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IMMUNOHISTOCHEMICAL EXPRESSION OF ESTROGEN RECEPTORS AS PROGNOSTIC MARKER AND CORRELATION WITH CLINICOPATHOLOGICAL PARAMETERS IN HUMAN ENDOMETRIAL ADENOCARCINOMA

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Abstract

In human steroid-sensitive tissue, Estrogen receptors (ERs) have been demonstrated at the mRNA and/or protein levels by using molecular biology and immunohistochemistry techniques. Estrogen receptors are present in endometrial hyperplasia and neoplastic endometrium. The present work aimed to compare the expression of ER as a prognostic biomarker in human endometrial adenocarcinoma versus benign tumors and normal endometrial tissues as well as their correlation with different pathological and histological parameters. The immunohistochemical technique was used to examine the expression of ER in normal, benign as well as in endometrial adenocarcinoma. Present results showed higher expression of ER in endometrial adenocarcinoma compared to normal and benign endometrial tissues.

Keywords: ER prognostic marker, Estrogen receptors, endometrial adenocarcinoma.

Introduction

Endometrial carcinoma is the most common malignancy in the female genital system [(1)] and it occupies the 7th place as a cause of death by cancer in women in Western Europe (1–2% of all deaths from cancer) [(2).] Endometrial cancer is symptomatic from early stages thus having a good prognosis in most patients, the overall five-year survival rate being relatively high [(3).]

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In human steroid-sensitive tissue, progesterone receptors (PRs) and estrogen receptors (ERs) have been demonstrated at the mRNA and/or protein levels by using molecular biology and immunohistochemistry techniques [(3).] Endometrial carcinoma is formed and develops in close relation to the plasma and tissue levels of sex steroidal hormones and their receptors. Estrogen and progesterone receptors are present in endometrial hyperplasia and neoplastic endometrium [(4).]

Estrogen receptors (ER) are among the steroid hormone that regulates angiogenesis. The presence and quantity of steroid receptors in endometrial cancer have been correlated with tumor grade, FIGO stage, and survival [(5).]

In the present study, the expression of ER in Endometrial adenocarcinoma was investigated using an immunohistochemical technique, and the intensity of immunostaining was quantitatively estimated using an image optical density (IOD) analyzer.

Material and Methods

The present study was carried out on 50 prospective biopsies obtained from El-shatby Hospital, Department of Gynecology and Obstetrics, Faculty of Medicine- Alexandria University, Egypt, during the period between September 2014 and April 2016. The specimens in the present study were classified as the following: Normal endometrial tissue (n=10), Benign endometrial hyperplasia (n=20), and Endometroid adenocarcinoma of different grades (n=20). All the cases were asked to freely volunteer for the study. They informed written consents were gathered before their inclusion in the study protocol, according to the ethical guidelines of the Faculty of Medicine, Alexandria University. Diagnosis of the specimens was made according to the WHO classification of the Tumors. Clinical parameters included patients' age, tumor size, and lymph node metastasis (LNM).

Immunohistochemical investigation of ER:

The immunohistochemical method was utilized to study the expression of ER in 50 paraffin-embedded endometrium tissues. In brief, paraffin-embedded specimens were cut into 5µm thick sections. The sections were deparaffinized using 2 changes of xylen and rehydrated. The sections were submerged in an antigen retrieval (citrate buffer saline pH 6) in an oven at 95°C for 20 minutes and then left at room temperature for 20 minutes to cool. The sections were treated with 3% H_2O_2 in PBS to quench the endogenous peroxidase activity and then incubated with a serum-blocking reagent for 30 minutes to block nonspecific binding. The sections were incubated with primary antibody for ER at 4ºC overnight. Sections were treated with conjugated 2nd antibody (ABC-HRP reagent) for 30 minutes, stained with diaminobenzidine (DAB), and counter-stained with hematoxylin. For negative controls, the antibody was replaced with PBS. Each step was followed by PBS washing. Evaluation of ER immunohistochemical results was arbitrarily graded as negative (0), weak (+1), moderate (+2), and strong (+3).

Statistical Analysis:

Data were normally distributed according to the Kolmogorov-Smirnov (K-S) normality test and then analyzed using the statistical software SPSS 20. P values≤ 0.05 were considered statistically significant.

