The Prognostic Value of Estrogen Receptor β Isoform With Correlation of Estrogen Receptor α Among Sudanese Breast Cancer Patients

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ABSTRACT: Two estrogen receptor isoforms (ERα and ERβ) have been characterized with variable and sometimes contrasting responses to estrogens, partially explained by different receptor signaling pathways in estrogen-sensitive tissues. This is a retrospective, descriptive, cross-sectional study, aiming to evaluate the expression pattern of ERβ, employing immunohistochemical techniques using specific monoclonal antibody for ERβ, to correlate its expression with that of ERα in a Sudanese population. Two-hundred and fifty formalin-fixed paraffin-wax-embedded breast tissue blocks were used in this study. Of these, 200 were taken from breast cancer patients ascertained as study cases, and the remaining 50 were noninvolved surgical margin considered as normal breast tissue. Receptor expression was demonstrated using immunohistochemical techniques. The immune expression of ERβ was detected in 57.5% of breast cancers. It was differentially expressed in breast tissues encompassing normal, noninvasive, as well as invasive carcinoma ($P = .02$). There was no evidence of a significant relationship between ERβ and ERα expression. Among the ERα-negative tumor, 60.4% expressed ERβ. The expression of ERβ among this subgroup was significantly associated with good clinicopathological parameters such as negative Her2/neu, lower grade, and negative lymph node metastasis ($P = .002$). This study concludes that ERβ was commonly expressed among Sudanese patients with breast cancer, either co-expressed with ERα or expressed alone. In the ERα-negative subgroup, it was associated with better tumor outcomes suggesting ERβ should be included in the diagnostic protocol as an independent marker for favorable prognosis.

KEYWORDS: Estrogen receptor β, estrogen receptor α, breast cancer, immunohistochemistry, Sudan

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Introduction

Globally, malignant tumors of the breast rank second behind lung cancers, as a major cause of female cancer-related mortality. In the United States, 268 600 new cases of invasive breast cancer were diagnosed in 2019.[1] In European woman, breast cancer was the most common malignancy with 523 000 cases, 13.4% of all cancer cases in 2018.[2]

Likewise, in Sudan, based on the National Cancer Registry, breast cancer had the highest incidence comprising 25.1 per 100 000 females among all registered cancer cases in 2009 to 2010.[3] In addition, between 2010 and 2016, there were 4630 breast cancer cases diagnosed in the Sudan with a prevalence rate of 3.9 cases per 100 000.[4]

Immunohistochemical examination of the estrogen receptor alpha (ERα)/progesterone receptor (PR) has been standardized and utilized in clinical laboratories as a prognostic marker that indicates a response to endocrine therapies. However, ERα status is not an ideal predictive marker for responsiveness to antiestrogen therapy as approximately 70% of ERα-positive as well as 10% of ERα-negative cases respond to tamoxifen.[5] According to recent studies, estrogen receptor beta (ERβ) has been implicated in the responsiveness of a subgroup of ERα-negative breast cancer patients to tamoxifen therapy, leading to longer disease-free survival.[6-8]

Sudan is one of the largest African countries with diverse ethnic background where breast cancer constitutes the main cancer among females in all of the Sudanese States which places a significant burden on the economy and heath care system. Research on breast cancer within Sudanese populations is limited and primarily focused on cancer distribution, etiology, and patient presentation. There is a knowledge deficit specifically in laboratory diagnosis and prognostic tumor markers, and no previous study in Sudan or its neighboring countries has assessed ERβ expression. Therefore, the objective of this study was to evaluate the expression pattern of ERβ via immunohistochemical techniques, using specific monoclonal antibody for ERβ, and to correlate its expression with the expression of ERα in a Sudanese breast cancer population. The results may affect clinical diagnostic protocols and guide patient management.

