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Comparative Study of Immunohistochemical Expression of ER and PR as Prognostic Markers and Correlation with Clinicopathological Parameters in Human Endometrial Adenocarcinoma

Dr. Saeed Mahmoud Saeed Mohamed[1*], Afaf Mosaad Amin[2], Aisha Mohmmed Osman salih[3], Sabah Ali Mugahed Al-Qadasi[4], Roa Mohmed Mahmoud Sultan[5]

[1]Assistant Professor of Histopathology and Cytology Department, Faculty of Medical Laboratory Sciences, West Kurdofan University, Sudan
[2]Professor of Histochemistry and Cell Biology Department, Medical Research Institute, University of Alexandria, Egypt
[3]Assistant Professor of Biology and Biotechnology Department (Animal Physiology), Faculty of Science and Technology, Al Neelain University, Khartoum-Sudan
[4]Assistant Professor of Histology, Anatomy and Histology Department, Faculty of medicine and Health sciences, Sana'a University, Sana'a, Yemen
[5]Lecturer of Histopathology and Cytology Department, Faculty of Medical Laboratory Sciences, Sudan International University, Khartoum- Sudan

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***Corresponding author:** Dr. Saeed Mahmoud Saeed Mohamed
Assistant Professor of Histopathology and Cytology Department, Faculty of Medical Laboratory Sciences, West Kurdofan University, Sudan

Abstract **Original Research Article**

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. Estrogen and progesterone receptors are present both in endometrial hyperplasia and in neoplastic endometrium. The aim of the present work was to compare the expression of ER and PR as prognostic biomarkers in human endometrial adenocarcinoma versus benign tumors and normal endometrial tissues as well as their correlation with different pathological and histological parameters. Immunohistochemical technique was used to examine the expression of ER and PR in normal, benign as well as in endometrial adenocarcinoma. Present results showed higher expression of ER and PR in endometrial adenocarcinoma comparing to normal and benign endometrial tissues.

Keywords: ER and PR prognostic marker.

INTRODUCTION

Endometrial cancer ranks as the second most common gynecological cancer and the sixth most common cancer overall among women in the United States [1]. Endometrial cancer appears most frequently during perimenopause and menopause, between the ages of 50 and 65. Overall, 75% of endometrial cancer occurs after menopause. Women younger than 40 make up 5% of endometrial cancer cases and 10–15% of cases occur in women under 50 years of age, This age group is at risk for developing ovarian cancer at the same time, The worldwide median age of diagnosis is 63 years of age [2].

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]. The endometrial carcinoma is formed and develops in close relation to the plasma and tissue levels of sex steroidal hormones and their receptors (65). Estrogen and progesterone receptors are present both in endometrial hyperplasia and in neoplastic endometrium [4].

Estrogen receptors (ER) and progesterone receptors (PR) are among the steroid hormones that regulate 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, expression of ER and PR in Endometrial adenocarcinoma was investigated using immunohistochemical technique and the intensity of

immunostaining was quantitatively estimated using image optical density (IOD) analyzer.

MATERIAL AND METHODS

The present study was carried out on 50 prospective biopsies obtained from El-shat by 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 to the study and informed written consents were gathered prior to their inclusion in the study protocol, according to the ethical guidelines of the Faculty of Medicine, Alexandria University. Hematoxlin and eosin (H&E) stained slides for each patient were reviewed by two pathologists. Diagnosis of the specimens was made according to the WHO classification of the Tumors. Clinical parameters included patients' age, tumor size, lymph node metastasis (LNM).

Immunohistochemical Investigation of ER and PR

Immunohistochemical method was utilized to study the expression of ER and PR 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 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 serum blocking reagent for 30 minutes to block nonspecific binding. The sections were incubated with primary antibody for ER and PR at 4ºC overnight. Sections were treated with conjugated 2nd antibody (ABC-HRP reagent) for 30 minutes, stained with diaminobenzedine (DAB) and counter stained with hematoxylin. For negative controls, antibody was replaced with PBS. Each step was followed by PBS washing. Evaluation of ER and PR 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 statistical software package SPSS 20. P values≤ 0.05 were considered statistically significant.

RESULTS

A -Histopathological Results

a. Haematoxylin and Eosin (H&E) Staining

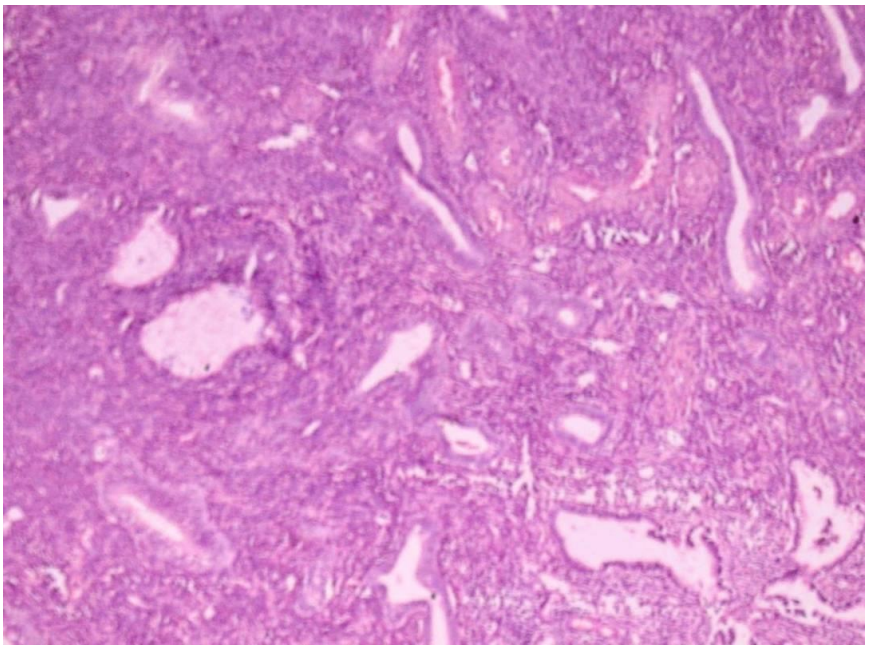

Fig. 1: Endometrial polyp showing proliferative glands within fibroblastic stroma entangling thick walled vessels (H&E X100)

