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GYNECOLOGIC ENDOCRINOLOGY AND REPRODUCTIVE MEDICINE

Examination of cervical swabs of patients with endometriosis using Fourier transform infrared spectroscopy

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Abstract

Purpose There is no established non-invasive method to diagnose patients with endometriosis. As a nondestructive type of radiation, infrared light might be used for discrimination by causing vibration of the covalent bonds of the molecules when absorbed by the tissues. The aim of the study was to test whether cervical swab can be used to diagnose women with endometriosis using Fourier transform infrared spectroscopy (FTIR).

Methods In this prospective case–control study, women between 18–45 years old and undergoing laparoscopy due to various reasons were recruited ($n = 20$). According to the findings during laparoscopy, patients were stratified as stage I–II or stage III–IV endometriosis groups. Women lacking any visible lesions of endometriosis were recruited as controls. A cervical swab was taken from all patients just before the surgical procedure and pulled into a tube containing saline solution. FTIR spectra were obtained and the fingerprint region (1750–850 cm^{-1}) was used for analyses.

Results Finally, three samples in stage I–II, five samples in stage III–IV and five samples in the control group were analyzed. Hierarchical cluster analysis and principal component analysis were performed as the chemometric method. A total of ten observable peaks were detected in the absorbance spectra of samples. The peaks at 1450 and 1405 cm^{-1} originating from lipids and proteins significantly increased in the stage III–IV endometriosis group when compared with controls. In addition, nucleic acid/carbohydrate ratio was significantly lower in the stage I–II group indicating that the alteration of the carbohydrate level might be important.

Conclusions Examination of cervical swab with FTIR spectroscopy might be a proper candidate for a non-invasive diagnostic approach of endometriosis.

Keywords Endometriosis · Cervical swab · Spectroscopy · Fourier transform infrared spectroscopy

Introduction

The incidence of endometriosis has been reported to be 1–7% in patients undergoing tubal sterilization, whereas it was noticed to be as high as 50% in women suffering from infertility [1–3]. Beside the poor quality of life due to the long-time use of medications for pain symptoms and necessity for several gynecological operations with high complication rate, the associated high financial burden is also an important issue. Although ultrasonography has sensitivity over 90% to define an endometrioma cyst with the typical visualization of an isoechogenic appearance [4], the diagnosis is highly problematic in women suspected to have early stage disease. Currently, for early stage disease, the only established method is obtaining a biopsy from implants for histological examination under laparoscopy which carries

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a complication rate of 0.1–10% in general [5]. Therefore, a non-invasive test is desperately required that is eligible for application in outpatient conditions either for the clinical practice or in the aim of research to define "unexplained" cohort of infertile women.

Cervical cytology has been mainly used for screening precancerous lesions and cervical cancer, but might potentially present epithelial cells from the endometrium [6]. As a matter of fact, cervical cytology might detect malign cells in 30–50% of patients with endometrial cancer [7]. Apart from cells, cervical fluids including proteins, lipids, nucleic acids, cytokines and various type of structures has been also a focus of research that might potentially reflect the upper part of female internal genitalia. In a study by Takata et al. [8], cervical fluid/serum human choriogonadotropin (hCG) concentration ratio was found to be a good predictor of miscarriage risk [8]. Since patients with endometriosis have already altered functional properties in the "eutopic" endometrium including significant aromatase activity and different hormonal environment from regular endometrial tissue in women without endometriosis [9, 10], investigation of secretions and cells that might be drawn from the cervix might be a candidate for the diagnosis of disease. In this context, Fourier transform infrared (FTIR) spectroscopy might be used to examine the molecular characterization of the samples taken from cervix by matching the wave numbers of unknown spectrum with references usually obtained from the literature, known as a fingerprinting process. Secondary structures of proteins can be also determined and compared by analyzing the second derivatives of amide peaks that originate from proteins [11, 12].

Infrared (IR) radiation is a nondestructive type of radiation and it causes vibration of the polar covalent bonds of molecules in the sample. Different vibrational modes have specific absorption characteristics. FTIR spectroscopy is a widely used method of IR spectroscopy due to its robustness and sensitivity [12]. Nevertheless, FTIR spectroscopy has recently emerged as a potential diagnostic method for gynecologic cancers as an alternative to the conventional screening methods [13–17].

