Expression of Estrogen and Progesterone Receptors among Sudanese Women with Breast Cancer: Immunohistochemical Study

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Abstract

Study design: This is a descriptive study to detect the level of Estrogen (ER) and Progesterone (PR) receptors in a sample of biopsies from Sudanese women with breast cancer presented at Khartoum teaching Hospital.

Material and Methods: Forty biopsies from breast cancer patients were examined with immuno-staining using anti-sera to ER and PR as markers to detect receptors.

Results: All the specimens showed the typical histopathologic features of breast cancer. Immunoreactivity testing revealed positive ER in thirty-six patients (90%) and positive PR in thirty-one patients (77.5%). Of the 36 ER positive samples, staining intensity was: strong in 20 (55.5 %) moderate in 10 (27.7 %) and weak in 6 (16.7 %). Of the 31 PR positive samples, 15 (48.4 %) showed strong staining, 7 (22.5 %) moderate and 9 (29.0 %) weak staining.

Conclusion: the studied specimens showed high level of positive ER and PR receptors.

Keywards: Immuno-staining, hormonal effect, African emales, monoclonal antibodies

Introduction

The estimated number of new cancer cases each year is expected to rise from 10 millions in 2000 to 15 millions by 2020. Some 60% of all these new cases will occur in the less developed parts of the world[1]. Breast cancer is the commonest cancer in women worldwide. Its incidence is rising at a rate of a proximately 2% per year in all populations[3].

The importance of the receptor level in the breast cancer as an indicator of hormone response has been extensively studied[4-7]. IHC method is a specific, sensitive, and economical method for determining ER and PR status[7]. The advances in the production of monoclonal antibodies and in antigen retrieval methods have greatly improved the ability to detect ER/PR in paraffin-embedded tissues[8-10] and results were consistent with results of frozen tissues[11].

Breast cancer in the Sudan

Female breast cancer is by far the leading cancer in Sudan. It accounts for 34.5% of all female cancer. The vast majority of patients suffering from these cancers were from the Northern parts of Sudan[12]

Materials and Methods

This is a descriptive study to evaluate the level of ER and PR receptors expression in 40 excisional or diagnostic biopsies taken from breasts of female patients diagnosed as having breast cancer who presented to the surgical department at Khartoum Teaching Hospital in the period from January 2000 to January 2001.

Biopsies were sent to the lab in 10% neutral buffered formalin.

Sample processing for histopathology:

Four sections of 5μm in thickness were obtained from formalin-fixed paraffin wax embedded tissues using a Rotary microtome. The sections were stained using haematoxylin and eosin (Mayer's procedure)[13].

Immunohistochemistry procedure:

Three sections of 5μm in thickness were obtained from formalin-fixed paraffin wax embedded tissues using a rotary microtome. Sections were retrieved by water-bath retrieval technique for 30 minutes and immunostained using monoclonal 1D5 and 1A6 antibodies in addition to negative control antisera. The staining system used in this technique based on the labeled streptavidin-biotin (LSAB) method and is optimized for paraffin-embedded tissues; after heat induced Target Retrieval was performed, endogenous peroxidase activity was quenched by incubating the specimen for five minutes with 3% hydrogen peroxide. Each specimen was then incubated with appropriate mouse monoclonal primary antibody, followed by sequential 10-minutes incubations with biotinylated link antibody and peroxidase labeled streptavidin. Staining was completed after five minutes incubation with a freshly prepared substrate-chromagen solution. Thereafter, conventional procedure for staining was followed.

Evaluation of staining intensity

Staining intensity was quantified using the Quick Score System, which was described before [13], as follows:

0= No nuclear staining.
1= Weak staining (<1% nuclei staining).
2= Moderate staining (1-10%).

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3= Strong staining (10-33% and More).

Results

A total of 40 breast cancer specimens from women who presented to Khartoum Teaching Hospital were studied. Their ages ranged between 20 to 75 years (mode 42).

36 specimens (90%) were ER positive (among which 30(75%) showed considerable expression and 6(15%) revealed weak staining) while 4 cases (10%) were negative. 31 specimens (70%) were PR positive. (among which 22 (55%) showed considerable expression and nine (15%) revealed weak staining). Only 9 cases (30%) were negative for progesterone status. Of the 36 ER positive, staining intensity was: 20 (55,5%) strong staining, 10 (27,7%) moderate and 6 (16.7%) showed weak staining. Of the 31 PR positive there were 15 (48.4%) strong, 7 (22.5%) moderate and 9 (29%) weak staining.

Discussions

Estrogen receptors (ER) are cellular proteins that bind estrogens with a high affinity and specificity. They are a necessary component for estrogen-mediated cellular activity[14]. The presence of progesterone receptors (PR) demonstrates an active ER mechanism for the induction of PR expression[10].

The treatment of breast cancer by anti-estrogen therapy is presently our front line defense against the disease, as well as progesterone. This will signify the important of detection of these receptors to reach accurate diagnosis of breast cancer and to plan a suitable treatment.

However, the immunohistochemical assessment of ER and PR receptor status in the present study has shown a high degree of expression, 75% and 55% for ER and PR respectively is in keeping with other similar studies conducted in paraffin-embedded tissues and in frozen tissues[8-11].

As the immunohistochemical staining was performed on formalin fixed paraffin wax processed tissues, in the present study, the little lack of sensitivity may be attributed to the fact that formalin fixation-paraffin wax processing masks or even destroys some antigenic epitopes. Retrieval of these masked antigenic epitopes using antigen recovery techniques depends on duration of fixation on formalin[13]. As some of specimens in our series have prolonged fixation time, poor retrievals were demonstrated for some sections when using the provided target time (30 minutes). Our results revealed low prevalence compared to the high incidence of ER-/PR+ breast cancer reported from India. This is most likely due to the use of suboptimal manual assays, rather than true genetic differences[14].

The use of immunocytochemical (ICC) assays were initially restricted to frozen section work but, the development of receptor antibodies such as 1D5, allow their use in routine formalin fixed paraffin embedded tissues. Assessment of reactivity is usually based on microscopical assessment of the proportion of tumor cells showing positive reactivity and on the degree of reactivity of the individual nuclei[15,16]. Therefore, immunocytochemistry (IHC) can be used, when conventional biochemical assay cannot be performed for hormone receptor evaluation, particularly on cytoponctions[17]. ICC determination of hormone receptors in routinely fixed smears obtained by FNAC is a simple method that correlates adequately with the results of IHC determinations, especially for ER[18].

Conclusions

IHC technique is important in diagnosis of breast lumps. Estrogen and progesterone receptors are recommended to be measured on breast cancer. The results would influence treatment planning. In view of the lack of studies that have used new methods to assess breast lesions reported from the Sudan, and because of the small number of patients in this study; further studies of large number of patients is needed for validation of this results.

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