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The immunoexpression profile of CyclinD1, Ki-67 and p53 in evaluation of Endometrial hyperplasia and Endometrial Carcinoma

Running title : Cyclin D1,Ki-67, p53 in endometrial pathology
Institutional ethics committee : DMIMS(DU)/IEC/Aug-2019/8256

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Abstract :-

Background: Endometrial carcinoma is a of globally perceived problem attributed to genomic molecular defects. Differentiating atypical endometrial hyperplasia from endometrial carcinoma specially in the samples of endometrial curettage is a diagnostic predicament for pathologists. Therefore, the studies of immunoexpression profile for proliferating markers and tumor suppressor genes ascertain the potential

malignant precursors. The present study was carried out with theobjectives to study cyclin D1, Ki-67 and p53 for inter variable comparisons in WHO categories of endometrial hyperplasia and type 1, endometrial carcinoma.

Methods - IHC was performed using monoclonal antibodies of cyclin D1, Ki-67 and p53 in WHO defined categories of endometrial hyperplasia and type 1 endometrial carcinoma. The results of IHC were statistically analysed and p value less than 0.05 was considered significant.

Results - The present study was carried out in Six groups with 15 cases in each group. The immunoexpression of cyclin D1, Ki-67 and p53 were in the increasing grade of hyperplasia and endometrial carcinoma.It is concluded that the IHC of cyclin D1, Ki-67 and p53 can derive conclusive immunostaining that enables the differentiation between atypical endometrial hyperplasia and endometrial carcinoma,type 1,low grade.

Key words –Endometrial carcinoma,type 1,endometrial hyperplasia,IHC, Cyclin D1, Ki-67 and p53.

INTRODUCTION

Endometrial carcinoma is major health problem affecting women for its morbidity and mortality. The incidence of endometrial carcinoma is 2.3 per lakh women in India[1].Endometrial carcinoma is showing upward trend at incidence in recent times as published on various data sites[2].

Atypical endometrial hyperplasia and endometrial intraepithelial neoplasia concepts merge to evolve model for pathogenesis of invasive endometrial carcinoma[3,4,5].

WHO offered the simplified version of endometrial hyperplasia that runs as follows; Benignhyperplasia without atypia category which included simple hyperplasia without atypia and complex hyperplasia without atypia and atypical hyperplasia category which included simple hyperplasia with atypia and complex hyperplasia with atypia[5]. These classes of endometrial hyperplasia conveys the risk of development of malignancies in it.

The histomorphological overlaps results in confusion of categorization of endometrial pathologies either to atypical endometrial hyperplasia or to endometrioid endometrial carcinoma(Type1).

The molecular basis of endometrial carcinoma, type 1 has been unravelled in past decade. A few of the molecular abnormalities have also been found to be shared by complex endometrial hyperplasia with atypia with that of the lesions of endometrial carcinoma, type 1. The tumor suppressor gene of PTEN has been found inactivated and mutated in substantial frequency in hyperplasia with atypia (approximately 50%) and endometrial carcinoma (more than 70%)[6]. These findings highlight that hyperplasia with atypia as the precursor lesions of endometrioid adenocarcinoma (Type 1, endometrial carcinoma)[6]. A cluster of molecular and mutational defects in BRCA1 or BRCA2[7], BRAF V600E[8], DACH1[9,10], CyclinD1[11,12,13,14], NF-Kβ[15]and β-catenin[15] pathway have also been suggested in underlying molecular pathology that modulates cell proliferation in type 1 endometrial carcinoma by upregulating Pin1 (propyl isomerase)[15,16].

The immune-expression of cyclinD1 (also called as BCL-1) is an essential molecule in a cell cycle for progression to G1 phase. Therefore, it remains a candidate proto-oncogene, the mutation, amplifications and overexpression of this gene alters the cell cycle progression and therefore contributes to tumorigenesis[17,18,19].

Ki-67 as a biomarker of active cell proliferation in the phases of G1, S and G2 has been known to correlate between the proliferative activity within hyperplasia, also the tumor grade but this marker lacks the consensus for prognostic value[20,21].

TP53 being a policeman of cell genome is a known tumor suppressor gene. The mutation of it results into unchecked proliferation of the tumor cells through the multipronged mechanisms[22].

Germline alteration in DNA mismatch repair genes and TP53 mutations pose high risk for Type 1 endometrial carcinoma[21,22]. A concomitant progressive high Ki-67 index have also been noted in endometrial hyperplasia as well as type 1 endometrial carcinoma[21,22,23]. The literature search on CyclinD1, Ki-67 and p53 defines the values which diagnostically differ for the lesions of endometrial hyperplasia with atypia and type 1 endometrial carcinoma. A few studies either propagates the use of the immune-expression of above markers to distinguish the endometrial hyperplasia with atypia specially of

complex types from that of type 1 endometrial carcinoma in the situations of histological overlaps [19,20,21,22,23].

Therefore, a study has been undertaken with the objective to know the immunoexpression of cyclin D1, Ki-67 and p53 in the endometrial hyperplasia pathology and endometrial carcinoma, type1, grade 1 tumor to understand its comparative expression and significance.

MATERIALS AND METHODS

A total of 90 subjects were recruited in the present study and their details were entered into a proforma that includedname, age, registration number, inpatients/outpatients, department, unit of care, occupation and address.The presenting symptoms were recorded pertaining to the endometrial pathology.

The history for menarche, menstrual cycle, menopause, abnormal uterine bleeding and others were also recorded. The additional investigations such as the findings of pelvic sonography specially for endometrial thickness and ovarian pathology were recorded. The findings of basic hematological investigations and related blood biochemistry were recorded.

The study design was observational when carried out prospectively over a period of two years and was sanctioned by the institutional ethics committee bearing number DMIMS(DU)/IEC/Aug-2019/8256.

Subject Characteristics

As titled the study subjects evaluated for their endometrium were grouped in six with each group having 15 cases each. Group Awas taken as control group withsamples of endometrial curettage histologically diagnosed as proliferative mid phase (PP).For study groups we divided the subjects into Group B withsamples of endometrial curettage histologically diagnosed as simple hyperplasia without atypia(SH) ; Group C withsamples of endometrial curettage histologically diagnosed as simple hyperplasia with atypia (SH with atypia) ; Group D with Samples of endometrial curettage histologically diagnosed as complex hyperplasia without atypia (CH) ; Group E with samples of endometrial curettage histologically diagnosed as complex hyperplasia with atypia (CH with atypia) ; Group F with Included the specimens of hysterectomy which were histologically diagnosed as endometrial carcinoma (EC) , endometrioid type, type I.

Laboratory methods and reporting

The samples of endometrial curettage were received in 10% formalin in the patients of groups of A, B, C, D and E. The endometrial curettage were grossly examined for theircolor, consistency and quantity and were processed in automated histokinette by standard methods [24]. The specimens of hysterectomy of the patients in Group F were examined and grossed by the standard protocol [25,26].These paraffin blocks sections were cut of 3-4 micron thickness on microtome.These paraffin sections were later processed for Hand E staining after deparaffinization. The sections of the endometrium for their histopathology were reported by standard text [25,26]**.**

Immunohistochemistry on the paraffin blocks for all the groups were performed for the immunostaining of cyclinD1 (PathNsitu,Rabbit monoclonal Ab), Ki-67 (PathNsitu,mouse monoclonal Ab) and p53 (DAKO,mouse monoclonal Ab) in a similar way as has been suggested in the standard references and text[24]. The negative and positive controls were also run with each batch of the immunomolecular staining.

