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Direct Mass Spectrometry Differentiation of Ectopic and Eutopic Endometrium in Patients with Endometriosis

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Author Contributions:

17 Leila Adamyan, MD Dr. Adamyan has nothing to disclose.
18 Natalia Starodubtseva, PhD Dr. Starodubtseva has nothing to disclose.
19 Anna Borisova, MD Dr. Borisov has nothing to disclose.
20 Assia Stepanian, MD Dr. Stepanian has nothing to disclose.
21 Vitaliy Chagovets, PhD Dr. Chagovets has nothing to disclose.
22 Dinara Salimova, Medical Student Dr. Salimova has nothing to disclose.
23 Zhihao Wang, Medical Student Dr. Wang has nothing to disclose.
Direct mass spectrometry identified 15 lipids with potential usefulness as biomarkers for real-time endometriotic tissue determination and differentiation during surgical treatment of endometriosis.
Abstract

Study objective: To introduce a method for rapid assessment of endometriotic tissues using direct mass spectrometry (MS)-based lipidomics.

Design: Prospective observational cohort study.

Design classification: II 2.

Setting: Department of Operative Gynecology of the Research Centre for Obstetrics, Gynecology and Perinatology.

Patients: Fifty patients with ovarian cysts and peritoneal endometriosis who underwent laparoscopic surgery between 2014 and 2016.

Intervention: Differences in mass spectrometric profiles of ectopic endometrium (endometriosis) and eutopic endometrium were analyzed for each patient in combination with morpho-histological evaluation. The lipidomic approach was applied using a direct high-resolution mass spectrometry method.

Measurements and Main Results: Out of 148 metabolites, 15 showed significant differences between endometriotic tissue and the comparison healthy endometrium of the same patient, considered as a control in this study. Main lipids prevalent in endometriotic tissues were: phosphoethanolamine (PE O-20:0), sphingomyelin (SM 34:1), diglycerides (DG 44:9), phosphatidylcholines (PC 32:1, PC O-36:3, PC 38:7, PC 38:6, PC 40:8, PC 40:7, PC 40:6, PC 40:9, PC O-42:1), and triglycerides (TG 41:2, TG 49:4, TG 52:3). Using PLS-DA models, MS demonstrated that the lipidomic profile of endometriotic tissue (peritoneal endometriosis and ovarian endometrioma) was clearly separated from eutopic endometrium, indicating tissue-type differentiation.

Conclusion: Our results suggest that direct mass spectrometry may play an important role for endometriotic tissue identification. Such approach has potential utility for real-time tissue
determination and differentiation during surgical treatment. Lipids of three important classes, sphingolipids, phospholipids, and the fatty acids, di- and tri-glycerides, were identified. Validation is required to determine whether these lipids can be used to discriminate between patients with endometriosis and those with other gynecological diseases.

Keywords: endometriosis, spectrometry, eutopic endometrium, endometrioma

1. Introduction

Endometriosis is a benign gynecological disorder characterized by the presence of endometrial tissue outside the uterine cavity [1]. It is one of the most common diseases in gynecology, affecting 10-15% of women of reproductive age [2]. Endometriosis, with its features of chronic inflammation, is associated with dysmenorrhea in up to 40–60% of women and infertility in approximately 20–30% [3]. The typical location of endometriosis is in the pelvis. However, endometriosis can be found in extra-pelvic or ectopic sites, including the gastrointestinal tract, anterior abdominal wall, surgical scars, diaphragm, lungs, urinary tract, and the musculoskeletal and nervous systems [4].

The diagnosis of endometriosis relies heavily upon direct visualization of suspected lesions during an invasive surgery coupled with histologic confirmation [5]. While the pathogenesis of endometriosis is still unknown, there is on average a 7-11 year delay in diagnosis following the onset of symptoms. Surgical removal of endometriotic foci remains the main method of treatment. Important issues continue to be quality control of the removal of the endometriotic foci as well as prediction and prevention of disease relapse (percentage of recurrence of external genital endometriosis varies greatly in different studies, from 6 to 67%) [6].