In this preliminary study, we primarily aimed to evaluate the usefulness and feasibility of FTIR spectroscopy as an alternative method in the diagnosis of patients with endometriosis using cervical fluid swab samples with FTIR spectroscopy.

Materials and methods

Participants

The current preliminary study was carried out in Department of Obstetrics and Gynaecology, Hacettepe University between January 2012 and June 2013. The Institutional Review Board of Hacettepe University approved the study and all participants gave informed consent for the trial. After completion of sample collection, FTIR analysis was performed in the Institute of Biotechnology, Ankara University in June 2014.

The exclusion criteria were (1) not in the age group 18–45 (2) menstrual irregularity (3) history of abnormal cervical cytology with cervical smear (4) current or past use of any hormonal drug (5) intravaginal medication in the last 1 month (6) presence of intrauterine device and (7) suspicious of leiomyoma and/or adenomyosis with ultrasonography.

Study protocol and sample collection

Prospectively, all women undergoing surgery between 18–45 years were informed and asked to participate in the current study before surgical intervention. A standardized interview-based medical form was used to obtain information regarding the age, obstetric history, last menstrual bleeding, menstrual regularity, gynecological history, medical diseases, medications and family history. During the surgical procedure, only patients on the late follicular phase of the menstrual period were recruited for the study to obtain standardization.

The cervical swab was taken from patients immediately after induction of anesthesia. Following insertion of speculum, vagina was gently wiped out with a dry sponge without using any solution for disinfection. The cervical swab was taken by inserting the cotton tip through the cervical canal until a resistance is felt in the level of internal os. Simultaneously, the tip was rotated two times clockwise and counter clockwise throughout the cervical canal and pulled into a tube containing 1 ml of physiological saline for 10 min. Of note, women presenting significant bleeding during cervical sampling were also excluded. After completion of the surgical procedure, the patient was assigned to one of the study groups (early or advanced stage endometriosis) or taken as controls, if no lesion was visualized. Revised American Society for Reproductive Medicine classification of endometriosis was used to stratify the patients to early (Stage I as minimal or Stage II as mild) or advanced (stage III as moderate or Stage IV as severe) stage endometriosis groups [18]. Diagnosis of endometriosis was confirmed histologically in all patients. FTIR analysis of the samples was performed blinded using coding system to surgical and histopathological results.

Regarding the study group, a total of 20 women were eligible for the cervical swab assessment. The reasons for women undergoing surgery were endometrioma cystectomy ($n = 10$), tubal sterilization ($n = 6$), non-endometrioma ovarian cyst excision ($n = 2$), suspicious of tubal

blockage with hysterosalpingography ($n=1$) and sacrocolpopexy ($n=1$). Of them, seven were excluded from the final analysis due to following reasons: being out of late follicular phase ($n=3$), notification of blood in the cervical swap ($n=3$) or failure to reproduce a waveform with FTIR spectroscopy ($n=1$). At last, the remaining 13 samples were used for the final analysis.

FTIR spectroscopy data collection and interpretation

Cervical fluid samples were lyophilized by a freeze-dryer (Millrock Tech. NY, USA). Infrared spectra were obtained by a Bruker Tensor 27 FTIR (Bruker Optics GmbH, Germany) equipped with a liquid N_2 cooled photovoltaic mercury cadmium telluride (MCT) detector and universal attenuated total reflectance (ATR) cell with a ZnSe crystal (Pike Technologies, Wisconsin, U.S.A). Continuously purging N_2 gas was provided during all the measurements. Lyophilized samples were placed onto the ATR crystal and pressed prior to the measurements. Physiological saline powder was also measured under the same condition and used as a background spectrum for subtraction. Air was recorded and subtracted automatically by the software before all the measurements. Spectra recorded in the mid-infrared region, between 4500–850 cm^{-1} wave numbers and interferograms were accumulated for 50 scans at 4 cm^{-1} resolutions at 22 °C in the single-bounce ATR mode. Each sample was measured three times, spectra were compared if they were identical and the average spectra were used for the further analyses.

