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Original Article

Obstetrics and Gynaecology Section

Diagnostic Characteristics, Histopathological and Aromatase Gene Polymorphism (rs ID-2470152) in Endometriosis Patients: A Case-control Study

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ABSTRACT

Introduction: Endometriosis is an oestrogen dependent gynaecological disease, having endometrial glands and stromal tissues outside the intrauterine locations. The aetiology and pathogenesis of endometriosis is still unclear and it affects a large proportion of reproductive age women. It's a heterogeneous disease and is found to be associated with hormonal and histological alterations. Studies indicate that mutation in aromatase (CYP19) gene is involved in a number of inflammatory diseases and CYP19 rs 2470152 site polymorphism may help to find its relation to susceptibility to endometriosis.

Aim: To ascertain the relationship between changes in histological architecture in endometrial cells during endometriosis with circulating hormone levels, stress parameters and aromatase (CYP19A1) gene polymorphism {Single Nucleotide Polymorphism (SNP) rs 2470152}.

Materials and Methods: This was a hospital-based case-control study where all patients and controls were recruited from the Outpatient Department (OPD) of the Sir Sunderlal Hospital, Department of Obstetrics and Gynaecology, Institute of Medical Sciences, Banaras Hindu University (BHU), Varanasi, India from March 2016-March 2019, and a total 300 subjects, 120 endometriosis patients and 180 healthy controls were studied. Histological studies were done by Haematoxylin and Eosin (H&E) staining in the endometrial tissues of patients and controls. Genotyping of SNP rs 2470152 was conducted by Polymerise Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method on genomic Deoxyribonucleic Acid (DNA) isolated from patients and control blood. Student's t-test was used to compare the mean for the two independent groups. Allele and genotype distribution among groups were evaluated using the Chi-squared test and Fisher's-Exact test.

Results: In the present study, all the subjects were in the age group of 20-50+ years where 20-40 years age group were premenopausal and 40-50+ year were perimenopausal. Significant histological changes were observed in the endometrial glands and stroma of the endometrium tissues of the diseased women compared to the healthy controls. Various pathological entities were altered in circulating blood plasma of patients than to control. For polymorphism studies allele (T,C) ($p=0.002^*$) and genotypic (TT, TC and TT) ($p<0.001^*$) frequencies were found significantly variable in endometriosis patients in comparison of controls.

Conclusion: The present study showed that endometrial tissue undergoes a lot of pathological changes during a disease and this may be due to significantly altered expression of aromatase gene leading to higher oestrogen level, causing this disease and its proliferation. Aromatase (CYP19A1) gene polymorphism was found significantly associated, and other factors may be affecting aromatase directly or indirectly in steroidogenic pathway.

Keywords: Hormones, Lipid peroxidation, Single nucleotide polymorphism, Superoxide dismutase

INTRODUCTION

Endometriosis is a disease, having endometrial glands and stromal tissues outside the intrauterine endometrial regions (pelvic peritoneum, fallopian tubes and on ovaries). It's an oestrogen dependent, gynaecological disorder occurring in approximately 5-10% reproductive and approximately 20-50% infertile women [1]. The aetiology and pathogenesis of endometriosis is unclear yet, but two main theories exist now for it. Retrograde menstruation theory, tells the endometrial cells adhere, invade and neovascularise the peritoneum resulting in the abnormal growth, while metaplasia theory describes, an abnormal cellular differentiation process occurring at the site, resulting in the endometrium like tissue proliferation [2,3]. It is hypothesised that the diseased condition develops as a result of any defect in clearance of menstrual efflux [4]. Women with symptoms of chronic pelvic pain, fatigue, dysmenorrhoea (pain during periods), dyspareunia (pain during intercourse), heavy menstruation, bleeding in between periods are mostly diagnosed with endometriosis [5]. Approximately 47% of infertile women suffer with endometriosis, most of the suspects suffer with chronic pelvic pain and subfertility, including four diagnostic areas: clinical manifestations (familial history and physical examination), imaging techniques such as Ultrasonography,Transvaginal Ultrasound (USG,TVS), endometriosis biomarkers and laparoscopic surgery [6,7]. On size, number and depth basis, staging of endometriotic lesions are done according to the revised American Society of Reproductive Medicine (rASRM) [1].

In recent time, studies include genetic, hormonal, immunological and inflammatory parameters, menstrual cyclicity, prostaglandin metabolism, to identify a putative disease biomarker. But socio-demographic, reproductive and gynaecological, co-morbidities, environmental and personal habits, are few more factors which have cumulative effect for development and progression of endometriosis [8]. Human endometrium, a dynamic tissue,having two distinguishing; glandular and stromal cells undergo hundreds of cycles of proliferation, differentiation and shedding during women's reproductive years [9]. The cyclic histological changes in the adult human endometrium have been described well [10]. In reproductive phase, uterine endometrium/mucosal lining is composed of simple columnar epithelium and a layer of connective tissue i.e. the endometrial stroma or called as lamina propria. The epithelium consisted of non ciliated, secretory columnar epithelium [11]. In early

menstrual phases the endometrial glands are simple straight tubes lined by columnar epithelium with large oval nuclei. In later phases these become curved and tortuous and subnucleolar vacuolisation was also observed.

