

Histopathological and Immunohistochemical Study of Endometrial Lesions Obtained from D&C and Hysterectomy Specimens at a Tertiary Care Hospital

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ABSTRACT

Background: Endometrial lesions are represented by a set of diversified disorders which has challenged clinicians for a long time. Due to its chances of progressing to malignant states, the condition needs prompt and focussed study, keeping clinical context in view.

Methods: This 12-month (June 2016-May 2017) cross-sectional study involved 100 specimens from dilatation and curettage (D & C) and hysterectomy specimens from female patients aged ≥ 18 years who presented with complain of pelvic pain, abnormal uterine bleeding, dysmenorrhoea, pelvic mass and infertility. For all the specimens received, histopathological and immunohistochemical assessment was done.

Results: Out of 100 cases of abnormal uterine bleeding 44 females showed physiological changes, 25 females showed benign lesions and 20 females had malignant lesions of endometrium. Out of 20 cases of endometrial carcinoma 50 % were well differentiated and 25% were moderately differentiated and 25% were poorly differentiated. Expression of Ki-67 was $>35\%$ in poorly differentiated carcinoma. Well differentiated carcinoma showed 80-85% positivity of ER, moderately differentiated showed 30-35% positivity and poorly differentiated carcinoma showed 6-12%. The association between benign and malignant endometrial lesion was found to be statistically significant with age-group, history of contraceptive use and chronic illnesses ($p < 0.05$).

Conclusion: Endometrial biopsy is one of the prompt tools in diagnosis and assessment of the benign and malignant diseases of endometrium. Immunohistochemical markers like ER (hormonal receptor) and Ki-67 (proliferative marker) play a major role in diagnosis, prognostication and therapeutic management of malignant cases.

Keywords: Endometrial Lesions, Histopathological, Immunohistochemical

Introduction

Women in reproductive age group use to suffer from numerous pathologies of the reproductive tract affecting the cervix, myometrium, endometrium, ovaries, fallopian tubes etc. Endometrium reflects the pathophysiological state of hypothalamic-pituitary-ovarian complex as well as serve as bed and bread of the early embryo.^[1] Endometrial lesions are quite common among reproductive as well as females in postmenopausal age groups. Abnormal uterine bleeding (AUB) is a common gynaecological complain associated with significant morbidity and hinders patients personal, family and social life as well.^[2]

Abnormal uterine bleeding (AUB) has a pattern of bleeding that doesn't correspond with duration, amount and frequency of the flow of a normal menstrual cycle. The causes of AUB in the reproductive age groups are commonly due to hormonal imbalance, while in peri-menopausal and post-menopausal women, hyperplasia's and malignancies mainly contributes to AUB.^[3] Endometrial lesions

manifest with symptoms of menorrhagia, polymenorrhoea, postmenopausal bleeding, infertility, dysmenorrhoea and intermenstrual bleeding.^[2]

Histopathological study of endometrial samples helps in assessing abnormal uterine bleeding, which accounts for 33% of outpatient gynaecological referrals.^[4] The proportion rises to 70% in perimenopausal and postmenopausal years.^[5] Out of the 18% of perimenopausal women who had menorrhagia or metrorrhagia, one-fifth are due to premalignant or malignant disease.^[6] Endometrial hyperplasia is observed in 5-10% of patients presenting with post menopausal bleeding while 10% of patients with post menopausal bleeding have endometrial cancer.^[6] Probability of endometrial cancer must be viewed among premenopausal women if abnormal bleeding is persistent or recurrent in nature. Hysteroscopic biopsy & D&C (dilatation and curettage) are mostly considered in routine for endometrial sampling in gynaecological cases.

In the present study endometrial specimens obtained from hysterectomy as well as D&C were processed and stained with Haematoxylin and Eosin stain. Histopathological assessment of specimens were done primarily and malignant cases were further assessed by both histopathological and Immunohistochemical methods - Estrogen receptors (ER) and Ki-67. A further attempt was made to correlate clinicopathological and epidemiological profile in studied cases.

Material & Methods

Study Design: Cross-sectional study

Study Period: The study was conducted from June 2016 to May 2017.

Study Settings: The study was conducted in Department of Obstetrics and Gynaecology of tertiary care institute and samples of endometrial lesions obtained from D&C and hysterectomy specimens were sent to Department of Pathology for further assessment of histopathological and Immunohistochemical pattern.