Results:

A -Histopathological results:

a. Haematoxylin and Eosin (H&E) staining

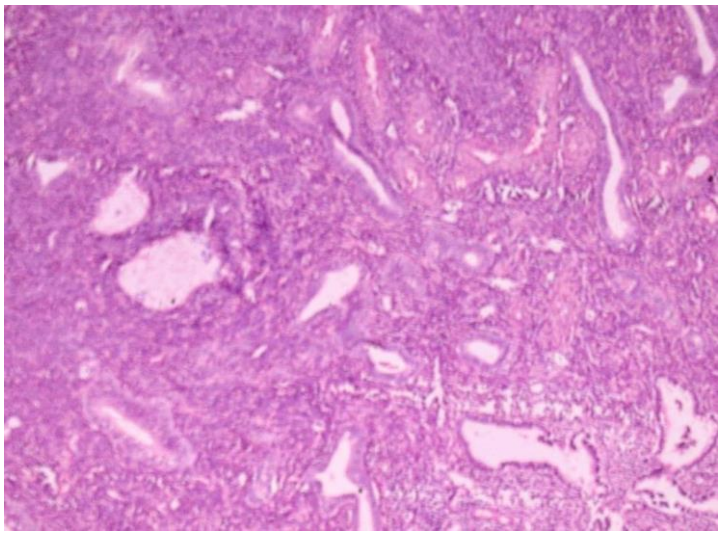

Fig. (1): Endometrial polyp showing proliferative glands within fibroblastic stroma entangling thick-walled vessels (H&E X100)

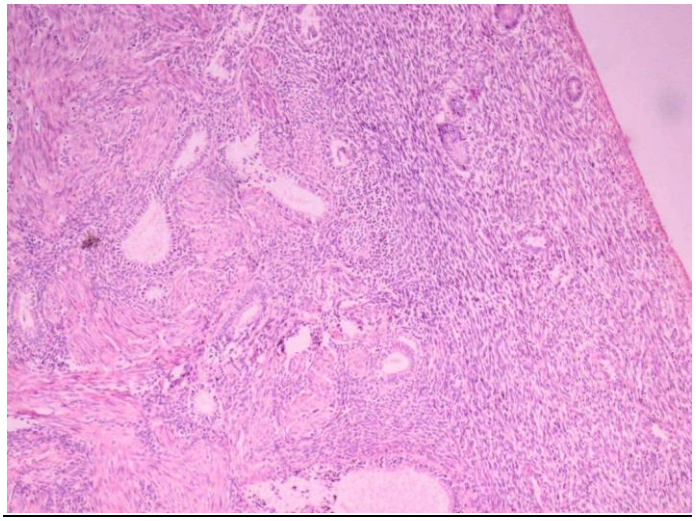

Fig. (2): Proliferative endometrial tissue showing few proliferative acini within the endometrial spindled stroma (H&E X100).

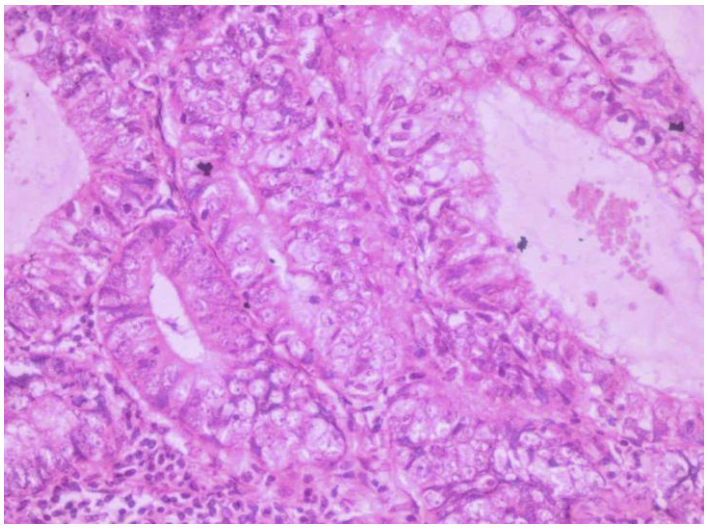

Fig. (3): Well-differentiated adenocarcinoma showing large fused acini lined by columnar cells with ample vacuolated eosinophilic cytoplasm (H&E X400).

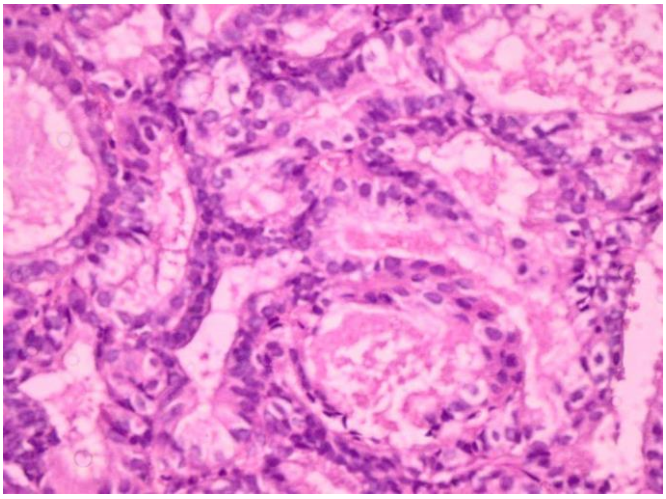

Fig. (4): A case of moderate differentiated endometrial adenocarcinoma showing multiple dilated closely packed and fused glands lined by malignant columnar cells with eosinophilic cytoplasm (H&E X400).

