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Immunohistochemical Expression of Oestrogen and Epidermal Growth Factor Receptors in Endometrial Cancerous in Sudanese Patients

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Abstract

Endometrial carcinoma is the most prevalent gynecologic cancer in industrialized countries and the second most common in developing countries after cervical cancer; it is the fourth most commonly diagnosed cancer. The purpose of this study is to evaluate the immunohistochemistry expression of oestrogen receptor (ER) and Her-2/neu in Sudanese patients with endometrial carcinoma (EMC). A retrospective descriptive study of archival formalin-fixed paraffin-embedded tissue from hysterectomy specimens acquired at Khartoum's Charity Teaching Hospital. Immunohistochemistry was performed on formalin-fixed, paraffin-embedded tissue samples from 40 patients with EMC. The immunohistochemistry method was used to examine the specimens for ER and Her2neu expression. SPSS was used to analyze the data, and the significance of the connection of these receptors' expression and histological grade of tumors was determined. In 30% of the cases, ER and Her-2/neu expressions were positive. Positive Her2neu expression was connected with the menopausal state (P-value ≤ 0.05), whereas ER was associated with Her2neu (P-value ≤ 0.05). The relationship between ER and Her2neu expression and histological grade and age in endometrial cancer was statistically insignificant. There is a statistically significant relationship between ER and Her2neu.

Keywords: Human epidermal growth factor receptor 2, Estrogen receptor, Estrogen, Epidermal growth factor

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Introduction

Endometrial carcinoma is the most frequent gynecologic malignancy in industrialized countries, the second most prevalent in developing countries after cervical cancer, and the fourth most common cancer in women (Siegel *et al.*, 2019). EMC is the third most frequent gynecological malignancy in South-East Asia, behind cervical and ovarian carcinomas (Srijaipracharoen *et al.*, 2010). EC has been on the rise in Europe, the United States, and other parts of the world in recent years. It is prevalent in high-resource countries, but its prevalence is increasing in low-resource countries as obesity rates rise and life expectancy increases (Linkov *et al.*, 2008).

ECs, or malignancies of the uterine lining, are classified into two histologic subgroups. The most frequent histologic form is type 1 endometrioid adenocarcinoma, which accounts for about 90% of endometrial cancer cases and is further differentiated based on the histological grade of tumor differentiation. Grade 1 (well-differentiated), 2 (moderately differentiated), and 3 (not differentiated) (poorly differentiated). Serous papillary and clear cell adenocarcinoma are examples of type 2 endometrial cancer. Grade 3 (poorly differentiated) More aggressive tumor biologic characteristics include adenocarcinoma, clear cell adenocarcinoma, and serous papillary (Freeman *et al.*, 2012).

Oestrogen, an essential mitogen, works by binding to its receptor; it is found in 50-80% of breast tumors. The effects of oestrogen are counteracted by endocrine therapies. Therapeutic hormones, such as Tamoxifen, function competitively as anti-oestrogen in breast tissue; it blocks oestrogen and so antagonizes transcriptional activation of genes essential for tumor growth. The existence of hormone receptors in tumor tissue is related to how well patients respond to hormone treatment and chemotherapy. Oestrogen hormone receptors positive for endometrial cancer, the hormone receptors cause endometrial cells to develop uncontrollably and cause the endometrium to thicken, promoting the proliferation of cancer cells (Linkov *et al.*, 2008; Halle *et al.*, 2018; Alhuzaim, *et al.*, 2020; Almehmadi, *et al.*, 2020).