Typical and subtle endometriotic lesions are histologically characterised by epithelium and stroma of the endometrial type [12]. The two anterior pituitary hormones, Lutenizing Hormone (LH) and Follicle Stimulating Hormone (FSH) called as gonadotropins are important for female reproductive function, where FSH stimulates growth of ovarian follicles, while an acute rise of LH triggers ovulation and development of the corpus luteum [13]. By the action of LH and FSH, two ovarian steroid hormones, oestrogen and progesterone, are synthesised from the ovarian follicles from the combined functions of the granulosa and theca cells and these further function in a coordinated fashion to support the reproductive activity of the female.The source for estradiol in premenopausal, is mostly ovarian but after menopause it's mainly peripheral, through conversion of androstenedione or C19 steroids from the adrenal cortex and ovaries. This occurs in skin, muscle, adipose tissue and the conversion is catalysed by the enzyme complex cytochrome P450 aromatase [14]. Progesterone, regulates female physiology, prepares and maintains endometrium, to allow for embryo implantation [15]. Cortisol, a hormone mainly released at the time of stress because of any disease condition, to increase blood pressure to distribute the glucose and nutrients to cells [16]. Thus, it helps the body to resist stress and reduces inflammatory responses. Interleukin-6 (IL-6), a pleotropic cytokine is produced in response to tissue damage and infections, that's associated with pro and anti-inflammatory effects [17].

In endometriotic implants aromatase activity was significantly stimulated by a Cyclic Adenosine Monophosphate (cAMP) analogue, PGE2 or a combination of a glucocorticoid and a cytokines {IL-6, IL-11, Tumour Necrosis Factor (TNF)} via cAMP pathway [18]. It is well known that free radicals and Reactive Oxygen Species (ROS) are continuously produced in all cells, to maintain the cell homeostasis and also during diseases [19]. Deleterious effects of ROS and Lipid Peroxidation (LPO) are counteracted by antioxidant defense system, which consists Antioxidant Molecules and Enzymes (AOE) such as Superoxide Dismutase (SOD) etc [20]. During endometriosis disease, significant increase in ROS and LPO may also occur, that will be counteracted by SOD. SOD and LPO assay in serum and endometriotic lesions will help in the disease diagnosis, prior to be chronic. Malondialdehyde (MDA) is the principal component or product of polyunsaturated fatty acid peroxidation (ie; LPO) and thus indicates the oxidative stress indirectly into the tissues or serum samples. Cholesterol, the main precursor molecule for oestrogen biosynthesis, moves from the cytosolic region of the cell to the mitochondria via Steroidogenic Acute Regulatory Protein (StAR). Six enzymes serially, catalyses the conversion of cholesterol molecule into Oestrogen, where aromatase, a key enzyme, converts androstenedione into biologically active oestrogen (E2-estradiol) [18]. Aromatase gene expression is highest in ovarian granulosa cells in premenopausal women but later after menopause adipose tissue becomes the major site [21]. It's immunolocalised exclusively in glandular cells cytoplasm but immunoreactivity was not detected in stroma [22]. It's present in the endoplasmic reticulum of cells and is composed of a specific form of cytochrome P450 (aromatase cytochrome P450). The P450 aromatase was also localised in the cytoplasm of glandular cells of adenomyotic and eutopic endometrium of endometriosis patients [18]. While disease free endometrium and myometrium, lack its expression [23].

Recently, SNP rs 2414096 A allele has been found to be associated with the activity of aromatase in PCOS [24]. Another study tells that rs 2470152 of the CYP19 gene is clearly associated with serum E2 and E1 levels in men [25]. These suggests that there may be a possible role of CYP19 gene polymorphisms in endometriosis initiation and progression, and any alternation occurs in its expression, may lead to endometriosis. Hence, the present study was conducted to determine the association between CYP19 rs 2470152 site polymorphism that can help to find its relation to susceptibility to endometriosis.