Study unit: A maximum of 100 females aged 18 years and above who presented with complain of pelvic pain, abnormal uterine bleeding, dysmenorrhoea, pelvic mass and infertility during the time frame of study were included in the study. Girls and married woman of age group less than 18 years, cases with clotting disorders or coagulopathy and cases of congenital anomalies of uterus were excluded from the study

Data collection: Before the data collection begins, written informed consent was taken from patients for participation in the study. Then after collecting detailed relevant clinical history and examination, other relevant investigations were done and complete set of data were made for each patient in pre-formed formats. In our study the proper history including age, medical history, history of taking oral contraceptives, intrauterine device, menopausal status, history of any RTI/STI, history of abortion, past history, duration of bleeding, nature of bleeding and history of parity were taken to correlate with the pathophysiological findings.

Histopathological and Immunohistochemical study of specimens For study of all the cases, D&C and hysterectomy specimens were received in 10% formalin. All samples obtained were processed using the conventional method of paraffin embedding and hematoxylin and eosin staining. Immunohistochemistry was done for further evaluation of estrogen receptor (ER) and Ki67. Estrogen receptor was determined by calculating the positivity index (PI) which represented the percentage of positive cells per 1000 cells counted on 40x power field. Cases with nuclear marker

presentation in at least 5% of cells were considered to be positive. Only the epithelial nuclear receptor expression was assessed, and not the stromal one. Ki67 immunoreactivity was quantified with the proliferation index, the percentage reporting the number of marked cells by the total number of cells counted in 40X power field. Also, a negative external control was used by omitting the primary antibody and positive external control, represented by sections of breast carcinoma.

Statistical Analysis: Collected data were entered directly into the Excel sheets primarily and then transferred after data cleaning and rechecking to Epi-Info software for analyzing according to aims and objectives. The descriptive results were presented in forms of frequency and means while the association were expressed using Chi-square test. P value < 0.05 was considered to be significant

Ethical clearance: Ethical clearance was obtained from institutional ethics committee before commencement of study.

Results

Maximum number of cases were seen in the age group of 41-50 years (39%) followed by 31-40 years (27%). Most of our study population belongs to rural area (64%). In the present study most of the patients belong to the premenopausal group (53%). Endometrial lesions were most common in premenopausal women in our study as compared to menopausal and postmenopausal women. Endometrial lesions were more common in population taking oral contraceptives. History of intrauterine device were present in 30% cases. Significant medical history (diabetes hypertension, PCOD and any chronic disorder) was present in 53 cases out of 100 cases. Out of 100 cases 56 cases presented with painless bleeding. Also 35 cases had bleeding for a duration of <15 days, while 31 had bleeding duration ranging from 15-30 days and 34 patients had bleeding >30 days. Most of the study population were presented with irregular bleeding. Postmenopausal bleeding was present mostly in endometrial carcinoma. Out of 100 cases hysterectomy specimens are more than D&C specimens. [Table no.1]

In the present study out of 100 cases of abnormal uterine bleeding 44 females showed physiological changes, 25 females showed benign lesions and 20 females had malignant lesions of endometrium. Also 11 females presented with pregnancy related complications [Table no.2]

Out of 20 cases of adenocarcinoma 50% were well differentiated and 25% were moderately differentiated and 25% were poorly differentiated. Expression of Ki-67 was

>35% in poorly differentiated carcinoma. Well differentiated carcinoma showed 80-85% positivity of ER, moderately differentiated showed 30-35% positivity and poorly differentiated carcinoma showed 6-12%. [Table no.3]

Most of the patients presents with the complaint of irregular bleeding (66%), out of which 25 cases were of normal physiological cycle, 11 cases were of pregnancy related complications, 17 cases were of benign and 13 cases were of malignant cases. Out of 44 cases of normal physiological cycle 18 cases were of age group 41-50yrs and followed by 14 cases of 31-40yrs. Benign cases were most common in the young age group and malignant cases were most common in the elderly patients. Patients that presented with irregular bleeding were most common in the age group of 41-50 yrs. Patients that presented with the

complaint of infertility were most common in young age group and postmenopausal bleeding were mainly present in elderly. [Table no.4]

Malignant cases were found to be more common in elderly patients (41-50yrs and >50 yrs) whereas benign cases were common in younger age group. It was found to be statistically significant ($p < 0.001$). χ^2 Malignancy was seen in only 4 cases of patients having history of IUCD and was statistically significant ($p < 0.001$). Malignancy was seen in only 6 cases of patients having history of oral contraceptives and was statistically significant ($p < 0.001$). Malignant cases were seen in 13 patients had medical history (diabetes, hypertension, obesity, PCOD) however it was statistically insignificant. [Table no.5]

Table 1: Distribution of study population on the basis of clinico-epidemiological profile (N=100)

Clinico-epidemiological Characteristics	Number (n=100)	Percentage(%)
Age-groups		
20-30	22	22
31-40	27	27
41-50	39	39
>50	12	12
Residence		
Rural	64	64
Urban	36	36
Menstrual status		
Premenopause	53	53
Menopause	35	35
Postmenopause	12	12
History of contraceptive		
Present	69	69
Absent	31	31
History of Intrauterine device		
Present	30	30
Absent	70	70
Presence of any relevant Medical history		
Present	53	53
Absent	47	47
Presence of history of RTI/STI		
Present	30	30
Absent	70	70
Specimen		
Hystrectomy	55	55
Dilatation & Curettage	45	45

Table 4: Relationship between age-group and chief complaints and histo-pathological pattern (N=100).

