

Immune-expression of Estrogen receptors Alpha in Oral mucosa among Sudanese patients with Breast cancer

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ABSTRACT

Background: The immune expression of Estrogen receptors (ERs) in oral mucosal cells is still controversial. This study aimed to detect ER alpha (ER α) in oral mucosa of Sudanese patients with breast cancer.

Methods: In this a retrospective case control study, the immune expression of ER α was evaluated by immunocytochemistry (ICC) using the avidin biotin technique on buccal smears obtained from 50 breast cancer patients (forty of them were known to be ERs positive in breast tissues) and 50 apparently healthy females.

Results: ER α expression was neither detected in the oral mucosacells of the patients with breast cancer nor in the study control group. 80% of the patients in this study were categorized as ERs positive in the breast biopsy by immunohistochemical method (IHC). The relationship between the ERs expression and the use of contraceptive pills was statistically significant ($P < 0.001$). The participants of some tribes showed 100% of ERs expression in their breast tissues. The invasive ductal carcinoma (IDC) cases showed positive ER immune expression more frequently compared to invasive lobular carcinoma (ILC) cases ($P < 0.001$).

Conclusion: ER α immunoeexpressing was not detected in the oral mucosal cells of the breast cancer patients by ICC. We recommended to use RT-PCR to detect the mRNA ERs expression in oral mucosa to get a conclusive result about the ERs expression in oral mucosa.

Keywords: Breast cancer, Estrogen receptors, ER α , Oral Mucosa, Immunocytochemistry, Immunohistochemistry, Sudan.

INTRODUCTION

Female breast cancer is one of the most frequently diagnosed cancer (11.6%). The expected number of new cases of breast cancer in 2040 in Sudan is expected to reach 11,254 compared to 5,677 in 2018 with the highest rate of mortality (17.1%) compared to other types of cancers according to the WHO Cancer country profiles 2020¹.

The biological effects of the female sex hormone Estrogens are primarily mediated by its binding and activation of ER α and ER β which act as a DNA binding transcription factor that regulates gene expression. There is an increasing evidence that estrogen is linked not only to mammary tumorigenesis, ovarian and endometrial carcinogenesis. But also, a play crucial role in regulating breast cancer progression and cancer stem cell fat².

Two hypotheses have been proposed to explain such relation: Firstly, binding of estrogen to the ER stimulates proliferation of mammary cells, with the resulting increase in cell division and DNA replication leading to mutations. Secondly, estrogen metabolism produces genotoxic waste. The result of both processes is disruption of cell cycle, apoptosis and DNA repair and therefore tumor formation³.

Both ERs are widely expressed in different tissue types e.g., in endometrium, breast cancer cells, ovarian stroma cells, hypothalamus, kidney, brain, bone, heart, lungs, intestinal mucosa, prostate, endothelial cells and oral tissues^{4,5}.

The histology of oral tissues looks like vaginal mucosa as well as its response to the sex hormones. ERs observed primarily in gingival tissue keratinocytes and salivary gland acinar and ductal cells this explain the effect of hormonal changes on the oral mucosa as well as on saliva secretion and composition in response to estrogen⁶.

Once the breast cancer is diagnosed, the most important step in the treatment strategy and prognosis is to test estrogen and progesterone receptors status. This test is usually done by getting a biopsy from the breast, which requires considerable experience from the specialist and is somewhat painful for patient. Buccal mucosal and salivary gland biopsies can be analyzed by real time-polymerase chain reaction (PCR) and Immunohistochemistry (IHC) to detect the expression of ERs as well⁷.

To the best of our knowledge, this is the first study in Sudan to test out the possibility of ER α detection in the smear of oral mucosa of patients with breast cancer using ICC, which is simple, painless, and does not require much time in processing.

