Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2017; 5(8A):2958-2966 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2017.v05i08.003

Original Research Article

Role of Morphometry and Proliferative Activity Markers in Assessment of Endometrial Glandular Lesions

Dr. Sucharita Sarkar[1], Dr. Mou Das[2], Dr. Manisha Sarkar[3], Dr. Asit Chakraborty[4], Dr. Chandradeep Mallik[5], Dr.Asim kumar Manna[6]

[1]Department Of Pathology, I.P.G.M.E&R, Kolkata, West Bengal, India.
[2,3]Assistant Professor, Department Of Pathology, I.P.G.M.E&R, Kolkata, West Bengal, India.
[4,5]Junior Resident, Department Of Pathology, I.P.G.M.E&R, Kolkata, West Bengal, India.
[6]Professor, Department Of Pathology, I.P.G.M.E&R, Kolkata, West Bengal, India.

***Corresponding author**
Dr. Mou Das
Email: drmoubanerjee75@gmail.com

Abstract: Both benign and malignant lesions of uterine glandular epithelium can have similar presenting feature of abnormal vaginal bleeding. At times, it is difficult to diagnose the nature of the lesion from haematoxylin and eosin stained sections. In such cases morphometry analysis and proliferative studies is expected to differentiate these glandular lesions of endometrium, especially when in dilemma in categorising a lesion benign, premalignant or malignant. This study aims to classify different glandular lesions of endometrium, their prevalence in perimenopausal and postmenopausal women, cellular morphometry in different types of lesions in connection with the clinical profile of the patient, analysis of the expression of proliferative markers like PCNA and Ki67 in these lesions that may have prognostic significance. Morphometric study using nuclear parameters like mean nuclear diameter (MND), mean nuclear area (MNA), mean nuclear perimeter (MNP) and N:C ratio was used to differentiate various benign, borderline endometrial glandular lesions from malignant endometrial glandular lesions in both perimenopausal and postmenopausal age group. In this study we found that morphometric parameters showed significant difference ($p<0.05$) between benign endometrial glandular lesions and malignant endometrial glandular lesions. In cases where proliferative index was high, age-old Morphometry technique emerged as a more useful tool in differentiating endometrial lesions into benign and malignant.
Keywords: Endometrial glandular lesions, morphometry, proliferative markers.

INTRODUCTION

Endometrial carcinoma is one of the common malignancies in developed countries with incidence of 12.9/100,000 women and mortality rate of 2.4/100,000 whereas in developing countries, it is second most common gynecological malignancy after cervical cancer with incidence of 5.9/100,000 and mortality rate of 1.7/100,000 [1]. The disease most commonly occurs in postmenopausal women who have had a long menstrual life and few pregnancies. Three-quarters of women presenting are over 50 years old, with few are under 40 years and a peak incidence of 61years.Nulliparas,accounting for 24-31% of patients with endometrial cancer, run twice the risk of developing the disease as women with one child and three times the risk of women with five or more children **[**2**].** Two distinct pathogenic types of Endometrial cancer exists-first and common variety seen in younger, perimenopausal women, is oestrogen dependent, starts in background of endometrial hyperplasia and has a favorable prognosis. Second type seen in older, postmenopausal, thin built women with no source of estrogenic stimulation, associated with endometrial atrophy and has a poor prognosis. Unopposed endogenous oestrogen, obesity, hypertension, diabetes mellitus have been found to increase the risk. Endometrioid endometrial carcinoma accounts for three fourth of endometrial cancers and is considered to develop following a continuum of premalignant lesions ranging from endometrial

hyperplasia without atypia, to hyperplasia with atypia and finally to well differentiated carcinoma.

The Endometrial glandular lesions includes - (a)Hormone induced changes(exogenous and endogenous), (b)Endometritis-both acute and chronic types, (c)Endometrial atrophy, (d)Endometrial metaplasia, (e)Endometrial polyps, (f)Endometrial hyperplasia(types are-simple and complex hyperplasia, with or without atypia), (h)Endometrial carcinoma of uterus.

