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Identifying serum microRNAs as biomarkers for endometriosis in adolescents and young adults

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Abstract

Background Endometriosis is a gynecological disorder that affects 190 million reproductive age women worldwide. Laparoscopic surgery is considered the gold standard to diagnose the disease, creating a barrier to diagnosis and leading to long delays in disease recognition. MicroRNAs may be useful in the diagnosis of this disease, however the ability to detect early disease in adolescents may be improved by identifying microRNAs specific to this population.

Methods This was a prospective clinical study evaluating adolescent patients with pelvic pain undergoing gynecologic surgery in an academic medical center. We enrolled 63 adolescent and young adult patients aged 13–26 years old undergoing gynecologic surgery between 2019 and 2024. Clinically relevant phenotypic and surgical information were recorded as well as evaluation of microRNAs abundance. We assessed microRNAs abundance by extracting total RNA from the serum samples and performed RNA-sequencing (RNAseq).

Results The mean age of adolescent women in the study was 16.3 and 15.9 for the endometriosis ($n=31$) and control groups ($n=20$), respectively. The mean BMI was 24.5 kg/m² and 29.0 kg/m² in the endometriosis and control groups, respectively. RNA-seq data analysis showed differential abundance of 859 microRNAs. Among 859 microRNAs, 488 were increased and 391 were decreased. We next selected those that were most distinct, with little overlap between subjects and controls. Four microRNAs were highly significantly increased while eighteen microRNAs were highly significantly decreased. We defined a signature of microRNAs that best distinguished subjects with endometriosis from controls.

Conclusions This is the first study to reveal the differential abundance of microRNAs specifically in adolescent patients with endometriosis. There are distinct differences from those identified in adult women with endometriosis. Our findings present a unique signature of microRNA found in the serum of adolescents with endometriosis. This finding may be useful as a noninvasive biomarker to diagnose early disease in adolescents. Non-invasive diagnosis may allow for early diagnosis and prevention of disease progression.

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Keywords Endometriosis, Adolescents, Young adults, MiRNA, Pelvic pain, Biomarker, MicroRNA

Background

Endometriosis is a chronic inflammatory, estrogen-dependent condition defined as the presence of endometrial-like glands and stroma outside the uterine cavity. The pathophysiology of endometriosis is poorly understood with multiple factors proposed such as retrograde menstruation, hormonal, genetic, epigenetic, immunologic factors, stem cells as well as activation of the disease associated macrophages [1, 2]. Adolescent endometriosis is a highly prevalent condition, present in approximately 65% of adolescents undergoing laparoscopy for pelvic pain and in 75% of adolescents having laparoscopy for chronic pelvic pain that was unresponsive to treatment. One study describes that more than 50% of adults report that their symptoms started during adolescence with nearly a fifth of them reporting that symptoms began before age 20 and two thirds report onset before age 30 [3].

While diagnostic laparoscopy remains the gold standard for endometriosis diagnosis, a clinical diagnosis is often sufficient and, perhaps, even preferred in young patients [4]. Whether endometriosis is confirmed surgically or suspected clinically, the mainstay of treatment are the non-steroidal anti-inflammatory drugs and various hormonal and non-hormonal preparations that reduce estrogen production and prevent endometrial proliferation [5]. The optimal medical management of endometriosis in adolescents has not been identified; a recent study demonstrated that wide spectrum of regimens, including progestins, combined oral contraceptives, levonorgestrel intrauterine device, GnRH agonists and antagonists as well as combinations of these agents were a common practice [6]. Non-invasive biomarker of endometriosis may add clarity and shorten the time to diagnosis and treatment. Previously, we reported that six microRNAs miR-125b-5p, miR-150-5p, miR-342-3p, miR-451a, miR-3613-5p, Let-7b in women ages 18 years and older could be used as a predictive test for endometriosis [7, 8]. This is the first report showing that microRNA biomarkers can differentiate between endometriosis and other gynecological pathologies in a clinical setting [8].