There are more than 100 proposed markers of endometriosis; however, none of which have demonstrated sufficient diagnostic predictive value [5]. According to the consensus of the World Endometriosis Society [6], the development of a reliable non-invasive test, such as the
discovery of a sufficiently sensitive and specific biomarker, is a top research priority. Until now, the search for markers of endometriosis has been mainly limited to targeted compounds, e.g., metabolites of arachidonic acid and steroids [5, 6]. However, the non-targeted screening of endometriosis has not been reported. One area of particular importance is the role of lipids inasmuch as differences in tissue lipids may be the key to understanding the process that occurs during the invasion and infiltrative growth of endometrial tissue in the ectopic sites.

Mass spectrometry (MS) is one of the most widely used and reliable techniques for the analysis of biological samples. MS tests have been introduced in clinical practice for the diagnosis and prognosis of chronic kidney disease (CE/MS) [7] and preeclampsia (SELDI-MS) [8], and for the rapid identification of microorganisms in clinical microbiology laboratories (MALDI-MS) [9]. MS-based proteomics have been also proposed in ovarian, breast, and prostate cancer diagnostics [10] and in newborn and prenatal screening programs, where it has been proposed to detect inherited inborn errors in metabolism [11]. In the past decade, novel improvements in MS have included the introduction of “ambient ionization” (direct MS), which stands out owing to its unique capability of direct analysis of complex samples with no or minimal pretreatment of the samples [12]; and direct-analysis real-time mass spectrometry (DART-MS), an established technique for rapid mass spectral analysis of a large variety of samples [13].

In our study, a modified spray-from-tissue ionization method was utilized, which was based on the findings previously reported for brain tissue analysis during neurosurgical procedures that demonstrated real-time lipid profile delineation between brain tumor tissue and surrounding healthy tissue [14]. The analysis of lipid profiles of resected endometriosis lesions (peritoneal endometriosis and ovarian endometrioma) and eutopic endometrium was undertaken in the current study to understand the possible dysregulation in the metabolism and fluxes of specific lipids in women affected by endometriosis. In a pilot experiment using the modified ionization method in 6 patients (data not published), mass spectrometric data demonstrated
apparent tissue type differentiation between endometriotic tissue (ectopic endometrium) and uterine endometrium. And as such, the aim of this study was to expand upon these early results.

2. Materials and Methods

2.1 Study design

Tissue samples were collected from 50 patients with ovarian endometriomas, infiltrative endometriosis, and peritoneal endometriosis who underwent laparoscopic surgery in the Department of a Gynecologic Surgery at the V. I. Kulakov Research Center in Moscow. All patients included in the study provided written informed consent. The Commission of Biomedical Ethics at V.I. Kulakov Research Center for Obstetrics Obstetrics, Gynecology, and Perinatology approved all procedures and study methods.

The inclusion criteria for all participants of the study were: reproductive age (15-45 years), histologically confirmed diagnosis of stage III or IV endometriosis, late proliferative/early secretory menstrual cycle (days 8 to 21), absence of any chronic pathology (including diabetes mellitus, kidney disease, cardiovascular disease, and inflammatory diseases), and absence of any hormone therapy over 6 months prior to surgery. The disease stage was determined according to the classification system of the American Society for Reproductive Medicine. Endometrioid ovarian cysts and infiltrative endometriosis were identified before surgery in all of cases by transvaginal ultrasound and MRI. Peritoneal endometriotic lesions were revealed during laparoscopic surgery.

