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Detection of P53, Estrogen Receptors and Progesterone Receptors in Breast Cancer

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ABSTRACT

This study aimed to detect P53, estrogen receptors (ER) and progesterone receptors (PR) in breast cancer patients using immunohistochemical method. Fifty paraffin embedded tissue blocks previously diagnosed as breast cancer were randomly selected, sectioned and stained immunohistochemically for the detection of P53 mutation, estrogen receptors and progesterone receptors. The obtained data were analyzed using SPSS computer program. The patients' information was collected from the archived files of patients. Histopathology diagnosis of study subjects revealed invasive ductal carcinoma in 41 (82%) samples, invasive mammary carcinoma in 2 (4%) samples, mucinous carcinoma in 3 (6%) samples, papillary carcinoma in 2 (4%) samples, tubulo lobular carcinoma in 1(2%) samples, and malignant stromal tumor in 1 (2%) sample. The majority were in grade 3, 25 (50%) patients while those with grade 2 were 14 (24%) patients and grade 1 only one patient (2%), and the not graded were 10 (20%) patients. Positive expression of p53 among study subjects represented 30 (60.0%) patients and the remaining 20 (40.0%) were negative with no association between p53 mutation and histopathological diagnosis (P.value>0.05). ER status showed positive expression in 24 (48.0%) patients and negative in 26 (52.0%), PR showed positive expression in 18 (36.0%) patients and negative in 32 (64.0%) patients. ER and PR showed insignificant association histopathological diagnosis (P.value>0.05). The study concludes that the mutated P53 is expressed in most breast cancer tissues with no association between p53 and histopathology diagnosis, also no association with ER and PR receptors.

INTRODUCTION

Breast cancer is a type of cancer originating from breast tissue, most commonly from the inner lining of milk ducts or the lobules that supply the ducts with milk. Cancers originating from ducts are known as ductal carcinomas, while those originating from lobules are known as lobular carcinomas. Breast cancer occurs in humans and other mammals. While the overwhelming majority of human cases occur in women, male breast cancer can also occur (Sariego, 2010).

Breast cancer is the most commonly diagnosed cancer in women worldwide (Chin and Suk, 2010), and is second only to lung cancer as a leading cause of mortality. It is found in high rates in developing countries as well as low middle income countries. 1 in 9 women is diagnosed with breast cancer worldwide (Elgaili *et al*., 2010). In Sudanese hospitals in 2000, cancer was the third leading cause of death after malaria and viral pneumonia, accounting for 5% of all deaths (Hussein, 2006) In Sudan it is more common at a young age contrary to the West where it is more common in old age (after 60 years) (Mahmood *et al*., 2006).

Breast cancer is more likely in women who have fibrocystic disease of the breast, particularly epithelial hyperplasia, women who have no children or who have their first child after the age of 30 years, women who have an early menarche and a late menopause, or both, and women who have cancer of endometrium, ovary, colon or contralateral breast (Stanley and Robbins, 2008). The strongest etiological factor is a positive family history; there is a definite increased risk if a female relative, i.e. mother, maternal grandmother or sister has had breast cancer (David *et al*., 2008).

Fine-needle aspiration (FNA) of a breast mass is a well-established diagnostic test. It is a simple office based procedure that is well accepted by women because it is sometimes less painful than a venipuncture (Fidel and Vern, 2010). Most types of breast cancer are easy to diagnose by microscopic analysis of the biopsy. Mammography and additional tests that are performed in special circumstances such as ultrasound or magnetic resonance imaging (MRI) are sufficient to warrant excisional biopsy as the definitive diagnostic and curative method. Other options for biopsy include core biopsy, and an excisional biopsy. In addition vacuum-assisted breast biopsy (VAB) may help diagnose breast cancer among patients with a mammographically detected breast in women (Yu *et al*., 2010).

Breast cancer is usually treated with surgery and then possibly with chemotherapy or radiation, or both. Hormone positive cancers are treated with long term hormone blocking therapy. Treatments are given with increasing aggressiveness according to the prognosis and risk of recurrence (Petit *et al*., 2011).