Clinico-epidemiological profile	Normal physiological cycle	Benign	Malignant	Pregnancy related complication
Age-group				
20-30 (n=22)	7 (15.90%)	6 (24%)	0 (0%)	9 (81.81%)
31-40 (n=27)	14 (31.81%)	11(45.83%)	0 (0%)	2 (18.18%)
41-50 (n=39)	18 (40.90%)	8 (32%)	13 (65%)	0 (0%)
>50 (n=12)	5 (11.36%)	0 (0%)	7 (35%)	0 (0%)
Chief complaint				
Infertility(n=11)	3 (27.27%)	8 (72.72%)	0 (0%)	0 (0%)
Irregular bleeding (n =66)	25 (37.87%)	17(25.75%)	13(19.69%)	11 (16.66%)
Postmenopausal bleeding(n=8)	1 (12.5%)	0 (0%)	7 (87.5%)	0 (0%)
UV prolapse (n=-15)	15 (100%)	0 (0%)	0 (0%)	0 (0%)

Table 5: Association of age group, contraceptive and medical history with benign and malignant lesions (N=45)

Variables	Endometrial lesions				Total
	Malignant		Benign		
	No.	%	No.	%	
Age Groups					
20-30	0	0%	6	100%	6
31-40	0	0%	11	100%	11
41-50	13	61.9%	8	38.1%	21
>50	7	100%	0	0%	7
χ^2 value- 24.93, df -3 ,p<0.001					
IUCD History					
Present	4	23.5%	13	76.5	17
Absent	16	57.1%	12	42.9%	28
χ^2 value- 4.840, df-1,p <0.001					
Oral Contraceptive History					
Present	6	26.1%	17	73.9%	23
Absent	14	63.6%	8	36.4%	22
χ^2 value- 6.42, df=1, p <0.05					
Medical history (excluding normal physiological cycle and pregnancy related complications)					
Present	13	48.1%	14	51.9%	27
Absent	7	31.9%	11	68.1%	18
$\chi^2 =0.375, df=1, pvalue =0.54$					

Discussion

In our study of endometrial lesions were most common in age groups were of 41-50 years. Similar results were reported by Dhadhanian et al.^[7] and Kaur et al.^[3] Dhadhanian et al. had studied the cases of DUB (dysfunctional uterine bleeding) and the commonest pattern in these patients were proliferative endometrium.^[7] Similarly in our study around 44% patients were of normal physiological cycle, out of which 19% were of proliferative. Commonest pattern of

benign endometrial pathology was simple hyperplasia in the study done by Dhadhanian et al;^[7] however in our study only 5% patients were diagnosed as simple hyperplasia. Sunila et al. studied endometrial histopathology in cases of abnormal uterine bleeding reported the most common to be physiological (23% of cases); however, in our study 44% of AUB were physiological in nature. Also the most of cases of AUB in the age group of 50-55 were malignant cases.^[8]

In a study by [Vaidya S et al.](#) on histopathology, normal cyclical endometrium was seen in 40.94% cases, followed by 13.40% cases of disordered proliferative endometrium and 10.92% cases of hyperplasia;^[9] however in our study normal cyclic endometrium, disordered proliferative and hyperplasia 44% , 1% and 11% respectively. [Sajita et al.](#) in their study found endometrial hyperplasia among 25% patients followed by secretory endometrium in 16.7% patients, proliferative phase pattern in 12.2% and disordered proliferative endometrium was seen in 12.2% patients and malignancy in 6.4% of cases with endometrial carcinoma to be the most common (4.5%) lesion. ^[21] However in our study endometrial hyperplasia was seen in 11% of patients and secretory was seen in 14% and proliferative was seen in 19%. Disordered proliferative endometrium was seen in only one case in our study while endometrial carcinoma was seen in 20% cases (20 out of 100).