2.2 Sample collection

Patients underwent laparoscopic surgery using the same laparoscope, Karl Storz Endoskope, Tuttlingen, Germany. Hysteroscopy was performed for sampling endometrial tissue for histological and mass spectrometric research. The biopsies of peritoneal endometriotic foci after excision (without thermal exposure), capsules of endometrioid cysts after cystectomy, and endometrium after diagnostic curettage of the uterine cavity were placed in separate sterile tubes
(1 ml) and immediately (at the operating room table upon excision) immersed in liquid nitrogen to prevent oxidation of tissue lipids. Samples were then transported to the laboratory, where the MS machine was located, and stored at –80°C. The tissue samples were divided into two segments. The first segment of each tissue sample was analyzed by histology and the second segment was studied by mass spectrometry. The diagnosis of endometriosis was confirmed histologically. Normal endometrial tissue from uterine sampling was also histologically assessed.

2.3 Direct MS analysis

The direct-spray-from-tissue method was used for molecular species extraction and simultaneous ionization. Sample wetting with organic solvent (methanol with 0.1% formic acid) was carried out constantly to provide a stable ion current (Fig. 1). All MS spectra were acquired by electrospray ionization at quadruple time of flight mass spectrometer (Maxis Impact, Bruker, Germany) in positive-mode. The scheme was designed with a spray directed to a small fragment of tissue at an angle selected with respect to the mass spectrometer. This design allows for biologic samples to tolerate complex sample matrixes, which makes it feasible to directly analyze biological samples with minimal pretreatment. After ionization, positively charged molecules were sampled through the ion optics system into the mass analyzer for further MS analysis.

2.4. Lipids identification and statistical analysis

Samples were investigated by the MS method to obtain information about their molecular composition. This information was further analyzed with multivariate data analysis (MDA) methods to find out if the MS data was sufficient for the classification of tissues and to find out which chemical compounds were involved in tissue differentiation. Acquired MS spectra were processed with a set of functions developed in R language [15]. Mass spectrometric peaks were filtered with a threshold of 200 arbitrary units to exclude noise signal; afterward, peak information was extracted from MS spectra for each tissue sample under investigation and
underwent MDA by the partial least squares discriminant analysis (PLS-DA) method with “ropls” software package [16, 17]. PLS-DA is a supervised modification of the principal components analysis (PCA), which is performed in order to enhance the separation between studied groups of tissue samples. Further, PCA is a statistical procedure that allows for the reduction of data to principal components, describing key aspects of data variance. Pareto scaling was applied to data before MDA [18].

As a result of PLS-DA, the statistical models for tissue separation were created. The characteristics of these models ($R^2$ and $Q^2$) showed the amount of data (%) that can be described using the latent variables ($R^2$) and the amount of data (%) that can be predicted by the model according to the cross validation of the values. Thus, $R^2$ and $Q^2$ show how accurately the model can be expected to separate new tissues. Models with $R^2$ and $Q^2$ of more than 60% are expected to have good predictive ability for tissue differentiation [17].

The $m/z$ variables that carried information about different tissue separation were determined based on the results of the PLS-DA study. Chemical compounds corresponding to the obtained $m/z$ were identified using accurately measured masses, with 5 – 10 ppm accuracy, and information from tandem mass spectra concerning characteristic fragmentation was obtained. Statistical analysis of the identified lipids was conducted using t-test with Bonferroni correction for multiple comparisons.

3. Results

3.1 Demographics data

Select demographic and clinical characteristics of patients (N=50) are presented in Table 1. On admission more than half of the women (60%) had complaints of chronic pelvic pain, 29% suffered from dysmenorrhea, and 9% had dyspareunia. Almost half of the women (48%) suffered from sterility, while infertility/miscarriages were reported in seven women (14%); irregular menstrual pattern and metrorrhagia were observed in 16% of patients. The majority of patients
had a normal BMI of 18.5 to 24.9; two patients were overweight, one was obese, and two were underweight. Prior to study enrollment, the duration of the clinical manifestations of disease was of $3.5 \pm 0.6$ years, ranging from 5 months to 10 years.