Endometrial hyperplasia is defined as a proliferation of glands of irregular size and shape with an increase in the gland/stroma ratio compared to proliferative endometrium [3]. The entity of disordered proliferation of endomerium, differs from hyperplasia without cytologic atypia by virtue of its relatively normal ratio of glands to stroma (1:1); that is, the significant shift in the glands-to stroma ratio (3:1) in favor of glands that is required for a diagnosis of hyperplasia is absent. Thus, disordered proliferation of endometrium serves as a morphologic bridge between normal proliferation and hyperplasia [4]. Detection of atypical hyperplasia in an endometrial biopsy specimen carries a high risk of undetected occult or subsequent carcinoma **[**5,6**].** In contrast, both simple hyperplasia and complex hyperplasia have low progression risks, are more common than atypical hyperplasia and have potential for over diagnosis and overtreatment [5].

Now a day, measurement of proliferative index is an extremely useful adjunct to histomorphology. Various aspects of proliferation within tissues can now be assessed by a multitude of modalities in tissue sections and cell suspensions. These methods include the determination of mitotic indices, proliferating cell nuclear antigen (PCNA) and Ki-67-positive cell populations by immunohistochemistry. This kind of assessment provides information about the number and type of cells in different parts of the cell cycle. Antibody directed against the DNA-binding nuclear protein, Ki-67 antigen, identify cells in most of G1 and all of S, G2, and M phases of the cell cycle, but not in the G0, or quiescent phase. Ki67 antibodies recognize a nuclear antigen of the cell proliferation, which has been shown in many studies to reflect the tumors aggressiveness [7]. However, in endometrial adenocarcinoma immunohistochemical staining with Ki-67 correlates with FIGO stage, grade, cell type, histologic subtype, and probability of survival [8]. Ki-67 thus, maybe considered a prognostic indicator of potential utility.

Proliferating cell nuclear antigen (PCNA) is a nuclear protein that is expressed in the late G1 phase, peaks in the S phase, and persists in the G2M phases of the cycle. A high PCNA index has been predictive of decreased survival by univariate analysis in some studies although, not by multivariate analysis **[**9**,** 10]. Quantification of the PCNA protein with immunohistochemistry techniques is thought to provide a measure of cell proliferation. A good correlation is seen between PCNA expression and other measures of cell proliferation, such as thymidine labeling index and the S-phase fraction. PCNA is also involved in DNA repair and there is evidence that PCNA immunostaining may occur in situation in which DNA repair rather than proliferation occurs.

The purpose of this current study is to see the frequency of glandular lesions of endometrium with focus on the avoidance of common diagnostic pitfalls from the correlation between histomorphological, morphometrically and immunohistochemically marker studies. The objective is to assess the role of proliferative activity in diagnosis of different endometrial glandular lesions in the premenopausal and postmenopausal age group.

MATERIALS AND METHODS

The Prospective & observational study was conducted in the Department of Pathology, with collected biopsy specimen of endometrium from the Department of Gynecology & Obstetrics. The patients included in the study group were:

- Those with complaints of irregular vaginal bleeding and discharge in premenopausal and postmenopausal age group.
- Ultrasonography showing increased endometrial thickness in premenopausal and postmenopausal women.

  100 such cases were included in these two years of study. These patients in premenopausal and postmenopausal age group attended the outpatient and indoor Department of Gynecology, with the complaints of abnormal vaginal bleeding.

Hematoxylin and eosin staining along with morphometry analysis and proliferative studies on

stained histopathology sections were done. Morphometric analysis of epithelial cells found in histology in terms of nuclear diameter, cytoplasmic diameter, mean nuclear area (MNA), nucleo cytoplasmic ratio (N:C), nuclear perimeter. Proliferative index of PCNA and Ki67 done in each of the cases. The ocular morphometer was calibrated using a calibration slide provided with it. One smallest division on the stage micrometer is equated with 0.01 mm. Ocular micrometer is equated with 2.5µm. 100 random nuclei from the most atypical area of the sections were subjected to analysis. Morphometric measurements using variables like mean nuclear diameter (MND), mean cytoplasmic diameter, mean nuclear area (MNA), N/C ratio, and nuclear perimeter were calculated in different sections.

The nuclear area (A) was calculated by the formula: A = π x a x b, where a and b are the semilargest and semi-smallest nuclear radius. Similar formula was applied for cell area. The nucleocytoplasmic ratio (N: C) was obtained from dividing nuclear area by cell area [11].