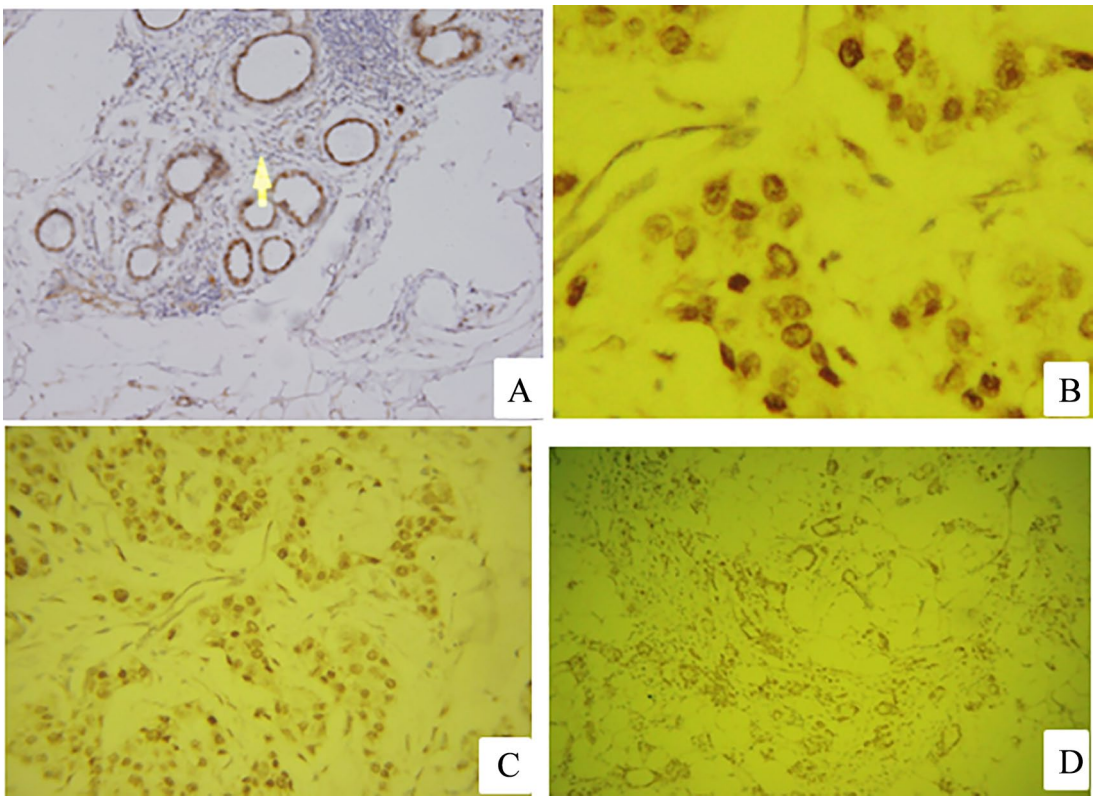

Figure 1. Immunohistochemical expression of ER-β: (A) normal breast tissue showing positive ERβ immunoexpression, (X40); (B) invasive breast cancer tissue showing positive ER-β immunoexpression, (X40); (C) invasive breast cancer tissue showing positive ER-β immunoexpression, (X20); (D) breast carcinoma in situ tissue showing positive ERβ immunoexpression, (X10).
ER indicates estrogen receptor.

Materials and Methods

Two-hundred and fifty formalin-fixed paraffin-wax-embedded breast tissue blocks were obtained from patient samples received between January 2014 and May 2019 in Radiation and Isotopes Center (Khartoum, Sudan). Two-hundred blocks were breast cancer cases, and the remaining 50 were noninvolved surgical margin considered as normal breast tissue. Ethical consent was obtained from the Ethics Committee of the Faculty Research Board and the Administrative Board of Radiation and Isotopes Center Khartoum, Sudan.

The primary diagnosis and clinicopathological data including tumor histological type, grade, and lymph node involvement were extracted from pathology reports, which were collected from the Radiation and Isotopes Center (Khartoum, Sudan) patient database.

PR and Her2/neu-stained slides were re-evaluated to confirm the previous diagnostic reports. Immunohistochemistry was performed on adjacent tissue sections which were previously confirmed by hematoxylin and eosin. Staining for ERβ and ERα were performed on 4-µm thick sections from the paraffin-embedded breast tissue blocks using the rabbit specific HRP/DAB (ABC) detection IHC (Abcam R, Cambridge, UK) as per manufacturer protocols.

The tissue sections were mounted on coated slides and dried for 1 hr at 60°C. Briefly, after dewaxing in xylene and rehydration in graded alcohol to water, the sections were exposed to Dako retrieval solution (PT link) for 30 min at 97°C. After cooling for 20 min at room temperature, the sections were placed in 3% hydrogen peroxide for 5 min to block the activity of endogenous peroxidase.

After phosphate-buffered saline (PBS) washing, blocking solution was used (Protein block Serum-free Ready to use, Abcam R) for 15 min. Next, the slides were washed with PBS. The primary antibodies of Rabbit monoclonal to estrogen receptor β (clone, EPR3778, cat.no ab133467; Abcam[R], Cambridge, UK) corresponding to residues on the C-terminus in human ERβ in 1:900 dilution, recognizes all isoforms of ERβ known to be expressed in breast cancer. Antiestrogen receptor alpha antibody (clone, E115, cat.no, ab32063, Abcam R, Cambridge, UK) in dilution 1:200 was applied to the sections for 45 min before washing in PBS and 20-min incubation with biotinylated goat antirabbit IgG (H + L). After washing in PBS, the slides were incubated for 20 min in streptavidin peroxidase.