Tumor development is influenced by gene changes, oncogene overexpression, and cell-cycle regulation. HER2 belongs to the human epidermal growth factor receptor (EGFR) family of transmembrane tyrosine kinases, which also includes EGFR

(HER1, ErB1), HER2/neu (ErB2), HER3 (ErB2), and HER4 (ErB4) (ErB2). Ligand interaction causes receptors to dimerize (homodimerize or heterodimerize), which causes phosphorylation of intracellular domains, which then activates multiple pathways involved in proliferation, survival, migrating, and development. Overexpression of HER2 causes ligand-independent dimer formation and constitutive activation of the kinase domain, resulting in increased cell proliferation. HER2 amplification and overexpression were believed to be involved in the pathogenesis of a variety of cancers, such as breast, ovarian, gastric, and esophageal cancer (Bancroft & Gamal, 2008; Konecny *et al.*, 2009; Diver *et al.*, 2015). Sudan has a high rate of EC (Yassin *et al.*, 2016). Cells divide and expand at an uncontrollable rate when the HER2 gene is faulty. EC can be caused by gene changes. EC with oestrogen hormone receptors and HER2 receptors causes endometrial cells to expand uncontrollably and the endometrium to thicken, promoting the formation of cancer cells. Knowing the status of these hormone receptors in endometrial cancer would thus be extremely beneficial in terms of therapy options and tumor prognosis (Wang *et al.*, 2020).

The immunohistochemistry expression of ER and Her2neu in endometrial cancer in Sudanese women in Khartoum State was examined in this study. Also, looking at the connection between tumor marker expression and age, menopausal state, and histopathological grading.

Materials and Methods

From January 2019 to September 2020, a retrospective facility-based cross-sectional study was conducted at Khartoum's Charity Teaching Hospital in Khartoum State, Sudan, on a sample of 40 women with endometrial cancer, on whom archival formalin-fixed paraffin-embedded tissue of hysterectomy specimen was performed.

After Hysterectomy tissues were collected in the operating room in 10% formalin, histopathological and immunohistochemical methods were conducted. Following fixation, excellent, well-prepared sections were taken. The tissue is contained within appropriately labeled plastic tissue cassettes. Tissue processing is carried out using an automated tissue processor machine for tissue dehydration, cleaning, and wax impregnation. Paraffin blocks are made using a paraffin embedding center.

Tissue sections were obtained on a slide, then de-waxed in xylene for two different changes for ten minutes each. They were rehydrated in different grades of alcohols, absolute alcohols (100 percent) for seven minutes, 90% for five minutes, and 70% for three minutes, before being washed in distilled water for two minutes. After re-hydration, slides were stained with hematoxylin for 10 minutes, then blued in running tap water for 15 minutes, counterstained with eosin for 7 minutes, then dehydrated with 70%, 90%, and 100% alcohols. After 2 minutes, each slide was air-dried, cleaned in xylene, and mounted with DPX (Bancroft & Gamal, 2008).

One section (3) of formalin-fixed, paraffin-embedded tutors was cut and mounted onto salinized slides (Thermo). Following xylene deparaffinization, slides are rehydrated in a graded series of alcohols before being placed in distilled water. Using a water bath, samples were steamed for antigen retrieval for ER and HER2. Endogenous peroxidase activity was inhibited for 10 minutes with 3% hydrogen peroxide and methanol. In a moisture chamber, slides were incubated with 100-200 l of primary antibodies for 20 minutes at room temperature. After 3 minutes of washing with PBS, antibody binding was detected by incubating for 20 minutes with dextran-labeled polymer (Thermo -ultra vision). Finally, the sections were washed three times in PBS before being stained with 3, 3 diaminobenzidine tetrahydrochloride (DAB) as a chromogen for up to 5 minutes to produce the typical brown stain for the visualization of the antibody/enzyme combination. Hematoxylin was used to stain the slides (Srijaipracharoen *et al.*, 2010).

Assessment of Immunohistochemical expression done by two pathologists blindly to their clinical examined all specimens and pathological manifestations, the reactivity of ER expressed in nuclei while for HER-2 expressed in membranous either positive for brown DAB chromogen and **n**egative for non-reactive.

