle phases and ARSM staging for analyses.

Wider implications of the findings:
The difference in concentration and size of exosomes in the patient groups is likely to translate into qualitative differences in exosome populations given genetic and phenotypic differences in endometriosis of different stages. This will be shown by the pending proteomics and RNA-seq results.
Trial registration number:
Not applicable.

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I have no potential conflict of interest to disclose

Keywords
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