Physiological saline spectrum was subtracted from each sample spectrum. Difference in the spectra were baselined using the rubber-band method and the baselined spectra were used for peak area integration calculations. The area under every absorbance peak in all the individual spectra was calculated and used for the statistical comparisons. Each peak was normalized by dividing its area value to the area of the whole spectrum to minimize the technical variation caused by the amount of the sample loaded onto ATR crystal or while taking swab samples. Min–max normalization was applied with respect to the Amide I peak (at 1651 cm^{-1}) for illustrative purposes. Second derivatives of the absorbance spectra, whose absorption maximal appear minimal, were calculated using the Savitzky–Golay algorithm with 13 smoothing points. Relative intensity values of second derivative peaks in the Amide I–II region (1700–1500 cm^{-1}) that was obtained by automated peak picking after vector normalization were used to compare protein secondary structures. All data collection, manipulation, pre-treatment and analyses were performed by OPUS 5.5 software (Bruker Optics).

Statistical analysis

The differences of the means or medians were compared by either one-way ANOVA test or the Kruskal–Wallis Test according to distribution characteristics using Statistical Package for Social Science (SPSS) 16.0 software. Levene's test for homogeneity of variances was performed prior to ANOVA. *P* values less than 0.05 were considered as significantly different and the results were expressed as means ± SD or median (minimum–maximum).

To achieve spectral differentiation according to the molecular fingerprints of the samples, hierarchical cluster analysis (HCA) and principal component analysis (PCA) were carried out using OPUS software and Chemostat standalone package [19], respectively. Ward's algorithm with Euclidian distances was used to construct the dendrograms in HCA.

Ethical approval

Ethical approval was received from Ethics Board and Commission of Hacettepe University and all participants gave informed consent for the trial.

Results

The mean age of the patients included in the final analysis was 34.3 ± 4.1 years. Though three swabs from patients with early stage disease had been analyzed, five samples had also been retrieved from women with advanced stage endometriosis. The number of women in the control group was five. The comparison of the baseline demographic features, reason for surgery and menstrual cycle pattern data of the patients with early stage endometriosis, advanced stage endometriosis and control are depicted in Table 1.

We obtained FTIR spectra of all the samples and used the fingerprint region (1750–850 cm^{-1}) for the analyses and excluded the region between 3800–3200 cm^{-1} wavenumbers, because of the strong overlapping peaks of physiological saline solution. Nevertheless, we observed ten peaks in the cervical swab samples between 1750–850 cm^{-1} wavenumbers (Fig. 1) that reflect assigned various bioorganic molecules such as proteins, lipids, nucleic acids and carbohydrates according to their chemical bond structures based on the information in the related literature (Table 2) [20–26].

We observed overlapping peaks of the study groups (Fig. 1). However, we detected some quantitative differences between control, early stage and advanced stage endometriosis groups as seen in Figs. 1 and 2. Means of the absorbance peak area values of each study group are demonstrated in Fig. 2 as a bar graphic. According to our results, the area of CH_2 bending/methyl asymmetric CH_3

Table 1 Demographic features of the patients in different groups

	Enarly stage endometriosis $n=3$	Advanced stage enndometriosis $n=5$	Cnontrol $n=5$	p
Age (years)	34 (33–38)	32 (28–36)	36 (33–40)	0.093
Weight (kg)	61 (59–64)	63 (56–64)	59 (48–64)	0.366
Length (cm)	165 (160–168)	160 (155–165)	159 (155–163)	0.145
BMI (kg/m^2)	22.7 (22.4–23.1)	23.5 (23.3–25.0)	23.3 (19.5–25.9)	0.280
Menstrual cycle	All regular[a]	All regular[a]	All regular[a]	NA
Reason for surgery	Benign non-endometrioma cyst ($n=2$) Chronic pelvic pain ($n=1$)	Endometrioma ($n=5$)	Benign non-endometrioma cyst ($n=2$) Diagnostic laparoscopy for infertility ($n=2$) Tubal ligation ($n=1$)	NA