MATERIALS AND METHODS

This was a hospital based case-control study where all patients and controls were recruited from the OPD of the Sir Sunderlal Hospital, Department of Obstetrics and Gynaecology, Institute of Medical Sciences, Banaras Hindu University (BHU), Varanasi, India from March 2016 to March 2019 (N=300). The ethical approval was granted by the Institutional Ethical Committee (Ref No: I. Sc./ ECM-IX/2 0 I 6-I7/04). Informed consent was obtained from all participants and questionnaire was filled up to record details of their Age at Menarche (AAM), Body Weight (BW), height, Body Mass Index (BMI), menstrual irregularities, blood sugar {fasting and Postprandial (PP)}, lifestyle, familial history, previous diseases and medications undergone. All the subjects belong to India and fall within same ethnic group.

Sample size calculation: Formula for sample collection was as follows; $\eta=[Z_{a/2}\,\sigma/E]^2$

Where, η=sample size, Za/2 represents the confidence level 95%, for standard normal (Z) distribution, σ=The population Standard deviation (SD) and E=margin of error (a 95% degree of confidence corresponds to the value of 1.96) [26].

Inclusion criteria: For case group: Endometriosis patient diagnosed to have endometriosis by ultrasonography. The subjects were also advised for advanced technology of imaging methods like {Magnetic Resonance Imaging (MRI) and laparoscopy} for the detection of deep infiltrating lesions and categorisation into different stages. The subjects had no endocrine, radiation, or chemical therapy, or taken oral contraceptives for three months prior to admission, and had no other disease history were included in the study.

For control group: In the control group, women who had a healthy medical examination {BMI, sugar levels (Fasting, PP), CA-125, hormone profiles in range}, normal menstrual cycle (28-32 days/ regular) having ≥2 pregnancies without history of pregnancy-related complications, in same age group to the case group were included.

Exclusion criteria: For case group: The endometriosis patient who had received hormonal therapy prior to admission, had severe heart, lung, liver, kidney, and/or another organ dysfunction were excluded from the study.

For control group: In the control group who had received hormone therapy, possessed heart, lung, liver, kidney, and/or other organ dysfunction previously, and were at gestation or at breastfeeding period were excluded.

Study Procedure

Sample collection and storage: Venous blood samples were collected from median cubital vein of subjects, during 3rd/5th day of menstrual cycle, between 08:00 am to 09:00 am after a 12 hour overnight fast. For serum, blood was kept at room temperature for 30-60 minutes, centrifuged (3000 rpm, 10 minutes, 4°C), isolated and stored (-80°C) further for hormone profiling. For genomic DNA, blood was collected in an Ethylenediamine Tetra-acetic Acid (EDTA) vacutainer, extraction was done via phenol-chloroform method and stored (-20ºC). Endometrial tissues were collected from biopsies.

Oxidative stress parameters: In order to determine the effect of oxidative stress in endometrial tissues, the antioxidant enzymes such as superoxide dismutase and lipid per oxidation assay were performed as the standard protocols [27,28]. A 10% tissue homogenate was prepared separately in ice cold Phosphate Buffer Solution (PBS), (pH-7.4) by using the homogeniser (IKA T10 basic, ULTRA-TURRAX), and further processed accordingly.

Histological examination: The excised endometrial tissue samples were fixed in Bouin's fluid for 24 hours and processed for paraffin embedding. Sections were cut at 6 µm and stained with Ehrlich's H&E, as the standard protocol [29]. The sections were dehydrated in alcohol grades, cleared in xylene and mounted in Dibutylphthalate Polystyrene Xylene (DPX). The sections were examined under Leica DM 2000 microscope, and images were taken with a Leica digital camera DFC 295 with 3 megapixel.

Serum profiling of hormones: From the collected serum, luteinizing hormone (LH, lot No.-LHG4061), estradiol (E2, lot No.-42K039-3), testosterone (T, lot No.-4510A), Cortisol (lot No.-3602) and Interleukin-6 (IL-6, lot No.-IL06001) were estimated by Enzyme Linked Immune Sorbent Assay (ELISA), following the manufacturer's protocol [30]. Absorbance was measured using iMarkTM Microplate Absorbance Reader (BioRad, USA) and the concentrations were expressed as ng/mL and ng/g. The intra-assay coefficients of variance for LH, E2, T, cortisol and IL-6 were ≤8.7%, ≤9.3%, ≤7%, ≤8.9%, ≤9.4% respectively. The FSH, Triiodothyronine (T3), Tetraiodothyronine (T4) and Thyroid Stimulating Hormone (TSH) levels were noted down from questionnaire.

Primer designing: Primers were designed according to the protocol, fp- forward primer, rp- reverse primer [31].

GAPDH: fp-5'CCATGGAGAAGGCTGGGG3',
rp-3'CAAAGTTGTCATGGATGACC5';

β-actin: fp-5'AAATCTGGCACCACACCTTC3',
rp-3'AGCACAGCCTGGATAGCAAC5';

CYP19: fp-5'CTGCCTTTGAGGAGCTTA3',
rp- 3'CTTCTCTGGCTTTCCCCTCT5'

Polymerase chain reaction: PCR was performed using the standard protocol, the annealing temperature was 58°C for GAPDH, β-actin and aromatase [32].