Cyclin D1 immunostaining for its interpretation was done as follows [27,28,29,30,31] :- The staining was evaluated for its brown distinct granularity in the nuclei. The minimum nuclei counted were 100 in high power fields. The nuclei with moderate to strong intensity staining were defined as positive for cyclin D1 immunoexpression. The immune-expression was later scored for their positive expressions as: 0 score for <10% staining, 1+ score for 11-30% staining, 2+ score for 31-60% and 3+ for >60% staining.Positive control for immunostaining for cyclin D1 was paraffin sections of invasive ductal carcinoma (IDC) processed with the similar steps of immunostaining of cyclin D1 [27].

Ki-67 immunoexpression interpretation was done by selecting minimum of 500 nuclei in high power field from the representative histopathological area from the section.The intensity of the nuclear staining was gauged.Following were the scores allotted for Ki-67 immune-expression [32]. 1+ if 10-39% cells stained positive for Ki-67 expression, 2+ for 40-69% staining and 3+ for >70% immunostaining. The positive

control used for the evaluation of immune-expression of Ki-67 was the paraffin sections from the carcinoma of colon [33].

The nuclear staining for p53 immune-expression was assessed in the representative area of pathology of all the groups in high power fields [34]and the scoring used was 0 score when <10% cells showing positive staining,1+ score – 11-30% cells showing positive staining, 2+ with 31-50% and 3+ with >51% staining. The positive control used for p53immunoexpression was the paraffin sections of serous ovarian carcinoma [33]. Flow chart for the methodology is shown in **Figure 1**.

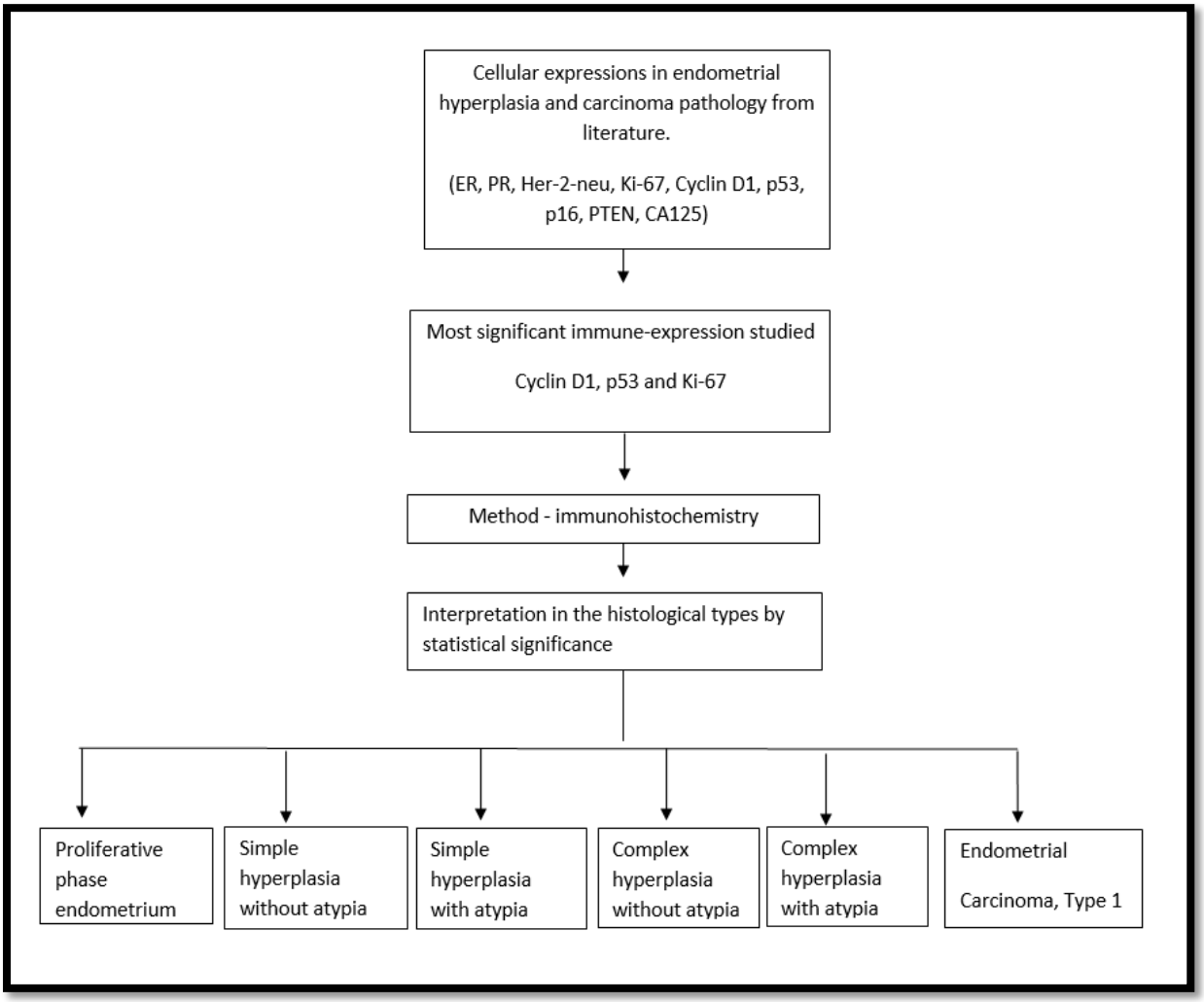

Figure 1 : Flow chart for methodology

Informed consent was obtained from all individual participants included in the study with a waiver by the institutional ethics committee bearing number DMIMS(DU)/IEC/Aug-2019/8256.

Statistics

The data obtained at the results of the present study was analyzed for correlation and comparison statistics. The statistical values of mean ± standard deviation, value of significance (P value) and chi-square test for each of the molecular expressions of cyclin D1, Ki-67 and p53 were carried out in each study group and compared with control group.

RESULTS

The mean age for group A (control) was 35.66±8.21. The mean age for group B was 39.60±7.47, Group C was 41.26±10.44, Group D was 45.20±10.20, Group E was 46.80±8.39 and Group F was 57.2±6.878. It was observed that higher mean age group was seen in Group F with 57.2±6.878.

The maximum cases of the present study were in Stage II, 08/15(53.33%). There were 04/15 cases (26.67%) and were equally distributed for substages of IA and IB each. There were 03/15 cases (20%) in stage III.

Histomorphology of complex hyperplasia with atypia is shown in **Figure 2.**The cyclin D1 immunoexpression in complex hyperplasia with atypia showed the distribution of 1+ in 2/15 cases (13.33%) **(Figure 3)**.The Ki-67 immunostaining was positive in all the cases with 2+ score in 6/15(40%) cases **(Figure 4)** and for p53 positive immunoexpression was seen in 3/15(20%) cases which showed a score of 1+ and 2/15(13.33%) cases showed a score of 2+ **(Figure 5)**.