There is considerable ongoing research attempting to identify a noninvasive diagnostic biomarker of endometriosis. A recent systematic literature review reports on 32 articles describing expression of 141 circulating microRNAs in patients with endometriosis, but studies are limited to participants 18 years of age and older [9]. There is concern about feasibility of the use of microRNAs as a biologic marker due to lack of replication and absence of validated differential expression. A review by Leonova et al. analyzed eighteen studies

which collectively described 63 different microRNAs that differentially expressed in the circulation of women with endometriosis compared with controls, with only 14 microRNAs duplicated in one or more studies [10]. According to this review, downregulation of the circulating levels of miR-17, miR-20a, miR-139-3p, miR-141, miR-320a, miR-3613, and let-7b was observed in more than one study, whereas, levels of miR-18a-5p, miR-122, miR-125, miR-199a, miR-451a, miR-150, and miR-342 were significantly upregulated in women with endometriosis compared with controls in more than one study as well [10–13]. Research dedicated to microRNAs as a potential biomarker specifically in adolescent population is lacking. Adolescent endometriosis is likely to be identified in the earlier stage of the disease and may be more readily detected with additional or different biomarkers. According to the Global Consortium of Investigators in Endometriosis, ongoing research in adolescent endometriosis should focus on biomarker specifically identified in adolescents [14].

The objective of this study was to evaluate whether microRNAs can be used as potential serum biomarkers for the diagnosis of adolescent endometriosis thus enabling early diagnosis. Here we report the differential expression of microRNAs in adolescent females with endometriosis.

Methods

Adolescent and young adult population

This study was approved by the Institutional review board of the Yale University School of Medicine (New Haven, CT, USA). The study was conducted at Yale-New Haven Hospital, a tertiary adult and children's hospital. Investigators obtained written informed assent from patients undergoing surgery for suspected benign indications or chronic pelvic pain; consent was obtained from the guardian if patient was a minor and directly from the patient if 18 years of age or older. The study period was between March 2018 and December 2021. Adolescent and young adult (AYA) women aged 13–26 years were included. Exclusion criteria consisted of inability to sign consent in English, history of intellectual or physical disability thus being unable to provide assent/consent, refusal to participate and pregnancy. Enrollment was concurrent with surgical intervention in all but three participants. Surgery was performed due to known adnexal or other pathology or due to chronic pelvic pain. All patients who underwent surgery had laparoscopy as surgical approach. Total enrolment was 63 patients who were stratified to the endometriosis group (when surgical findings and/or histology confirmed endometriosis

and also included three subjects who had endometriomas as determined by magnetic resonance imaging-MRI) and control group (no evidence of endometriosis but allowing other pathology, most commonly, paratubal cysts). Serum samples were collected from participants typically on the day of laparoscopy and microRNA expression analysis was performed blinded to the surgical findings. For participants who had intraoperative findings of endometriosis, the revised American Society of Reproductive Medicine (rASRM) classification was utilized to assigned stage of endometriosis as showed in Table 1. [15].

Table 1 Sociodemographic and medical characteristics of participants

Baseline characteristic	Endometriosis (N=31)	Control (N=20)	P-value
Age, years	16.3 ± 1.9	15.9 ± 3.2	0.152
BMI, kg/m ²	24.5 ± 5.0	29.0 ± 8.9	0.016
Race			
White	26 (83.9)	15 (75.0)	
Black/African American	1 (3.2)	2 (10.0)	
Asian	2 (6.5)	0 (0)	
Other	2 (6.5)	3 (15.0)	
Phase of Menstrual Cycle			
Proliferative	8 (25.8)	6 (30.0)	
Secretory	3 (9.7)	5 (25.0)	
Unable to Determine***	20 (64.5)	9 (45.0)	
rASRM endometriosis stage			
1	18 (58.1)	-	
2	4 (12.9)	-	
3	4 (12.9)	-	
4	2 (6.5)	-	
No surgery	3 (9.7)	0 (0.0)	
Control Diagnosis			
No Abnormality	-	3 (15.0)	
Cystadenoma	-	3 (15.0)	
Teratoma	-	5 (25.0)	
Paratubal Cyst	-	7 (35.0)	
Mucinous Borderline	-	1 (5.0)	
Cystadenoma			
Cholecystitis	-	1 (5.0)	
Hormonal Treatment			
Combined OCP	13 (41.9)	6 (30.0)	
Progestin	11 (35.4)	3 (15.0)	
GnRH Agonist	1 (3.2)	0 (0)	
LNG IUD	1 (3.2)	1 (5.0)	
No treatment	3 (9.7)	10 (50.0)	
Transdermal patch	2 (6.5)	0 (0)	