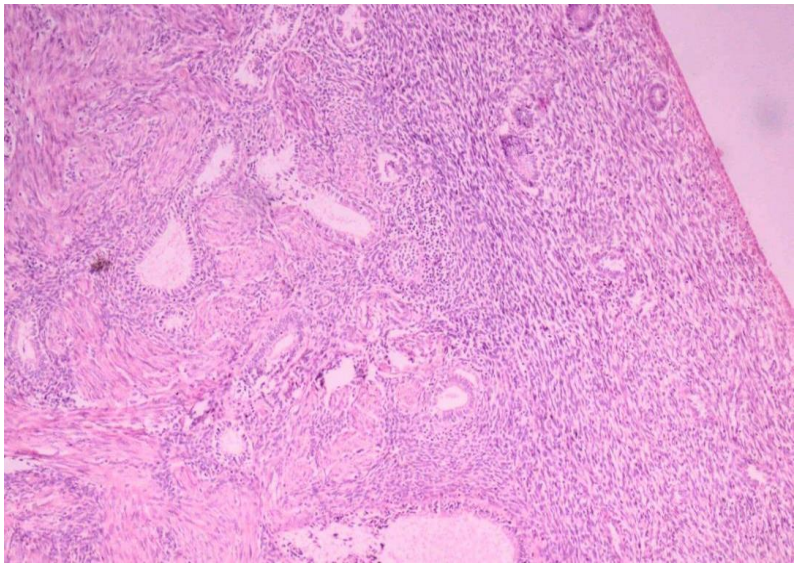

Fig. 2: Proliferative endometrial tissue showing few proliferative acini within endometrial spindled stroma (H&E X100)

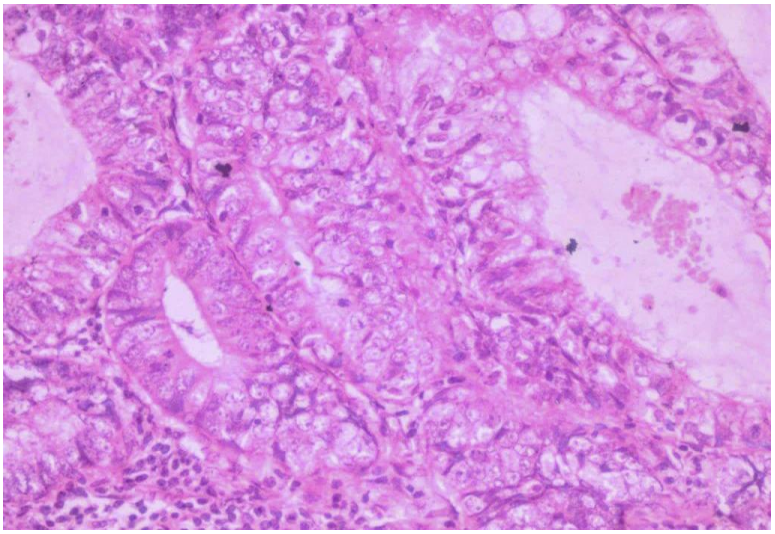

Fig. 3: Well differentiated adenocarcinoma showing large fused acini lined by columnar cells with ample vacuolated eosinophelic cytoplasm (H&E X400)

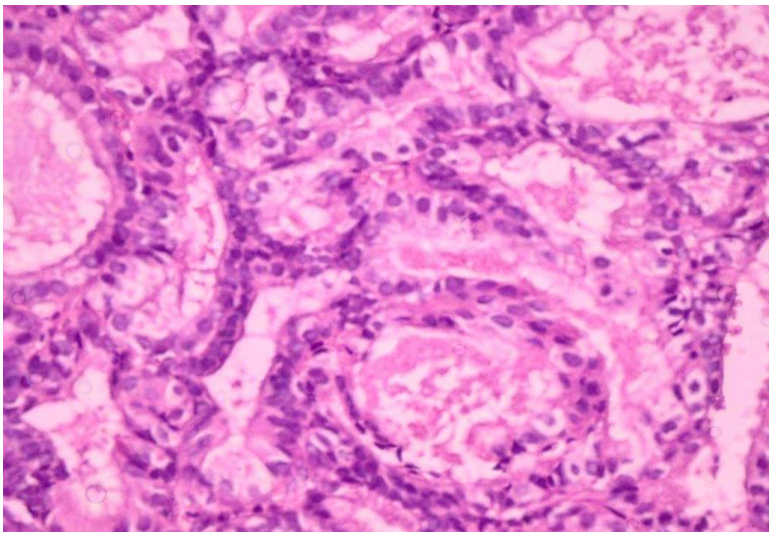

Fig. 4: A case of moderate differentiated endometrial adenocarcinoma showing multiple dilated closely packed and fused glands lined by malignant columnar cells with eosinophilic cytoplasm (H&E X400)

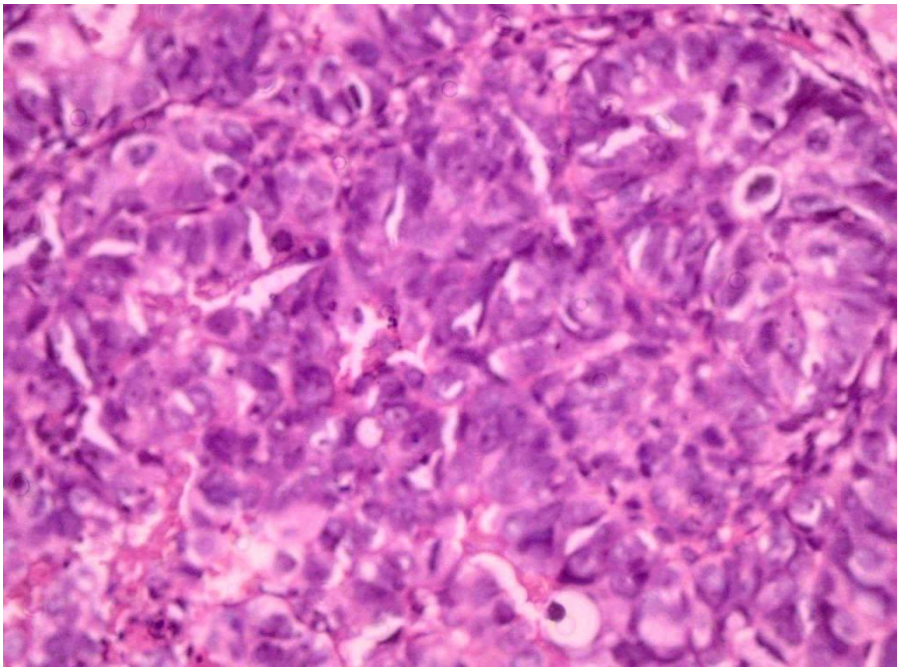

Fig. 5: A case of poorly differentiated endometriod adenocarcinoma composed of few glands and solid areas of malignant epithelial cells (H&E X400)

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.