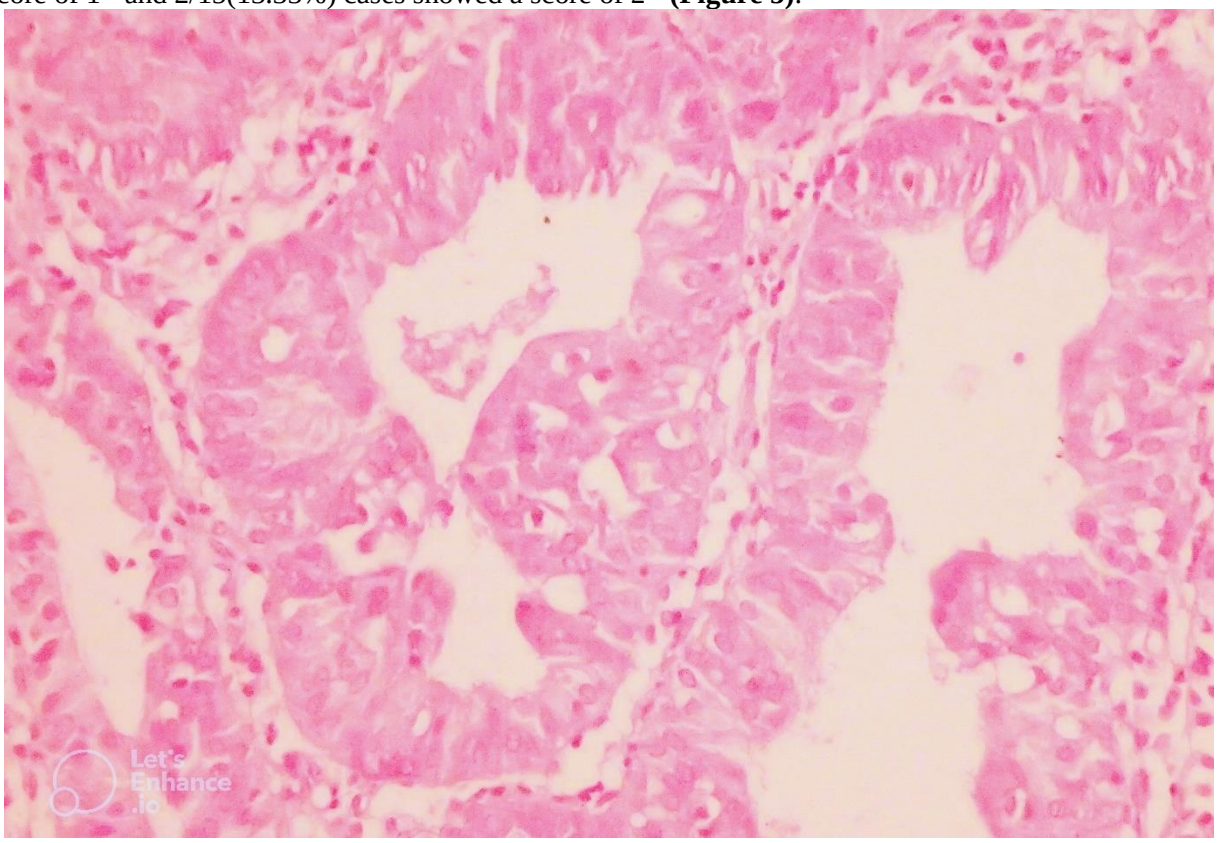

Figure 2: Photomicrograph of Complex hyperplasia with atypia showing numerous endometrial glands arranged back to back in complex hyperplastic state with features of atypia (HP) ; 40x

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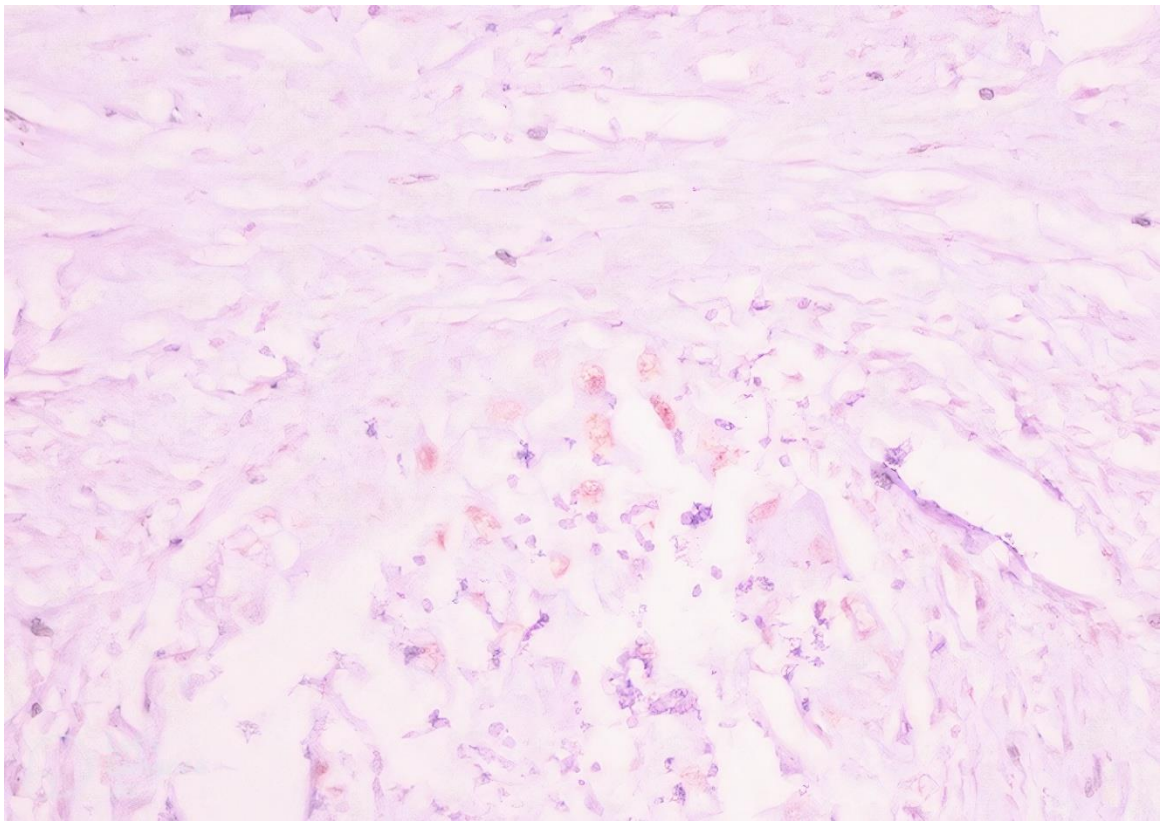

Figure 3: Photomicrograph of Complex hyperplasia with atypia showing nuclear immunostaining; cyclin D1 (IHC) : Score-1+ ;40x