Visualization was performed with 3,3α-diaminobenzidenetetrahydro-chloride (DAB) containing H_2O_2 as substrate, applied for 1 min. Sections were counter stained with Mayer's heamatoxylin, dehydrated in alcohol, cleared in Xylene, then mounted and cover slipped. The negative control was stained simultaneously with cases, but the primary antibody was omitted. Sections from invasive breast cancer tissues known to be expressing the ERβ were used as a positive control.

Interpretation of the result

Cancer cells that showed moderate to strong brown stain of nuclei, irrespective of the presence of cytoplasmic staining, were considered as ERβ positive. The whole slide was examined, and the percentage of positively stained epithelial cells was expressed as a proportion of the total number of epithelial cells present. The slide was scored as positive only when at least 20% or more of tumor cells were stained as previously described by other reports.[9-12]

For ERα, the guidelines of ASCO-CAP were followed to considerer ⩾1% ER-positive tumor cells as cutoff to distinguish positive from negative cases[13] and the percentage of positive cells was determined by visual estimation. Interpretation of the immunohistochemical staining results was evaluated independently by pathologists and biomedical scientists.

Statistical data analysis

Analysis of data was performed using Statistical Package for Social Sciences software. Pearson chi-square test was used. The level of significance was set at $p < .05$% and 95% confidence interval.

Result

This is a retrospective, descriptive; cross-sectional study that evaluated the expression of ERβ in breast cancer using immunohistochemical techniques in 250 samples (200 tissues from cases with breast carcinoma, and 50 were noninvolved surgical margin considered as normal breast tissue). Their ages ranging from 24 to 90 years with a mean of 49.8 years, the majority 170 (68%) of the study populations were >40 years old, and the remaining 80 (32%) were in age ⩽40 years old. The demographic and clinicopathologic characteristics of the patient population are described in Table 1. No evidence of significant relationship between the age group and the immune expression of ERβ, ($P = .945$) was seen. ERβ was differentially expressed in breast tissues from normal 36 (72%), noninvasive 11 (84.6%) and invasive carcinoma 104 (55.6%; $p < .0001$), Table 2 and Figure 1. Twenty-nine samples (80.5%) and 83 (72.2%) from normal and breast cancer tissue showed >50% ERβ positivity, respectively, Table 3.

The ERβ was expressed independently of ERα, as the highest expression was detected among the ERα-negative breast cancer cases (64, 32%). Besides this, a proportion of ERα-positive breast carcinoma at 43 samples (21.5%) were ERβ negative ($P = .382$). Similarly, with PR, a not significant relationship was found ($P = .210$; Table 4).

The expression of ERβ among the ERα-negative population was found to be significantly associated with better prognosis such as Her2/neu-negative 50 samples (25%) and negative lymph node metastasis 43 samples (21.5%). However, in breast carcinoma, co-expression of both estrogen receptors was significantly associated with progesterone receptors 44 (22%). The worst outcomes including PR-negative 41 samples (20.5%), overexpression of Her2/neu 24 samples (12%), as well as samples positive for lymph node metastasis 32 (16%) which

Table 1. Demographic and clinicopathologic characteristics of the study cases.

PATIENTS CHARACTERISTICS	FREQUENCY
Age (years)	
⩽40	63 (31.5%)
>40	137 (68.5%)
Menopausal status	
Premenopausal	115 (57.5%)
Postmenopausal	85 (42.5)
Histological subtype	
Invasive ductal carcinoma	133 (66.5%)
Invasive lobular carcinoma	27 (13.5%)
Others	40 (20%)
Lymph node metastasis	
Yes	95 (47.5%)
No	105 (52.5%)
Histological grades (IDC only)	
Grade I	37 (27.9)
Grade II	54 (40.6%)
Grade III	42 (31.5%)
ERβ	
Positive	115 (57.5)
Negative	85 (42.5)
ERα	
Positive	94 (47%)
Negative	106 (53%)
PR	
Positive	92 (46%)
Negative	108 (54%)
Her2/neu expression	
Negative	108 (54%)
Expression + 1	37 (18.5%)
Expression + 2	25 (12.5%)
Expression + 3	30 (15%)

Abbreviations: ER, estrogen receptor; PR, progesterone receptor; IDC, invasive ductal carcinoma.