Similar study on abnormal uterine bleeding by [Saraswathi et al.](#) showed that most common pattern was normal cyclic phase of endometrium on histopathological examination (28.4%) and the most common age group presenting with abnormal uterine bleeding was 41-50 years; ^[10] a finding coherent with our study result. Also in AUB cases, among pregnancy related complications about 20.4% of the cases were of partial mole (2 out of 11), however [Saraswathi et al.](#) in their study reported only about 3% (3 out of 93) cases of partial moles among complications of pregnancy. ^[10] Similar to the findings reported by [Rajshri et al.](#) incidence of hyperplasia was most common among perimenopausal women; ^[11] but in contradiction to that [Susan et al.](#) found the incidence of hyperplasia highest during early postmenopausal years and in the early 60s, respectively. ^[12]

In present study among 11%(11 out of 100) of the patients presenting with infertility, tuberculosis was found among 5 cases and while 4 cases were diagnosed as cases of chronic endometritis and 2 cases were of normal cyclic endometrium. In contradiction to that study done by [Sharma et al.](#) found 40% cases endometrial tuberculosis (2 out of 50) among patients who presented with infertility. ^[13]

[S. Salim et al.](#) reported endometrial polyps to account for 39% of all abnormal vaginal bleeding in premenopausal woman; ^[14] however, in our study 4% of cases diagnosed as polyp presented with irregular bleeding and menorrhagia and patients were mostly of perimenopausal groups.

Similar to the findings of other studies the most common histological pattern was proliferative endometrium. ^[11,15,16,17 & 18]

The risk of development of endometrial carcinoma decreases by about 50% among oral contraceptive users.

^[19] In line with that similar inference could be drawn from present study as most of the cases of malignant endometrial lesions were not having history of oral contraceptives (Out of 20 cases of endometrial carcinoma 14 patients never had oral contraceptives).

[Aune et al.](#) concluded that hypertension may increase the risk of endometrial carcinoma by 61%. ^[20] Similarly in our study out of 20 cases 13 had medical history of chronic disorders like hypertension, however association was found to be statistically insignificant. **D. Cibula**

[Kounelis et al.](#) studied 61 cases of hysterectomy specimens in which 65.5% were of endometrioid carcinoma and 34.4% were of serous papillary. ^[21] In present study out of 55 case of hysterectomy specimens, 20 cases were of endometrial carcinoma. Out of carcinoma cases eighteen (90%) were of endometrioid type, one (5%) was serous papillary and one (5%) was of clear cell type.

[Modi et al.](#) in their study reported most common endometrial carcinoma were well differentiated adenocarcinoma (41%), followed by moderately differentiated adenocarcinoma (27%) and then poorly differentiated adenocarcinoma (10%). ^[22] Similar patterns were also observed in our study also.

In our study 100% of the cases of endometrial carcinoma showed positive expression of ER. However studies by [Suthipintawong et al;](#) ^[23] [Suamchock et al;](#) ^[24] [Markova et al;](#) ^[25] [Sidonia et al;](#) ^[26] [Yu CG et al;](#) ^[27] [Pasam et al;](#) ^[28] [Musfera et al.](#) ^[29] ER expression was present in 76%, 59.3%, 79.3%, 86.3%, 44.7%, 68% and 60.7% of the cases respectively.

[Stoian](#) has studied the clinical, histological and Immunohistochemical factors involved in endometrial carcinogenesis and found Ki67 expression was positive in 100% of cases. ^[26] However, in present study only 25% cases of carcinoma (5 out of 20) showed >35% expression of Ki67. Expression of Ki67 was positive in 64.1% and 41.5% cases of endometrial carcinoma in the study done by [Cui-Ge Yu et al.](#) ^[27] and [Suthipintawong C et al.](#) ^[23]

However the small sample size was one of the major limitation of the present study. Since the study was conducted at a private tertiary care institution and a convenient sample was taken through complete enumeration process (during the time frame of study), the findings are subjected to selection bias. Therefore the results might not be generalised to the whole population.

Conclusions

Most common histopathological pattern was of normal physiological cycle (44%) and about 5% cases

diagnosed as tubercular endometritis and 4% cases were of chronic endometritis. About 4% cases were of adenomyomatous polyp. Out of 20 malignant cases in the present study, the positive expression of Ki-67 was >35% in poorly differentiated carcinoma, 11-35% in moderately differentiated carcinoma and <10% in well differentiated carcinoma indicating that Ki-67 expression goes up successively from well differentiated to poorly differentiated carcinoma. The positive expression of hormonal receptor ER was 80-85% in well differentiated, 30-35% in moderately and 6-12% in poorly differentiated endometrial carcinoma indicating ER expression decreases as the tumor grade increases. Endometrial biopsy is a prompt diagnosing tool assessing the benign and malignant diseases of endometrium and in this era of IHC, the markers ER (hormonal receptor) and ki-67 (proliferative marker) play a major role in diagnosis, prognostication and therapeutic management of malignant cases.

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