Genotyping: Genotyping of SNP (-1562C/T) CYP19 gene was done by PCR-RFLP. PCR amplification of the -1562C/T mutation region in the aromatase gene was performed using primers (forward: 5'-CTGCCTTTGAGGAGCTTA-3'; reverse: 5'CTTCTCTGG CTTTCCCCTCT-3'). PCR conditions for 30 cycles were denaturation (94°C, 1 minute), annealing (58°C, 1 minute) and extension (72°C, 1 minute). For RFLP analysis, restriction digestion was done for 278-bp fragment with HPY CH4 IV (restriction enzyme) at 37°C overnight. If there was a T→C transition, the 278-bp fragment after digestion generates two restriction products of 179 and 99 bp. Thus, homozygous wild type phenotype (TT) shows a single thick band of 278 bp, heterozygous (TC) 2-bands of 179 and 99 bp, and homozygous recessive (CC) a single band of 99 bp [33]. Socio-economic status was assessed using modified BG Prasad socio-economic classification [34].

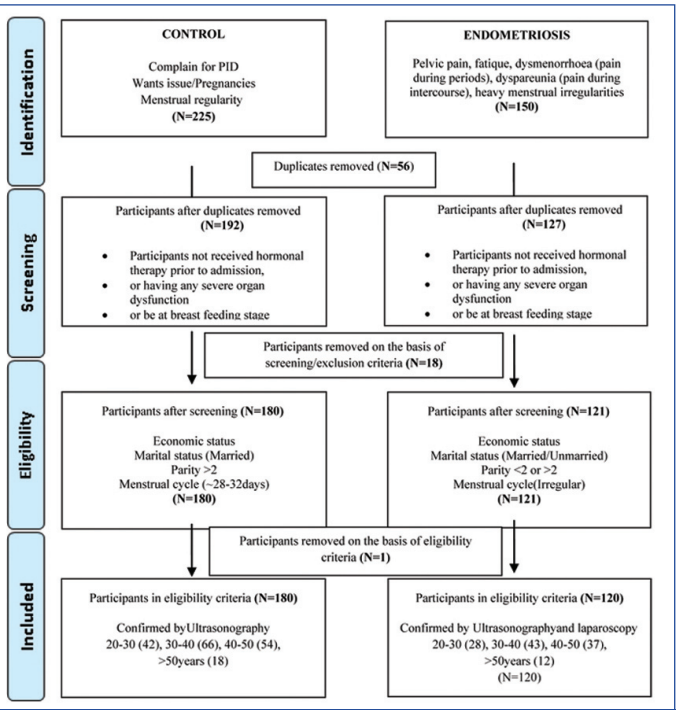
STATISTICAL ANALYSIS

Statistical analysis were performed using the Graphpad Prism software, version 5.0. The data was presented as Mean±SD. Student's t-test was used to compare the mean for the two independent groups. Allele and genotype distribution among groups were evaluated using the Chi-square test and Fisher's-Exact test. The difference in frequencies between the case and control groups was analysed for statistical significance at the 95% Confidence Interval (CI) using the Chi-square test. The p-value <0.05 was considered significant in all the analysis.

RESULTS

In the present study, all the subjects were in the age group of 20-50+ years where 20-40 years age group were premenopausal and 40-50+ year were perimenopausal. Survey details of total 375 samples were done, and further identification, screening and inclusion of 300 subjects was done on the basis of different criteria undergone. Then control (180) and endometriosis patients (120) were charted and numbers of different age group of control and endometriosis patients were calculated.

The distribution of collected samples according to the selection criteria (lifestyle, familial history, history of previous diseases and medication undergone) is shown in [Table/Fig-1]. In the endometriosis patient's disease symptoms (chronic pelvic pain, fatigue, dysmenorrhoea, dyspareunia, heavy menstruation, menstruation and bleeding in between periods) were found to be most common in 43 women between the age of 30-40 years group.



[Table/Fig-1]: Flow chart to depict the algorithm for control and endometriosis sample collection and selection criteria for its further analysis.

All the included subjects in the present study belonged to lower, middle and higher class and there was not any association of occurrence of endometriosis with their economic status. All the fertile women assigned to control subjects were married, having regular menstrual cycle, ≥2 parity, and were examined for the cause of chronic pelvic pain without any pelvic abnormalities determined by laparoscopy. But the endometriosis subjects were mostly including married women at the time of their diagnosis who have visited the hospital because of their menstrual irregularities. The marital status, menstrual irregularities and parity in endometriosis patients showed significant values (p-value <0.001) in respect to control [Table/Fig-2].