Table 2. Immunoexpression of estrogen receptor beta among normal breast tissue and breast carcinoma tissue.

ESTROGEN RECEPTOR BETA STATUS	NORMAL BREAST TISSUE N (%)	NONINVASIVE BREAST CARCINOMA TISSUE	INVASIVE BREAST CARCINOMA TISSUE N (%)
Positive	36 (72)[a]	**11 (84.6)**[a]	**104 (55.6)**[a]
Negative	14 (28)	**2 (15.4)**	**83 (44.4)**
Total	50 (20)	13 (5.2)	187 (74.8)

Significance determined by comparison of each group with controls indices.
[a]$P < .0001$.

Table 3. The percentage of positivity of estrogen receptor beta among the normal and breast cancer tissue.

TYPE OF BREAST SAMPLE	PERCENTAGE OF CELLS SHOWING ESTROGEN RECEPTOR BETA EXPRESSION	
	20-50%	MORE THAN 50%
Normal breast tissue	7 (19.5)	29 (80.5)
Breast carcinoma tissue	32 (27.8)	83 (72.2)

Table 4. The relationship between ERβ status and other hormone receptors status among the breast carcinoma cases.

HORMONE RECEPTOR STATUS	ESTROGEN RECEPTOR BETA STATUS		TOTAL
	ERβ POSITIVE	ERβ NEGATIVE	
Estrogen receptor alpha			
ERα positive	51 (25.5)[a]	43 (21.5)	94 (47)
ERα negative	64 (32)[a]	42 (21)	106 (53)
Progesterone receptor			
PR positive	56 (28)[b]	36 (18)	92 (46)
PR negative	59 (29.5)[b]	49 (24.5)	108 (54)

Abbreviations: ER, estrogen receptor; PR, progesterone receptor.
Significance determined by comparison of each group with controls indices.
[a]$P = .382$.
[b]$P = .210$.

were significantly associated with breast carcinomas which are ERα and ERβ negative. This association was found to be statistically significant ($P < .0001$) for PR, Her2/neu, and lymph node metastasis, as shown in Table 5.

ERβ expression was significantly elevated in 69 invasive ductal carcinoma samples (34.5%) compared to other histological subtypes, ($P = .04$). Among this group, it was expressed more commonly in grade I tumors compared to grade II and III, ($P < .0001$), as shown in Table 6.

There is a significant inverse relationship between the ERβ expression and Her2/neu expression. The ERβ expression decreased with increasing expression of Her2/neu. The highest expression of ERβ 74 samples (64.3%) was detected among the cases with negative Her2/neu expression. However, 20 samples (17.4%) were ERβ positive in +1 expression of Her2/neu, 10 (8.7%) in +2 expression and 11 (9.6%) in +3 expression. This was found to be statistically significant, ($P = .003$; see Table 7).

Discussion

The assessment of hormone receptor levels, determined by ERα expression, is an important step in formulating a management plan for breast cancer patients. About 30% of ERα-positive tumors do not respond to tamoxifen, thus ERα alone is imprecise in predicting responsiveness to antiestrogen.[14] On the contrary, approximately 5-10% of ERα-negative breast cancers do show response to tamoxifen;[8] this suggests that ERβ may be involved in mediating the responsiveness of endocrine-sensitive tumors to hormonal agents. To the best of our knowledge, this is the first study which evaluates the ERβ expression among Sudanese patients with breast cancer using immunohistochemical techniques.

In this study, ERβ was found to be expressed in 57.5% of breast cancer tissues. Although no previous studies have reported on patients from the Sudan or neighboring countries, this result is in line with many studies in more developed countries, either prospective or retrospective, regarding the frequency of ERβ expression in breast cancer.[15]

ERβ was detected in 48% of samples from 67 breast cancer patients.[16] In comparison, a study by Gruvberger-Saal et al,[11] investigated 353 breast tumors, concluding that 74% of the cases stained positive for ERβ. Furthermore, positive expression was detected by immunohistochemistry for ERβ in 77% of the 80 breast carcinoma cases analyzed.[17] Moreover, samples from 3093 breast cancers were examined and it was reported that 68% were ERβ positive.[18]

In this study, the expression pattern of ERβ was significantly different among 72% of normal breast tissues. About 84.6% of those with noninvasive carcinomas, as well as 55.6% of invasive carcinomas, showed expression. This result was similar to that obtained by Bozkurt and Kapucuoglu,[19] who detected ERβ positivity in 70% of normal breast, 100% of ductal carcinoma in situ, and 84.1% of cases with invasive ductal carcinoma.