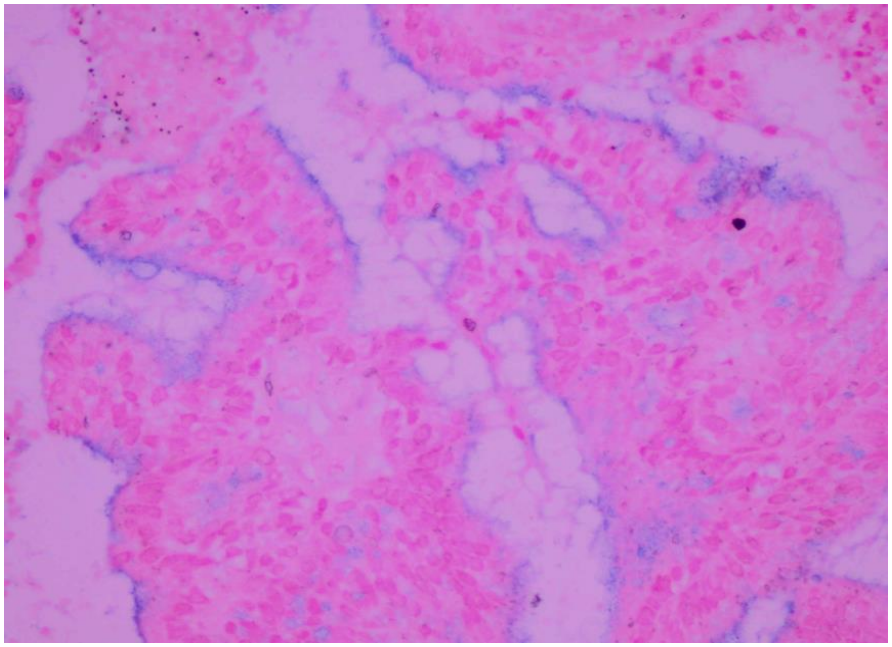

Fig. 6: Proliferative endometrium stained with alcian blue showing low number of the goblet cells with weak mucin activity(X400)

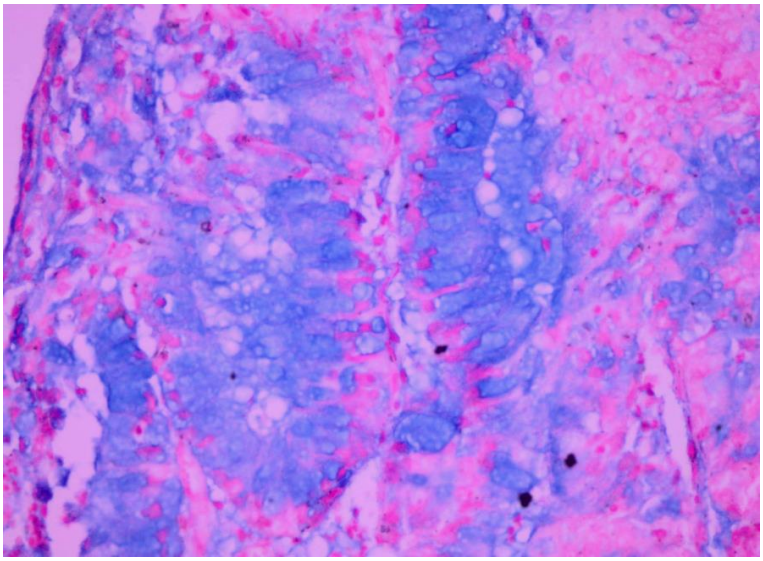

Fig. 7: Hyperplastic end polyp stained with alcian blue showing increase number of the goblet cells with moderate mucin staining(X400)

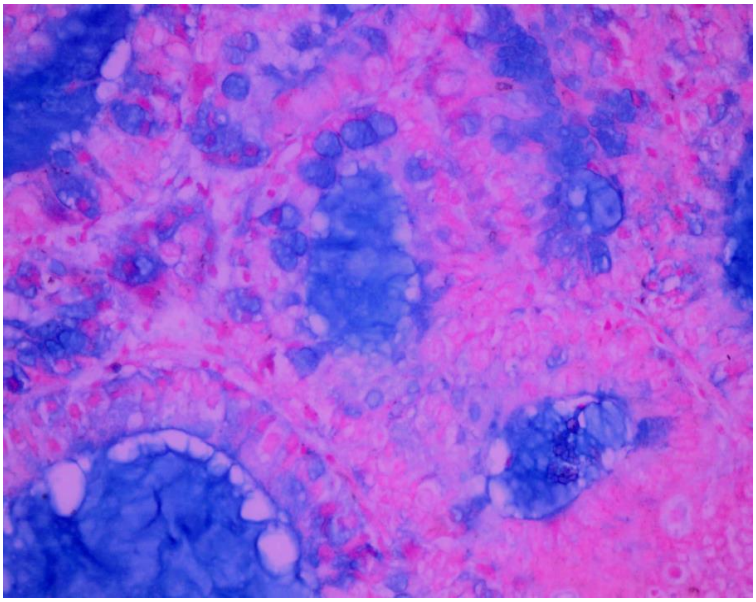

Fig. 8: Endometrial carcinoma stained with alcian blue showing high increase in number of the goblet cells with strong mucin secretion(X400)

Quantitative Evaluation

Staining of mucin secretion was performed on endometrial tissues using 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 high 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	5.342*	0.022*
Mean ± SD.	152.61 ± 30.01	164.35 ± 11.10	190.76 ± 6.92		
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 Post Hoc Test (LSD)
*: Statistically significant at $p \leq 0.05$