*** Phase of the menstrual cycle could not be determined due to participants taking various hormonal preparations for menstrual suppression and contraception

Abbreviations: BMI Body Mass Index, OCP Oral Contraceptive Pill, GnRH Gonadotropin-Releasing Hormone, rASRM revised American Society for Reproductive Medicine, LNG IUD Levonorgestrel Intrauterine Device

Sample collection

Samples were collected prior to surgery. Blood (5–10 ml) was drawn from the subjects and collected in sterile tubes (BD, Franklin Lakes, NJ, USA). Serum was collected immediately by centrifuging at 2500 rpm for min at 4 °C and stored at −80 °C.

MicroRNA seq quality control

Total RNA was extracted from the serum samples using Quiagen kit miRNeasy Mini kit (cat. #17004, Qia-gen, Germantown, MD, USA) and subjected to RNA. Seq. Analysis. Total RNA quality was determined by estimating the A260/A280 and A260/A230 ratios by nano-drop. RNA integrity is determined by running an Agilent Bioanalyzer gel or Agilent Fragment Analyzer, which measures the ratio of the ribosomal peaks. Samples with RIN values of 7 or greater are recommended for library prep.

MicroRNA seq library Prep

Qiagen microRNA Library Prep Kit (Cat# 331505) was used for the miRNA Seq library preparation. RNA was normalized to a starting input between 1ng-500ng. Adapters were ligated sequentially to the 3' and 5' ends of microRNAs prior to cDNA synthesis with unique molecular identifiers (UMIs) assignment, cDNA cleanup, amplification, and final library cleanup. The library was run on the Agilent Tapestation and size selection was performed if there is excess adapter dimer. The library is then quantified by qRT-PCR using a commercially available kit (KAPA Biosystems).

Flow cell preparation and sequencing

Sample concentrations were normalized to 1.2 nM and loaded onto an Illumina NovaSeq flow cell at a concentration that yields 10–25 million passing filter clusters per sample. Samples were sequenced using 100 bp paired-end sequencing on an Illumina NovaSeq according to Illumina protocols. The 8 bp unique dual index was read during additional sequencing reads that automatically follow the completion of read 1. Data generated during sequencing runs were simultaneously transferred to the Yale Center for Genome Analysis high-performance computing cluster. A positive control (prepared bacteriophage Phi X library) provided by Illumina was spiked into every lane at a concentration of 0.3% to monitor sequencing quality in real time. miRNA abundance was estimated using miRge3 using human small RNA reference database (https://sourceforge.net/projects/mirge3/files/miRge3_Lib/) [16]. The miRge3 analysis resulted in miRNA raw counts. The raw counts from mirGe3 analysis were used to estimate differentially expressed miRNAs.

Primary analysis

Sample de-multiplexing and alignment to the human genome was performed using Illumina's CASAVA 1.8.2 software suite. The data was checked for the sample error rate, confirming a rate of less than 2% and assuring that the distribution of reads per sample in a lane was within standard tolerance.

Data analysis and storage

Differentially expressed miRNAs were identified using DESeq2 (v 1.22.1) [17]. Wald test was used for comparing the groups. Benjamini-Hochberg (BH) method was used to control the false discovery rate (FDR). We used p -value < 0.05 as a cutoff for significantly expressed miRNAs.

Signal intensities were converted to individual base calls during a run using the system's Real Time Analysis (RTA) software. Base calls are transferred from the machine's dedicated personal computer to the Yale High Performance Computing cluster via a 1 Gigabit network mount for downstream analysis.