Similarly, a further study demonstrated the expression of ERβ in most of the normal tissues and 20-30% of invasive breast cancer.[20] In comparison, Skliris et al[21] found ERβ was continuously detected in 100% of normal breast epithelium and ductal carcinoma in situ, but only in 80% of invasive breast carcinoma cases. In addition, Huang et al[22] demonstrated ERβ expression in more than 70% of normal breast tissues, reduced expression among ductal carcinoma *in situ*

Table 5. The relationship between ERβ/ERα expression status and clinicopathological parameters among the breast carcinoma cases.

ESTROGEN RECEPTORS EXPRESSION	PROGESTERONE RECEPTOR		HER2/NEU EXPRESSION		LYMPH NODE METASTASIS	
	PR POSITIVE	PR NEGATIVE	YES	NO	YES	NO
ERα–/ERβ– (n=42)	1 (0.5)	41 (20.5)[a]	24 (12)[a]	18 (9)	32 (16)[a]	10 (5)
ERα+/ERβ+ (n=51)	44 (22)[a]	7 (3.5)	27 (13.5)[b]	24 (12)	22 (11)	29 (14.5)[a]
ERα–/ERβ+ (n=64)	12 (6)	52 (26)[a]	14 (7)	50 (25)[a]	21 (10.5)	43 (21.5)[a]
ERα+/ERβ– (n=43)	35 (17.5)[a]	8 (4)	27 (13.5)[a]	16 (8)	20 (10)	23 (11.5)[b]
Total	92 (46)	108 (54)[a]	92 (46)	108 (54)[a]	95 (47.5)	105(52.5)[a]

Abbreviations: ER, estrogen receptor; PR, progesterone receptor.
Significance determined by comparison of progesterone receptor (PR positive with PR negative, comparison of Her2/neu expression (Yes with NO) and comparison of lymph node metastasis (Yes with NO).
[a]$P < .0001$.
[b]$P < .0001$.

Table 6. Expression of ERβ among the 3 histological grades of invasive ductal carcinoma.

ESTROGEN RECEPTOR BETA STATUS	GRADE I (%)	GRADE II (%)	GRADE III (%)
ERβ positive	30 (43.5)[a]	23 (33.3)	16 (23.2)
ERβ negative	7 (11)	31 (48.4)[a]	26 (40.6)[a]
Total	37 (27.9)	54 (40.6)	42 (31.5)

Abbreviation: ER, estrogen receptor.
Significance determined by comparison of each grade (ERβ positive with ERβ negative).
[a]$P < .0001$.

Table 7. The relationship between ERβ expression and the expression of Her2/neu among the breast carcinoma cases.

HER2/NEU EXPRESSION	ESTROGEN RECEPTOR BETA STATUS		TOTAL
	ERβ POSITIVE (%)	ERβ NEGATIVE (%)	
Negative	**74 (64.3)**[a]	34 (40)	108 (54)
Expression +1	20 (17.4)[a]	17 (20)	37 (18.5)
Expression +2	10 (8.7)[a]	15 (17.6)	25 (12.5)
Expression +3	11 (9.6)[a]	19 (22.4)	30 (15)

Abbreviation: ER, estrogen receptor.
Significance determined by comparison of Negative Her2/neu expression (ERβ positive with ERβ negative); Expression +1—Her2/neu expression (ERβ positive with ERβ negative); Expression +2—Her2/neu expression (ERβ positive with ERβ negative), and Expression +3—Her2/neu expression (ERβ positive with ERβ negative).
[a]$P = .003$.

used and completely lost in invasive carcinoma. This discrepancy between studies could be attributed to differences in the methods for assessment, that is, polymerase chain reaction (PCR), tissue microarray and IHC. In addition to this, differences in primary antibodies employed produce different staining patterns, and variation in the protocol for tissue preparation. Thus, all these studies found a higher frequency of ER-β than we found in Sudan. This study used rabbit monoclonal antibody clone EPR3778 which is a synthetic peptide corresponding to residues on the C-terminus of ER-β. The same antibody was used by other studies[23–25] and showed positive, specific staining for ERβ.