Values are given as median (minimum–maximum), unless stated otherwise

BMI body mass index, *NA* non-applicable

[a] indicating woman with menstrual bleeding in every 21–35 days

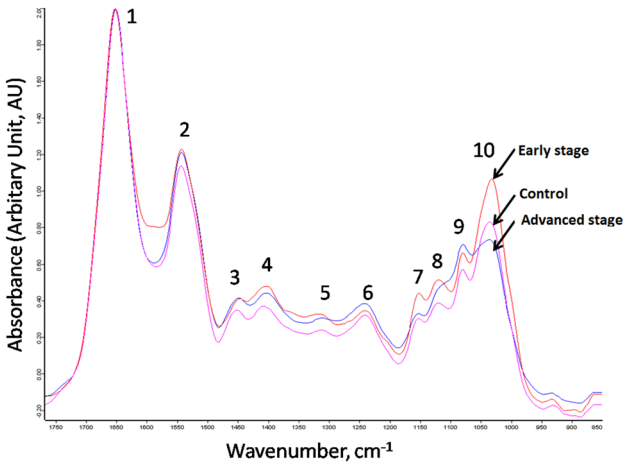

Fig. 1 Overlayed average FTIR spectra of cervical swab samples belonging to three study groups (control, early and advanced stage endometriosis) between 1750–850 cm^{-1} wavenumbers. Peaks 1, 2 and 5 originate from different vibrational modes of proteins; peaks 3 and 4 originate from lipids and proteins; peaks 6, 8 and 9 arise from mainly nucleic acids; peaks 7 and 10 are from carbohydrates and nucleic acids. Refer to Table 2 for detailed assignment of each numbered peak. Min–max normalization was applied with respect to the Amid I peak between 1700–1600 cm^{-1} for illustrative purposes. Alterations in terms of peak areas were observed between the study groups. Change in the absorbance intensity of a particular peak means alterations in the corresponding molecule. Increase of the lipid/protein peaks (number 3 and 4) in advanced stage compared to control group was statistically significant

bending peak at 1450 cm^{-1} originating from lipids and proteins and the CH_3 asymmetric deformation lipid peak at 1405 cm^{-1} in the samples were significantly increased ($P<0.05$) in early stage endometriosis group, compared to controls (Figs. 1 and 2). The area of the peak at 1450 cm^{-1} was also increased in early stage endometriosis group, but the change was statistically insignificant. Additionally, we observed some other changes which did not reach statistical significance such as the area of the Amid I peak that indicates proteins gradually decreased from control to advanced stage endometriosis group. The area of the peak at 1035 cm^{-1} originates from carbohydrates with a contribution from nucleic acids that increased in early stage I–II group compared with the control and decreased in advanced stage endometriosis group (Figs. 1 and 2).

We also compared the peak area ratios of the different components, since differences in the ratios are important in FTIR spectroscopy-based studies [14, 23]. We calculated glycogen/protein (peak 10/1), nucleic acid/protein (peak 9/1), glycogen/nucleic acid (peak 10/9) and nucleic acid/carbohydrate (peak 9/7) ratios (Supplementary Figure I). Only the decrease of the nucleic acid/carbohydrate ratio (peak 9/7) in early stage endometriosis group compared to controls was found to be statistically significant ($P<0.05$).

For HCA and PCA analysis, various regions of absorbance and second derivative spectra were used. However, multivariate analyses did not yield an observable distinct classification between the study groups (data not given).

For the second derivative spectrum of the Amide I and Amide II peaks (at 1651 and 1541 cm^{-1}), derivatization resulted in five sub-peaks (Fig. 3) and they were assigned to the various protein secondary structures (Table 3) based on the available literature [11, 12, 25, 27]. The mean intensities of the sub-peaks are demonstrated in Fig. 4 as bar graphics. As shown in Figs. 3 and 4, we observed some slight differences in terms of peak shifts and intensities, but none of the differences was statistically significant.

Table 2 Peak assignments of the absorbance peaks detected in the present study based on the literature

Peak number	Wavenumber (cm^{-1})	Definition	Organic compound
1	1651	Amide I; 80% C=O stretching, 10% N–H bending, 10% C–N stretching	Proteins
2	1541	Amide II; 60% N–H bending, 40% C–N stretching	Proteins
3	1450	CH_2 bending, asymmetric CH_3 bending of the methyl groups	Lipids, proteins
4	1405	CH_3 asymmetric deformation	Lipids, collagen
5	1313	Amide III; N–H bending, C–H stretching	Proteins
6	1241	PO_2^- antisymmetric stretching (non H-bonded)	Nucleic acids (mainly), phosphorylated proteins and phospholipids
7	1153	C–OH stretching	Carbohydrates/glycogen, nucleic acids
8	1119	PO_2^- symmetric stretching	Nucleic acids (RNA)
9	1079	PO_2^- ionized symmetric stretching (fully H-bonded) of phosphodiester groups	Nucleic acids, phospholipids
10	1035	C–O stretching, coupled with C–O bending of the C–OH groups of carbohydrates, skeletal *trans*/*cis* conformation of DNA	Oligosaccharides, polysaccharides, glycogen, nucleic acids