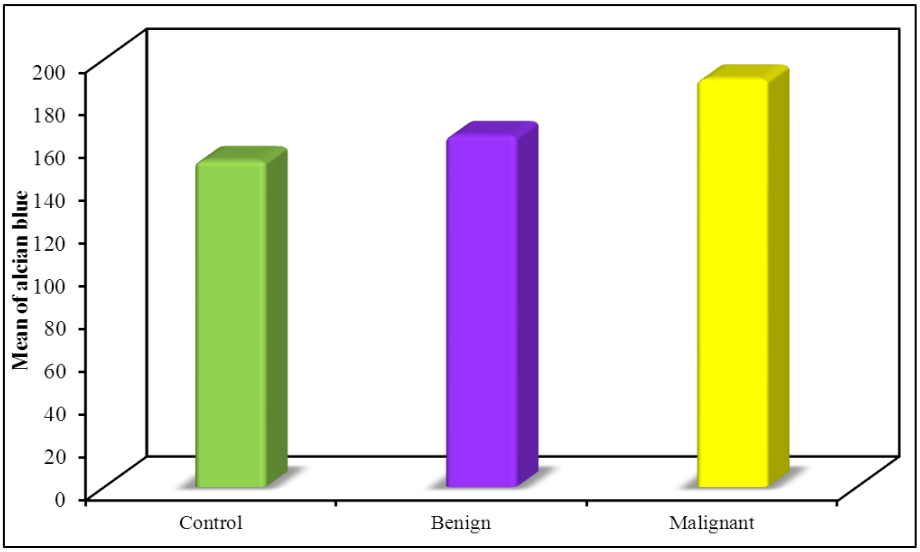

Fig. 9: Comparison between the three studied groups stained with alcian blue

C. Immunohistochemical Results

1. Estrogen Receptor(ER)

The immunohistochemical staining results were 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 was done using Chi square test

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

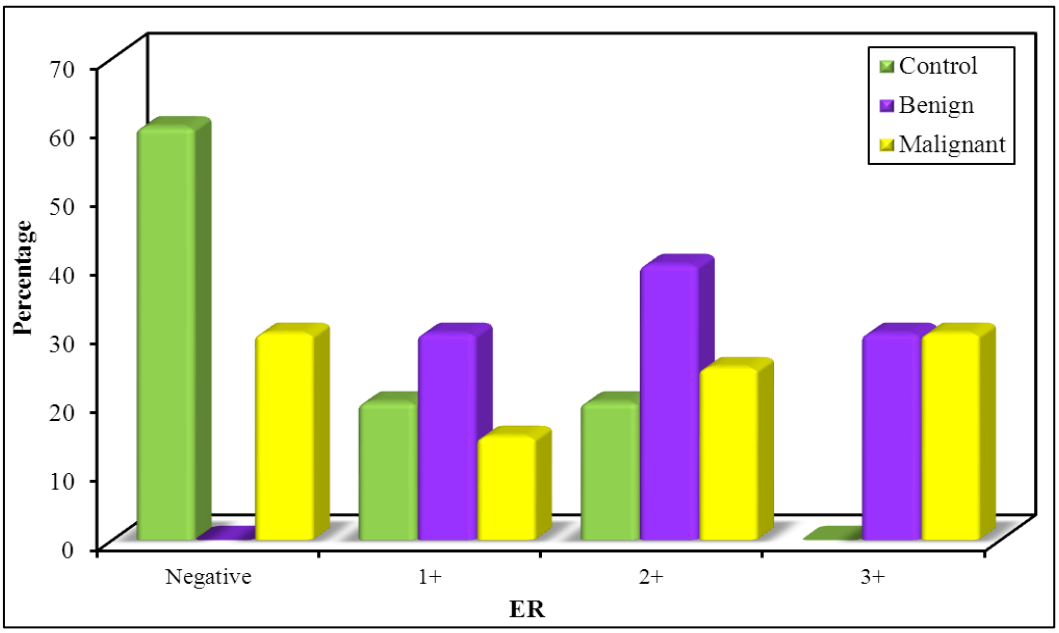

Fig. 10: Comparison between ER of the three studied groups

Relation between Tumor Grade and ER in Malignant Groups

As regards the grade of malignant groups, the immunohistochemical staining results were illustrated in table (3) and figure (11). There was 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 case. The results illustrated no significant difference between the three grades.

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

	Grade						χ^2	^{MC}p
Estrogen Receptor(ER)	**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		

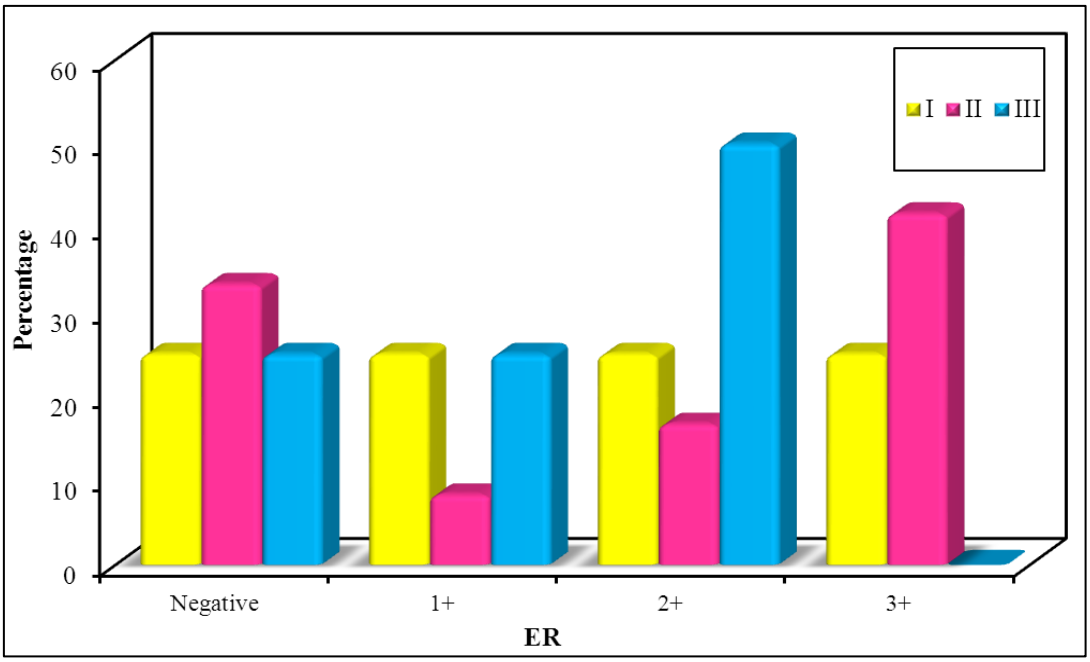

Fig. 11: Relation between tumor grade and ER in malignant groups (n = 20)

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)

	Tumor size								χ^2	^{MC}p
Estrogen receptor(ER)	**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		