Results

During the study period we recruited 63 participants of which 12 were excluded due to low yield of microRNAs in the samples. We detected no miRNAs in these samples as miRNAs might have degraded during sample processing or was otherwise not detected. The final sample included 51 participants, 31 of which were designated to the endometriosis group and 20 assigned to the control group as showed in Table 1. In the endometriosis group 28 (90%) patients had surgical intervention and all 20 patients in the control group had surgery. Patients in the endometriosis group who did not have surgery had evidence of endometriosis on pelvic MRI. All surgeries were done laparoscopically by surgeons highly skilled in recognition of adolescent endometriosis. The indication for surgical intervention in the endometriosis group was chronic pelvic pain, with a diagnosis of endometriosis allowing inclusion. The most common reason for surgery in the control group was adnexal pathology as well as one subject who underwent elective cholecystectomy. Paratubal cysts were the most common adnexal pathology in the control group with seven (36.4%) participants having paratubal cysts followed by five cases (26.3%) of benign teratomas. There was no significant age difference between endometriosis and control group (16.3 ± 1.9 vs. 16.0 ± 3.2). The majority of participants in both groups were White, including 26 (83.9%) in endometriosis group and 15 (78.9%) in control group. In line with previous studies, we observed a significant difference in participants' Body Mass Index (BMI) at $24.5 \text{ kg/m}^2 \pm 5.0$ for the endometriosis group and $28.4 \text{ kg/m}^2 \pm 8.6$ in control group ($p = 0.028$). The vast majority of participants in endometriosis group had stage I endometriosis based

on the rASRM staging criteria [15]. We were unable to determine phase of the menstrual cycle in 20 out of 31 participants with endometriosis as they were treated with various hormonal preparations for menstrual suppression. Menstrual phase of the cycle was available in only 9 out of 20 controls due to majority of the participants using hormonal preparation for various reasons. The full characteristics of the adolescent subjects who participated ($N = 63$) in this study, including control subjects without endometriosis ($N = 20$) and subjects with endometriosis ($N = 31$), are shown in Table 1.

Analysis of microRNA-sequencing data demonstrated a total of 859 microRNAs in the serum of adolescents with endometriosis (Fig. 1).

In both the control group as well as the endometriosis group similar microRNAs were identified, however in the disease condition some of them were differentially abundant compared to the control group. Among 859 microRNAs detected, 468 microRNAs were increased while 391 were decreased compared to controls. Of these most showed small differences in abundance with considerable overlap between groups. This is consistent with our previously reported microarray analysis of microRNAs in the serum from adult women with endometriosis [7, 18]. We found that the majority of the microRNAs identified in adolescent patients with endometriosis were the same as previously identified in our prior microarray analysis of adults with endometriosis (Table 2).

We first examined microRNAs that were found in increased abundance among adolescents with endometriosis. Among upregulated microRNAs that we had not previously identified in adult endometriosis, there were four microRNAs that were significantly differentially expressed between control subjects and subjects with endometriosis, with sufficient separation to be considered as a potential biomarker in adolescents. These four microRNAs, specifically, miR-501-5p, miR-19b-3p, miR-3940-3p, and miR-1255b-5p, are listed in Table 3.

Of these, miR-19b-3p has been reported in adult patients with EM-associated infertility, leaving three as potential adolescent specific biomarkers [19]. Among decreased microRNAs newly identified here, eighteen microRNAs were highly significantly differentially expressed and able to discriminate endometriosis from controls. These eighteen microRNAs are miR-2116-5p, miR-455-3p, miR-4686, miR-7850-5p, miR-34c-5p, miR-451b-5p, miR-1277-5p, miR-2355-3p, miR-5589-5p, miR-6513-5p, miR-3064-5p, miR-27b-5p, miR-9-5p, miR-760, miR-204-5p, miR-195-5p, miR-21-3p, and miR-34a-5p and are listed in Table 4.

Among these 18 microRNAs, six microRNAs have been reported previously in adult women with endometriosis. MiR-34c-5p has a previously described role in endometrial receptivity [20] and a role in the development of