S. No.	Factors		Endometriosis (n=120)	Control (n=180)	p-value
1.	Economic status	Low	37 (30.8%)	45 (25.0%)	0.312
		Middle	43 (35.8%)	60 (33.3%)	
		High	40 (33.4%)	75 (41.7%)	
2.	Marital status	Unmarried	23 (19.2%)	0	<0.001
		Married	97 (80.8%)	180 (100%)	
3.	Menstrual cycle	Regular	8 (6.7%)	180 (100%)	<0.001
		Irregular	112 (93.3%)	0	
4.	Parity	<2	97 (80.8%)	0	<0.001
		≥2	23 (19.2%)	180 (100%)	

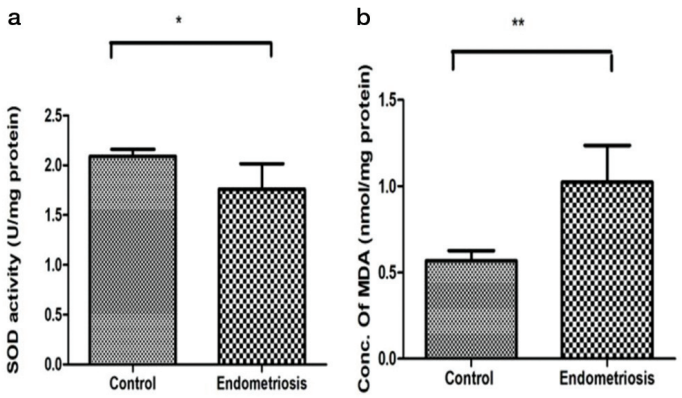
[Table/Fig-2]: Socio-demographic characteristics of the total subjects (Total N=300). Chi-square test and Fisher's exact test were used to study the significance of difference between proportions. Two-tailed p-values less than 0.05 were considered statistically significant

The variables AAM, fasting blood sugar, PRL, T4, FSH, T were not found to be significantly variable (p>0.05) in patients than to normal control women, and its shown in [Table/Fig-3]. But PP blood sugar, T3, LH were significant (p<0.005), while BMI, CA-125, TSH, LH/FSH, E2, E2/T, IL-6 and cortisol were significantly higher (p<0.001) in endometriosis subjects than those of control.

Significantly low (p<0.01) level of Superoxide Dismutase (SOD) and increased level (p<0.05) of MDA (Malondialdehyde) was seen in [Table/Fig-4]. This was the principal component of lipid peroxidation; the two stress parameters in tissue samples collected from endometriosis suffering women as compared to the control.

S. No.	Variables	Endometriosis (n=120)	Control (n=180)	p-value
1.	BMI (kg/m^2)	25.00±1.80	19.99±0.98	<0.001
2.	AAM (years)	14.64±1.24	14.03±1.17	0.3626
3.	CA-125 (kU/L)	52.56±14.76	23.47±4.83	<0.001
4.	Sugar (fasting) (mmol/L)	102.4±49.11	90.23±5.58	0.3027
5.	Sugar (PP) mmol/L	143.5±69.43	106.3±18.9	0.0265
6.	PRL (ng/dL)	14.25±9.16	8.15±0.90	0.0508
7.	T3 (ng/dL)	131.6±34.71	102.5±9.21	0.0103
8.	T4 (ug/dL)	8.99±3.19	8.62±0.90	0.7017
9.	TSH (µlu/mL)	4.63±0.87	2.29±0.65	<0.001
10.	LH (miu/mL)	19.71±11.02	4.94 ± 1.55	0.002
11.	FSH (miu/mL)	10.35±5.49	6.63±1.75	0.0712
12.	LH/FSH	1.70±0.37	0.73±0.10	<0.001
13.	Estradiol E2 (pg/mL)	421.0±18.76	47.22±2.65	<0.001
14.	Testosterone (ng/dL)	0.46±0.08	0.43±0.07	0.4309
15.	E2/T ratio	925.3±194.9	112.1±25.82	<0.001
16.	IL-6 (pg/mL)	434.2±26.24	16.13±1.24	<0.001
17.	Cortisol (nmol/L)	106.6±7.40	91.97±2.74	<0.001

[Table/Fig-3]: Significance of variable factors in endometriosis patients with respect to control.
Student's t-test was used to compare the mean for the endometriosis and control subjects. Two-tailed p-values less than 0.05 were considered statistically significant



[Table/Fig-4]: Biochemical assay of SOD (A; Superoxide Dismutase) and LPO (B; Lipid Peroxidase) in endometrium tissues of control and patient samples; p>0.05, *p<0.05, **p<0.01, ***p<0.001.