MATERIALS AND METHODS

This is a retrospective case control study, carried out among breast cancer patients attending the Radiation and Isotopes Center of Khartoum (RICK). A total of 100 participants were examined in this study, 50 breast cancer patients. The control group involved 50 apparently healthy, single females of childbearing age as control. All participants were informed about the aim of the study and

asked for their approval before taking the sample. Tongue depressors were used to obtain cytological smears from participants. By gently scraping the inside of cheek and gingival epithelium, the cells were collected, and smeared on clean, Silanized slides. After air drying at room temperature for 1-3 hours, all smears were fixed in absolute acetone for 30 minutes, air dried, and then immunostained using monoclonal antibodies (M7047- Estrogen Receptor α Dako™) by avidin biotin technique for detection of ERs. Smears were treated with peroxidase blocking solution for 10 mins, washed in phosphate buffer saline (PBS) of PH 7.2, and then incubated in primary antibody for 1 hour (monoclonal mouse antihuman). After washing with PBS, smears incubated in biotinylated bridge reagent for 30 mins, then repeatedly washed with PBS and incubated in labeled streptavidin for 30 mins. Diaminobenzidine tetrahydrochloride (DAB) substrate solution was then added and left for 7 mins, before washing for counter stain with Mayer's hematoxylin for 1-2 min. It was then dehydrated, cleared, and mounted. All smears underwent cytological assessment, the ERs located in the nucleus, if present were seen as a brown color against the blue background.

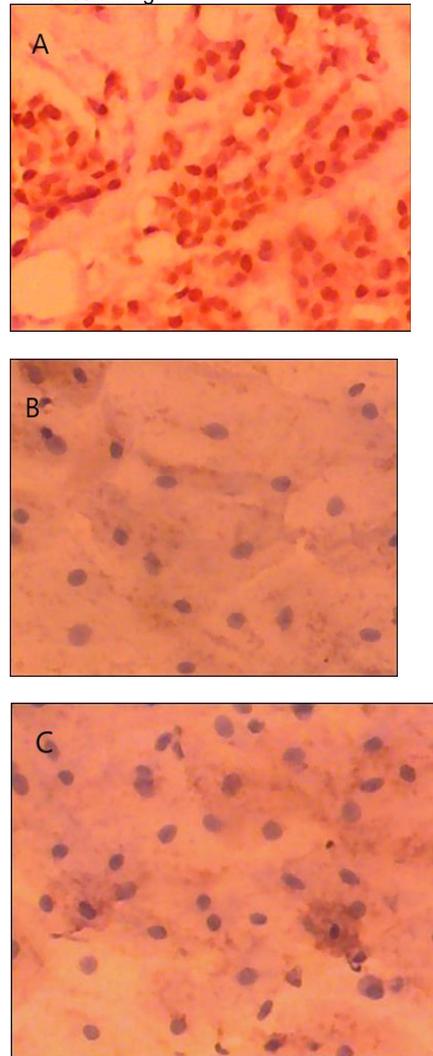
Formalin-fixed paraffin-embedded pretreatment breast biopsy specimens were sectioned at 5- μ m thickness, deparaffinized, and retrieved by water-bath retrieval technique for 30 minutes. For IHC, Using the same monoclonal antibodies (M7047- Estrogen Receptor α Dako™) in addition to negative control antisera were used; The endogenous peroxidase activity was quenched by incubating the specimen for five minutes with 3% hydrogen peroxide. Each specimen was then incubated with appropriate monoclonal primary antibody, followed by sequential 10-minute incubations with biotinylated link antibody and peroxidase labeled streptavidin. Staining was completed after five minutes incubation with a freshly prepared substrate chromagen solution. The slides were then counterstained in Mayer's hematoxylin, dehydrated, and prepared for microscopy. The staining intensity was scored, the ERs positive appeared as a brown color against the blue background.

The obtained results were analyzed by using the Statistical Package for the SPSS. Descriptive statistics were generated according to case-control results.

RESULTS

ER α expression was neither detected in the oral mucosa cells of the patients with breast cancer nor in the study control group. Sensitivity was 53% (95% confidence interval [CI], 44–61%) and specificity 93% (95% CI, 81–99%). Forty patients (80%) in this study were categorized as ERs positive in the breast biopsy by immunohistochemical methods (Figure 1). The mean age of the study subjects was 35 year. The peak expression was detected in the age group between 41-50 years (Table 1).