Transvaginal ultrasound and subsequent laparoscopy revealed that 32 women (64%) had no concomitant gynecological pathology, ten women (20%) were diagnosed with adenomyosis, and eight women (16%) with uterine fibroids; 12 women (24%) had undergone previous surgery for endometriosis (ovarian resection and excision and coagulation of the peritoneal endometriosis foci). After the first surgical treatment, 7 out of 12 women (58%) received postoperative hormonal therapy for 3-6 months (GnRH agonists, synthetic progestins).

### 3.2. Molecular composition

Samples of peritoneal endometriosis and ovarian endometriomas were analyzed in all 50 cases. We identified that 26 women had healthy endometrium, with no signs of inflammation or other endometrial pathology. From these women with healthy endometrium, we collected tissue from the capsule of the cyst (n=28) and peritoneal foci (n= 27). Using PLS-DA models, we observed that endometriotic tissue samples (peritoneal endometriosis and ovarian endometriomas) were clearly separated from eutopic endometrium. In the score plots shown in Figure 2, endometriosis is represented by red dots and eutopic endometrium is represented by grey dots. The graphs show a comparative analysis of eutopic endometrium with ovarian endometrioma (A) and peritoneal foci (B), respectively. Each dot on the graph corresponds to one sample (mass spectrum).

The PLS-DA models for tissue differentiation were developed. The PLS-DA statistical model ($R^2 = 80\%$ and $Q^2 = 66\%$) showed good predictive ability for tissue classification in cases of separation of the ovarian endometrioma vs. eutopic endometrium. The PLS-DA statistical model for the separation of the peritoneal endometriosis vs. eutopic endometrium ($R^2 = 94\%$ and $Q^2 = 83\%$) had even better accuracy in tissue differentiation. Sensitivity and specificity of such
classification method for pelvic endometriotic tissue versus eutopic (normal) endometrium are 0.93 and 0.97, respectively; and for the ovarian cysts compared to the eutopic endometrium are 0.90 and 0.92. The variable importance of the projection (VIP) values were obtained from PLS-DA models and determined that 15 compounds proved beneficial for tissue differentiation. Identification of these compounds was performed in accordance with accurate mass and characteristic MS/MS spectra. Most of the determined chemical compounds in the direct MS spectra from tissues were found to be lipids, and their distribution provided sufficient information to distinguish between endometriosis and eutopic endometrium.

3.3. Lipids

Figure 3 shows the typical masses of lipids that were obtained from the three types of tissues: ovarian endometrioma, peritoneal endometriosis, and eutopic endometrium. Among the most important lipids were: phosphoethanolamine (PE O-20:0), sphingomyelin (SM 34:1), diglycerides (DG 44:9), phosphatidylcholines (PC 32:1, PC O-36:3, PC 38:7, PC 38:6, PC 40:8, PC 40:7, PC 40:6, PC 40:9, PC O-42:1), and triglycerides (TG 41:2, TG 49:4, TG 52:3). It should be noted that the amount of some lipids were particularly elevated in endometriotic tissues: sphingomyelin SM 34:1, phosphoethanolamine PE O-20:0, triglyceride TG 41:2 and phosphatidylcholines - PC 38:7, PC 40:8, PC 40:7.

The level of some types of phosphatidylcholine (PC 38:6, PC 40:6, PC 40:9, PC 32:1) was higher in eutopic endometrium than in endometriotic tissue. More than 70% of the identified lipids included some polyunsaturated fatty acids. The amount of phospholipid (PC O-42:1) and triglyceride (TG 52:3) in peritoneal endometriosis foci was 10 times higher in the capsules of endometrioma and normal endometrium. We considered that such a high level of triglycerides (TG 52:3) might have been caused by inclusion of some fragment of adipose tissue into the sample during the excision of peritoneal lesions (fat cells are known to consist of up to 85% triglycerides). Statistical analysis of the identified lipids was conducted using a t-test with
Bonferroni correction (Table 2). This analysis showed that despite significant difference (p<.05) between endometriosis and eutopic endometrium as it relates to individual lipids present in the tissue, there were no such significant differences between the tissues of endometrioid cyst of the ovaries and peritoneal endometriosis. These results suggest that the observed lipids are specific for the endometriotic process and not for peritoneal tissue or the ovary separately.