Peak numbers are illustrated in Fig. 1

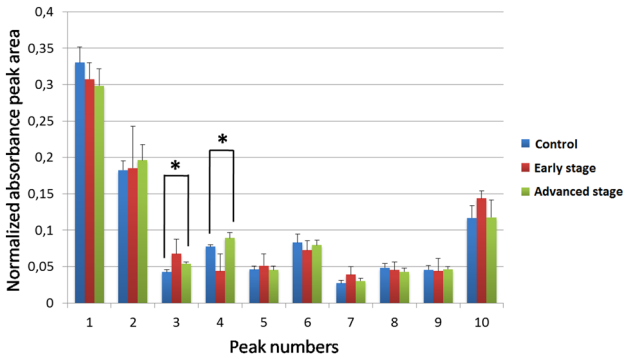

Fig. 2 Bar graphics demonstrating the mean values (±SD) of the normalized absorbance peak areas of control, stage I–II (early) and stage III–IV (advanced) endometriosis groups based on our results. Peaks 3 and 4, which showed statistically significant difference (*=$P<0.05$) between control and advanced stage endometriosis arise from lipids and proteins. Carbohydrate peaks (number 7 and 10) increased in early stage group, compared to controls. Peaks 1, 2, and 5 originate from proteins and peaks 6, 8 and 9 arise from mainly nucleic acids

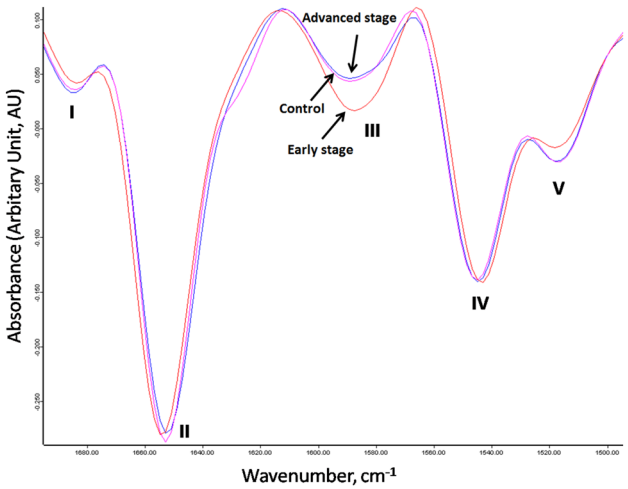

Fig. 3 Second derivative mean spectra of Amide I and II absorption peaks of the study groups (control, early and advanced stage endometriosis) between 1700–1500 cm^{-1} wavenumbers (with centers at 1651 and 1541cm^{-1}). Second derivative sub-peaks indicate protein secondary structures. Absorption maxima appear as minima and the spectra were vector normalized. Peak I arises from β-sheet structure; peaks II and IV are from α-helix structure; peaks III and V originate from glutamine and tyrosine side chains, respectively. Statistically non-significant alterations in terms of band area and band shifts were observed between the study groups. The second derivative spectrum of Amide I–II peak is useful to detect structural changes of the proteins in samples, as well as the amount of amino acids such as glutamine and tyrosine

Discussion

Cervical swab samples obtained from endometriosis patients and controls were studied using FTIR-ATR spectroscopy to introduce the technique and evaluate the potential application of molecular IR fingerprints as a non-invasive alternative method for the diagnosis of endometriosis. To our knowledge, for the first time, our procedure allowed us to characterize major biomolecules of the cells in cervical swab samples in a qualitative and quantitative manner in patients with endometriosis. The FTIR spectral patterns of the cervical samples obtained in the present study were similar to the results in the previous studies looking to discriminate cervical cancer cells [14–17] or eutopic endometrial tissues from patients with or without endometriosis [13]. Notably, there appear to

Table 3 Peak assignments of the sub-peaks obtained by the second derivatization of Amide I peak indicating the protein secondary structures