The study found that 32% of breast cancer cases expressed ERβ but not ERα, 25.5% co-expressed both ERα and ERβ, while 21.5% and 21% expressed ERα alone or were negative for both estrogen receptors, respectively. In contrast, it has been reported that 13% of breast cancers were ERβ positive and ERα negative, 55% co-expressed ERα and ERβ, and 22% expressed ERα but not ERβ.[18] There is no evidence of a significant relationship between ERβ and ERα expression in the not present study. This finding is supported by other studies which found a measurable but not significant correlation between the expression of ERβ and ERα.[11,26] However, Marotti et al[18] found significant association between the expression of ERβ and ERα. Similarly, other studies have detected a positive correlation between ERβ and ERα.[19,27]

In addition, the association between the expression of ERβ and PR was nonsignificant. This finding agrees with other published studies that concluded that ERβ was not statistically associated with PR expression.[28,29] However, contrary findings have reported strong associations between ERβ and PR.[9,18]

In addition, this study confirms that there is a subset of ERα-negative cancers that express ERβ. Of the ERα-negative tumors, 60.4% expressed ERβ. This finding is substantially similar to a study which stated that 60% of 196 ERα-negative breast carcinomas studied were ERβ positive.[21] Moreover, another study reported that 56% of ERα-negative tumors were positive for ERβ.[18] The expression of ERβ among this subgroup was found to be significantly associated with better

clinicopathological parameters such as Her2/neu-negative, lower-grade, and negative for lymph node metastasis. These findings suggest that ERβ expression could be a predictor of good prognosis in breast carcinomas. Interestingly, co-expression of both ERβ and ERα was significantly associated with positive PR.

This study shows that ERβ expression was significantly different among the various histological types of breast carcinoma. Most of the cases expressing ERβ belonged to the invasive ductal carcinoma subtype. This might be due to the predominance of ductal carcinoma among the study cases. However, when comparing the frequency of expression within each histological subtype; the vast majority (81%) of invasive lobular carcinoma expressed ERβ, comparing to 51.9% of invasive ductal carcinoma. This finding is in accordance with that of Marotti et al[18] who found that frequency of ERβ expression was 87% in invasive lobular carcinomas and 63% in invasive ductal carcinomas. Furthermore, Skliris et al[21] found a strong association between lobular carcinomas and ERβ expression in comparison with ductal and other types of carcinoma. In addition, Huang et al[22] reported that invasive lobular carcinoma strongly expressed ERβ, but there was no evidence of ERβ expression in most invasive ductal carcinomas.

Of the ductal carcinoma cases, a statistically significant inverse relationship was detected between ERβ and tumor grade, with ERβ expressed in 81.1% of grade I, 42.6% of grade II, and 38.1% of grade III tumors. This finding may support the role of ERβ as inhibitor of cancer proliferation and invasion, and its action as tumor suppressor. This finding is in agreement with results reported by Marotti et al,[18] where 85% of grade I, 71% of grade II, and 49% of grade III breast cancers showed positive ERβ staining. Moreover, another study concluded that ERβ was associated with lower tumor grade;[15] however, others have shown no correlation between the presence of ERβ and better grade.[30]

The expression of ERβ was significantly associated with negative lymph node involvement as the highest level of expression (62.6%) was detected among node-negative breast cancer. This finding may support the hypotheses that loss of ERβ expression reflects an aggressive behavior of a tumor with high capability to metastasis. This is supported by Jarvinen et al[9] and Rosa et al[17] who found a significant correlation between ERβ expression and negative lymph node status. In contrast, Skliris et al[21] observed a statistical association with lymph-node-positive breast cancer. However, Miyoshi et al[10] and Shaw et al[30] failed to demonstrate any significant association with nodal involvement.[10,21,30]

This study found no significant association between the expression of ERβ and PR. This finding agrees with other published studies which conclude that ERβ was not statistically associated with PR expression.[28,29] On the contrary, others have reported strong associations between ERβ and PR.[9,18,31]

There was a statistically significant inverse association between ERβ and Her2/neu overexpression, the highest ERβ expression being detected among those tumors not overexpressing Her2/neu. This finding indicates that the expression of ERβ might be a good prognostic marker. The result agrees with previous studies, which documented an inverse relationship between ERβ and overexpression of Her2/neu.[18,32] However, some studies found a positive association between ERβ and Her2/neu overexpression.[31,33,34] Furthermore, others found no significant association, positive or negative, between ERβ and Her2/neu overexpression.[19,21]

There was no evidence of significant relationship between ERβ expression and age group in this study. This result is in agreement with studies,[15,21] which found that the expression of ERβ was normally distributed irrespective of patient age.