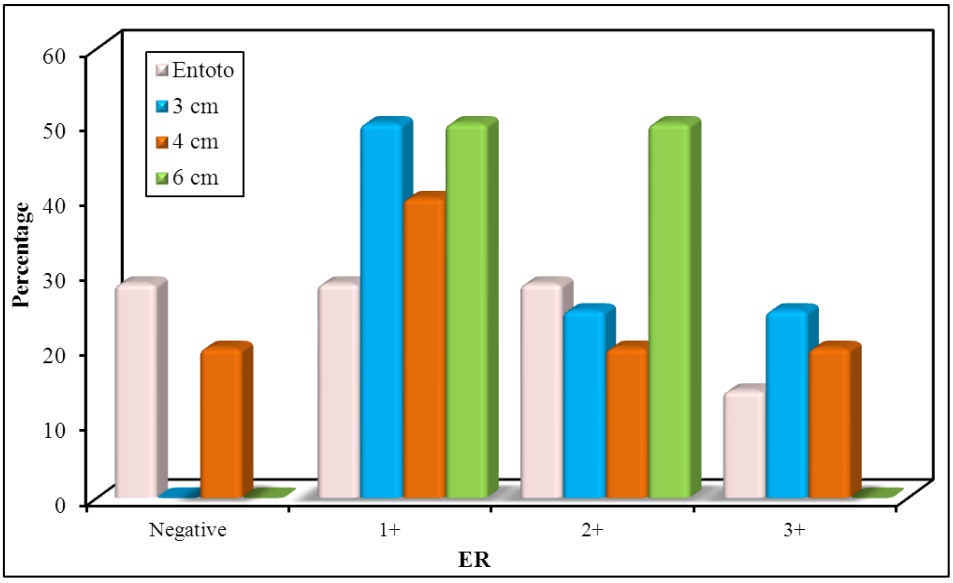

Fig. 12: Relation between tumor size and ER in malignant groups (n = 20)

Immunohistochemical Results of Estrogen Receptor (ER)

Immunohistochemical staining of estrogen receptor are present in both normal endometrial tissue and endometrial cancer.

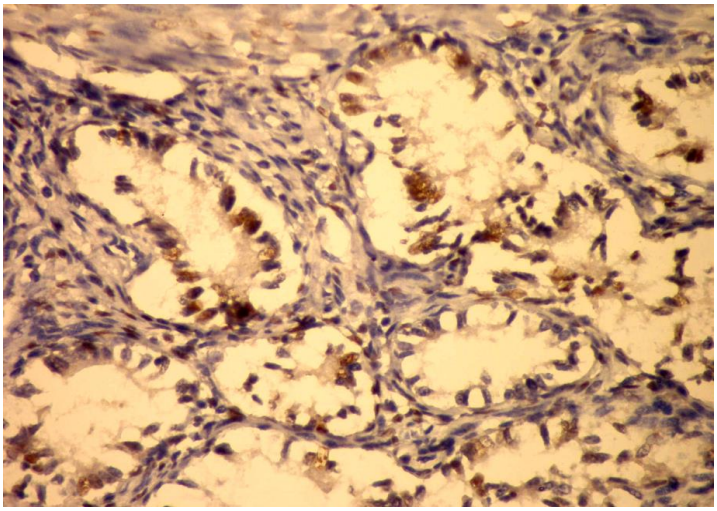

Fig. 13: A case of proliferative endometrium shows weak nuclear expression in endometrial glands for ER receptor (X400)

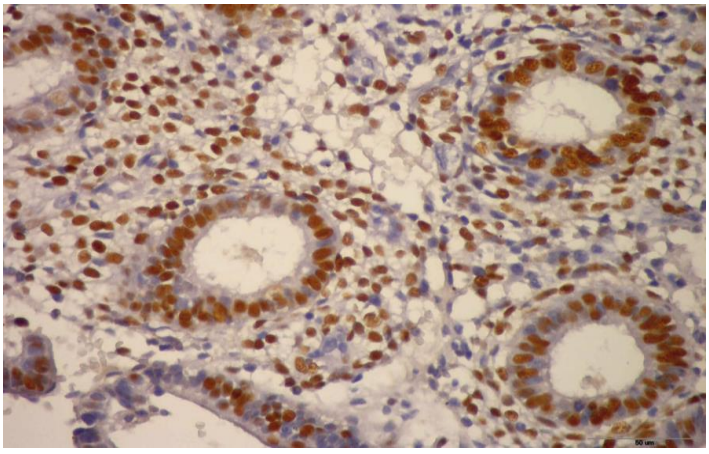

Fig. 14: A case of simple hyperplasia shows moderate nuclear expression in endometrial glands for ER receptor (X400)

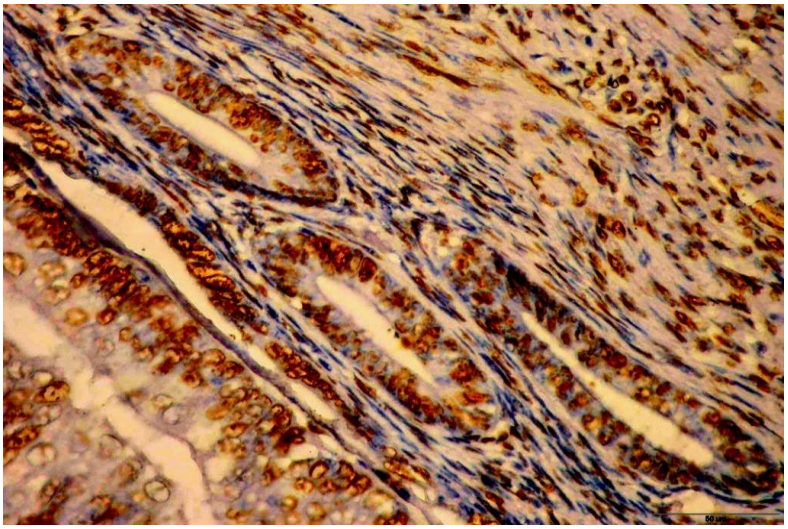

Fig. 15: A case of endometriod adenocarcinoma shows strong nuclear expression in endometrial glands for ER receptor (X400)

2-Progesterone Receptor (PR)

Immunohistochemical staining results of PR showed that 60% (6/10) of control cases were PR weak positive, while 55% (11/20) of benign were PR strong positive (3+) and40%(8/20) of malignant cases were PR strong positive (3+) (Table 5 figure 16).

