IMMUNOHISTOCHEMICAL DETECTION OF KI67 AND HER2 IN CERVICAL TUMORS AMONG SUDANESE WOMEN

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Abstract: This is a descriptive study aimed to detect the expression of Ki67 and Her2 tumor markers in cervical tumors. Forty-five formalin fixed paraffin blocks were randomly selected from women samples previously diagnosed as cervical tumors (Thirty-five samples were malignant tumors and ten samples were benign tumors). The paraffin blocks were cut by rotary microtome and stained by immunohistochemical method for detection of Ki67 and Her2. The data obtained was analyzed using SPSS program. The malignant tumors included 29 (83%) samples were squamous cell carcinoma and remaining 6 (17%) samples were adenocarcinoma.

The patient age ranged from 27 to 85 years with mean age of 57 years. From the samples 14 (31%) samples were Ki67 positive (all of them were malignant) and 31 (69%) samples were Ki67 negative (P value <0.05). The results also showed 15 (33%) samples Her2 positive (all of them were malignant) and 30 (67%) samples were Her2 negative (P value <0.05). Regarding malignant tumors 14 (40%) positive samples and 15 (43%) negative samples were squamous cell carcinoma and no positive result was found of Ki67 expression for adenocarcinoma (P value >0.05). And 10 (29%) positive samples and 19 (54%) negative samples of Her2 results were squamous cell carcinoma and 5.0 (14%) positive samples and 1 (3.0%) negative sample of Her2 results were adenocarcinoma (P value >0.05). This study concluded that the expression of Ki67 and Her2 is associated with malignant tumors of the cervix, also concluded that there was significant different between Ki67 and histopathological grade while there were no significant different between Her2 expression and the histopathological grade.

Keywords— Cervical tumors; Ki67; Her 2.

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1. INTRODUCTION

Cervical cancer is a malignant neoplasm that develops in the squamous or glandular cells of the female uterine cervix [1]. Cancer of the cervix is the second most common cancer in women worldwide after cancer of the breast. Based upon projection from the American cancer society, more than 555,000 new cases were diagnosed annually and nearly 310,000 women die from the disease. More than 473,000 of the new cases diagnosed in women living in developing countries where cervical cancer is the leading cause of the cancer death (more than 270,000 deaths per year). Cervical cancer is much less common in developed countries, affecting 87,500 women and accounting for approximately 40,000 deaths per year [2].

The cervix cancer is the second most common cancer in

Sudan estimated from 12-15.5%. Expected cancer cases 8,000-10,000 new cases every year [3].

The main risk factors for cervical cancer include exposure to human papilloma virus, smoking, parity and immune suppression. Other factors that have been linked with cervical cancer are race, socioeconomic status and sexually transmitted infections [4].

Fortunately cervical cancer can be detected even before it become cancer by the Papanicolau test which is evaluated for the presence of precancerous cervical lesion and cervical cancer can be identified, also other test include colposcopy, biopsy, large loop excision of the transformation zone (LLETZ), cone biopsy, chest X-ray, computerized temoghraphy (CT) scan [5].

The surgery and combination of chemotherapy and radiotherapy are the most common treatment for cervical cancer [6].

Immunohistochemistry is a technique for identifying cellular or tissue constituents (Antigen) by mean of antigen-antibody interaction. The recent introduction of prognostic and predictive markers in immunohistochemistry has made a tremendous impact on patient treatment and management, this has been made possible by the gradual development of immunohistochemical methodologies over the past 60 years, which allow the identification of specific or highly selective cellular epitopes in formalin fixed paraffin processed tissue with an antibody and appropriate labeling system [7].

Ki67, is a proliferation marker known as predictive factor for tumor development, define as a nuclear antigen (associated with hetro and euchromatin) expressed during

all active phases of all cell cycle (G1,S,G2,M) except G0, the level of Ki67 expression is used to determine the cell proliferation status [8].

Her2, (also called cerb2 or neu) is a receptor tyrosine kinase involved in normal cell growth. Her2 over expression is correlated with shortened disease-free and overall survival and it is adverse prognostic factor associated with poorly differentiated, high-grade tumor; high rates of cell

proliferation; and lymph node involvement [9].

2. Materials and methods:

2.1 Materials:

Formalin fixed paraffin blocks were used in this study.

2.2 Study design

This is a hospital based descriptive retrospective case study, aimed to detect the expression of Ki67 and Her2 tumor markers in cervical tumors

3. Methods:

3.1 Sample processing:

Two sections of 5µm thickness was obtained from formalin fixed paraffin embedded tissue using a rotary microtome, sections required for immunohistochemistry taken in thermal coated slides and dried in hot plate oven at 80ºC for one hour (7)

3.2 Immunohistochemical stain

Sections for immunohistochemistry were taken to water and retrieved using water bath retrieval technique at 97ºC, then treated with hydrogen peroxide solution for fifteen minutes, then washed in phosphate buffer saline (PH 7.4) for five minutes, then treated with anti- Ki 67 and Her2 primary antibodies for thirty minutes, then rinsed in phosphate buffer saline, then treated with secondary polymer conjugate for thirty minutes, then rinsed in phosphate buffer saline, then treated with DAB for seven minutes, then washed in phosphate buffer saline for five minutes, then counterstained in Mayer's haematoxylin for one minute, then washed in water and blued in 0.05% ammoniated water for 16 second, then wash in tap water, then dehydrated through ascending of ethanol (50%, 70%, 90%, 100%) two minutes for each then cleared in two change of xylene two minutes for each, and mounted in DPX mounting media [7].