Nuclear perimeter (P), calculated from mathematical formula: P = 2 $\pi\sqrt{a2+b2/2}$ [11]. For PCNA and Ki-67, all identifiable nuclear staining in cells was recorded as positive regardless of intensity. At least 1000 cells were observed under a microscope (objective lens x 40) and counted vertically from one corner to another. The percentage of stained cells was evaluated 3 times independently by 2 examiners. The result of PCNA and Ki-67 immunostaining index were interpreted as labelling index = Number of nuclei showing positive staining (brown color)/Total number of nuclei (1000) x100%.

RESULT & ANALYSIS

In this study, morphometric parameters showed no significant difference ($p>0.05$) between benign endometrial glandular lesions (exogenous hormone and persistent exposure to endogenous estrogen induced hormonal changes of endometrium, chronic endometritis, atrophic endometrium, endometrial polyp, secretory endometrium with focal atypia) and borderline endometrial glandular lesions (simple hyperplasia without atypia, complex hyperplasia with atypia). Whereas, morphometric parameters showed significant difference ($p<0.05$) between benign endometrial glandular lesions and malignant endometrial glandular lesions (endometrioid adenocarcinoma of endometrium, serous papillary carcinoma of endometrium). Also morphometric parameters showed significant difference ($p<0.05$) between borderline endometrial glandular lesions and malignant endometrial glandular lesions.

In this study, mean nuclear diameter >7.339µm indicating probability of malignancy with sensitivity 85.71(95% confidence level 57.2-98.20%) and specificity 89.47(95% confidence level 75.20-97.10%). Mean nuclear area >42.329µm2 indicating probability of malignancy with sensitivity 85.71(95% confidence level 57.20-98.20%) and specificity 89.47(95% confidence level 75.20-97.10%). Mean nuclear perimeter >23.065µm indicating probability of malignancy with sensitivity 85.71(95% confidence level 57.20-98.20%) and specificity 89.47(95% confidence level 75.20-97.10%). Mean N/C ratio >0.552 indicating probability of malignancy with sensitivity 100 (95% confidence level 76.80-100%) and specificity 100 (95% confidence level 90.70-100). Correlation coefficient r = 0.668 showing good correlation between Mean nuclear diameter and N/C ratio.

In this study, Proliferative index (Ki67 and PCNA) showed significant difference ($p<0.05$) between benign, borderline and malignant endometrial glandular lesions from each other. Mean Ki67 index was highest in proliferative endometrium (28%) followed by serous papillary carcinoma of endometrium (23%) and endometrioid adenocarcinoma of uterus (20.17%) followed by simple hyperplasia without atypia of endometrium (11.44%) and complex hyperplasia with atypia of endometrium(10.15%). Mean Ki67 index was lowest in atrophic endometrium (2%). Mean PCNA index was highest in proliferative endometrium (25.50%) followed by serous papillary carcinoma of endometrium (20.40%) and endometrioid adenocarcinoma of uterus (18.17%) followed by complex hyperplasia with atypia of endometrium (12.10%) and simple hyperplasia without atypia of endometrium (10.16%). Mean PCNA index was lowest in atrophic endometrium (2%). Correlation coefficient r = 0.789 showing strong correlation between Mean nuclear diameter and PCNA index. Correlation coefficient r = 0.832 showing strong correlation between N/C ratio and Mean PCNA index. Correlation coefficient r = 0.712 showing strong correlation between Mean nuclear diameter and Ki 67 index. Correlation coefficient r = 0.802 showing strong correlation between N/C ratio and Ki 67 index.