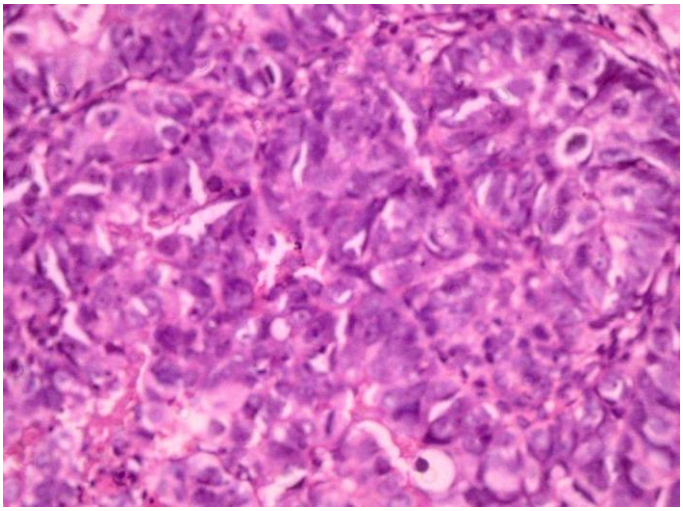

Fig. (5): A case of poorly differentiated endometriod adenocarcinoma composed of few glands and solid areas of malignant epithelial cells (H&E X400).

B. Histochemical results:

1. **Alcian blue stain:** Alcian blue histochemical stain was applied to detect endometrial mucin content in the glandular epithelium of the secretory phase and to differentiate between non-neoplastic and neoplastic endometrium.

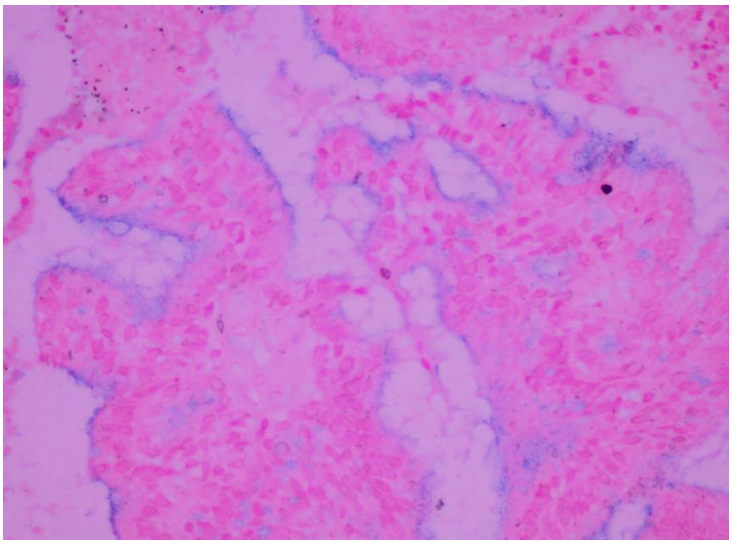

Fig. (6): Proliferative endometrium stained with alcian blue showing a low number of goblet cells with weak mucin activity(X400).

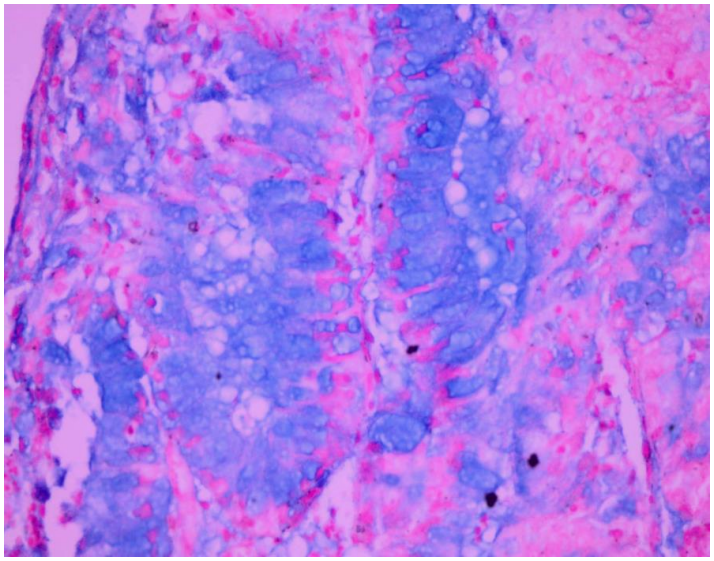

Fig. (7): Hyperplastic end polyp stained with alcian blue showing an increased number of goblet cells with moderate mucin staining(X400).