4. Discussion

Direct mass-spectrometry is actively used for the analysis of cancer-involved tissues with high specificity and sensitivity [19, 20]. However, this method has not been studied in women with endometriosis to establish the extent of excision or for the intraoperative identification of lesions questionable for presence of endometriosis. Lipids play an integral role in the development of fundamental reactions underlying almost any pathological process, such as inflammation, oxidative stress, proliferation, and angiogenesis; all of which are involved in the pathogenesis of endometriosis [21, 22]. In the framework of this study, we proposed a new principle of utilizing tissue analysis and a new ionization source based on electrospray for patients with endometriosis.

We first set out to establish that differences in lipids between healthy endometrium and endometriosis exists; and then, to identify the lipids that demonstrate such difference. Direct mass spectrometry is a unique technique for analyzing a sample without any sample preparation. The time required for the analysis of one sample takes 3-6 minutes. Currently available method of tissue freezing, sectioning, and staining, followed by microscopic examination is time-consuming, limiting the number of samples that can be processed in a timely manner. It is, therefore, not feasible for rapid guidance of excision of endometriosis margins. In view of this, the utilization of a method for express analysis of tissues involved with endometriosis, by using such a highly sensitive method as mass spectrometry, is extremely appealing. Because we did not have access to a portable mass spectrometry machine (our machine is large and located within our laboratory department), all tissues excised for analysis were frozen in the operating
room (OR) in liquid nitrogen to prevent oxidation of lipids, which can occur within 30 minutes if
not frozen, and then transported to the laboratory. Despite the fact that delivery of the specimen
from the OR to the laboratory uniformly took place within 15 minutes and hence the specimen
quality would probably not have been affected without freezing, we wanted to provide standard
conditions for all specimens. Therefore, each specimen was frozen at the OR table site
immediately upon excision. This step would not be necessary for existing centers in the world
where mass spectrometers are installed directly in operating rooms.

The purpose of our study was to identify the lipid composition of endometriotic tissues.
We found that the mass spectrometry method allowed identification, with high sensitivity, of
endometriotic tissue from that of eutopic endometrium in an individual patient with
endometriosis. We identified 148 lipids, but significance was shown only for a panel consisting
of 15 lipids. As demonstrated by PLS-DA (Fig. 2), there was a significant difference in lipid
content between endometriosis and eutopic endometrium, but not in the composition between
pelvic endometriosis and ovarian endometrioma. This observation suggests that these lipids may
be specific for the endometriotic process per se, and not for differentiation between peritoneal
and ovarian endometriosis. Three classes of lipids showed marked differences between
endometriotic and endometrial tissue in studied patients – sphingolipids, phospholipids, and the
fatty acids, di- and tri-glycerides.

As is the case of all of the lipids identified in this study, the role of sphingolipids in
endometriosis has not been fully investigated; however, they are a distinctive and highly
important class of lipids functioning in different biological processes, including signal
transduction and cell fate determination [23]. As such, sphingolipids are increasingly known to
be important bioactive signaling molecules [24-29]. Since endometriosis enhances cellular
growth, it can be postulated that an imbalance in the sphingolipid metabolic pathway may result
in intrinsic accelerated proliferation of endometrial cells. This may cause implantation and
growth of lesions at ectopic sites. Additionally, the bioactive sphingolipid metabolite,
sphingosine-1-phosphate (S1P), enhances the proliferative potential of the cells, provides their resistance to apoptosis, and stimulates angiogenesis and cell migration. S1P stimulates expression of the local immune cells TNFα and IL8, which activate COX2 and prostaglandin synthesis. Prostaglandins are known to participate in the development of pain and infertility in endometriosis [30]. Additionally, Vouk et al demonstrated that continuing processes of denervation followed by re-innervation in ectopic endometrium are connected with elevated levels of sphingomyelins [29], strongly suggesting that elevated levels of sphingomyelins are the primary source of pain in patients with endometriosis. These observations suggest that the presence of elevated levels of sphingolipids and phosphatidylycholines, and imbalances in their metabolic pathways in the endometriotic tissue, may be directly involved in the implantation as well as the heightened proliferative and decreased apoptotic properties of endometriotic tissue, with a resultant growth of lesions and associated pain.