P53 mutation remains the most common genetic change identified in human neoplasia. In breast cancer, p53 mutation is associated with more aggressive disease and worse overall survival. Molecular pathological analysis of the structure and expression of constituents of the p53 pathway is likely to have value in diagnosis, in prognostic assessment and, ultimately, in treatment of breast cancer (Gasco *et al*., 2002).

Immunohistochemical assessment of ER and PR is part of the standard clinical workup of newly diagnosed breast carcinomas. ER status predicts response to hormonal therapies such as tamoxifen and aromatase inhibitors, but is a relatively weak prognostic biomarker, as it is related to tumor histology and grade (Anderson *et al*., 2002).

The objective of this study was to detect oncogenic marker p53 and therapeutic markers (ER and PR) in breast cancer patients using immunohistochemical method.

MATERIALS AND METHODS

Sample collection*:* Paraffin embedded tissue blocks previously diagnosed as breast cancers were collected for this study.

Slides preparation*:* Three sections of 5µm thickness were obtained from each formalin fixed paraffin embedded tissue using a rotary microtome for immunohistochemistry which is then taken in thermal coated slides and dried in hot plate oven at 80ºC for one hour.

Immunohistocheical staining*:* Sections were brought to water and retrieved using water bath retrieval technique at 97ºC, then treated with hydrogen peroxide solution for 15 minutes, then washed in phosphate buffer saline (pH 7.4) for 5 minutes, then treated with anti P53, ER and PR primary antibodies (for separate sections) for 30 minutes, then rinsed in phosphate buffer saline, then treated with secondary polymer conjugate for 30 minutes, then rinsed in phosphate buffer saline, then treated with DAB for 7 minutes, then washed in phosphate buffer saline for 5 minutes, then counterstained in Mayer's haematoxylin for 1 minute, then washed in water and blued in 0.05% ammoniated water for 16 second, then washed in tap water, then dehydrated through ascending of ethanol (50%, 70%, 90%, 100%) 2 minutes for each then cleared in 2 change of xylene 2 minutes for each, and mounted in DPX mounting media (Bancroft and Marilyn, 2008).

RESULTS

Histopathology diagnosis of study subjects revealed invasive ductal carcinoma in 41 (82%) samples, invasive mammary carcinoma in 2 (4%) samples, mucinous carcinoma in 3 (6%) samples, papillary carcinoma in 2 (4%) samples, tubulo lobular carcinoma in 1(2%) samples, and malignant stromal tumor in 1 (2%) sample (Table 1). Among study subjects, the majority were in grade 3, representing 25 (50%) of the patients (Table 2).

Table 1: Distribution of study population by histopathological diagnosis of breast cancer

Diagnosis	Frequency	Percent%
Invasive ductal carcinoma	41	82.0
Invasive mammary carcinoma	2	4.0
Mucinous carcinoma	3	6.0
Papillary carcinoma	2	4.0
Tubulo lobular carcinoma	1	2.0
Malignant stromal tumor	1	2.0
Total	50	100.0

Table 2: Distribution of study population by pathological grade of breast cancer

Grade	Frequency	Percent%
Grade 1	1	2
Grade 2	14	28
Grade 3	25	50
Not graded	10	20
Total	50	100

P53 mutation was positive in 30 (60%) patients, and negative in 20 (40%) patients (11 (22%) of them showed hetrogenous reaction and 19 (38%) were homogenous. 24 (48%) patients were ER positive, and the remaining 26 (52%) patients were ER negative. 18 (36%) patients were PR positive, and 32 (64%) patients were negative. The relation between p53 and histopathological diagnosis showed no statistical association (Tables 3 and 4).

Table 3: Distribution of p53 mutation among breast cancer patients

Marker	Positive (N/%)	Negative (N/%)
P53	30 (60)	20 (40)
ER	24 (48)	26 (52)
PR	18 (36)	32 (64)

Table 4: Relation between p53 mutation and histopathological diagnosis among breast cancer patients

P53	Histopathology diagnosis						Total
	Invasive ductal carcinoma	Invasive mammary carcinoma	Mucinous carcinoma	Papillary carcinoma	Tubulo lobular carcinoma	Malignant stromal tumor	
Positive	25 (50%)	1(2%)	2 (4%)	1(2%)	0(0%)	1(2%)	30(60%)
Negative	16(32%)	1(2%)	1(2%)	1(2%)	1(2%)	0(0%)	20(40%)
Total	41(82%)	2(4%)	3(6%)	2(4%)	1(2%)	1(2%)	50(100%)
P.value				0.405			

DISCUSSION

The present result showed that there was an increase in susceptibility of breast cancer involvement with increase in age as in the study of Elgaili *et al*., (2010) who reported that about (74%) of women with breast cancer in Sudan were older than 50 years old.