This study demonstrated no significant relationship between ERβ expression and menopausal status among breast carcinoma cases. This finding is in agreement with a study which detected that ERβ expression did not correlate significantly with menopausal status.[19] However, one study did find that ERβ was significantly more common in premenopausal than in postmenopausal patients.[9]

Conclusion and Recommendation

The ERβ was commonly expressed among Sudanese patients with breast cancer. It was expressed alone or co-expressed with ERα. In the former subset which reported as ER-negative patients, its expression was associated with Her2/neu negativity, low histological grade, and no lymph node infiltration. These findings suggest that ERβ is an independent predictor for good prognosis, and loss of expression might be indicator of an aggressive tumor with high metastatic capability. Additional prospective studies are needed with larger sample sizes and long-term follow-up to firmly establish the relation between ERβ and clinical outcomes, such as disease-free survival rate and overall survival. In addition, the development of more selective anti-ERβ antibodies and better-validated protocols may help to resolve the discrepancy of result among different clinical studies.

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Author Contributions

Manar G Shalabi, Anass M Abbas, and Mohamed A Kheirelseid had designed, conducted the study and stastistical analysis. Jeremy Mills and Abozer Y Elderdery revised the results and managed this manuscript in term of prepration, writing and editing.

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Availability of Data and Materials

The data that support the findings of this study are available from the corresponding author (A.Y.E.) upon reasonable request.