Tubular uterine glands (A); pseudostratified cells with mitotic activity (B), a feature of proliferative endometrium, subnuclear vacuolisation (C); prominent spiral arteriole (D), a feature of secretary endometrium of premenopausal women; and E showed low columnar cells with no secretary activity in endometrium of a postmenopausal women [11]. These features are shown in [Table/Fig-5a].

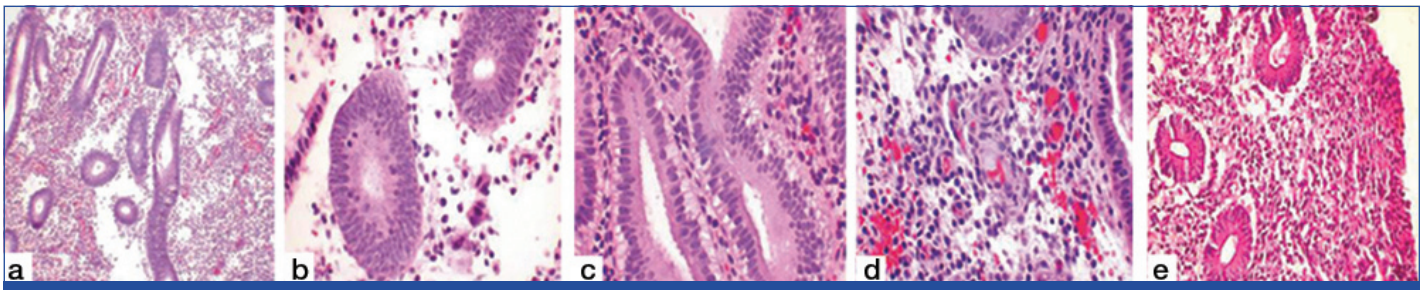
Features of secretary phase endometrium; tortures endometrial glands (UG), (A, 10X), wider lumen (L), high subnuclear vacuolisation (arrow) and more Spiral Arterioles (SA) in endometrioma tissues of premenopausal (reproductively active) women suffering with endometriosis disease. While endometrioma tissues of perimenopausal (around the phase of menopause) women suffering with endometriosis disease showed less tortures endometrial glands (UG) (F,10X), less wide Lumen (L), luminal vacuolisation (arrow) and lesser number of spiral arterioles (G, H, I, J, 40X) compared to reproductively active women but a prominent features of secretary endometrium were seen in [Table/Fig-5b]. Gel electrophoresis for GAPDH, β-actin and aromatase have been shown in [Table/Fig-6].

Allele (p=0.002*) and genotypic frequencies (p<0.001*) were found significantly different in patients than to controls. It has been shown that the genotypic distribution (TT, TC and TT) in women with endometriosis (0.167, 0.566 and 0.267) was differ from that of the controls (0.333, 0.60 and 0.67), (p<0.001). The rs 2470152 (T,C) allele frequency in endometriosis patients (0.45,0.55) was not similar to that in controls (0.633,0.367) (p=0.002) [Table/Fig-7].