Figure 1: Results of positive and negative estrogen receptor immunostaining:



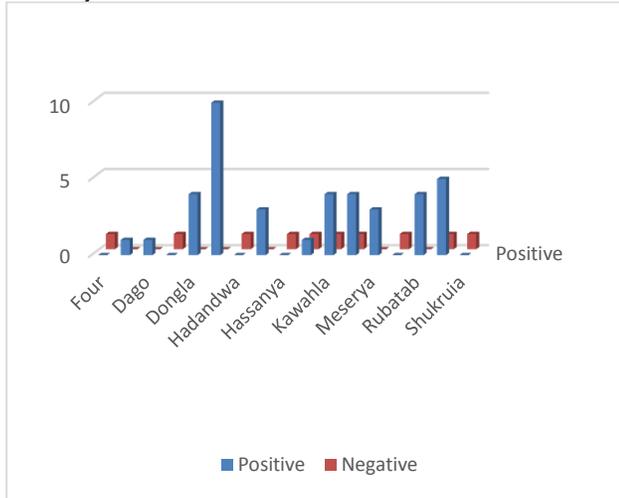
A: ERs positive staining (IHC) in breast biopsy of one patient. B: Smear from buccal cavity of normal female (control) shows negative ER α immunostaining (ICC). C: Smear from buccal cavity of one breast cancer patient shows negative ER α immunostaining (ICC).

Table 1: Estrogen receptor expression among cases by age

Age group	ER status in breast		Total
	Positive	Negative	
< 30	0	2	2
30-50	27	6	33
51>	13	2	15
Total	40	10	50

The ERs expression among patients from different Sudanese tribes was varied. The participants of some tribes showed 100% of ERs expression in their breast tissues. While others were negative. The frequency of the participant tribes was shown in figure 2.

Figure 2: ERs expression in the issues of patients with breast cancer by ethnic tribes



The relationship between the ERs expression and the use of contraceptive pills was statistically significant ($P < 0.001$). 14 (28%) of cases had been reported using contraceptive pills at some point during their lifetime. 24% of them showed immunoeexpressing of ERs. 72% of cases had never used contraceptive pills, 56% were ERs positive. Concerning the age at which cases reach the menarche: 96% of cases attained puberty at ≥ 12 years, while only 4% reached menarche before 12 years. Seemingly having more than 2 children does not protect against breast cancer. Most patients (74%) had more than 2 children while the rest had less than 2.

20% of cases had a family history of breast cancer in their first-degree relatives, all of them (100%) expressed the ERs in their breast tissues. 30 patients showed positive ER immune expression without family history. The relationship between family history and ER expression is statistically significant ($P < 0.001$).

The relationship between type of breast cancer and ER expression is also statistically significant ($P < 0.001$). 90% of cases were diagnosed with invasive ductal carcinoma (IDC), 74% showed positive ER immune expression and 16% were negative. Meanwhile, only 5 patients had invasive lobular carcinoma (ILC), 6% of them were ERs positive (Table 2).

Table 2: Relationship between the expression of ERs in the breast tissues of the patients and different conditions

Status	ERs status in breast		Total	P values*
	Positive	Negative		
Contraceptive pills				
Yes	12(24%)	2(4%)	14	$P < 0.001$
No	28(56%)	8(16%)	36	
Family history				
Yes	10(20%)	0	10	$P < 0.001$
No	30(60%)	10(20%)	40	
Type of breast cancer				
Yes	37(74%)	8(16%)	45	$P < 0.001$
No	3(6%)	2(4%)	5	

*P values were calculated using a Chi square test

**IDC: invasive ductal carcinoma

***ILC: invasive lobular carcinoma

DISCUSSION

The prevalence of breast cancer in Sudan was found to be 3.9 cases per 100,000 female populations⁸. It is essential to examine the estrogen and progesterone receptors status in the affected tissue for the prognosis and treatment follow up.