Table 5: Comparison between PR of the three studied groups

Progesterone Receptor(PR)	Control (n = 10)		Benign (n = 20)		Malignant (n = 20)		χ^2	^{MC}p
	No.	%	No.	%	No.	%		
Negative(-ve)	0	0.0	0	0.0	4	20.0	24.707*	<0.001*
Weak positive(1+)	6	60.0	6	30.0	0	0.0		
Moderate positive(2+)	4	40.0	3	15.0	8	40.0		
Strong positive(3+)	0	0.0	11	55.0	8	40.0		
Sig. bet. grps.	$^{MC}p_1$= 0.009*, $^{MC}p_2$<0.001*, $^{MC}p_3$= 0.002*							

χ2, p:χ2 and p values for Chi square test, sig. bet. Groups were done using Chi square test

*: Statistically significant at p ≤ 0.05

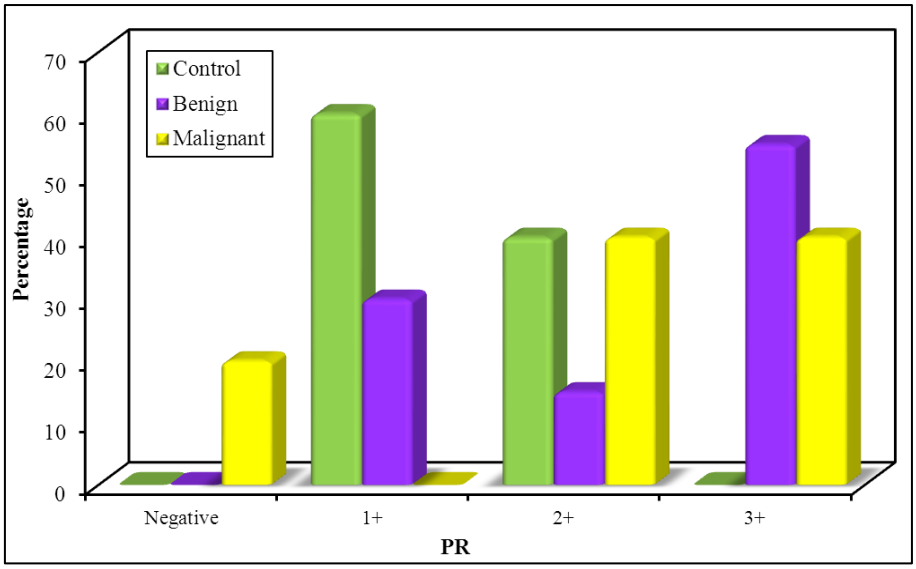

Fig. 16: Comparison between PR of the three studied groups

Relation between Grade and PR in Malignant Groups

Immunohistochemical staining results illustrated in table (6) and figure (17), 50% (2/4) of grade I cases were PR moderate positive (2+), while 50% (6/12) of grade II were PR strong positive (3+) and50%(2/4) of grade III were PR strong positive (3+) .

Table 6: Relation between grade and PR in malignant groups (n = 20)

	Grade						χ^2	^{MC}p
Progesterone Receptor (PR)	**I (n=4)**		**II (n=12)**		**III (n=4)**			
	No.	**%**	**No.**	**%**	**No.**	**%**		
Negative(-ve)	2	50.0	1	8.3	1	25.0	5.111	0.239
Weak positive(1+)	0	0.0	0	0.0	0	0.0		
Moderate positive(2+)	2	50.0	5	41.7	1	25.0		
Strong positive(+3)	0	0.0	6	50.0	2	50.0		

χ2, p:χ2 and p values for Chi square test
MCp: p value for Monte Carlo for Chi square test

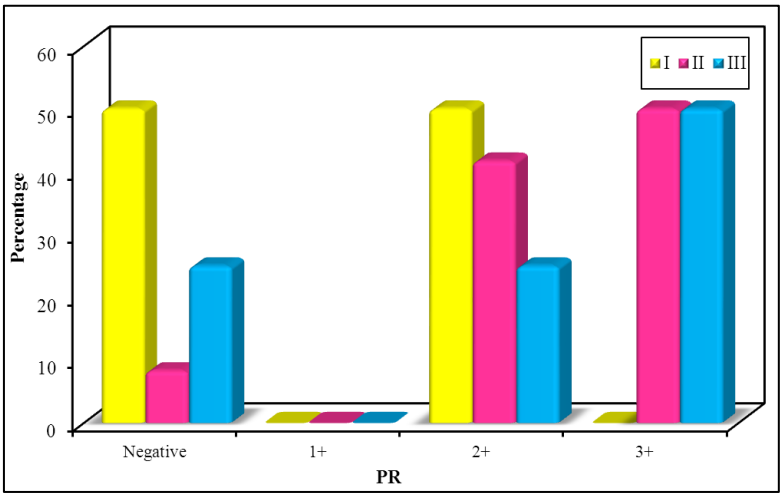

Fig. 17: Relation between grade and PR in malignant groups (n = 20)

Relation between Tumor Size and PR in Malignant Groups

The immunohistochemical staining results illustrated in table (7) and figure (18), 57%(4/7) of entoto cases were PR strong positive (3+), while 50%(2/4) of 3cm were PR moderate positive (2+),40%(2/5) of 4cm were PR strong positive (3+) and 50%(2/4) of 6cm were PR strong positive (3+).

Table 7: Relation between tumor size and PR in malignant groups (n = 20)

	Tumor size								χ^2	^{MC}p
Progesterone Receptor(PR)	**Entoto (n = 7)**		**3 cm (n = 4)**		**4 cm (n = 5)**		**6 cm (n = 4)**			
	No.	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**		
Negative(-ve)	0	0.0	2	50.0	2	40.0	0	0.0	7.451	0.264
Weak positive(1+)	0	0.0	0	0.0	0	0.0	0	0.0		
Moderate positive(2+)	3	42.9	2	50.0	1	20.0	2	50.0		
Strong positive(3+)	4	57.1	0	0.0	2	40.0	2	50.0		