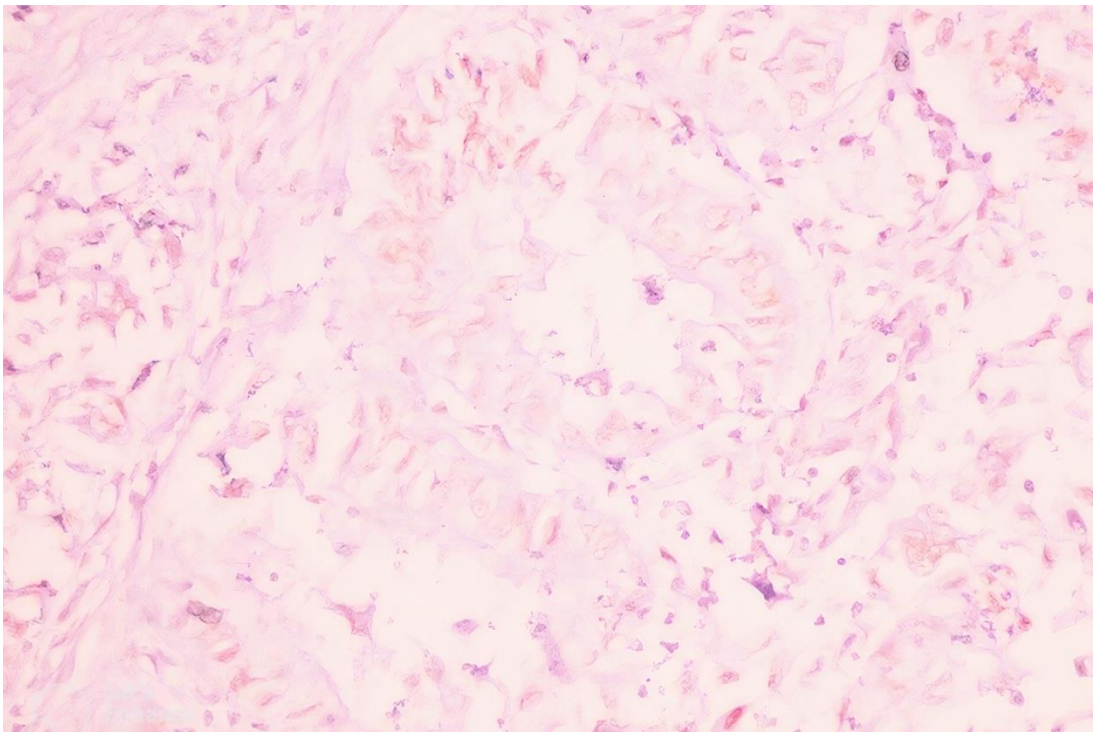

Figure 4 : Photomicrograph of Complex hyperplasia with atypia showing nuclear immunostaining; Ki-67 (IHC) : Score-2+ ;40x

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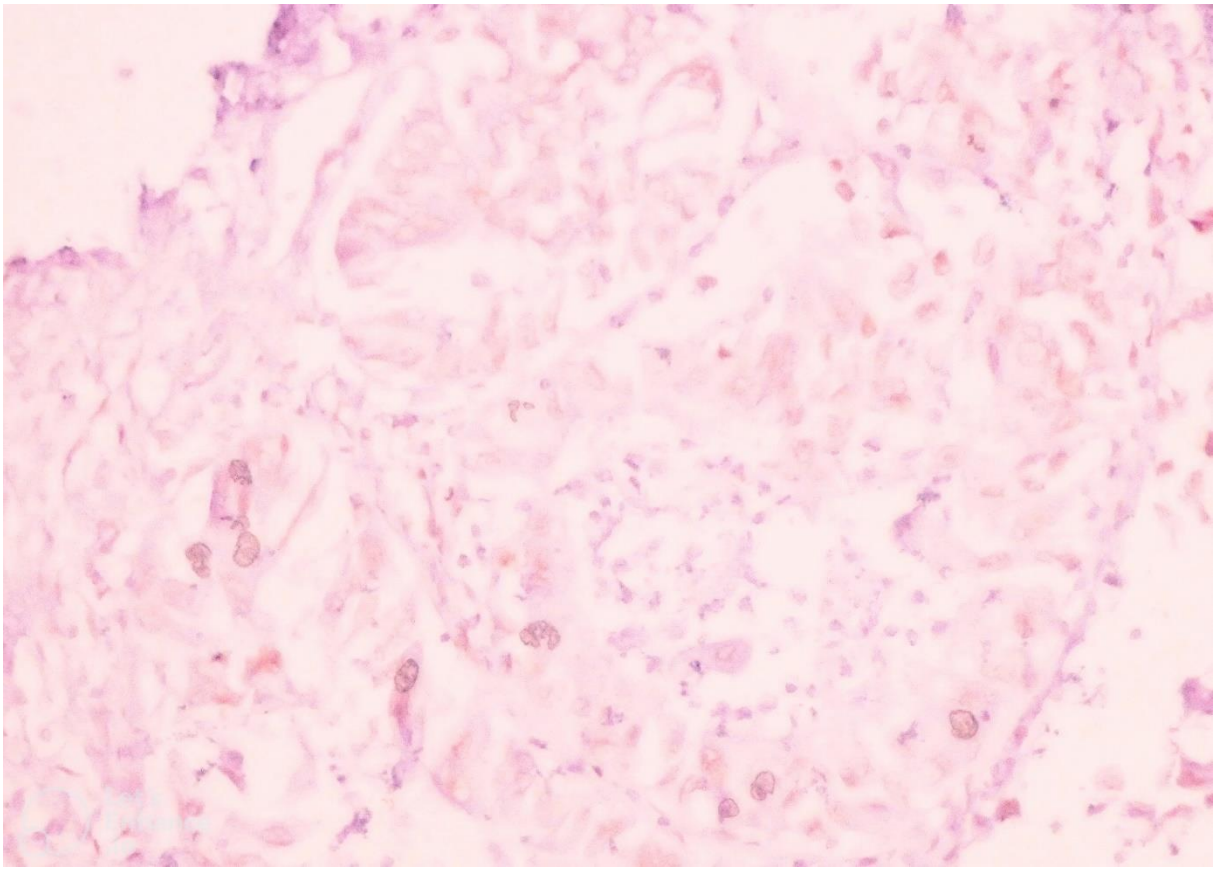

Figure 5 : Photomicrograph of Complex hyperplasia with Atypia showing nuclear immunostaining; p53 (IHC) : Score-2+ ;40x

Histology of endometrial carcinoma, type 1, grade 1 is shown in **Figure 6**. There were 2/15(13.33%) cases of endometrial carcinoma who had cyclin D1 negative staining while 13/15(86.67%) cases had positive staining out of which 3+ score was observed in 8/15(53.33%) cases **(Figure 7)**.