Figure 1

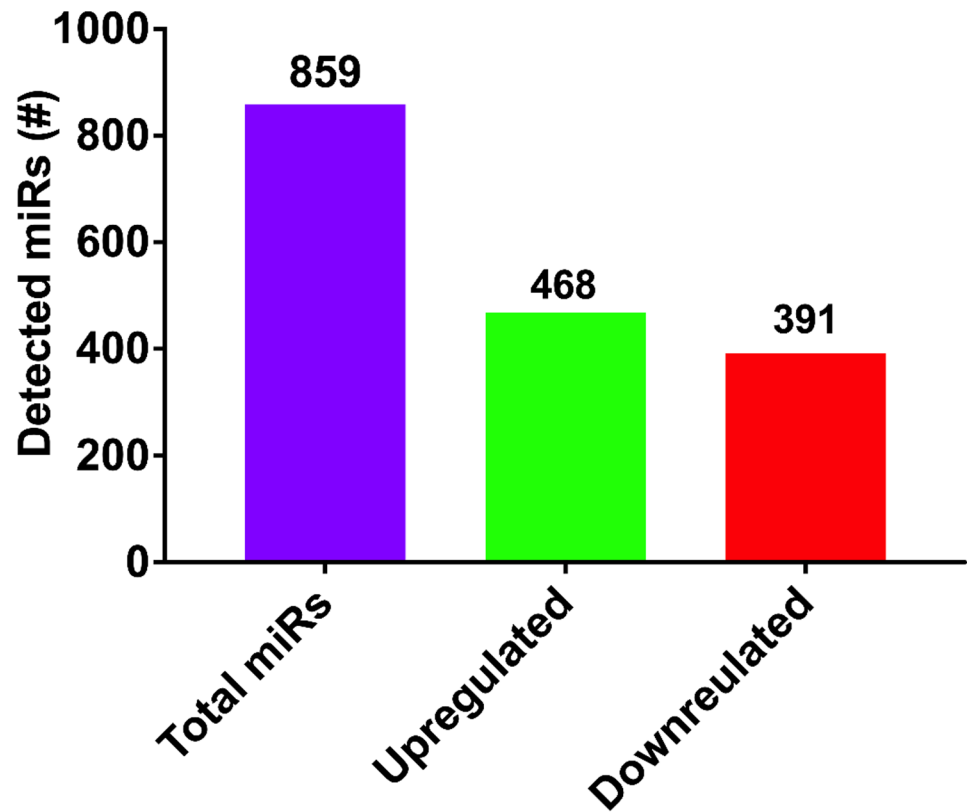


Fig. 1 RNA-seq data identified a total 859 microRNAs detected in endometriosis. 468 microRNAs were upregulated and 391 microRNAs were downregulated compared to controls without endometriosis

Table 2 Common MicroRNAs between adult and adolescent endometriosis

No	microRNAs (matched)
1	Let-7b-5p
2	Let-7a-5p
3	Let-7c-5p
4	Let-7d-5p
5	Let-7e-5p
6	let-7f-5p
7	miR-135a
8	miR-125b-5p
9	miR-150-5p
10	miR-342-3p
11	miR-145-5p
12	miR-143-3p
13	miR-500a-3p
14	miR-451a
15	miR-18a-5p
16	miR-3613-5p
17	miR-6755-3p

Table 3 Upregulated MicroRNAs

No.	microRNAs	Fold Change	P-Value
1	hsa-miR-501-5p*	1.8	0.034
2	hsa-miR-19b-3p*	1.6	0.023
3	hsa-miR-3940-3p*	1.5	0.037
4	hsa-miR-1255b-5p*	1.4	0.043

*Significant ($p < 0.05$)

endometriosis [21]. The other microRNAs miR-451 [21, 22], miR-204-5p [23], miR-21-3p [24], miR-34a-5p [25, 26], miR-19b-3p [19] also have a previously reported function in the pathophysiology of endometriosis. The remaining 11 microRNAs miR-2116-5p, miR-455-3p, miR-4686, miR-7850-5p, miR-1277-5p, miR-2355-3p, miR-5589-5p, miR-6513-5p, miR-3064-5p, miR-27b-5p, miR-9-5p, miR-760, miR-204-5p, miR-501-5p, miR-3940-3p, and miR-1255b-5p are uniquely differentially expressed in adolescent patients.

Table 4 Downregulated MicroRNAs

No.	microRNAs	Fold Change	P-Value
1	hsa-miR-2116-5p*	-Inf	0.047
2	hsa-miR-455-3p*	-Inf	0.050
3	hsa-miR-4686*	-Inf	0.029
4	hsa-miR-7850-5p*	-32.4	0.036
5	hsa-miR-34c-5p*	-25.6	0.018
6	hsa-miR-451b-5p*	-17.6	0.011
7	hsa-miR-1277-5p*	-7.3	0.046
8	hsa-miR-2355-3p*	-6.3	0.037
9	hsa-miR-5589-5p*	-6.2	0.029
10	hsa-miR-6513-5p*	-5.9	0.005
11	hsa-miR-3064-5p*	-5.7	0.000
12	hsa-miR-27b-5p*	-4.1	0.009
13	hsa-miR-9-5p*	-3.1	0.044
14	hsa-miR-760*	-3.3	0.027
15	hsa-miR-204-5p*	-3.0	0.045
16	hsa-miR-195-5p*	-2.4	0.000
17	hsa-miR-21-3p*	-2.2	0.039
18	hsa-miR-34a-5p*	-2.0	0.039