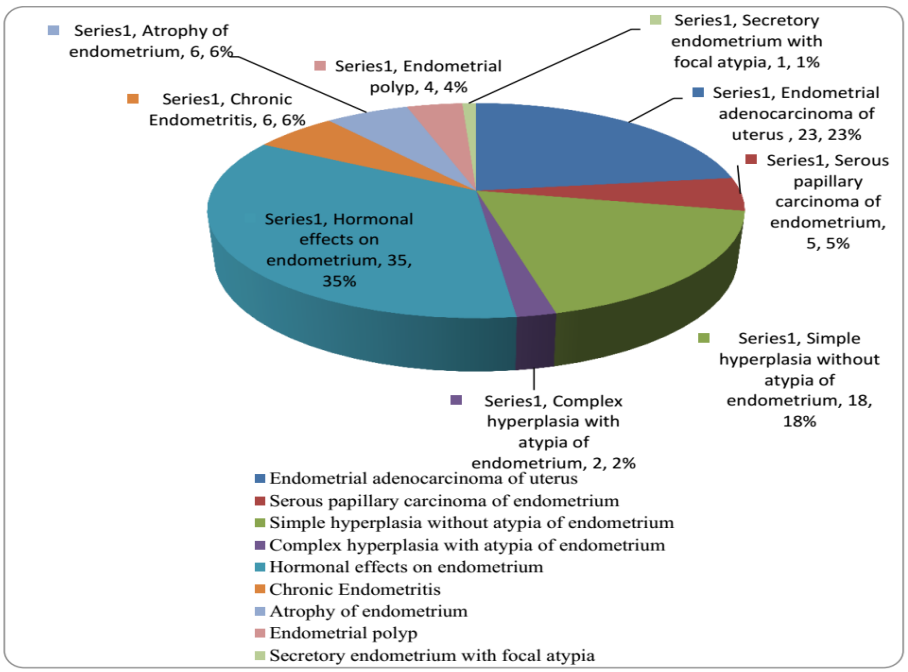
Correlation coefficient r =0.930 showing strong correlation between Ki67 index and PCNA.

Table−1: Result of morphometric study (on H& E stained sections)

Histopathological Diagnosis	**MMND** (μm)	**MMCD** (μm)	**MMNA** ($\mu^2 m$)	**M(N:C)**	**MNP** (μm)
Endometrioid adenocarcinoma of uterus	8.556	10.295	65.539	0.761	27.396
Serous papillary carcinoma of endometrium	10.059	12.063	84.408	0.695	32.036
Simple hyperplasia without atypia of endometrium	6.209	11.290	30.282	0.321	20.837
Complex hyperplasia with atypia of endometrium	6.803	12.671	37.134	0.298	21.484
Hormonal effect on endometrium	5.850	11.808	28.245	0.253	18.406
Chronic endometritis	5.858	15.115	27.398	0.184	18.375
Atrophy of endometrium	5.493	10.388	24.015	0.281	17.374
Endometrial polyp	6.094	12.223	34.649	0.208	19.944
SecretorySSS endometrium with focal atypia	5.601	13.492	24.652	0.172	17.601

Table−2: Distribution of cases according to mean ki-67 index & mean PCNA index among premenopausal & postmenopausal age group

Histopathological Diagnosis	**Immunohistochemistry**	
	Mean Ki67 index (%)	**Mean PCNA index (%)**
Proliferative Endometrium	28.00	25.50
Secretory Endometrium	4.10	5.15
Endometrioid adenocarcinoma of uterus	20.17	18.17
Serous papillary carcinoma of endometrium	23.00	20.40
Simple hyperplasia without atypia of endometrium	11.44	10.16
Complex hyperplasia with atypia of endometrium	10.15	12.10
Hormonal effect on endometrium	6.50	7.20
Chronic Endometritis	6.08	6.42
Secretory endometrium with focal atypia	5.10	5.70
Endometrial Polyp	5.58	6.08
Atrophy of endometrium	2.00	2.00