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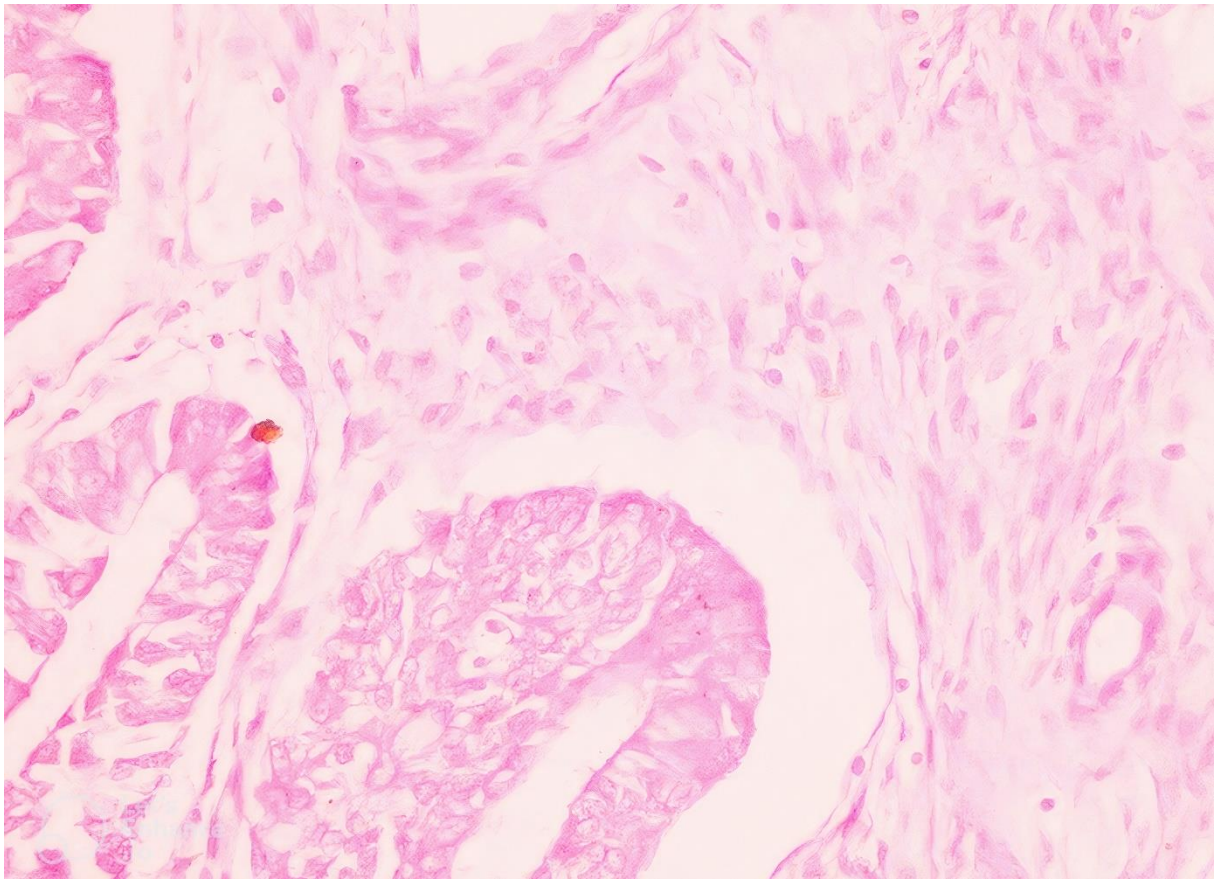

Figure 6 : Photomicrograph of Endometrial carcinoma, Type 1 showing numerous endometrial glands of complex nature with mild to moderate degree of pleomorphism with invasion into the myometrium (HP); 40x

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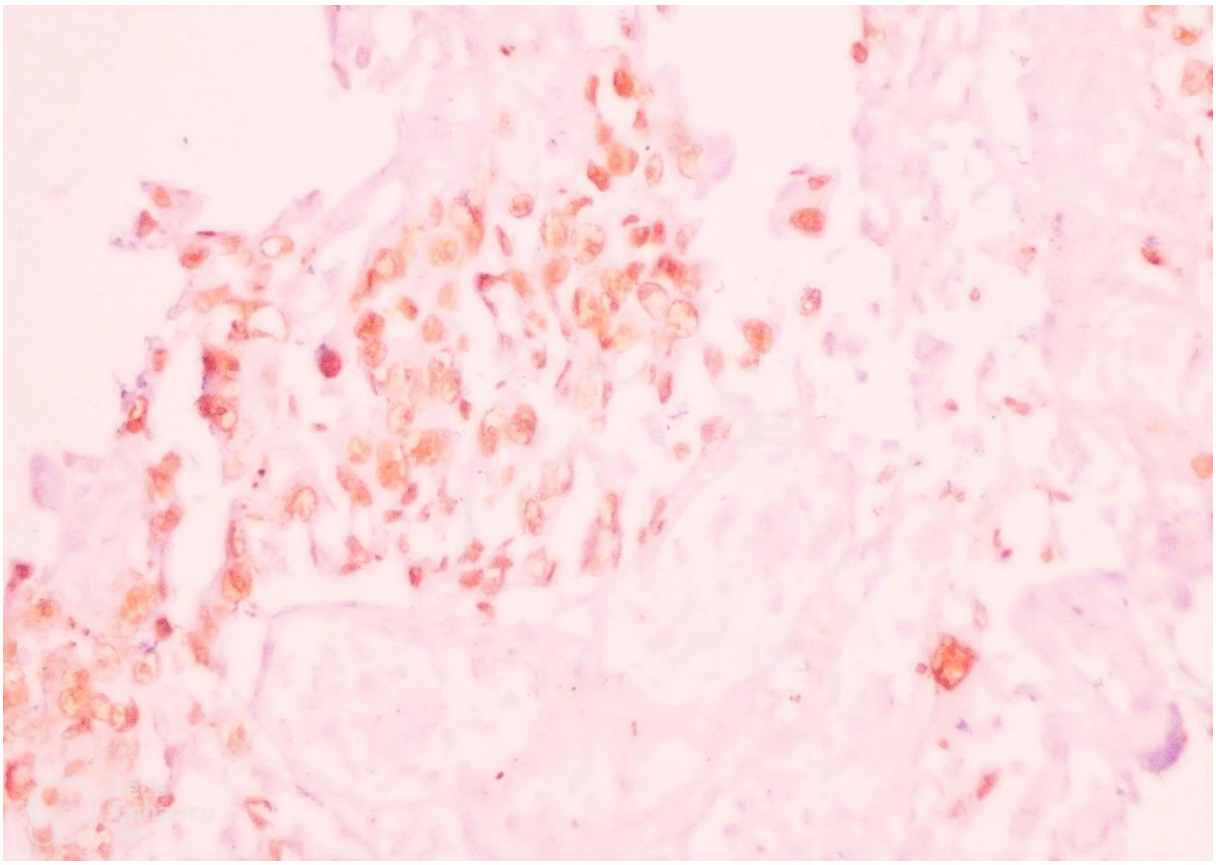

Figure 7 : Photomicrograph of Endometrial carcinoma, Type 1 showing nuclear immunostaining; cyclin D1 (IHC) : Score-3+ ;40x

The immunostaining for Ki-67 was positive for all cases (100%) of endometrial carcinoma of which 6/15(40%) cases showed a score of 3+ was observed **(Figure 8)**. The immunostaining for p53 in group F was positive for all cases (100%). 3/15(20%) cases had a score of 3+ for p53 immunostaining **(Figure 9).**