Peak number	Wavenumber (cm^{-1})	Protein secondary structure
I	1684	Aggregated strand, antiparallel β-sheets, β-turns
II	1653	α-helix
III	1587	Glutamine side chain
IV	1544	α-helix
V	1517	Tyrosine side chain

Refer to the Fig. 3 for peak numbers

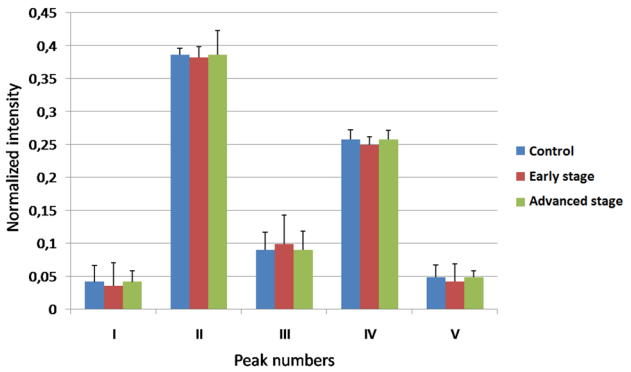

Fig. 4 Bar graphics demonstrating the mean values (±SD) of the vector normalized intensities of second derivative sub-peaks in the Amid I–II region (1700–150 cm^{-1}). Peak I arises from β-sheet structure; peaks II and IV are from α-helix structure; peaks III and V originate from glutamine and tyrosine side chains, respectively. Slight differences in terms of peak frequencies and intensities were observed between the study groups

be some differences in the FTIR spectra between groups indicating the molecular alterations in the swab samples. Lipid–protein peak at 1450 cm^{-1} and lipid–collagen peak at 1405 cm^{-1} were found to be increased in women with advanced stage endometriosis compared to controls. Lipid peak at 1450 cm^{-1} was also increased in early stage group. Additionally, we observed a significant decrease of the nucleic acid/carbohydrate ratio in the early stage group indicating that the alteration of the nucleic acid and carbohydrate level might be important. Recent studies using the endometrium tissue as a material have shown that endometriosis condition may alter lipid profiles [28–29], corroborating our findings, which showed altered lipid profiles between groups. Phospholipids drew the attention in these mass spectrometry-based studies and considered as potential biomarkers. These results suggest that a cervical swab might reflect the altered microenvironment within the endometrium in patients with endometriosis. The differences regarding the peak area associated with lipids/proteins and carbohydrates need explanation with further studies.

The results of the present study indicated that the proteins in the cells in our swab samples had dominantly α-helix secondary structure. However, secondary structures showed slight and statistically insignificant conformational changes with regard to protein among groups. Therefore, the second derivative spectrum of the Amid I–II region does not appear to be sensitive enough to detect possible alterations related to the disease condition, but that observation might be related with small sample size. But still, the main purpose of this preliminary study is to assess the potential of FTIR-ATR spectroscopy in the diagnosis of endometriosis using swab samples and these findings might be useful for the method's establishment as a reference work.

Recently, FTIR spectroscopy and its combination with microscopy has been investigated not only to understand the molecular pathology of the diseases but also to evaluate its suitability as a non-invasive diagnostic test in gynecological conditions as well as other diseases [30, 31]. In that context, 800 cervical scraping samples were investigated by FTIR spectroscopy and the results were compared with cervical cytology in a study by El–Tawil et al. [14]. Their results showed that FTIR spectroscopy was capable of differentiating normal cells from abnormal cervical cells as defined by high/low-grade squamous intraepithelial lesion and cancer.

Although the diagnostic potential of FTIR spectroscopy in cervical cancer was evaluated in a number of papers, there has been little information concerning its potential use in the diagnosis of endometriosis. In a study by Cheung et al. [13], the biochemical differences between endometriotic tissues growing outside the uterus (ectopic), endometrial tissue of the uterus (eutopic) and endometrial tissue from the endometriosis-free women were investigated by FTIR-ATR spectroscopy and transmission FTIR microspectroscopy but using paraffin sections as a material. They used PCA with linear discriminant analysis as a chemometric analysis and focused on the fingerprint region (1800–900 cm^{-1}) similar to the approach applied in the present study. Although the authors obtained mostly overlapping spectra between groups like our results, their chemometric analysis resulted in a classification between groups and the peaks responsible for the segregation were determined. Their results showed that PO_2^- symmetric stretching peaks at 1121 and 1070 cm^{-1} were distinguishing wave numbers segregating samples with endometriosis from endometriosis-free samples. Notably, the same peaks were also observed in our study at 1119 cm^{-1} as peak 8 and 1079 cm^{-1} as peak 9 originating from nucleic acids and phospholipids. Additionally, the decrease of the nucleic acid/carbohydrate ratio (peak 9/7) in early stage endometriosis group compared to controls was found statistically significant in our study and PO_2^- symmetric stretching at 1119 cm^{-1} which arise from nucleic acids showed