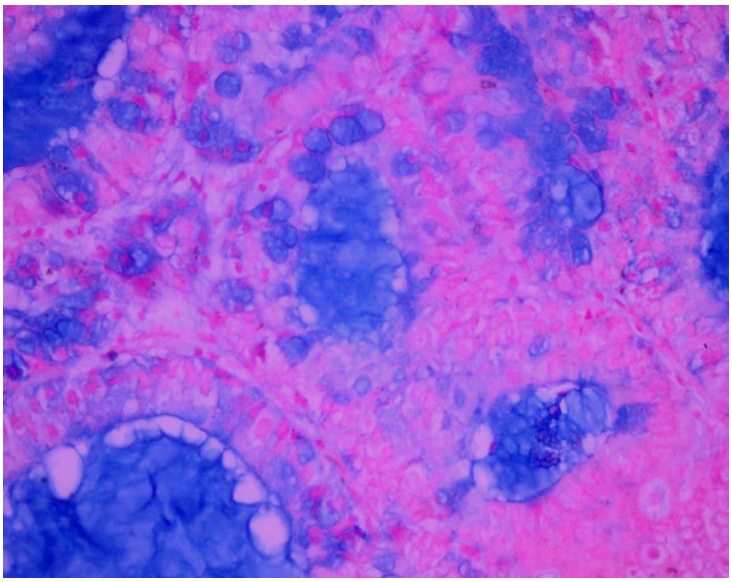

Fig. (8): Endometrial carcinoma stained with alcian blue showing a high increase in number of the goblet cells with strong mucin secretion(X400).

Quantitative evaluation:

Staining of mucin secretion was performed on endometrial tissues using an alcian blue stain. It was found to be proportional to the density of the blue color. Using Image J software, the mean ranges of mucin content per field by pixel were 190.76 ± 6.92, 164.35 ± 11.10 and152.61 ± 30.01 area 180.7 – 197.16, 152.45 – 176.6 and 99.28 – 169.7, in malignant, benign and control cases respectively. These results represented a highly significant difference between the malignant cases compared to benign and control ($p \leq 0.02$) with median of 191.85%, 166.16% and 166.60%. (table 1 and figure 9).

Table (1): Comparison between the three studied groups stained with Alcian blue:

Alcian blue	**Control (n = 5)**	**Benign (n = 5)**	**Malignant (n = 5)**	**F**	**P**
Min. – Max.	99.28 – 169.7	152.45 – 176.6	180.7 – 197.16		
Mean ± SD.	152.61 ± 30.01	164.35 ± 11.10	190.76 ± 6.92	5.342*	0.022*
Median	166.60	166.16	191.85		
Sig. bet. grps.	p_1= 0.346, p_2= 0.008*, p_3= 0.047*				

F,p: F and p values for **ANOVA test**, Sig. bet. grps was done using a **Post Hoc Test (LSD)**
*: Statistically significant at $p \leq 0.05$

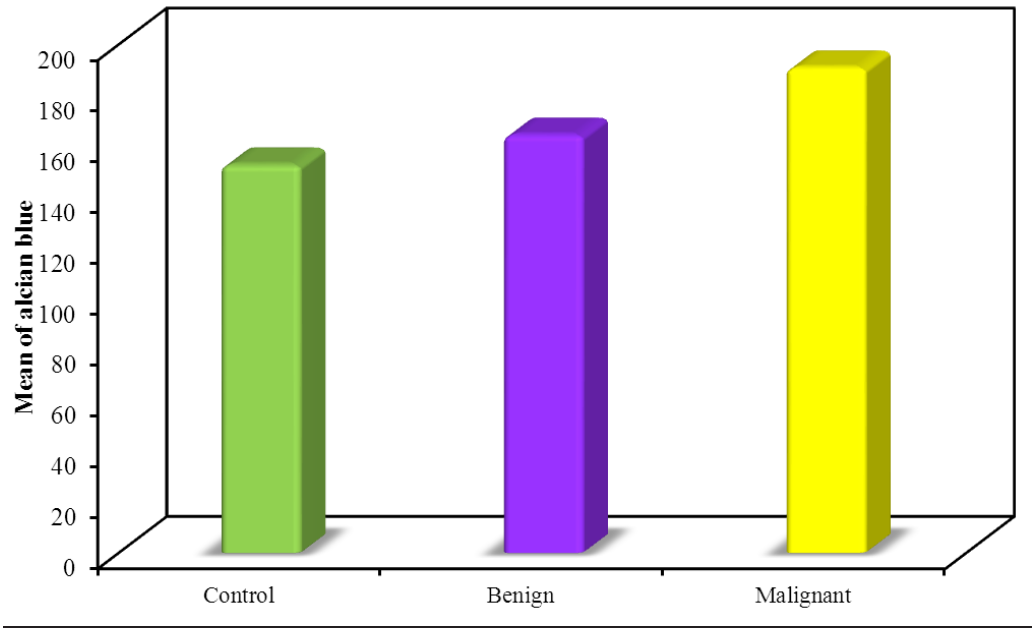

Fig. (9): Comparison between the three studied groups stained with alcian blue.