*Significant ($p < 0.05$)

Discussion

The diagnosis of endometriosis is typically delayed by 8–10 years, with the delay most pronounced in adolescents [27, 28]. The delay in diagnosis of endometriosis reaches nearly 14 years in women aged 9–19 years [29, 30]. The diagnostic process in adolescents is also more complex and the awareness of endometriosis in adolescents among medical professionals and caregivers of adolescents is low. Greene et al. described 4334 women with surgically confirmed endometriosis, reporting that women who first experienced symptoms as adolescents waited three times as long before seeking care compared to adults (6 vs. 2 years); it took longer before a diagnosis was made (5.4 vs. 1.9 years), and they reported not being taken seriously (65.2% vs. 48.9%) or told that nothing was wrong (69.6% vs. 49.8%) more often than women experiencing first symptoms as adults [31].

This is one of the few studies in young women with endometriosis to examine the differential abundance of circulating microRNAs in the blood. We identified a unique set of microRNAs in a subset of adolescent and young adult women with and without endometriosis.

RNA-sequence analysis of microRNAs in the serum of adolescent and young women with endometriosis demonstrated that a new set of microRNAs exist in circulation that are differed from microRNAs from the serum of adult women with endometriosis. Among four upregulated microRNAs that were significantly expressed in adolescent patients, three microRNAs miR-501-5p, miR-3940-3p, and miR-1255b-5p were never reported previously in women with endometriosis. Among 18 microRNAs that were downregulated significantly in

adolescent patients, six microRNAs miR-34c-5p [21, 32], miR-204-5p [23], miR-21-3p [24], miR-451b-5p [33], miR-195-5p [34] and miR-34a-5p [25, 26] have been reported previously in women with endometriosis. In our study miR-9-5p expression was reduced significantly in contrast Zheng et al. [35] reported that it is upregulated in women with endometriosis. The remaining 11 microRNAs miR-2116-5p, miR-455-3p, miR-4686, miR-7850-5p, miR-1277-5p, miR-2355-3p, miR-5589-5p, miR-6513-5p, miR-3064-5p, miR-27b-5p, and miR-760 have never been reported previously in women with endometriosis. Therefore, this signature of microRNAs may have the potential to serve as a biomarker panel for adolescent patients with endometriosis. Expression of different microRNAs in adolescents might stem from earlier stages of the disease in younger patients. Our research complements discovery of 49 miRNAs that were differentially expressed between endometriosis cases and controls by Brady et al. [36]. In the same study authors completed neural network analysis that revealed 5 miRNAs in the final analysis (miR-542-3p, let-7b-3p, miR-548i, miR-769-5p, miR-30c-1-3p).

We reported previously the differential expression of circulating microRNAs in the serum of women with endometriosis [7, 8, 18]. Recently, we described a panel of six microRNAs as a biomarker to diagnose women with endometriosis [8]. Out of six microRNAs four microRNAs miR-125b, miR-150-5p, miR-342-3p, and miR-451a were significantly increased in patients with endometriosis while miR-3613-5p and let-7b were significantly decreased. Furthermore, our group demonstrated differential methylation of the Let-7b coding region in ectopic samples compared with eutopic endometrium from patients with endometriosis, suggesting the epigenetic nature of the differential regulation of microRNAs in endometriosis [37]. Circulating microRNA biomarkers, including miR-122 and miR-199a in serum [38] and miR-31 and miR-145 in plasma [39] have also reported as potential biomarkers to diagnose the adult women with endometriosis.

We have also demonstrated that targeting aberrantly produced microRNA is potential treatment of endometriosis [40, 41]. Targeting abnormally expressed microRNAs showed a robust respond to therapy with the reduction of lesion volume after endometriosis treatment [40, 42, 43]. MicroRNA analogs or inhibitors have potential as therapeutic agents in this disease, further demonstrating the utility of identifying the microRNA signature of adolescents with endometriosis. The distinct microRNAs signatures produced in adult women with endometriosis differ from microRNAs produced by adolescent women with endometriosis, suggesting the potential for therapy aimed specifically at this population.