Statistical Analysis

The collected data were computerized using an Epi-Info 7 template and analyzed using SPSS 23. The information was summarized quantitatively (mean, standard deviation, and median) as well as graphically (frequency, tables, and graphics). The Fisher test was used to analyze the relationship between categorical variables. All statistical tests were judged significant when the P-value was less than 0.05.

Results and Discussion

This study assessed the status of receptors (her2neu and ER) in endometrial cancer. The connection of receptor status (ER and Her2neu) with histological kinds and grade of endometrial cancer was investigated in this study. Endometrial adenocarcinoma was found in all of the cases investigated. Participants aged 40 years to 79 years with a mean age of 63 years ±11. The majority (72.5%, 29/40) were aged ≥ 60 years and the remaining 27.5% (11/40) were < 60 years old. Most of the participants were postmenopausal (42.5%, 17/40), 30.0% (12/40) were menopause and 27.5% (11/40) were pre-menopausal. According to histopathological grading, 6 patients (15%) had grade 1 tumors; 12 patients (30%) had grade 2 tumors, while 22 (55%) patients had grade 3 tumors. It has been revealed that 30.0% (12/40) of the patients showed positive Her2neu expression, while 70.0% (28/40) did not. In addition, 12/40 patients showed positive expression on ER, 28/40 patients showed no expression of the marker **(Table 1)**.

A statistically significant association (P-value = 0.021) was found between ER and Her2neu receptors. Whereas, the association between ER, age groups, and histological grade were insignificant with a p-value of respectively 0.876 and -0.999, as shown in **Table 2**.

Table 3 displayed that a significant association (P-value = 0.040)

was found between her2neu and menopausal status. Whereas, the association between Her2neu, age groups, and histological grading were insignificant (P-value =0.278 and 0.140 respectively).

Oestrogen (ER) nuclear expression is positive in endometrial cancer (Grade 2 moderately differentiated). 40x, as shown in **Figure 1**. **Figure 2** exhibited nuclear Oestrogen (ER) expression in endometrial cancer (grade 1 well-differentiated), whereas, **Figure 3** represented membrane Her2neu expression in endometrial adenocarcinoma. (Poorly differentiated grade 3). Membranous expression of Her2neu in endometrial adenocarcinoma (Grade 2 moderately differentiated) as shown in **Figure 4**.

Table 1. The distribution of age group, menopausal status, histological grade, and ER in endometrial carcinoma

Parameter	No (%)
Age groups	
40-49 years	8 (20%)
50-59 years	3 (7.5%)
60-69 years	13 (32.5)
70-79 years	16 (40%)
Menopausal status	
Pre-menopausal	11 (27.5%)
Menopausal	12 (30%)
Post-menopausal	17 (42.5%)
H&E histological grading	
Well-differentiated	6 (15%)
Moderately differentiated	12 (30%)
Poorly differentiated	22 (55%)
HER-2 in endometrial carcinoma	
Positive	12 (30%)
Negative	28 (70%)

Table 2. Frequency of ER receptors and their associations with age groups, menopausal status, H&E histological grading of the tumor, and Her2Neu (n=40).

Variables		ER		Fisher's Exact Test P-value
		Positive	Negative	
Age groups	40-49 years	2	6	0.876
		25.00%	75.00%	
	50-59 years	1	2	
		33.30%	66.70%	
	60-69 years	5	8	
		38.50%	61.50%	
	70-79 years	4	12	
		25.00%	75.00%	
Menopausal status	Premenopausal	3	8	0.619
		27.30%	72.70%	
	Menopausal	5	7	
		41.70%	58.30%	
	Post-menopausal	4	13	
		23.50%	76.50%	
H&E histological grading	Well-differentiated	2	4	0.999
		33.30%	66.70%	
	Moderately differentiated	3	9	
		25.00%	75.00%	
	Poorly differentiated	7	15	
		31.80%	68.20%	
HER-2	Positive	7	5	0.021*
		58.30%	41.70%	
	Negative	5	23	
		17.90%	82.10%	

*P≤ 0.05

Table 3. Frequency of Her2neu receptors and their associations with age groups, menopausal status, H&E histological grading of the tumors (n=40).