Fig–1: Distribution of Endometrial lesions

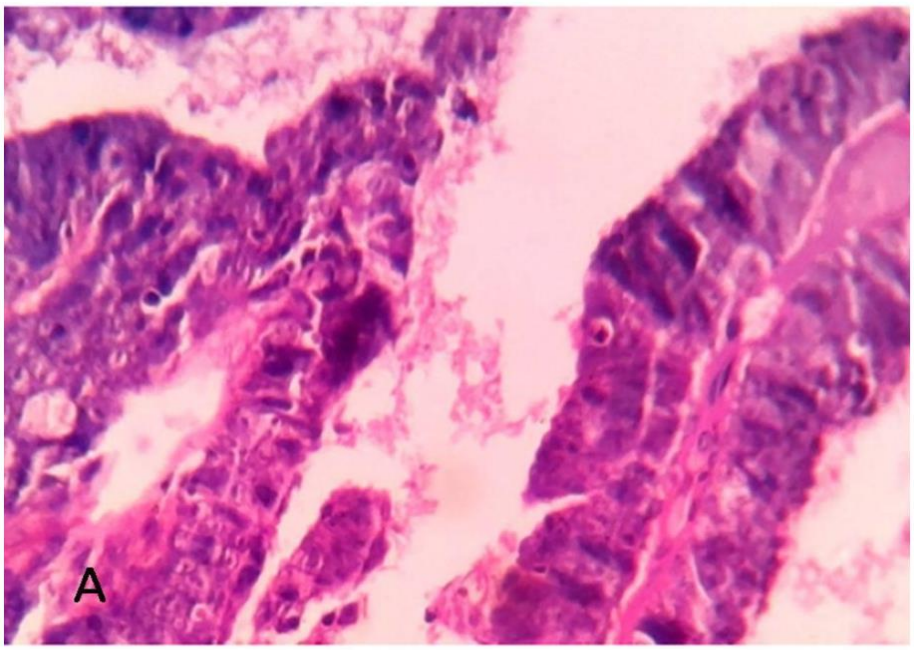


Fig-A: Serous papillary carcinoma of endometrium atypia & high mitotic rate along with complex papillary architecture (H/E, 400X) showing positive nuclear staining

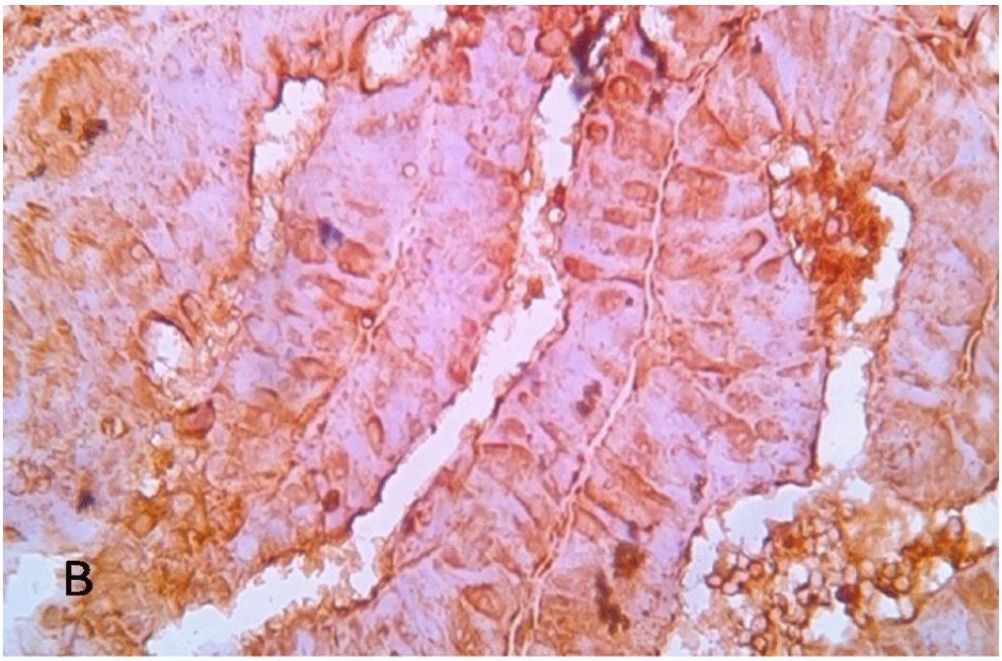


Fig-B: Serous papillary carcinoma of endometrium (IHC for Ki-67 x400) showing positive nuclear staining