REFERENCES

1. DeSantis CE, Ma J, Gaudet MM, et al. Breast cancer statistics, 2019. *CA Cancer J Clin*. 2019;69:438-451.
2. Ferlay J, Colombet M, Soerjomataram I, et al. Cancer incidence and mortality patterns in Europe: estimates for 40 countries and 25 major cancers in 2018. *Eur J Cancer*. 2018;103:356-387.
3. Saeed IE, Weng HY, Mohamed KH, Mohammed SI. Cancer incidence in Khartoum, Sudan: first results from the Cancer Registry, 2009-2010. *Cancer Med*. 2014;3:1075-1084.
4. Elbasheer MMA, Alkhidir AGA, Mohammed SMA, et al. Spatial distribution of breast cancer in Sudan 2010-2016. *PLoS ONE*. 2019;14:e0211085.
5. Warner M, Gustafsson JA. The role of estrogen receptor beta (ERbeta) in malignant diseases—a new potential target for antiproliferative drugs in prevention and treatment of cancer. *Biochem Biophys Res Commun*. 2010;396:63-66.
6. Sirinian C, Papanastasiou AD, Schizas M, et al. RANK-c attenuates aggressive properties of ER-negative breast cancer by inhibiting NF-kappa B activation and EGFR signaling. *Oncogene*. 2018;37:5101-5114.
7. Tan W, Li Q, Chen K, Su F, Song E, Gong C. Estrogen receptor beta as a prognostic factor in breast cancer patients: a systematic review and meta-analysis. *Oncotarget*. 2016;7:10373-10385.
8. Manna S, Holz MK. Tamoxifen action in ER-negative breast cancer. *Sign Transduct Insights*. 2016;5:1-7.
9. Jarvinen TA, Pelto-Huikko M, Holli K, Isola J. Estrogen receptor beta is coexpressed with ER alpha and PR and associated with nodal status, grade, and proliferation rate in breast cancer. *Am J Pathol*. 2000;156:29-35.
10. Miyoshi Y, Taguchi T, Gustafsson JA, Noguchi S. Clinicopathological characteristics of estrogen receptor-beta-positive human breast cancers. *Jpn J Cancer Res*. 2001;92:1057-1061.
11. Gruvberger-Saal SK, Bendahl PO, Saal LH, et al. Estrogen receptor beta expression is associated with tamoxifen response in ER alpha-negative breast carcinoma. *Clin Cancer Res*. 2007;13:1987-1994.
12. Yan M, Rayoo M, Takano EA, Fox SB. Nuclear and cytoplasmic expressions of ERb1 and ERb2 are predictive of response to therapy and alters prognosis in familial breast cancers. *Breast Cancer Res Treat*. 2011;126:395-405.
13. Hammond MEH, Daniel H, Dowsett M, Allred C, Hagerty K, et al. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for immunohistochemical testing of estrogen and progesterone receptors in breast cancer. *Arch Pathol Lab Med*. 2010;134:907-922.
14. Szostakowska M, Trebinska-Stryjewska A, Grzybowska EA, Fabisiewicz A. Resistance to endocrine therapy in breast cancer: molecular mechanisms and future goals. *Breast Cancer Res Treat*. 2019;173:489-497.
15. Badowska-Kozakiewicz AM, Patera J, Sobol M, Przybylski J. The role of oestrogen and progesterone receptors in breast cancer—immunohistochemical evaluation of oestrogen and progesterone receptor expression in invasive breast cancer in women. *Contemp Oncol (Pozn)*. 2015;19:220-225.
16. Litwiniuk M, Filas V, Dziekan B, Moczko J, Bręborowicz J. The role of oestrogen receptor β in invasive breast cancer. *Rep Pract Oncol Radiother*. 2005;10:173-177.
17. Rosa FE, Caldeira JR, Felipes J, et al. Evaluation of estrogen receptor alpha and beta and progesterone receptor expression and correlation with clinicopathologic factors and proliferative marker Ki-67 in breast cancers. *Hum Pathol*. 2008;39:720-730.
18. Marotti JD, Collins LC, Hu R, Tamimi RM. Estrogen receptor-beta expression in invasive breast cancer in relation to molecular phenotype: results from the Nurses' Health Study. *Mod Pathol*. 2010;23:197-204.
19. Bozkurt KK, Kapucuoglu N. Investigation of immunohistochemical ER alpha, ER beta and ER beta CX expressions in normal and neoplastic breast tissues. *Pathol Res Pract*. 2012;208:133-139.
20. Hawse JR, Carter JM, Aspros KGM, et al. Optimized immunohistochemical detection of estrogen receptor beta using two validated monoclonal antibodies confirms its expression in normal and malignant breast tissues. *Breast Cancer Res Treat*. 2020;179:241-249.
21. Skliris GP, Munot K, Bell SM, et al. Reduced expression of oestrogen receptor beta in invasive breast cancer and its re-expression using DNA methyl transferase inhibitors in a cell line model. *J Pathol*. 2003;201:213-220.
22. Huang B, Omoto Y, Iwase H, et al. Differential expression of estrogen receptor alpha, beta1, and beta2 in lobular and ductal breast cancer. *Proc Natl Acad Sci USA*. 2014;111:1933-1938.
23. Su Y-X, Du G-L, Shen H-L, et al. Increased expression of aromatase cytochrome P450 enzyme is associated with prolactinoma invasiveness in post-menopausal women. *J Int Med Res*. 2019;47:3115-3126.
24. Hryciuk B, Pęksa R, Bieńkowski M, et al. Expression of female sex hormone receptors, connective tissue growth factor and HER2 in gallbladder cancer. *Sci Rep*. 2020;10:1871.
25. Andersson S, Sundberg M, Pristovsek N, et al. Insufficient antibody validation challenges oestrogen receptor beta research. *Nat Commun*. 2017;8:15840.
26. Guo L, Meng J, Yilamu D, et al. Significance of ER beta expression in different molecular subtypes of breast cancer. *Diagn Pathol*. 2014;9:20.
27. Borgquist S, Holm C, Stendahl M, Anagnostaki L, Landberg G, Jirstrom K. Oestrogen receptors alpha and beta show different associations to clinicopathological parameters and their co-expression might predict a better response to endocrine treatment in breast cancer. *J Clin Pathol*. 2008;61:197-203.
28. Esslimani-Sahla M, Simony-Lafontaine J, Kramar A, et al. Estrogen receptor beta (ER beta) level but not its ER beta CX variant helps to predict tamoxifen resistance in breast cancer. *Clin Cancer Res*. 2004;10:5769-5776.
29. Hongzhang C, Liying L. Unpolluted fractionation of wheat straw by steam explosion and ethanol extraction. *Bioresour Technol*. 2007;98:666-676.
30. Shaw JA, Udokang K, Mosquera JM, Chauhan H, Jones JL, Walker RA. Oestrogen receptors alpha and beta differ in normal human breast and breast carcinomas. *J Pathol*. 2002;198:450-457.
31. Umekita Y, Souda M, Ohi Y, et al. Expression of wild-type estrogen receptor beta protein in human breast cancer: specific correlation with HER2/neu overexpression. *Pathol Int*. 2006;56:423-427.
32. Nakopoulou L, Lazaris AC, Panayotopoulou EG, et al. The favourable prognostic value of oestrogen receptor beta immunohistochemical expression in breast cancer. *J Clin Pathol*. 2004;57:523-528.
33. Choi Y, Pinto M. Estrogen receptor beta in breast cancer: associations between ER beta, hormonal receptors, and other prognostic biomarkers. *Appl Immunohistochem Mol Morphol*. 2005;13:19-24.
34. Kim GE, Lee JS, Choi YD, et al. Expression of matrix metalloproteinases and their inhibitors in different immunohistochemical-based molecular subtypes of breast cancer. *BMC Cancer*. 2014;14:959.