In this study, we aimed to test out the immune expression ER in oral mucosa of breast cancer patients by using simple buccal smear to get rid of the complications associated with the breast biopsy or even FNA⁹ which occasionally led to some post procedure complication e.g., pain, bruising and rarely hematoma, infection, and pneumothorax¹⁰. Buccal smears are relatively hypercellular and does not require much training. Contrary to what we had hoped, the ER α expression was neither detected in the oral mucosa cells of the patients with breast cancer nor in the study control group. Our result is in accordance with Leimola *et al*, they could not detect ERs in oral tissues by IHC; however, the ER mRNA expression was detected by RT-PCR⁷. Perhaps because of a very low level of expressed protein or difficulties in recognizing the epitopes by IHC.

The positive ERs expression in the breast tissue was detected in 54% of the patients with breast cancer of age between 30 -50 years in the current study. This is in agreement with the study conducted by Al-Sweer *et al*, they found that the most females (70%) of 50 years or younger expressed ERs more than those above 50 years¹¹. This finding opposes Alvarez *et al*. who found higher ERs positivity among Cuban women over age 50¹².

Our finding revealed that there was variation of ERs expression among patients from different Sudanese tribes. An ethnic difference in hormone receptor defined subtypes of breast cancer was reported, and ER status varied significantly across racial/ethnic groups even within the same tumor stage¹³.

Associations between oral contraceptive use and breast cancer incidence have been reported. 28% of cases using contraceptive pills, 24% showed positive ERs immune expression. This statistically significant relationship is consistent with Tewarim *et al* and Lower *et al*^{14,15}. They found significantly more ERs positive expression in breast cancer among premenopausal and postmenopausal users of exogenous estrogen (oral contraceptive or hormone replacement therapy) compared to non-users.

Number of child births and age at menarche are hormonal factors thought to influence breast cancer risk. In this study, most patients 74%, had a great number of pregnancies (2+) and 96% attain puberty at 12 years and above. These findings suggest no relationship between these factors and breast cancer risk in Sudanese women. On the other hand, Yavari *et al*, conclude that number of births was significantly associated with breast cancer although early menarche was not a significant risk factor among Iranian women¹⁶. Ewertz and Duffy concluded that the risk decreased by increasing monarchical age and there was increased risk associated with never being pregnant¹⁷. Likewise, Kishk detected increasing risk with decreasing age at menarche but no significant risk regarding number of births in the Egyptian women¹⁸.

Intriguingly, all patients who had family history of breast cancer were expressed ERs in their breast tissues. This statistically significant relationship disagrees with Tuter *et al.* and Hines *et al.*^[19,20] They conclude that family history of breast cancer was associated with increased risk for all receptor-defined subtypes of breast cancer except ER positive tumors. This difference in findings can be attributed to the contribution of family history in the development of breast cancer subtypes varying between different ethnic populations.

Ninety percent of patients with breast cancer in this study was IDC type, (82.2%) of them showed an express of ERs in their breast tissues. The result is in accordance with Wilfred *et al.*, they found that in patients with IDC, 86% of the tumors showed an ER expression level of 90% or more.^[21] Our result was not corresponding with the result obtained by Lee *et al.*, the ILC group showed more ERs expression; however, the frequency of ILC was relatively low in Korean breast cancer patient was similar to our findings about the frequency of the same type among Sudanese breast cancer patients²².

CONCLUSION AND RECOMMENDATION

In summary, ER α immunoexpressing was not detected in the oral mucosal cells of the breast cancer patients by ICC. We recommended to use RT-PCR to detect the mRNA ERs expression in oral mucosa to get a conclusive result about the ERs expression in oral mucosa.

Funding: None.

Conflict of interest: The authors declared no conflict of interests.

Acknowledgement: Our special thanks and appreciation to all those who helped us through their assistance and guidance in performing our study. We would like to thank specially; our colleagues in the Surgical Pathology lab and Oncology Department at Khartoum Radiation and Isotopes Oncology hospital; Ministry of health for their sincere assistance.

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