Endometriotic lesions secrete some of these microRNAs directly, while some microRNAs are produced in response to the endometriosis by other tissues. Further, multiple organs in the body produce microRNAs in response to inflammation associated with endometriosis. Some of these microRNAs are released into the circulation where they have an effect on organs that may be far removed from the location of the endometriosis [1, 42, 44, 45]. Those microRNAs that are produced directly by endometriosis are more likely to be therapeutic targets. Other microRNA may account to the diffuse multiorgan effects of endometriosis and may be targeted to reduce these systemic effects.

Identifying microRNAs that are differentially expressed in adolescent endometriosis will broaden our understanding the role of microRNAs in the pathophysiology of early endometriosis. These microRNAs may provide novel biomarkers for endometriosis diagnosis and potential for treatment. While these findings must be confirmed in a large independent population, this work may produce a noninvasive serum biomarker of this chronic and debilitating disease at an early stage. Ability to diagnose endometriosis sooner will lead to improved quality of life and likely improved long-term outcomes as a result of more rapid and early treatment.

Strengths and limitation of the study

This study has several strengths. Firstly, we evaluated participants prospectively and included other pelvic pathology, suggesting that these markers not only distinguish women with endometriosis from healthy individuals, but also can distinguish endometriosis from other common pathologies. Second, the investigators who were evaluating microRNA levels were blinded to the clinical or surgical diagnosis. Third, all but three subjects in the endometriosis group had surgically and histologically confirmed endometriosis and all participants in control group had surgery thus using the “gold standard” of surgical diagnosis.

We recognize a few limitations of our study. The limited number of participants precluded us from stratifying levels of microRNAs according to the severity or stage of the disease. The study was conducted at one institution comprising of participants of one geographic area. Our sample was uniform with predominance of Caucasian participants, which does not fully reflect the demographic makeup of the catchment area. Majority of participants used various hormonal preparations as the 1st line of treatment for pelvic pain with resultant amenorrhea which precluded us from accurately documenting the phase of menstrual cycle. Similarly, use of various hormonal preparations may have a confounding effect on the composition of circulating microRNAs. Lastly, our control group included patients with various histological

adnexal masses which could have an impact on the composition of microRNA levels.

Conclusions

This prospective cohort study carried out in adolescent women with endometriosis and identifies a distinct set of microRNAs that are differentially expressed compared to microRNAs reported in adult women with endometriosis. Further studies will include a larger sample with goal to validate these findings. Circulating microRNAs provides a hope for non-invasive method to diagnose endometriosis. Such a diagnostic tool would enable us to avoid surgery simply for diagnosis and to reduce the prolonged delay in detection of endometriosis. Earlier diagnosis could reduce the surgical risk, years of discomfort, hospitalizations and healthcare costs, disease progression and associated co-morbidities.

Abbreviations

BMI	Body Mass Index
OCP	Oral Contraceptive Pill
GnRH	Gonadotropin-Releasing Hormone
rASRM	revised American Society for Reproductive Medicine
LNG IUD	Levonorgestrel Intrauterine Device

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Disclosure

Statement: HST is the shareholder for the DotLab.

The rest of the authors have no financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript.

Authors' contributions

AVM created the idea of the research study and implemented it, performed acquisition, analysis and interpretation of data for the work; drafted and reviewed the entire manuscript; participated in the approval of the final version.- RM created the idea of the research study and implemented it, performed acquisition, analysis and interpretation of data for the work; drafted and reviewed the entire manuscript; participated in the approval of the final version.- KP performed analysis and interpretation of data for the work; drafted and reviewed the entire manuscript and participated in the approval of the final version.- DM performed analysis and interpretation of data for the work; drafted and reviewed the entire manuscript and participated in the approval of the final version.- HST created the idea of the research study and implemented it, performed acquisition, analysis and interpretation of data for the work; drafted and reviewed the entire manuscript; participated in the approval of the final version. Provided mentorship to AVM throughout the research process and writing of the manuscript.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

Ethics approval and consent was granted by the Institutional Review Board Protocol#1608018237 in accordance with the Declaration of Helsinki.

Consent for publication

not applicable.

Competing interests

The authors declare no competing interests.

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