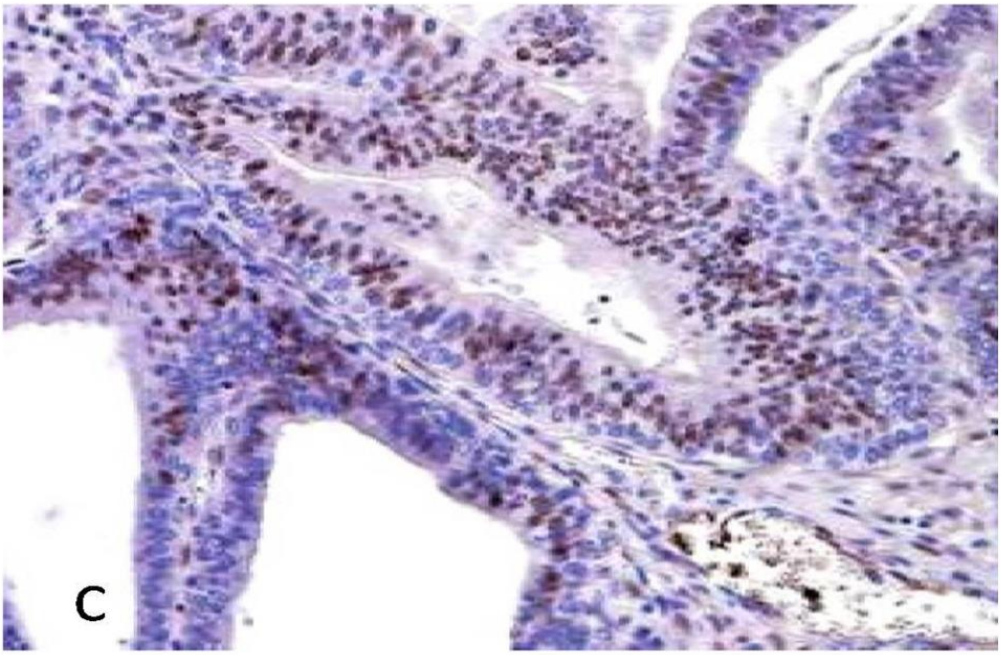


Fig-C: Serous papillary carcinoma of endometrium (IHC for PCNA x400) showing positive nuclear staining

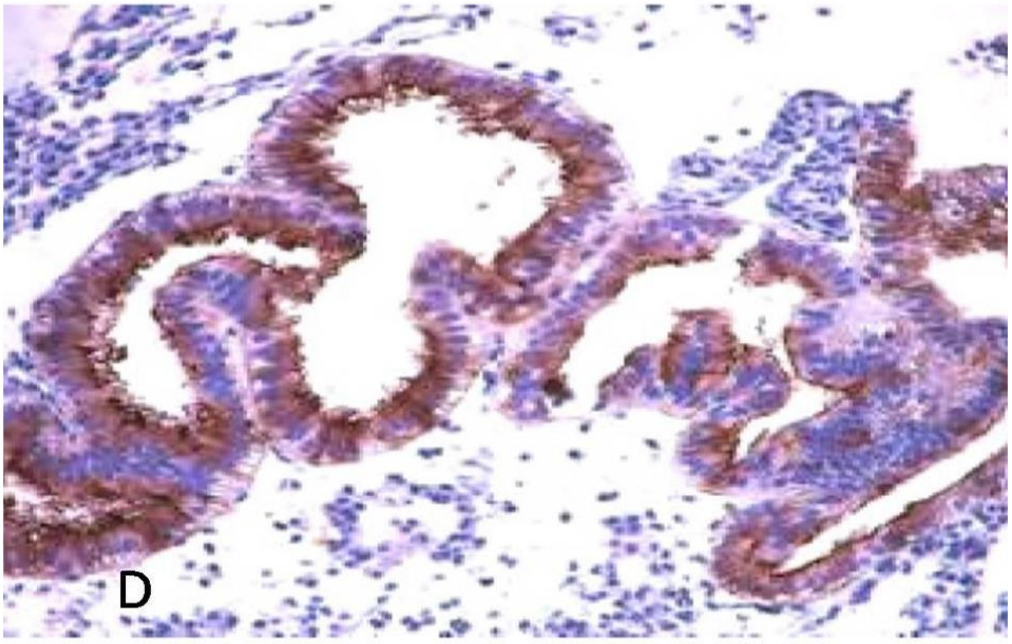


Fig-D: Complex hyperplasia with atypia (IHC for PCNA X400) showing positive nuclear staining