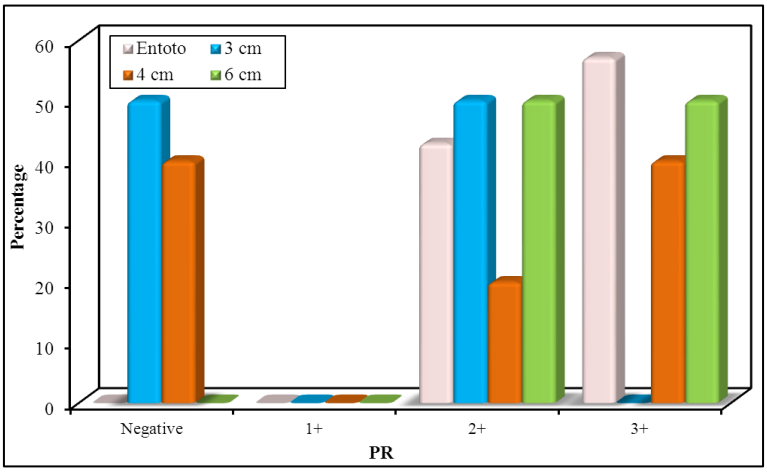

Fig. 18: Relation between tumor size and PR in malignant groups (n = 20)

Immunohistochemical Results of Progesterone Receptor

Immunohistochemical staining of progesterone receptor present in both normal endometrial tissue and endometrial cancer.

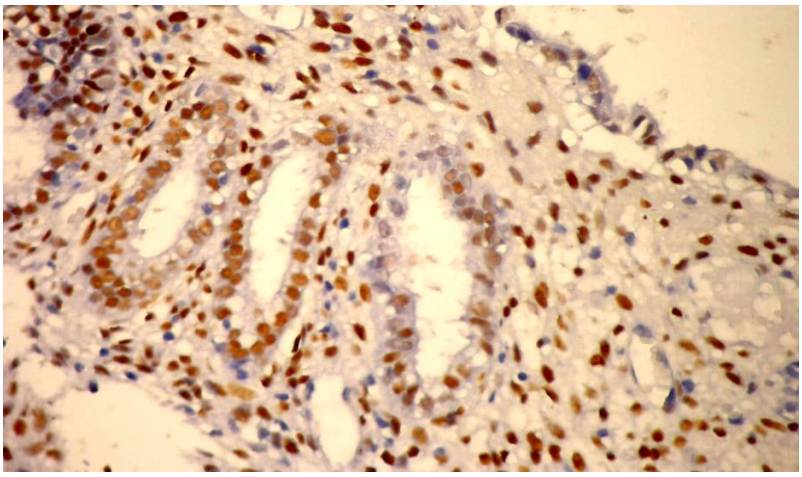

Fig. 19: A case of proliferative endometrium shows weak nuclear positivity of PR receptor(x400)

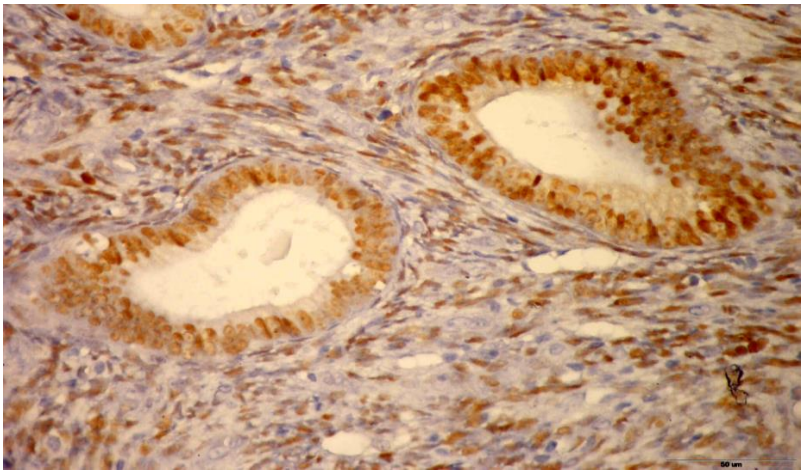

Fig. 20: A case of simple hyperplasia shows moderate nuclear positivity of PR receptor (x400)

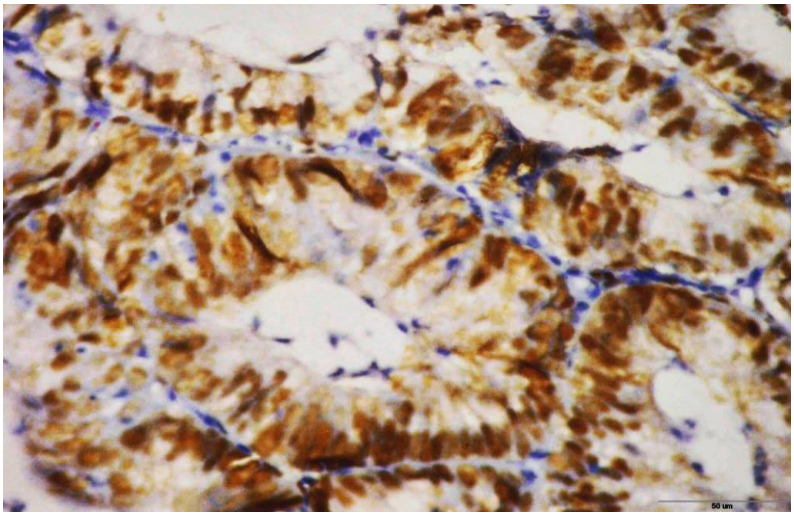

Fig. 21: Strong nuclear positivity of PR receptor in secretory carcinoma (x400)

DISCUSSION

Endometrial carcinoma (EC) has been described as one of the common malignant tumors of the female genital organs in the 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%) diagnosed as grade II, while (4/20, 20%) as grade I and 20% (4/20) of the malignant cases were grade III. This result is supported by 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 at age range >31-40 years,45.5% (9/20) of the benign group cases were at age range 41-50 and 55% (15/20)of the malignant group cases were at age ranges>60 years. In another study done by Singh, *et al.,* (2011) [10] revealed that the median age of malignant cases was 60 years, this is in accordance with the results of the present work.

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

Immunohistochemical staining method proved to be effective for clinical determination of antibody proteins 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 and PR 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 and PR) in both normal and hyperplastic endometrium indicated their important role in carcinogenesis of endometrial cancer associated with estrogen stimulation in conditions unopposed by progesterone. The highest expression of ER and PR was demonstrated by the endometrioid subtype of endometrial cancer [16]. The present result revealed that immunohistochemical staining of ER & PR illustrated a significant difference ($P>0.007$) ($P>0.001$) respectively.

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 a 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 and progesterone positive receptors correlated significantly with early stage and well differentiated tumors.