C. Immunohistochemical results:

1. Estrogen Receptor (ER):

The immunohistochemical staining results are illustrated in Table (2) and Figure (10). Sixty percent (6/10) of control cases were ER-negative (-ve), 40% (8/20) of benign ER moderate positive (2+) while 30% (6/20) of malignant cases were ER strong positive (3+).

Table (2): Comparison between ER of the three studied groups

Estrogen Receptor (ER)	Control (n = 10)		Benign (n = 20)		Malignant (n = 20)		χ^2	^{MC}p
	No.	%	No.	%	No.	%		
Negative (-ve)	6	60.0	0	0.0	6	30.0	16.556*	0.007*
Weak positive (1+)	2	20.0	6	30.0	3	15.0		
Moderate positive (2+)	2	20.0	8	40.0	5	25.0		
Strong positive (3+)	0	0.0	6	30.0	6	30.0		
Sig. bet. grps.	$^{MC}p_1$= 0.001*, $^{MC}p_2$= 0.214, $^{MC}p_3$= 0.051							

χ^2, p:χ^2 and p values for **Chi-square test**, sig. bet. Groups were done using the **Chi-square test.**

*: Statistically significant at $p \leq 0.05$.

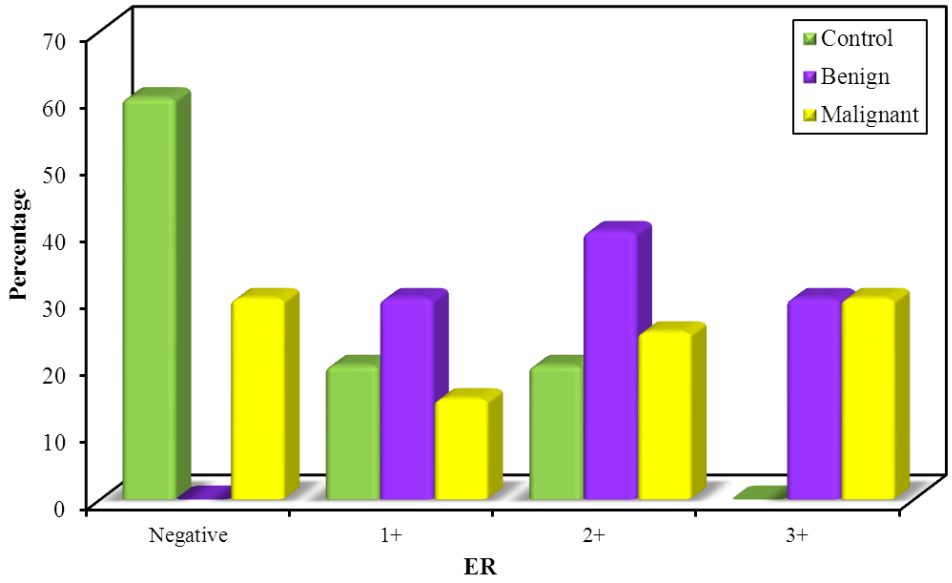

Fig. (10): Comparison between ER of the three studied groups.

Relation between tumor grade and ER in malignant groups:

As regards the grade of malignant groups, the immunohistochemical staining results are illustrated in Table (3) and Figure (11). There were 25% (1/4) moderate positive (2+) ER of grade I, 41% (5/12) ER strong positive (3+) of grade II, and 50% (2/4) ER moderate positive (2+) of grade III cases. The results illustrated no significant difference between the three grades.

Table (3): Relation between tumor grade and ER in malignant groups (n = 20)

Estrogen Receptor (ER)	Grade						χ^2	^{MC}p
	I (n = 4)		II (n = 12)		III (n = 4)			
	No.	%	No.	%	No.	%		
Negative(-ve)	1	25.0	4	33.3	1	25.0	4.783	0.687
Weak positive (1+)	1	25.0	1	8.3	1	25.0		
Moderate positive (2+)	1	25.0	2	16.7	2	50.0		
Strong positive (3+)	1	25.0	5	41.7	0	0.0		