In this study, we also observed elevated levels of various phospholipid (PL) subclasses in patients with endometriosis. Biologically active PLs implement a variety of cellular functions such as enzymatic regulation, transcription, signal transduction of second messengers, as well as transport substances [31]. Phosphatidylcholine (ChoGpl) is the most important lipid in the human body. It plays an important role in the formation of the membrane structure and cell signaling. The increase of ChoGpl is the basis for the synthesis of phospholipase A2 (PA2), an enzyme that is overexpressed in endometriotic lesions. PA2 is, in turn, responsible for the production of lysophosphaticidic acid, a lipid involved in cell proliferation related to cancer and endometriosis [32]. ChoGpl is considered a marker of high proliferation of malignant tissue; and as such, it can be postulated as a potential biomarker for endometriosis.

Additionally, the role of diacylglycerol (DG) in the metabolism of glycerolipids is of primary importance; it is also a precursor of numerous lipid molecules, including phosphatidylycholine, phosphoethanolamine, triacylglycerol, and phosphatidic acid [33]. Moreover, DG is important in the signaling of glycerolipids since it activates protein kinase C,
which plays an important role in various biological processes, including cell differentiation and proliferation [34]. Interestingly, DG counteracts the effect ceramides, bioactive sphingolipids, have against apoptosis, but not against the cell cycle, which suggests the protective role of diglyceride [35]. Changes in the levels of ceramide and diglyceride can cause prolonged and presumably permanent reprogramming of cell function through the regulatory mechanism of apoptosis and the cell cycle [36]. Most often and most noticeably, DG mediates agonist-induced stimulation of cell growth and proliferation, while ceramides contribute transduction agonists activation in antiproliferative pathways [37]. Consequently, having the concentration of ceramide and diacylglycerol determined can be important in regulating cell growth and viability in that disturbance of the ratio of DG to ceramides may lead to abnormal cellular responses and cause malignant disorders, aging processes, and, presumably, endometriosis [38].

In this study, we compared direct mass spectrometry-based lipidomics of the healthy endometrium of women with endometriosis with that of excised endometriotic tissue (peritoneal endometriosis and ovarian endometrioma). We considered that the best way to isolate a specific substance in targeted tissue (endometriosis) is to compare it with the tissue with least variability from the tissue studied (endometrium). Furthermore, while the endometrium of a healthy woman without endometriosis could be a better control, the endometrium of the same woman allows for the least lipidomic differences as compared with her own endometrial tissue of different position and quality (endometriosis). The differences in biochemical composition of the endometriosis and endometrium are minimized in a sample originating from the same host. We therefore consider eutopic endometrium of a woman with endometriosis to be the best control/comparator for her endometriosis lesions. Studies involving the inclusion of tissues from healthy patients may be of additional benefit.

We do not conclude, based on our findings, that surgeons can replace conventional histological methods with the mass spectrometric method outlined herein. We continue to consider histopathology to be the gold-standard in evaluation of excised tissue. Based on our
review of the literature, a lipid analysis using mass spectrometry to compare endometriotic tissue with eutopic endometrium has been carried out for the first time in our study. These findings lay the groundwork for possible intraoperative clinical applications. Future studies focusing on comparisons between healthy tissue and the same tissue involved with endometriosis are needed to further confirm and/or refine the lipidomic criteria for establishing the presence or absence of the disease and to corroborate our results and conclusions. Continued exploration may bring us closer to understanding the pathobiology of the disease and to elaborating new ways of early diagnostics and heightened surgical capacity for tissue discernment as it relates to surgery for endometriosis.