3.3 Result interpretation:

The slide with nuclear heterogeneous brown spot was considered as positive.

3.4 Data analysis:

Data were analyzed by SPSS statistics computer program. Frequencies, means and chi square were calculated.

3.5 Ethical consideration:

Samples collected after ethical acceptance from the hospital.

4. Results

In this study patient's ages was classified into two age groups; 18 (40%) of them were less than 50 years and 27 (60%) were more than 50 years (Table 1). The correlation between immunohistochemical expression of Ki67 and Her2 and cervical tumor showed in (Table 2). Table (3) showed the correlation between immunohistochemical expression of Ki67 and Her2 and cervical cancer. The correlation between expression of Ki67 and Her2 and histopathological grade showed in (Table 4).

Table (1): Distribution of age among the study samples.

Age group (year)	Frequency	Percent (%)
Less than 50 years	18	40
More than50 years	27	60
Total	45	100

Table (2): Correlation between Ki67 and Her2 expression and the study samples.

Tumor markers	Malignant		Benign		P. value
	Positive N (%)	Negativ e N (%)	Positiv e N (%)	Negativ e N (%)	
Ki67	14(31%)	21 (69%)	0(0%)	10 (22%)	0.016
Her2	15 (33%)	20 (67%)	0(0%)	10 (22%)	0.011

Table (3): Correlation between Ki67 and Her2 expression and type of malignant tumors.

Type of malignant tumors	Ki67 expression		Her2 expression	
	Positiv e N (%)	Negativ e N (%)	Positiv e N (%)	Negativ e N (%)
Squamous cell carcinoma	14 (40%)	15 (43%)	10 (29%)	19 (54%)
Adenocarcinom a	0(0%)	6 (17%)	5 (14%)	1 (3%)

P. value	0.028	0.028

Table (4): Correlation between expression of Ki67 and Her2 and histopathological grade.

Histopathologica l grade	Ki67 expression		Her2 expression	
	Positiv e	Negativ e	Positiv e	Negative
	N (%)	N (%)	N (%)	N (%)
Well Differentiated tumors	3(10%)	2 (7%)	1 (3%)	4 (13%)
Moderatelly differentiated tumors	4 (13%)	12 (40%)	8 (27%)	8 (27%)
Poorly differentiated tumors	7 (23%)	2 (7%)	1 (3%)	8 (27%)
P. value	0.032		0.111	

5. DISCUSSION

Cancer of the cervix is one of the common cancers in women, especially in developing countries [10], and the second common cancer in Sudan, and is a major cause of premature death in middle-aged and older women [3].

The age group among study samples showed that 40% of patients were less than 50 years and 60% were found more than 50 years. This indicates that the risk of developing cervical tumor is increased with increasing age. This result is agree with Rositch, *et al* [11] who reported that the higher age-specific cervical cancer incidence rates, in the peak incidence to older women given high rate of cervical cancer in women over the age of 60 to 65 years.

The relation between Ki67 and Her2 and histological diagnosis, there was relation between positive expression and malignant tumors, although all benign tumor showed negative expression, this is due high proliferation rate associated with malignant tumors. This result showed that there is significant different between Ki67 and Her2 expression and histological diagnosis (P.value 0.016 and 0.011 respectively) for Ki67 and Her2. This result agrees with Son, *et al* [12] who reported that the expression of Ki67 was significant higher in malignant lesion (squamous cell carcinoma than benign lesion). In addition, these results agree with Gupta, *et al* [13] who

reported that the higher expression of Her2 was noted in malignant lesion as compared to benign lesion.

The relation between Ki67 and type of malignant tumors, there were strong relationship between the appearance of this tumor marker and squamous cell carcinoma while adenocarcinoma showed no expression, this explained by the normal presence of Ki67 which is found in exocervical epithelia, it's only expressed in the suprabasal layer, and in CIN cases throughout the different epithelial layers .This result showed significant different between Ki67 and type of malignant tumors (P.value 0.028). This result consistence with Son, *et al* [12] who reported that the specimen containing squamous cell carcinoma was found to have higher frequency of Ki67 expression.

The results also showed that there was significant difference in tumor marker Her 2 expression and tumor type related to most adenocarcinnomas (P.value 0.028). This result consolidated with Lee and Lee [14] who stated that there was significant increased expression of Her2neu with adenocarcinoma.

Concerning the relation between expression of Ki67 and histopathological grade, there is increasing in expression with more aggressive form of tumor. Ki67 correlate with histologic grade that high level of Ki67 is associated with poorly differentiated tumors. Overall, Ki67 appears to be correlated with decreased disease-free survival, but independent significance is thought to be modest (P. value 0.032). These results agree with Carreras, *et al* [15] who concluded that there was increased expression of Ki67, according to the degree of squamous neoplasia and useful in

distinguishing the different grades of neoplasia.

The relation between expression of Her 2 and histopathological grade, the results showed that there is no significant different between Her2 expression and histopathological grade (P. value 0.111). These results disagree and discrepant with Gupta, *et al* [13] who deduced that the expression of Her2 was related to high grade, more aggressive and having poor prognosis in cervical carcinoma.

6. CONCLUSTION

This study concluded that the expression of Ki67 and Her2 is associated with malignant tumors of the cervix, also concluded that there was significant different between Ki67 and histopathological grade while there were no significant different between Her2 expression and the histopathological grade.

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