DISCUSSION

The study was done to assess the role of morphometry in this modern era of immunohistochemistry. In routine day to day practice, it is very common to encounter difficulties in diagnosing glandular lesions of endometrium lying in the borderline between benign and malignant. Among 100 cases, highest number of cases (35%) belonged to hormone induced changes (both exogenous and endogenous) of endometrium. Cases of endometroid adenocarcinoma and serous papillary carcinoma of endometrium were 23% and 5% respectively. Among the total 28 cases of endometrial adenocarcinoma of uterus, endometrioid adenocarcinoma were 23 cases and constituted the major percentage of cases (82.14%) followed by serous papillary carcinoma(17.85% i.e.5/28). 2 cases of endometrioid adenocarcinoma with squamous differentiation noted out of 28 cases of endometrial adenocarcinoma of uterus (7.14%) [Figure1]. In this study maximum cases (57.14%) of endometrial adenocarcinoma of uterus were found between 50 to 60 years of age with a mean age of 58.14 yrs. In this study, mean age of endometrial hyperplasia was 46.25 yrs; mean age of simple hyperplasia without atypia was 45.83 yrs whereas mean age of complex hyperplasia with atypia was 50 yrs. Out of 20 cases of endometrial hyperplasia, 15 cases of simple endometrial hyperplasia without atypia were found between 40 to 49 yrs. Mean of Mean Nuclear Diameter (MMND) were 8.56, 10.06, 6.21, 6.80, 5.85, 5.86, 5.49, 6.09, 5.60 micron ;Mean of Mean Nuclear Area (MMNA) were 65.54, 84.41, 30.28, 37.13, 28.25, 27.40, 24.02, 34.65 and 24.65 square micron; Mean of Mean Nuclear Perimeter (MMNP) were 27.39, 32.04, 20.84, 21.48, 18.41, 18.38, 17.37, 19.94, 17.60 micron & N/C ratio were 0.76,0.70, 0.32, 0.30, 0.25, 0.18, 0.28, 0.21, 0.17 in endometrioid adenocarcinoma of endometrium, serous papillary carcinoma of endometrium, simple hyperplasia without atypia, complex hyperplasia with atypia, hormonal induced effect on endometrium(both exogenous and endogenous), chronic endometritis, atrophic endometrium, endometrial polyp, secretory endometrium with focal atypia respectively.[Table 1]Although morphometric parameters showed no significant difference ($p>0.05$) between benign & borderline endometrial glandular lesions but shows significant difference ($p<0.05$) between benign and malignant endometrial glandular lesions.

The most useful morphometric parameter in classifying different glandular lesions of endometrium, especially when in dilemma in concluding a lesion benign, premalignant or malignant, was nucleus: cytoplasmic ratio (N/C). George L.mutter, Jan P.A.

Baak *et al.* [12] showed that morphometric analysis reproducibly and precisely identifies monoclonal endometrial precancers from histological section. In this study, Mean Ki67 index (%) were 28.00, 4.10, 20.17, 23.00, 11.44, 10.15, 6.50, 6.08, 5.10, 5.58, 2.00 & Mean PCNA index(%) were 25.50, 5.15, 18.17, 20.40, 10.16, 12.10, 7.2,6.42, 5.70, 6.08, 2.00 in proliferative endometrium, secretory endometrium, endometrioid adenocarcinoma of uterus, serous papillary carcinoma of endometrium, simple hyperplasia without atypia of endometrium, complex hyperplasia with atypia of endometrium, hormone induced changes (both exogenous and endogenous) of endometrium, chronic endometritis, secretory endometrium with focal atypia, endometrial polyp, atrophy of endometrium respectively. [Table 2] Proliferative index (Ki67 index and PCNA index) showed significant difference ($p<0.05$) between benign, borderline and malignant endometrial glandular lesions Risberg, Bjorn *et al.* conducted a study on expression of Ki-67 in various endometrial glandular lesions [13]. Where mean Ki67 index was highest in proliferative endometrium and Ki-67 score was significantly higher in endometrial carcinomas than in hyperplasia. Another study by F Abike *et al*. showed that immunoreactivity of PCNA was found to be significantly higher in atypical complex hyperplasia as compared to all other groups ($p < 0.05$) i.e. simple hyperplasias, simple hyperplasia with atypical changes, complex hyperplasias, proliferative endometrium and secretory endometrium [14]. Thus we can see morphometrical analysis of adenomatous hyperplasia can predict the majority of those patients, who will develop frank carcinoma in their later course. Morphometric analysis has revealed the diagnostic significance of several quantitative microscopical features. Routine application in diagnostic gynaecology of this selective type of morphometry over a period has regularly corrected the original subjective histopathological grade.