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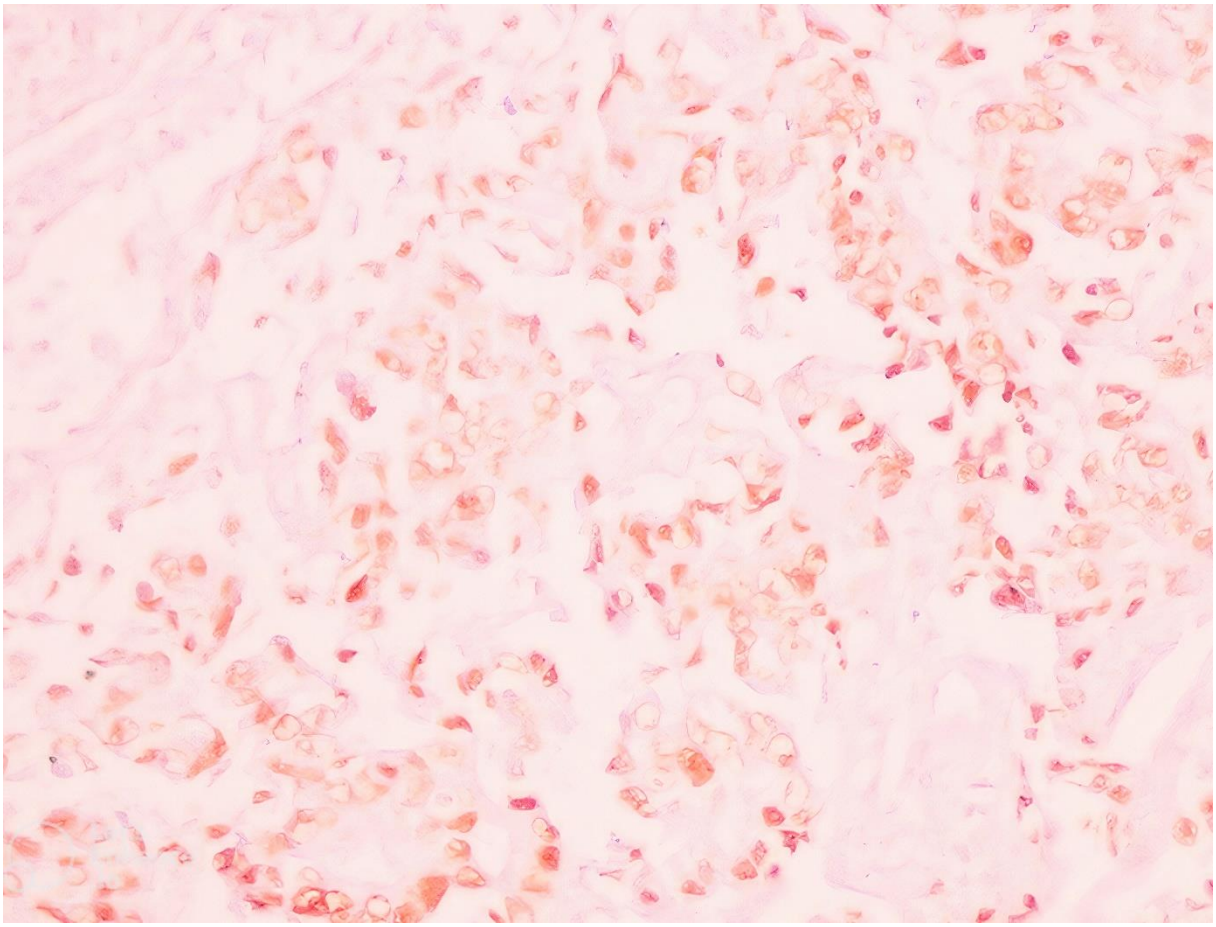

Figure 8 : Photomicrograph of Endometrial carcinoma, Type 1 showing nuclear immunostaining; Ki-67 (IHC) : Score-3+ ;40x

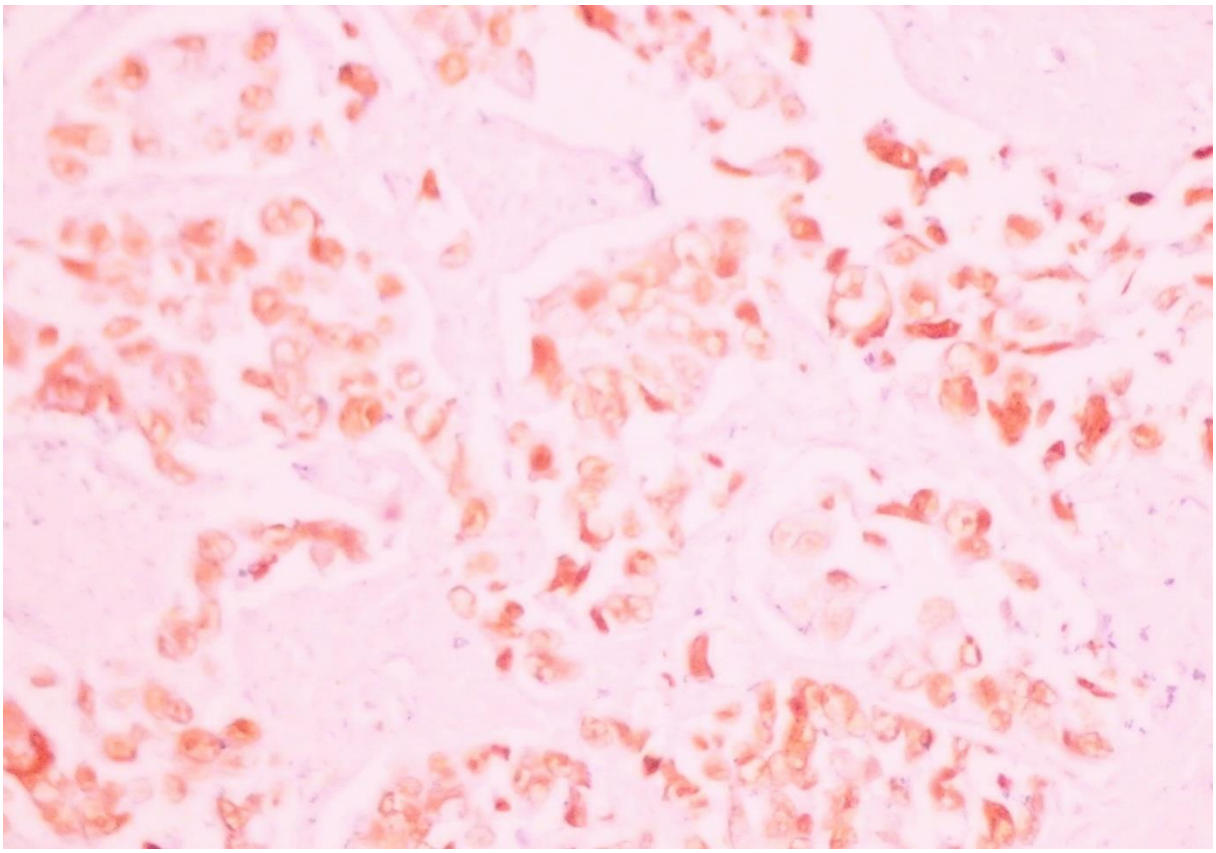

Figure 9 : Photomicrograph of Endometrial carcinoma, Type 1 showing nuclear immunostaining; p53 (IHC) : Score-3+ ;40x

IHC expressions for cyclin D1, Ki-67 and p53 done across all the groups along with the intergroup comparisons are mentioned in **Table 1, 2 and 3**.