Variables		HER-2		Fisher's Exact Test P-value
		Positive	Negative	
Age groups	40-49 years	1	7	0.278
		12.50%	87.50%	
	50-59 years	2	1	
		66.70%	33.30%	
	60-69 years	5	8	
		38.50%	61.50%	
	70-79 years	4	12	
		25.00%	75.00%	
Menopausal status	Pre-menopausal	1	10	0.040*
		9.10%	90.90%	
	Menopausal	7	5	
		58.30%	41.70%	
	Post-menopausal	4	13	
		23.50%	76.50%	
H&E histological grading	Well-differentiated	2	4	0.140
		33.30%	66.70%	
	Moderately differentiated	1	11	
		8.30%	91.70%	
	Poorly differentiated	9	13	
		40.90%	59.10%	

*P≤ 0.05

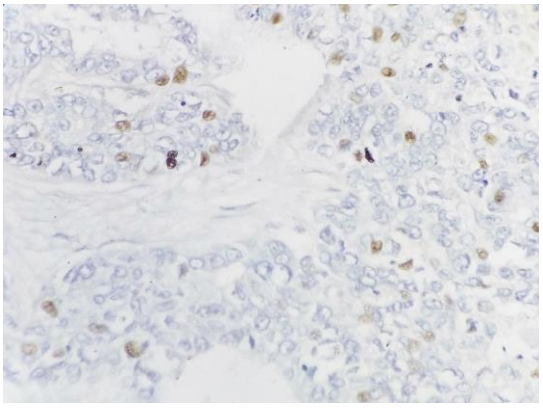

Figure 1. Photograph Show positive nuclear expression of Oestrogen (ER) in endometrial adenocarcinoma (Grade 2 moderately differentiated). 40x

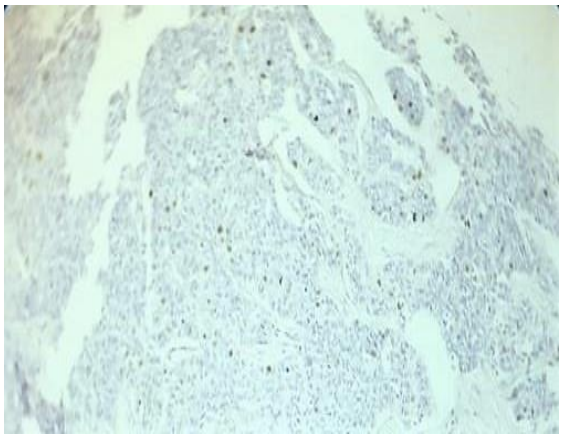

Figure 2. Photograph shows the nuclear expression of Estrogen (ER) in endometrial adenocarcinoma (grade 1 well-differentiated) 10x

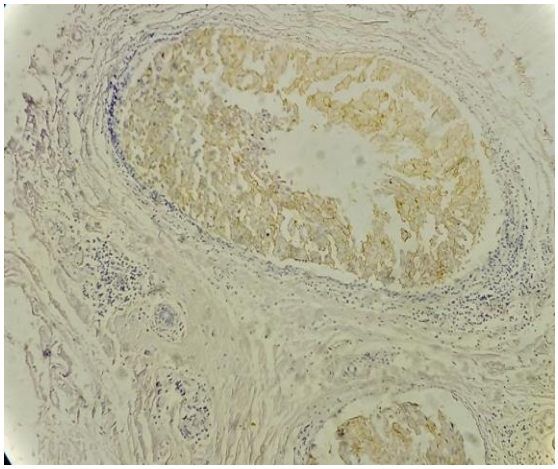

Figure 3. The photograph shows the membranous expression of Her2neu in endometrial adenocarcinoma. (Grade 3 poorly differentiated) 10x.