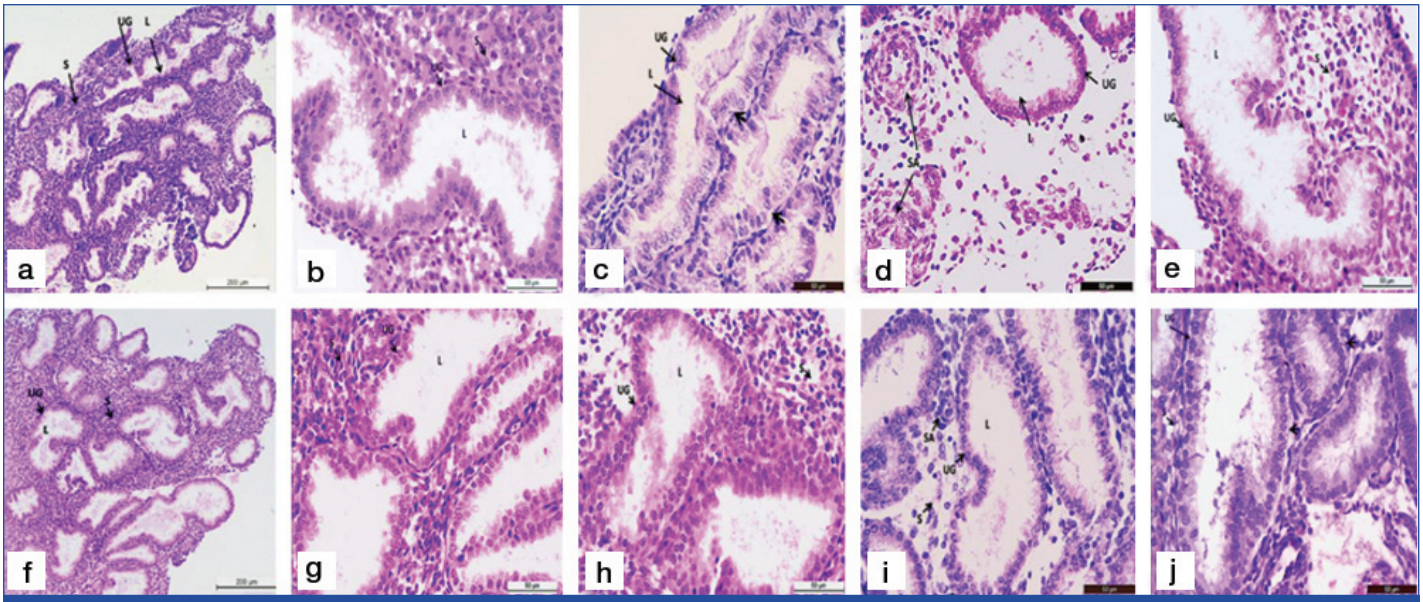
DISCUSSION

Endometriosis, is an oestrogen dependent disease. For novel, effective and early diagnostic biomarkers, there is a great need to do an extensive research to assess the role of different factors playing a role in disease progression. This disease is still a challenging clinical problem, and the relevance of the findings may be exemplified for the successful treatment of severe or recurrent cases of the disease. The present study revealed that the age (reproductively active), marital status, parity and menstrual irregularities had a relationship with incidence of endometriosis in Indian women, which is consistent with previous results and as it may be because of women with menstrual irregularities visit the hospitals more often after their marriage, if they have any issues regarding pregnancy and then these have been diagnosed to have endometriosis in the developing countries like India. Further BMI, CA-125 (a well-known tumour marker), TSH, LH/FSH, IL-6 were significantly higher (p<0.001) in the sera of endometriosis patients, and it's evidenced that intramural aromatase is expressed in stromal cells of endometroid tissues and aromatase synthesis is all regulated by hormones TSH, LH and FSH (steroidogenic pathway), while IL-6 is an inflammatory biomarker and increases with stages of endometriosis disease progression [35,36].

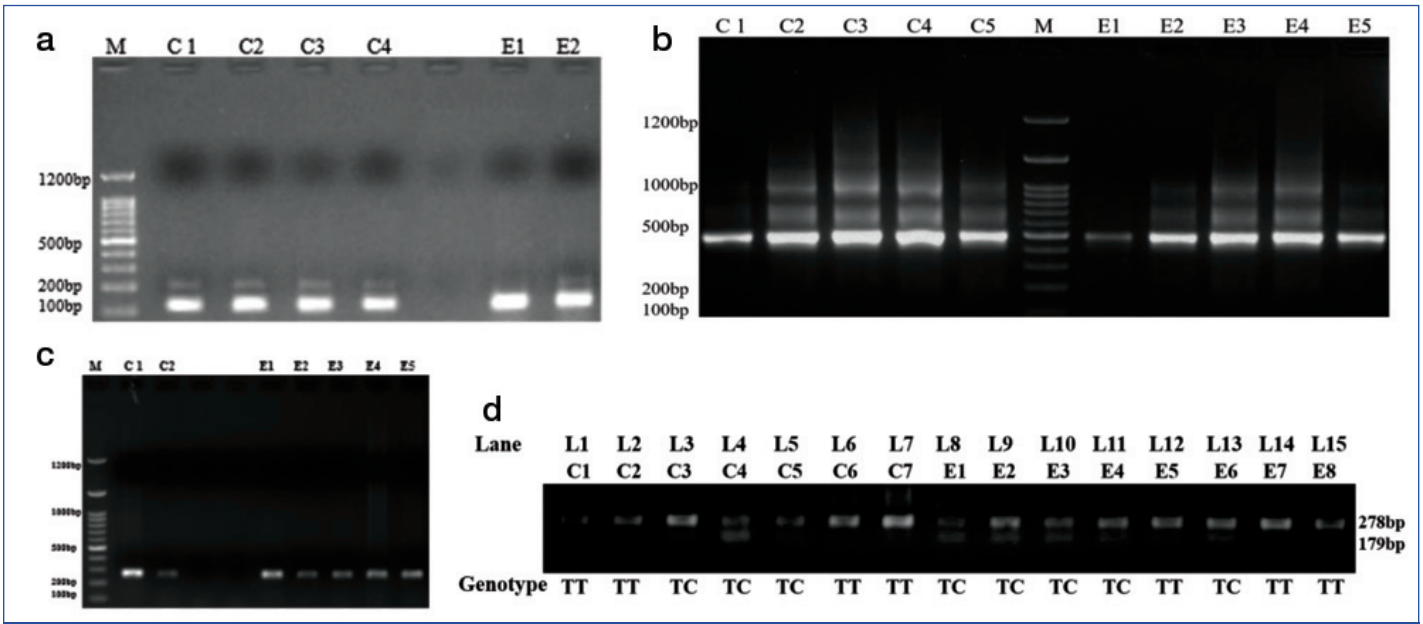
As its now known by studies, that oestrogen level is high in endometriosis suffering women leading to inflammation and since



[Table/Fig-5a]: Histological architecture of the uterus (endometrial lining, cellular structures, thickness and organisation of its endometrial tissues) in normal ovulating women (A, B, C, D) and postmenopausal women (E), (H&E staining, A-10x,B to E-40x) [11].