Whereas there were no significant difference between the ER immunohistochemical staining and the tumor size. Our results showed that 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 by the different tumor grades. The same results were illustrated in the correlation of immunohistochemical staining of the PR with the tumor size and tumor grades. These findings consistent with those reported by Maniketh, *et al.,* (2014) [18] who illustrated that estrogen and progesterone hormones may play a role in detecting the cancer cases of the endometrial injury.

The pattern of the number positive cells and the degree of immunoreactivity intensity for ER and PR was important in high-grade endometrioid tumors [19]. Therefore, the steroid hormones, estrogen and

progesterone, play a significant role in the pathogenesis of endometrial carcinoma, the most frequently type which account more than 80% of cases. Estrogenic action unopposed by progesterone induced sequential malignant changes in the endometrium by atypical hyperplastic changes [20].

REFERENCES

1. Asyikeen, W. A. W. N., Jalil, N. A. C., Zin, A. A. M., & Othman, N. H. (2016). Median Survival Time of Endometrial Cancer Patients with Lymphovascular Invasion at the Hospital Universiti Sains Malaysia. *The Malaysian journal of medical sciences: MJMS*, *23*(6), 44-51.
2. Soliman, P. T., & Lu, K. H. (2013). Neoplastic diseases of the uterus. In: Lentz GM, Lobo RA, Gershenson DM, Katz VL (Eds). Comprehensive gynecology. 6th ed. Philadelphia: Elsevier Mosby; 23-39.
3. Kuiper, G. G., Shughrue, P. J., Merchenthaler, I., & Gustafsson, J. Å. (1998). The estrogen receptor β subtype: a novel mediator of estrogen action in neuroendocrine systems. *Frontiers in neuroendocrinology*, *19*(4), 253-286.
4. Sivridis, E., Giatromanolaki, A., Koukourakis, M., & Anastasiadis, P. (2001). Endometrial carcinoma: association of steroid hormone receptor expression with low angiogenesis and bcl-2 expression. *Virchows Archiv*, *438*(5), 470-477.
5. Montejo, M., Werner, T. L., & Gaffney, D. (2009). Current challenges in clinical management of endometrial cancer. *Advanced drug delivery reviews*, *61*(10), 883-889.
6. Jemal, A., Siegel, R., Ward, E., Hao, Y., Xu, J., Murray, T., & Thun, M. J. (2008). Cancer statistics, 2008. *CA: a cancer journal for clinicians*, *58*(2), 71-96.
7. Goncharenko, V. M., Beniuk, V. A., Kalenska, O. V., Demchenko, O. M., Spivak, M. Y., & Bubnov, R. V. (2013). Predictive diagnosis of endometrial hyperplasia and personalized therapeutic strategy in women of fertile age. *EPMA Journal*, *4*(1), 1-20.
8. Reed, S. D., Newton, K. M., Garcia, R. L., Allison, K. H., Voigt, L. F., Jordan, C. D., ... & Weiss, N. S. (2010). Complex hyperplasia with and without atypia: clinical outcomes and implications of progestin therapy. *Obstetrics and gynecology*, *116*(2 Pt 1), 365-73.
9. Davidson, B. A., Foote, J., Clark, L. H., Broadwater, G., Ehrisman, J., Gehrig, P., ... & Havrilesky, L. J. (2016). Tumor grade and chemotherapy response in endometrioid endometrial cancer. *Gynecologic Oncology Reports*, *17*, 3-6.
10. Singh, M., Darcy, K. M., Brady, W. E., Clubwala, R., Weber, Z., Rittenbach, J. V., ... & Leslie, K. K. (2011). Cadherins, catenins and cell cycle regulators: impact on survival in a Gynecologic Oncology Group phase II endometrial cancer trial. *Gynecologic oncology*, *123*(2), 320-328.
11. Wira, C. R., Grant-Tschudy, K. S., & Crane-Godreau, M. A. (2005). Epithelial cells in the female reproductive tract: a central role as sentinels of immune protection. *American journal of reproductive immunology*, *53*(2), 65-76.
12. Sorvari, T. E. (1969). A histochemical study of epithelial mucosubstances in endometrial and cervical adenocarcinomas. With reference to normal endometrium and cervical mucosa. *Acta pathologica et microbiologica Scandinavica. Supplement*, *207*, 1+.
13. Linden, S. K., Sutton, P., Karlsson, N. G., Korolik, V., & McGuckin, M. A. (2008). Mucins in the mucosal barrier to infection. *Mucosal immunology*, *1*(3), 183-197.
14. Al-Kapten, I. A. H. (2005). Study of mucins in epithelial ovarian tumors. *J Fac Med Baghdad*, 47, 4.
15. Ramos-Vara, J. A., & Miller, M. A. (2014). When tissue antigens and antibodies get along: revisiting the technical aspects of immunohistochemistry—the red, brown, and blue technique. *Veterinary pathology*, *51*(1), 42-87.
16. Carcangiu, M. L., Chambers, J. T., Voynick, I. M., Pirro, M., & Schwartz, P. E. (1990). Immunohistochemical evaluation of estrogen and progesterone receptor content in 183 patients with endometrial carcinoma: part I: clinical and histologic correlations. *American journal of clinical pathology*, *94*(3), 247-254.
17. Stoian, S. C., Simionescu, C., Margaritescu, C. L., Stepan, A., & Nurciu, M. (2011). Endometrial carcinomas: correlation between ER, PR, Ki67 status and histopathological prognostic parameters. *Rom J Morphol Embryol*, *52*(2), 631-636.
18. Maniketh, I., Ravikumar, G., Crasta, J. A., Prabhu, R., & Vallikad, E. (2014). Estrogen and progesterone receptor expression in endometrioid endometrial carcinomas: a clinicopathological study. *Middle East Journal of Cancer*, *5*(2), 67-73.
19. Wei, J. J., Paintal, A., & Keh, P. (2013). Histologic and immunohistochemical analyses of endometrial carcinomas: experiences from endometrial biopsies in 358 consultation cases. *Archives of Pathology and Laboratory Medicine*, *137*(11), 1574-1583.
20. Pozharisskiĭ, K. M., Samsonova, E. A., Ten, V. P., Maksimova, N. A., & Urmancheeva, A. F. (2005). Immunohistochemical profile of endometrioid adenocarcinoma of the uterus: ER, PR, HER-2, Ki-67 and their prognostic value. *Arkhiv patologii*, *67*(2), 13-17.