CONCLUSION

Histomorphology is the keystone in the evaluation of the different endometrial glandular lesions, but morphometric evaluation and immunohistochemistry can also be helpful in establishing the correct diagnosis. Hematoxylin and eosin study cannot differentiate some of the 'gray zone lesions', here morph metric analysis is helpful in differentiating these lesions. Morphometric measurements using variables like mean nuclear diameter, mean cytoplasmic diameter, mean nuclear area, N/C ratio, and nuclear perimeter help in classifying different glandular lesions of endometrium, especially in doubt about concluding a lesion benign, premalignant or malignant. Morphometric study along with proliferative study may help to distinguish between very close histological lesions like-complex atypical hyperplasia and well differentiated adenocarcinoma. Now a day, measurement of proliferative index is an extremely useful adjunct to Morphometric analysis has revealed the diagnostic significance of several quantitative microscopically features. Routine application in diagnostic gynecology of this selective type of morphometry over a period has regularly corrected the original subjective histopathological grade. Morphometrically analysis of adenomatous hyperplasia can predict the majority of those patients, who will develop frank carcinoma in their later course. Few limitations of this study were that survival analysis could not be done as follow up of the cases was not possible due to the limited time span and also progression of different types of endometrial hyperplasia to carcinoma could not been assessed due to lack of follow-up.

Abbreviations

MMND - mean of mean nuclear diameter
MMCD – mean cytoplasmic diameter
MMNA - mean of mean nuclear area
MND –mean nuclear diameter
MNP – mean nuclear perimeter

REFERENCES

1. Jemal A, Bray F, Center MM, Ferlay J, Ward E, Forman D. Global cancer statistics. CA: a cancer journal for clinicians. 2011 Mar 1; 61 (2):69-90.
2. Dickens C, Whitfield R. Nicholas Nickelby. Blackstone Audiobooks; 1999.
3. Kurman RJ, Kaminski PF, Norris HJ. The behavior of endometrial hyperplasia. A long-term study of "untreated" hyperplasia in 170 patients. Cancer. 1985 Jul 15; 56 (2):403-12.
4. Trimble CL, Kauderer J, Zaino R, Silverberg S, Lim PC, Burke JJ, Alberts D, Curtin J. Concurrent endometrial carcinoma in women with a biopsy diagnosis of atypical endometrial hyperplasia. Cancer. 2006 Feb 15; 106(4):812-9.
5. Kurman RJ, Mazur MT. Benign diseases of the endometrium. InBlaustein's pathology of the female genital tract 1994 (pp. 367-409). Springer New York.

6. Lacey Jr JV, Ioffe OB, Ronnett BM, Rush BB, Richesson DA, Chatterjee N, Langholz B, Glass AG, Sherman ME. Endometrial carcinoma risk among women diagnosed with endometrial hyperplasia: the 34-year experience in a large health plan. British journal of cancer. 2008 Jan 8; 98 (1):45.
7. Salvesen HB, Iversen OE, Akslen LA. Identification of high-risk patients by assessment of nuclear Ki-67 expression in a prospective study of endometrial carcinomas. Clinical cancer research. 1998 Nov 1; 4 (11):2779-85.
8. Garzetti GG, Ciavattini A, Goteri G, De Nictolis M, Romanini C. Proliferating cell nuclear antigen in endometrial carcinoma: pretreatment identification of high-risk patients. Gynecologic oncology. 1996 Apr 30;61(1):16-21.
9. Hamel NW, Sebo TJ, Wilson TO, Keeney GL, Roche PC, Suman VJ, Hu TC, Podratz KC. Prognostic value of p53 and proliferating cell nuclear antigen expression in endometrial carcinoma. Gynecologic oncology. 1996 Aug 31;62 (2):192-8.
10. Todd JC. Clinical diagnosis and management by laboratory methods.
11. Mutter GL, Baak J, Crum CP, Richart RM, Ferenczy A, Faquin WC. Endometrial precancer diagnosis by histopathology, clonal analysis, and computerized morphometry. The Journal of pathology. 2000 Mar 1;190 (4):462-9.
12. Risberg B, Karlsson K, Abeler V, Lagrelius A, Davidson B, Karlsson MG. Dissociated expression of Bcl-2 and Ki-67 in endometrial lesions: diagnostic and histogenetic implications. International journal of gynecological pathology. 2002 Apr 1;21(2):155-60.
13. Abike F, Tapisiz OL, Zergeroglu S, Dunder I, Temizkan I, Payasli A. PCNA and Ki-67 in endometrial hyperplasias and evaluation of the potential of malignancy. European journal of gynaecological oncology. 2011 Jan 1; 32 (1):77.