[Table/Fig-5b]: Histopathological changes related to endometrial lining, cellular structures, thickness and organisation of endometrial tissues in women suffering with endometriosis disease, (H&E staining, A and F-10x, B to E, G to J-40x).
UG: Uterine gland; L: Lumen; S: Stroma; SA: Spiral arteriole; ↖=Sub nuclear vacuolisation



[Table/Fig-6]: Gel electrophoresis for GAPDH (A), β-actin (B), aromatase (C) gene in DNA samples and (D) Restriction enzyme (HpyCH4IV) digestion of CYP19 gene mapping.
Here, M=100bp ladder, C=Control and E=Endometriosis samples.A, B and C having 157bp, 498bp, 278bp band on gel, showing genes have been amplified in all samples. D; is showing Lane L1, L2, L6, L7, L12, L14, L15=Wild type (278bp) and Lane L3, L4, L5, L8, L9, L10, L11, L13=Heterozygotes (278, 179 and 99 bp)

	Alleles N (%)		p-value	Genotype N (%)			p-value
rs 2470152	T	C		TT	TC	CC	
Control	114 (63.3)	66 (36.7)	0.002[a]	60 (33.3)	108 (60.0)	12 (6.70)	<0.001[b]
Endometriosis	54 (45.0)	66 (55.0)		20 (16.7)	68 (56.6)	32 (26.7)	

[Table/Fig-7]: Frequency distribution of CYP19 rs 2470152 in women with endometriosis and control.
Where, a-Based on the allele frequencies of endometriosis vs. control, b-based on the genotype frequencies of endometriosis vs. control. Allele and genotype distribution among groups were evaluated using the Chi-squared test

the conversion of Androgens (T) to oestrogens (E2) is catalysed by aromatase, the E2/T ratio may be a marker of aromatase activity with a positive correlation with the disease progression [23].

Increased oxidative stress parameters SOD, LPO, cortisol and prolactin in the endometriosis as compared to normal ovulating women were indicated here, and these finding are similar to previous one. And it's probable that stress may together with hormones and other inflammatory factors contributes for the development and progression of the disease [37,38].

The morphological features of endometrium lining of uterus at different stages of the active bleeding phase of menstruation were studied previously and detailed as in early menstrual phase (reproductive), endometrial glands are simple straight tubes lined by columnar epithelium with large nuclei, but later glands become curved or tortuous with subnuclear vacuolisation, while its thin, atrophic (sparse and inactive or no mitotic activity) and composed of basalis only [11,39]. The knowledge of normal histological architecture of the uterus will be helpful to isolate any pathological changes in the endometrial tissues collected from endometriosis patients in respect to it. In the present study morphology of collected tissues were analysed, compared and after evaluation it has been found to demonstrate a features of secretary endometrium, depicting that these tissue adhesions have been a part of secretary phase endometrium during endometriosis.

Previous studies on aromatase gene polymorphisms in Tianjin for SNP rs 10046 and in Iranian women (24) for SNP rs 2414096 revealed that these site polymorphisms were related to an individual's susceptibility to develop breast cancer and PCOS respectively,

the two prominent diseases related to effect of oestrogen on reproductive health of a women [33]. But CYP19 rs 2470152 polymorphism which is located in the intronic region of aromatase gene and not changes the amino acid sequences, was found to be strongly associated with serum oestrogen levels and susceptibility of PCOS [25,40]. So far, rs 2470152 polymorphism study was done in Indian population, for a hypothesis that however, variations in the introns may affect regulatory sequences in close proximity, leading to the endometriosis disease.

Limitation(s)

The study does not include a separate study for genotypes, disease staging and hormone profiling as different disease suffering women have different hormone profiles and the table shows the average of all the data sets [Table/Fig-3]. It also does not include the study of other genes of steroidogenic pathway except aromatase, a gene playing key role in oestrogen biosynthesis.

CONCLUSION(S)

Endometriosis is a multifactorial pathology where more than one factors are responsible for the disease condition. Histological examination of endometrial biopsies now may be a major diagnostic tool for evaluation and comparison of disease conditions. Further, a specific diagnostic biomarker will help the physician to plan a therapy for successful management of this disease.

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