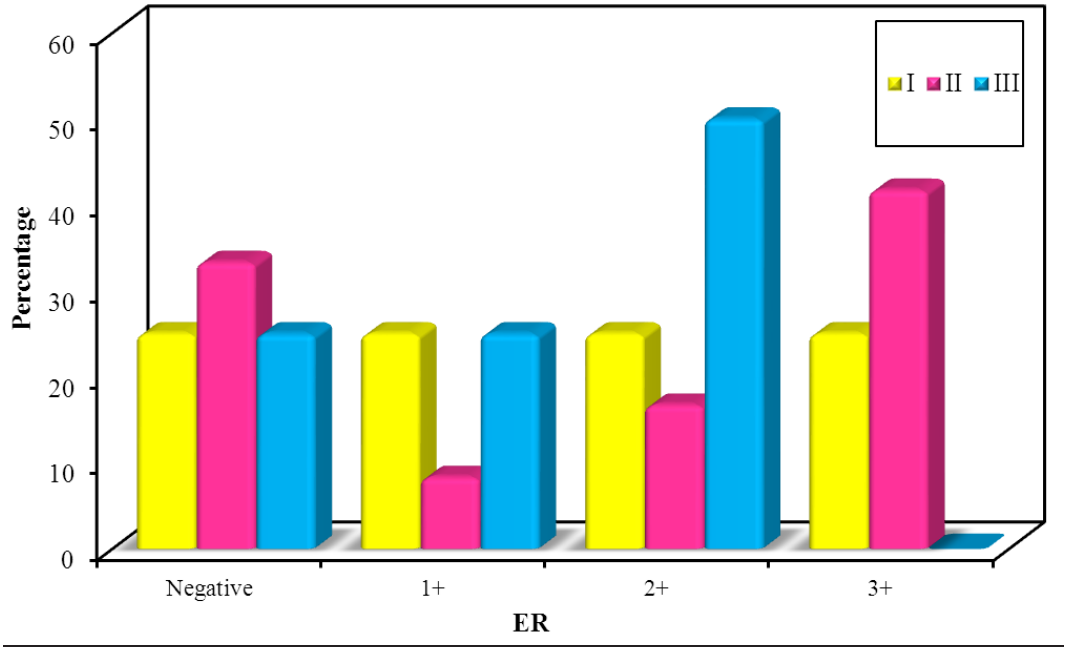

Fig. (11): Relation between tumor grade and ER in malignant groups (n = 20).

Relation between tumor size and ER in malignant groups

According to the immunohistochemical staining results illustrated in table (4) and figure (12), 28% (2/7) of entoto cases showed ER moderate expression (2+), while 50% (2/4) of 3cm were ER weak positive (1+), 40% (2/5) of 4cm were ER weak positive (1+) and 50% (2/4) of 6cm were ER weak positive (1+).

Table (4): Relation between tumor size and ER in malignant groups (n = 20)

Estrogen receptor (ER)	Tumor size								χ^2	^{MC}p
	Entoto (n = 7)		3 cm (n = 4)		4 cm (n = 5)		6 cm (n = 4)			
	No.	%	No.	%	No.	%	No.	%		
Negative(-ve)	2	28.6	0	0.0	1	20.0	0	0.0	4.709	0.979
Weak positive (+1)	2	28.6	2	50.0	2	40.0	2	50.0		
Moderate positive (2+)	2	28.6	1	25.0	1	20.0	2	50.0		
Strong positive (3+)	1	14.3	1	25.0	1	20.0	0	0.0		

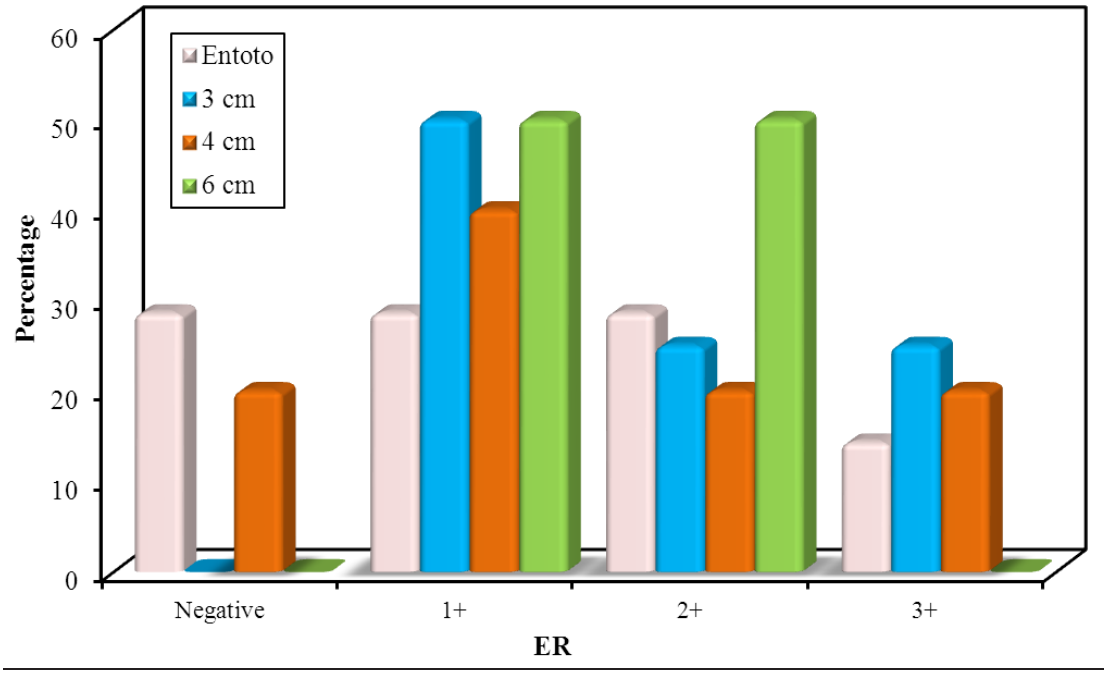

Fig. (12): Relation between tumor size and ER in malignant groups (n = 20).