Table 1:-Immunoexpression of cyclin D1 across the groups

Groups	Number of cases		Degree of freedom	P value	Intercomparison Tukey test
	Positive Expression n(%)	Negative Expression n(%)			

Group A	2(13.33%)	13(86.67%)	1	0.0001,S	Group A VsB:0.10**,NS** Group A VsC:0.02**,S** Group A VsD:0.05**,NS** Group A VsE:0.02**,S** Group A VsF:0.0001**,S** Group B VsC:0.71**,NS** Group B VsD:1.00**,NS** Group B VsE:0.71**,NS** Group B VsF:0.05**,NS** Group C VsD:1.00**,NS** Group C VsE:1.00**,NS** Group C VsF:0.21**,NS** Group D VsE:1.00**,NS** Group D VsF:0.10**,NS** Group E Vs F: 0.21**,NS**
Group B	7(46.67%)	8(53.33%)	1	0.47,NS	
Group C	9(60%)	6(40%)	1	0.0071,S	
Group D	8(53.33%)	7(46.67%)	1	0.47,NS	
Group E	9(60%)	6(40%)	1	0.0071,S	
Group F	13(86.67%)	2(13.33%)	1	0.0001,S	

Table 2 :- Immunoexpression of Ki-67 across the groups

Groups	Number of cases		Degree of freedom	P value	Intercomparison Tukey test
	Positive Expression n(%)	Negative Expression n(%)			
Group A	1(6.67%)	14(93.33%)	1	0.0001,S	Group A VsB:0.59**,NS** Group A VsC:0.005**,S** Group A VsD:0.0005**,S** Group A VsE:0.0001**,S** Group A VsF:0.0001**,S** Group B VsC:0.06**,NS** Group B VsD:0.009**,S** Group B VsE:0.0001**,S** Group B VsF:0.0001**,S** Group C VsD:0.69**,NS** Group C VsE:0.01**,S** Group C VsF:0.01**,S** Group D VsE:0.09**,NS** Group D VsF:0.09**,S** Group E Vs F: -1.00,NS
Group B	3(20%)	12(80%)	1	0.0001,S	
Group C	9(60%)	6(40%)	1	0.0001,S	
Group D	11(73.33%)	4(26.67%)	1	0.0001,S	
Group E	15(100%)	0(0%)	1	0.0001,S	
Group F	15(100%)	0(0%)	1	0.0001,S	

Table 3 :- Immunoexpression of p53 across the groups

Groups	Number of cases		Degree of freedom	P value	Intercomparison Tukey test
	Positive Expression n(%)	Negative Expression n(%)			
Group A	0(0%)	15(100%)	1	0.0001,S	Group A VsB:1.00,**NS** Group A VsC:0.48,**NS** Group A VsD:0.48,**NS** Group A VsE:0.04,**S** Group A VsF:0.0001,**S** Group B VsC:0.48,**NS** Group B VsD:0.48,**NS** Group B VsE:0.04,**S** Group B VsF:0.0001,**S** Group C VsD:1.00,**NS** Group C VsE:0.38,**NS** Group C VsF:0.0001,**S** Group D VsE:0.48,**NS** Group D VsF:0.0001,**S** Group E Vs F: 0.0002,**S**
Group B	0(0%)	15(100%)	1	0.0001,S	
Group C	2(13.33%)	13(86.67%)	1	0.0001,S	
Group D	2(13.33%)	13(86.67%)	1	0.0001,S	
Group E	5(33.33%)	10(66.67%)	1	0.0001,S	
Group F	15(100%)	0(0%)	1	0.0001,S	

The intergroupcomparison for cyclin D1 expression mostly revealed a positive cyclin D1 staining maximizing in Group F (Endometrial carcinoma, Type 1) followed by Group E (Complex hyperplasia with atypia). The tukey test performed for intergroup comparisons revealed significant values when Group A results were compared to Group C, Group E and Group F with respect to p values 0.02, 0.02 and 0.0001 respectively.The other intergroup comparison for cyclin D1 yielded Non significant values. Thus, it was observed that the immune-expression of cyclin D1 linearly increased with the increased grade of hyperplasia and with endometrial carcinoma**(Table 1).**

The intergroup comparison for Ki-67 expression revealed that cases of Group E (Complex hyperplasia with atypia) and Group F (Endometrial carcinoma, Type 1) show 100% immune-expression to Ki-67 closely followed by Group D (Complex hyperplasia without atypia) with 11/15(73.33%) cases showing positive immune-expression to Ki-67**(Table 2).**

For p53, the intergroup comparison revealed maximum expression in Group F (Endometrial carcinoma, Type 1) of 15/15 cases (100%) followed by Group E (Complex hyperplasia with atypia) with 5/15 cases (33.33%) showing positive expression for p53. The tukey test performed for intergroup comparisons revealed significant P values when results of Group A were compared with the results of Group E and F with p values of 0.04 and 0.0001 respectively.Similarly, results of tukey test for Group B when compared to results of group E and F came out to be statistically significant with p values of 0.04 and 0.0001. Group F results were statistically significant with that of group A, C, D and E with p values of 0.0001, 0.0001, 0.0001 and 0.0002 respectively.**(Table 3)**

The comparative expressions of IHC staining with cyclin D1, Ki-67 and p53 along with their score across all the groups are mentioned in **Table 4**.

The cyclin D1 appeared to increase for their immunoexpression with increasing grade of hyperplasia. It was observed that group F (Endometrial carcinoma, Type 1) showed the highest number of cases i.e.

13/15(86.67%) expressing cyclin D1. This group also had the highest number of cases i.e. 8/15(53.33%) with 3+ score. Group A, B and C had no cases that expressed cyclin D1 with a score of 3+.
The Ki-67 expression was found to be associated with increasing proliferative activity of the cells. In Group E and F all the cases expressed Ki-67 with 6/15(40%) cases and 9/15(60%) cases showing score of 2+ and 1/15(6.67%) case and 6/15(40%) cases showing score of 3+ respectively. Ki-67 expression was found to be increasing in reference to the control with increasing grade of hyperplasia and endometrial carcinoma.
p53 immunoexpression was completely absent in Group A and B patient sample. It was lowest with 1+ score in Group C and D with 2/15(13.33%) cases each. But in group E, p53 was expressed as 1+ in 3/15(20%) cases and 2+ in 2/15(13.33%) cases. All the cases in group F were immunostained for p53 with 1+ score in 4/15(26.67%) cases, 2+ score in 8/15(53.33%) cases and 3+ score in 3/15(20%) cases. The observations in this group showed that p53 immunoexpression was found to be associated with hyperplasia with atypia but in low percent for its appreciation. But its strikingly being appreciated for the change of value of expression for the malignant group of endometrium.
The comparison of immunoexpression in between Group A to F with values expressed with the score showed a significant p value of 0.0001.**(Table 4).**