difference in terms of the spectral pattern. This peak was present in the control and early stage endometriosis group, while it was reduced forming a shoulder in the advanced stage endometriosis group (Fig. 1). Those findings, together with those by Cheung et al. [13] suggest that PO_2^- symmetric stretching peaks may be useful for the diagnosis of endometriosis and endometriosis-related key transcriptional differences and phospholipid alterations can be detected using FTIR spectroscopy from cervical swabs.

Cheung et al. [13] also analyzed the surrounding stromal tissue and identified carbohydrate peaks at 1155 and 1024 cm^{-1} as major contributing wavenumbers in the segregation of endometriotic eutopic and benign eutopic endometrium tissues. We detected the same bond vibrations of carbohydrates at 1153 and 1035 cm^{-1} in cervical swab samples. Similarly, we also observed alterations in the level of carbohydrates according to our results (Fig. 2, Supplementary Figure I). The area of these two carbohydrate peaks increased first in early stage endometriosis and decreased in advanced stage group, compared to controls. These results suggest that progression of endometriosis may affect glycosylation patterns and carbohydrate metabolism in cells and FTIR spectroscopy is a sensitive method to detect these kinds of endometriosis-related molecular alterations both in the endometrial tissues and cervical swabs.

The main drawback of the current study is the small sample size. Although more samples were retrieved from participants, relatively less samples were left particularly after the optimization process of the cervical swab technique. Therefore, since this is a preliminary study for determining the validity of cervical swab in the diagnosis of endometriosis, the findings should be confirmed with further trials having a larger sample size. There is also the need to optimize the technique used to take cervical swab to decrease the rate of blood contamination, since our results revealed that blood contamination affected the results.

FTIR (micro) spectroscopy followed by chemometric analysis has recently emerged as a promising non-invasive diagnostic tool for different types of cancer [13, 16, 30, 31]. It is a sensitive, robust and high-throughput analysis that is suitable for routine use. However, standardization of the sampling process and normalization of the spectral data are important. Generally, tissue biopsy samples and body fluids are used for measurements. Since the major component of cells and body fluids is water, it is important to subtract water signals using an appropriate method (within the experimental procedure or by spectral subtraction methods) and standardize the process. Since the general chemical fingerprint of all the biomolecules is obtained by FTIR, it is inevitable to use strong multivariate statistical analyses (such as principal component analysis, hierarchical cluster analysis). Although there are many successful applications of FTIR spectroscopy for the discrimination of disease condition from normal tissues, its use in clinics to analyze tissue samples still needs further scientific data and improvement in its in vivo use.

In conclusion, our study showed that FTIR spectroscopy had the potential to provide molecular information about endometriosis from the cervical swab cells and FTIR-based methods may be useful for the non-invasive diagnosis of endometriosis. There is no universally accepted non-invasive method for the diagnosis of endometriosis and our study showed that FTIR-ATR spectroscopy-based methods may be useful as a non-invasive, quick and sensitive approach. Since this is a preliminary study for assessing the availability of cervical swab in the diagnosis of endometriosis using FTIR-ATR spectroscopy, further large-scale studies are needed to perform detailed chemometric analyses and determine possible biomarker peaks particularly in patients with early stage endometriosis.

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Author contributions GB: Project development, data collection, manuscript writing. NI: protocol development, data management, data analysis. PC: data collection, manuscript writing. BA: protocol development, data management, data analysis. DOD: project development, data analysis, manuscript writing. SM: data analysis, manuscript writing. HY: data collection, manuscript writing/editing.

Compliance with ethical standards

Conflict of interest Authors state that there is no conflict of interest.

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