5. Conclusions

This study identified, using modified spray-from-tissue ionization mass spectrometry, a consistent lipid profile present in histopathologically-confirmed endometriotic tissue that was different than that of the comparison healthy endometrium of the same woman. In this study, endometriotic tissue was shown to be associated with elevated levels of lipids of three important classes: sphingolipids, phospholipids, and the fatty acids, di- and tri-glycerides. These lipids have been previously described as directly involved in the implantation and the heightened proliferative and decreased apoptotic properties of endometriotic tissue, as well as contributing to pain syndromes of affected patients. Validation of these lipids as biomarkers in future studies is warranted.

Acknowledgements

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(PCSIRT) (no. IRT13054), the Science and Technology Planning Project at the Ministry of Science and Technology of Jiangxi Province, China (no. 20152ACB21013).

References


Fig. 1 Scheme of the ion source for Direct Tissue Analysis.

Fig. 2 PLS-DA score plots for MS data on tissue samples of ectopic and eutopic endometrium:
A) ovarian endometrioma (red dots) and eutopic endometrium (grey dots);
B) pelvic endometriosis (red dots) and eutopic endometrium (grey dots).

Fig. 3 Comparison of the substances of three types of tissue (red – pelvic endometriosis; yellow – endometrioid ovarian cysts (endometriomas); green – eutopic endometrium).
**Table 1** Clinical and demographic data of the patients

<table>
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<th>Age category</th>
<th>Patients (N=50)</th>
<th>Frequency (%)</th>
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<tr>
<td>&lt;25 years</td>
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<td>20</td>
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<tr>
<td>26–29.9 years</td>
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<td>24</td>
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<tr>
<td>30–35.9 years</td>
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<td>36–40.9 years</td>
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<tr>
<td>&gt;41 years</td>
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**Menstrual phase**

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<td>Proliferative</td>
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<tr>
<td>Late proliferative/early secretory</td>
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<td>92</td>
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<tr>
<td>Secretory</td>
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<td>4</td>
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**Concomitant gynecologic pathology**

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<td>Adenomyosis</td>
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<tr>
<td>Myoma uteri</td>
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<td>16</td>
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<tr>
<td>None</td>
<td>32</td>
<td>64</td>
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**Ethnicity**

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<td>Caucasian</td>
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<tr>
<td>Others</td>
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**Reproductive function**

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<td>Infertility/previous miscarriage</td>
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<td>14</td>
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<tr>
<td>No willingness to conceive</td>
<td>12</td>
<td>24</td>
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**Recurrence of endometriosis**

<table>
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<tr>
<th>Recurrence</th>
<th>Patients (N=50)</th>
<th>Frequency (%)</th>
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<tr>
<td>Previously operated for endometriosis</td>
<td>12</td>
<td>24</td>
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Table 2. The results of the Student test of tissue types under investigation.

<table>
<thead>
<tr>
<th>Lipid ID</th>
<th>Pelvic vs ovarian</th>
<th>Pelvic vs endometrium</th>
<th>Ovarian vs endometrium</th>
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<tr>
<td>PE O-20:0</td>
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<td>0.00</td>
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<td>SM 34:1</td>
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<td>0.00</td>
<td>0.01</td>
</tr>
<tr>
<td>TG 41:2</td>
<td>0.79</td>
<td>0.00</td>
<td>0.00</td>
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<td>DG 44:9</td>
<td>0.03</td>
<td>0.91</td>
<td>0.01</td>
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<td>PC 32:1</td>
<td>0.62</td>
<td>0.01</td>
<td>0.02</td>
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<td>PC O-36:3</td>
<td>0.06</td>
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Figure 1.tiff
Figure 3.tiff