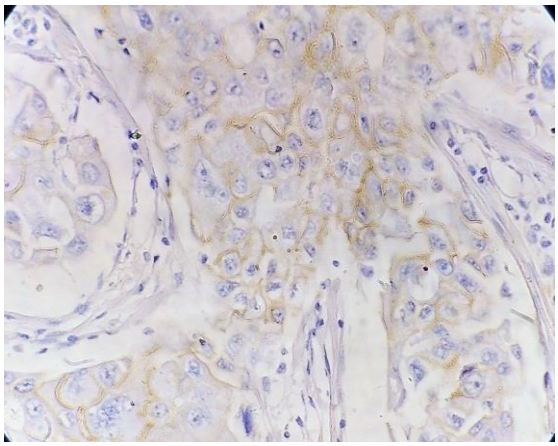

Figure 4. The photograph shows the membranous expression of Her2neu in endometrial adenocarcinoma (Grade 2 moderately differentiated). 40x

This research investigated the status of receptor expression (her2neu and ER) in endometrial cancer. The relationship between receptor status (ER and Her2neu) and the histological type and grade of endometrial cancer was studied. Endometrial adenocarcinoma was found in all of the cases investigated. 29 patients (72.5 %) were 60 and older, whereas 11 patients (27.5%) were younger than 60 years old. This finding is consistent with the findings of Salama *et al.*, 2019 who found that endometrial adenocarcinoma was more common in the postmenopausal period (42.5 percent), that the association between ER and Her2neu receptors was statistically significant, but not between ER and age groups, or between ER and histological grade. This finding is in accordance with Sirijaipracharoen *et al.*, 2010 finding that ER association with age group was statistically insignificant, as well as Waqar *et al.*, 2018 who reported that ER association with histological grading was not statistically significant.

Her2neu and menopausal status were shown to have a significant association, but Her2neu and age groups, as well as Her2neu and histological grading, were found to be insignificant with P values of 0.278 and 0.140, respectively. Sirijaipracharoen *et al.*, 2010 affirmed these findings in a study on ER, PR, and Her-2/neu expression and their association with tumor histological grade, concluding that ER expression is a good prognostic factor, Her-2/neu expression appears to be a poor indicator for both disease-free and overall survival, and PR showed impact for only disease-free survival of Thai EMCs (Srijaipracharoen *et al.*, 2010). Another study concluded that the associations of PR and ER with histological types, grades, and stages were not significant. However, there is a significant association of Her-2/neu receptors with a high grade of Endometroid and Serous carcinoma (Salama *et al.*, 2019). Furthermore, two studies on the expressions of these hormones (Carcangiu *et al.*, 1990; Mohapatra, 2019) concluded that overexpression of HER2 and ki67 and low expression of ER and PR indicate a more malignant EC behavior, whereas expression of ER, PR, and Her2neu, similar to breast cancer, may be useful to determine treatment and prognosis, particularly in developing countries.

In endometrial tumorigenesis, the understanding of the different pathways that work could lead to a new method of anticancer drugs target molecules. Currently, Anti-EGFR targeted therapies are available for breast cancer. New anticancer medicines targeted at molecular targets on endometrial cancer cells are required, and certain molecules, such as ER and HER-2, have been suggested. ER and HER-2 are strong candidates for a potential molecular target for monoclonal therapies against these receptors in endometrial cancer because of the high expression of this receptor in endometrial cancer, the invasion, and dissemination, the biological role on tumor growth, the cell membrane position, and the influence on patient prognosis (Wang *et al.*, 2020).

Conclusion

According to the findings of this investigation, adenocarcinoma was the most common type of cancer. The expression of ER and Her-2/neu increased with age and menopausal status; it also enhanced the prevalence of endometrial cancer. ER and Her-2/neu were shown to be more abundant in poorly differentiated cells (Grade 3). There was a link between ER expression and Her2neu,

which could assist with diagnosis. In poorly differentiated tumors, ER and Her-2/neu are abundantly expressed.

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