Immunohistochemical results of estrogen receptor (ER):

Immunohistochemical staining of estrogen receptors is present in both normal endometrial tissue and endometrial cancer.

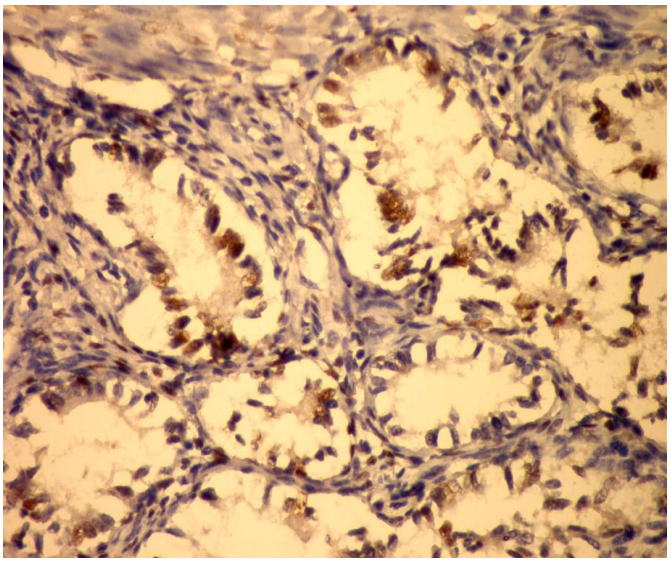

Fig. (13): A case of proliferative endometrium shows weak nuclear expression in endometrial glands for ER receptor (X400).

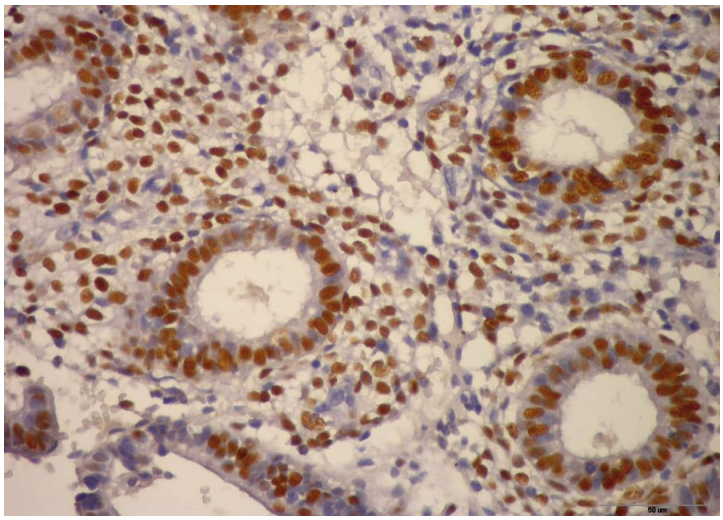

Fig. (14): A case of simple hyperplasia shows moderate nuclear expression in endometrial glands for ER receptor (X400).

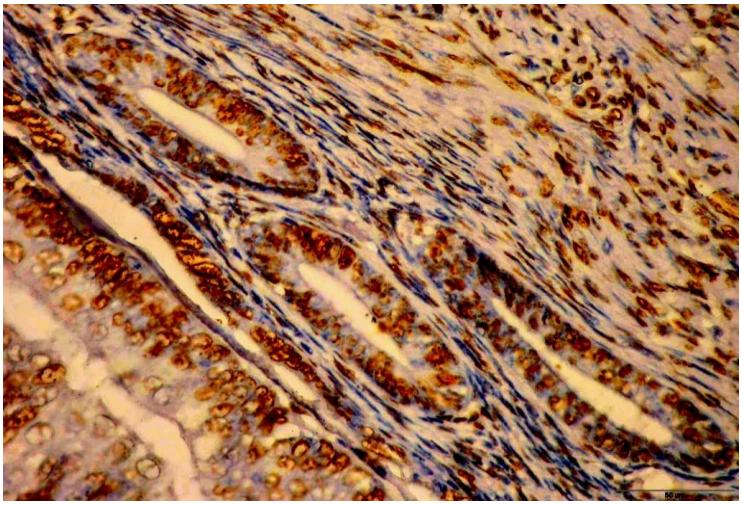

Fig. (15): A case of endometriod adenocarcinoma shows strong nuclear expression in endometrial glands for ER receptor (X400).