Table 4 : Comparative expression with score in all groups

Groups	Cases	CyclinD1 n=15(%)				Ki-67 n=15(%)				p53 n=15(%)				P value
		0	1+	2+	3+	0	1+	2+	3+	0	1+	2+	3+	
A	15	13 (86.67%)	2 (13.33%)	0 (0%)	0 (0%)	14 (93.33%)	1 (6.67%)	0 (0%)	0 (0%)	15 (100%)	0 (0%)	0 (0%)	0 (0%)	0.0001 S
B	15	8 (53.33%)	5 (33.33%)	2 (13.33%)	0 (0%)	12 (80%)	3 (20%)	0 (0%)	0 (0%)	15 (100%)	0 (0%)	0 (0%)	0 (0%)	
C	15	6 (40%)	5 (33.33%)	4 (26.67%)	0 (0%)	6 (40%)	8 (53.33%)	1 (6.67%)	0 (0%)	13 (86.67%)	2 (13.33%)	0 (0%)	0 (0%)	
D	15	7 (46.67%)	3 (20%)	3 (20%)	2 (13.33%)	4 (26.67%)	6 (40%)	5 (33.33%)	0 (0%)	13 (86.67%)	2 (13.33%)	0 (0%)	0 (0%)	
E	15	6 (40%)	2 (13.33%)	3 (20%)	4 (26.67%)	0 (0%)	8 (53.33%)	6 (40%)	1 (6.67%)	10 (66.67%)	3 (20%)	2 (13.33%)	0 (0%)	
F	15	2 (13.33%)	2 (13.33%)	3 (20%)	8 (53.33%)	0 (0%)	0 (0%)	9 (60%)	6 (40%)	0 (0%)	4 (26.67%)	8 (53.33%)	3 (20%)	

Cyclin D1 was positive in 8/15(53.33%) cases of stage II disease out of total 13/15(86.67%) cases of cyclin D1 positive results and 3/15(20%) cases of stage IIIB disease.
Ki-67 was maximally expressed in stage II disease in 8/15(53.33%) followed by 3/15(20%) cases in stage IIIB and 2/15(13.33%) cases each in stage IA and IB disease.
The p53 immunoexpression was also maximally positive with 8/15(53.33%) cases of stage II disease followed by 3/15(20%) cases of stage III disease and 2/15(13.33%) cases of stage IA and IB each.
The TNM interstage comparison for immunoexpression of cyclin D1, Ki-67 and p53 yielded non significant values (p value=0.98, NS)

DISCUSSION

Cyclin D1, Ki-67 and p53 in various pathologies of the endometrium in comparison to its normal physiological state of proliferative phase has been performed previously [1,12,14,17,32]. The objectives

of such comparisons is to know its value at differentiating the lesions of endometrium with overlapping histomorphologies as well as to know its propensity as a precursor lesion for endometrial carcinoma.

The studies of Ozuysal et al[11], Shevra et al[17], Masjeed et al[21], Quddus et al[27], Shawana et al[29],Kundu et al[30]and Nishimura et al[31]have carried out such comparisons in establishing the values and its diagnostic and prognostic significance by cyclin D1, Ki-67 and p53 immunostaining. The present study conquers to the objectives of aforesaid studies and has similarly carried out the immunoexpression profiles of endometrial cells for cyclin D1, Ki-67 and p53 in the lesions of simple hyperplasia, simple hyperplasia with atypia, complex hyperplasia, complex hyperplasia with atypia and endometrial carcinoma and compared with the control cases of proliferative phase.

The present study observed the age range for endometrial carcinoma (Type 1) in Group F was of 47-70 years with mean age of 57.13±7.16. The study of Ahmed et al[22]observed endometrial carcinoma above the age of 60 years in 55% cases and below the age of 60 years in 45% cases.

Stage II was the frequently observed stage when patients consulted and presented for the treatment. This observation is contrary to presentation at treatment to the studies of Khabaz et al[12], Moreno bueno et al[13], Nikaido et al[14]andNishumura et al[31] wherein maximum cases were observed in stage I disease. The observations of the present study could not be compared for the grading of endometrioid adenocarcinoma as the inclusion criteria for the present study was Grade 1 Endometrial carcinoma.

The present study observed the following percentages of expression of cyclin D1 : Group A (PP)-13.33%, Group B (SH)-46.67%, Group C (SH with atypia)- 60%, Group D(CH)- 53.33%, Group E (CH with atypia)-60% and Group F (EC)-86.67%. These observations are consistent with the observations of studies of Ozuysal et al[11], Khabaz et al[12], Yildrim et al[18] and Liang et al[28], for the immunoexpression of cyclin D1.

The values and the comparisons of the Ki-67 for the individual histomorphological groups of endometrial pathologies and/or comparisons in the groups has been done byShevra et al[19] and Masjeed et al[21]. The present study conquers with the observations of the above authors **t**hat the percentage of immunoexpression of Ki-67 is high and much similar in complex hyperplasia with atypia and endometrial carcinoma.

The present study observed no p53 expression in Group A and B while in Group C and D it was 13.33% in each. The Group E revealed 33.33% immunoexpression for p53 while Group F of endometrial carcinoma had 100% immunoexpression for p53. These observations of the present study confirmed the utility of p53 immunoexpression that segregated the malignant endometrial lesions versus hyperplastic lesions and physiological lesions of proliferative state. A little higher immunoexpression of p53 of 33.33% was observed in Group E of complex hyperplasia with atypia suggesting its relationship as a precursor for overt endometrial carcinoma. Such observations for p53 immunoexpression with histological classes of endometrial lesions has also been quoted in the studies ofNikaido et al[14], Masjeed et al[21], Ahmed et al[22], Suthipintawong et al[23], Ragni et al[34], Panwar et al[35], Opric et al[36] and Stavropoulos et al[37]

The present study observed significant P value of 0.005 in relation with cyclin D1 with that of the higher stage of the disease which has also been quoted in the studies of Khabaz et al[12], Nikaido et al[14] and Yan et al[16],. Significant P value of 0.00001,S was observed in the present study when Ki-67 immunoexpression was correlated with the stage of endometrial carcinoma. This observation was similar to the study of Stoian et al[38]. In the present study significant P value (0.00001, S) were observed in relation with p53 expression and stage of endometrial carcinoma which has also been quoted in studies of Ahmed et al[22] and Opric et al[36].

The present study when carried out the comparative statistics to correlate the expression of all three (Cyclin D1, Ki-67 and p53) molecules with the stage of disease yielded insignificant P value (0.98, NS).

CONCLUSION

The present study concludes that the immunoexpressions of cyclin D1, Ki-67 and p53 differs for each of the WHO category of endometrial hyperplasia and endometrial carcinoma, type 1. p53 immunoexpression was consistent with group of endometrial carcinoma,type 1 and complex endometrial hyperplasia with atypia. It is further concluded that the tissue profiling of endometrial biopsies of cyclin D1, Ki-67 and p53

is adjuvant in differentiating lookalike lesions such as endometrial hyperplasia with atypia and low grade endometrioid endometrial carcinoma.
It's a single centre study and wider data evaluation of immunoprofiling is required.

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Tables number	List of Tables with legends
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