Our study demonstrates that there was an increased incidence with invasive ductal carcinoma among Sudanese patients with breast cancer. A result that is in agreement with as Neville, (1998) who reported that invasive ductal carcinoma is by far the commonest variety of invasive breast cancer (greater than 80% of cases). The majority of patients were demonstrated at late stage (grade 3) representing 50% (25 patients) and this finding may be attributed to the fact that patients ignore the symptoms of the disease and do not access medical care at early stages.

Bacchi *et al*., (2009) reported that the invasive ductal carcinoma is more frequent type of breast cancer representing (97.5%). Elgaili *et al*., (2010) reported that the invasive ductal carcinoma is more frequent type of breast tumors in Sudan.

Concerning this study, it is clear that the positive expression of p53 among study subjects represented 30 (60.0%) patients and the remaining 20 (40.0%) were negative, this means the positive expression is predominant and this

finding may be attributed to that the majority of patients are diagnosed as high-grade ductal carcinomas . This finding is supported by the study of Done *et al*., (2001) who reported that low-grade ductal carcinoma in situ (DCIS) is essentially devoid of mutations, whereas mutations are more common in high-grade DCIS.

The relation between p53 and histopathological diagnosis and pathological grade showed insignificant statistical association (P. value > 0.05). This finding is not consistent with the study of Keiichi, *et al*, (2005) who reported that the nuclear p53 immunoreaction was closely associated with high histological grade, advanced clinical stage. Nuclear p53 immnoreaction was significantly associated with shorter overall survival of patients. This incompatible finding may be attributed to the small sample size and the high percent of patients that are not graded (20%).

In this study ER status showed positive expression in 24 (48.0%) patients,. and negative in 26 (52.0%) patients and this result is incompatible with that of Elgaili *et al*., (2010) who reported that tumor tissues in Sudanese patients with breast cancer were found to be predominately ER negative representing (68%). ER receptors status provides limited prognostic information; currently, the major clinical value of determining ER receptor status is to assess the likelihood that a patient will respond to endocrine therapy, and are unlikely to gain additional benefit from adjuvant chemotherapy (Colleoni *et al*., 2000, Poole *et al*., 2006). Many researchers have concluded that ER receptors is probably the most powerful single predictive factor identified in breast cancer (Sorlie *et al*., 2003, Oh *et al*., 2006).

PR receptors positive tumors comprise the majority of breast cancer cases; multiple studies have provided evidence for the prognostic and predictive importance of PR receptors assessment in breast cancer (Colomer *et al*., 2005). PR receptors positive cancers have been shown to have a better prognosis than PR receptors negative tumors, and there are some data to suggest that PR receptors status can help to predict respond to hormone treatment, both in patients with metastatic disease (Regan *et al*., 2006). PR receptor expression is activated by ER receptor. Low PR receptor is associated with up-regulated growth factor signaling and aggressive tumors (Cui, *et al*. 2005). PR receptor expression may define a subpopulation of breast cancer patients having a stronger dependence on hormone receptor associated with biological growth, and therefore superior response to hormone therapy (Hede, 2008).

In this study PR showed positive expression in 18 (36.0%) patients and negative in 32 (64.0%) patients and this result is not consistent with that of Elgaili *et al*., (2010) result who reported that tumor tissues in Sudanese patients with breast cancer were found to be predominately PR negative representing (70%).

It seemed that PR receptors negative tumors are most frequent subtype of breast carcinoma identified. This study also agreed with Shuzhen *et al*., (2010), who reported that PR positive receptors is not more frequent which represents 51% cases.

The study concludes that P53 is expressed in more than half of breast cancer tissues, while the expression of ER and PR is less frequent.

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