DISCUSSION

Endometrial carcinoma (EC) has been described as one of the most common malignant tumors of the female genital organs in industrial countries [(6)]. Endometrial hyperplasia is a common gynecological disorder, mainly due to prolonged unopposed estrogen stimulation. Microscopic studies showed varieties of endometrial hyperplasia which are classified as simple or complex hyperplasia and atypical hyperplasia that may progress to endometrial cancer [(7)].

In the current study, 50% (10/20) of benign cases were diagnosed as simple hyperplasia (S.H), while 40% (8/20) cases were simple endometrial hyperplasia with atypia (S.E.H with Atypia), 5% (1/20) cases were complex hyperplasia without atypia and 5% (1/20) cases were hyperplastic end polyp. The results were in agreement with Reed, et al (2010) [(8)] who reported that the majority of cases (73.3%) had atypical endometrial hyperplasia.

The studied endometrial tumor grade of the malignant cases (12/20, 60%) was diagnosed as grade II, while (4/20, 20%) was grade I and 20%

(4/20) of the malignant cases were grade III. This result is supported by a recent study by Davidson, et al, 2016[9] who demonstrated that most of the endometrial cancer cases undergoing surgical resection were at grade II and III.

The results of the present study showed that 40% (4/10) of the control group cases were in the age range >31-40 years, 45.5% (9/20) of the benign group cases were in the age range 41-50 and 55% (15/20) of the malignant group cases were at age ranges>60 years. Another study done by Singh, et al (2011) [10] revealed that the median age of malignant cases was 60 years, this follows the results of the present work.

Marked variation in tumor size in the present study was determined in 20 patients with endometrial malignant tumors. The smallest size entoto was 35% (7/20), while 25% (5/20) of cases were 4cm and two similar groups of 20% (4/20) were 3cm and 6 cm in size respectively.

Reproductive tract lining epithelium was characterized by the presence of a thick apical glycocalyx. Mucin appeared to serve a general function in protecting reproductive mucosa from bacterial pathogenesis [11]. Endometrial mucosa excretes mucin which is a glycoprotein rich in acid and neutral mucopolysaccharides. This mucin is most prominent in the apical border of the epithelial cells, where it can be demonstrated as a narrow rim or as luminal tips [12].

The results of alcian blue staining showed increased numbers of the goblet cells of the secretory lining of the endometrium with a marked density of mucin secretion as a diffuse reaction in the malignant groups. This finding suggested that the mucosal epithelial cells form a contiguous lining that acts as a barrier between the moist exterior environment and the remainder of the host. Similar data were also reported by Linden et al 2008[13] who demonstrated a statistically significant increase in malignant endometrial groups versus control and benign endometrial groups ($p<0.022$). This finding is in agreement with Al-Kapten IAH, (2005) [14] who indicated that mucin production was increased in malignant tumors compared to benign tumors.

The immunohistochemical staining method proved to be effective for the clinical determination of antibody protein expression owing to specific targeting of tumor cells. Nowadays, it is used in the investigation of a broad range of disease processes with applications in diagnosis, prognosis, and therapeutic decisions [15]. The present study was undertaken to assess the immunohistochemical expression of ER in human endometrial adenocarcinoma versus normal control and benign endometrial tumor and to investigate the correlation of their expressions with clinicopathological parameters.

Expression of hormone receptors (ER) in both normal and hyperplastic endometrium indicated their important role in the carcinogenesis of endometrial cancer associated with estrogen stimulation in conditions unopposed by progesterone. The highest expression of ER was demonstrated by the endometrioid subtype of endometrial cancer [16]. The present result revealed that immunohistochemical staining of ER illustrated a significant difference ($P>0.007$).

Furthermore, the present result revealed 60% (6/10) of control cases with ER-negative (-ve), 40% (8/20) of benign have ER moderate positive (2+) while 30% (6/20) of malignant cases were ER strong positive (3+). These findings were in agreement with those reported by Stoian, et al (2011) [17] who reported that the estrogen-positive receptors correlated significantly with early-stage and well-differentiated tumors.

Whereas there was no significant difference between the ER immunohistochemical staining and the tumor size. Our results showed that in the entoto cases 28% (2/7) showed ER moderate expression (2+), 50% (2/4) of 3cm and 6cm were ER weak positive (1+) and 40% (2/5) of 4cm showed the same ER weak positivity (1+). Also, no significant difference was revealed in the different tumor

grades. These findings were consistent with those reported by Maniketh, et al, (2014) [18] who illustrated that estrogen hormone may play a role in detecting the cancer cases of endometrial injury.

Competing interests:

Authors declare that they have no competing interests, financials, or others.

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