ejpmr, 2016,3(3), 60-67

SJIF Impact Factor 3.628

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 3294-3211

EJPMR

ASSOCIATION BETWEEN HER-2/NEU IHC AND P53, ESTROGEN, PROGESTERONE RECEPTOR (ER, PR) AND KI67 BIOMARKERS

Osman Mohammed Elmahi[1], Elwaleed Mohamed Elamin[1], Lamya Ahmed Mohamed El Hassan[2], Ahmed Mohamed El-Hassan[3], Hisham Ali Waggiallah*[4]

[1]Department of Histopathology, Medical Laboratory Sciences college, Al-Zaeim Al Azhari Univesity, Khartoum, Sudan.

[2]Deaprtment of Pathology, College of Medicine, Ahfad University for Women, Omdurman, Sudan.

[3]Institute of Endemic Diseases, University of Khartoum, Khartoum, Sudan.

[4]Deaprtment of Clinical Laboratory Science, AlGhad International Colleges for Health Sciences. Alriyadh, Saudia Arabia.

***Author for Correspondence: Dr. Hisham Ali Waggiallah**
Deaprtment of Clinical Laboratory Science, AlGhad International Colleges for Health Sciences. Alriyadh, Saudia Arabia.

Article Received on 08/01/2016 **Article Revised on 29/01/2016** **Article Accepted on 20/02/2016**

ABSTRACT

Human epidermal growth factor receptor 2 (Her-2/neu) has been evaluated in endometrial cancer. **Aim:** This study was conducted to evaluate Association between HER-2/neu IHC and p53, oesrogen, progesterone receptor (ER, PR) and Ki67 biomarkers among Sudanese women. **Methods**: This was a descriptive cross sectional retrospective cohort study conducted in Khartoum state, Sudan. Eighty one samples of Archival formalin fixed paraffin embedded blocks referred to the histopathology lab in the period from 2007 to 2013 were used in this study. Tissue microarray (TMA) technique was employed in which paraffin blocks were prepared before subjected to different immunostains. **Results**: This study demonstrated that there is 12(16.3%) of over expression and amplification of Her-2/neu oncoprotein in endometrial carcinoma. No association found between HER-2/neu receptor and other negative prognostic factors of the endometrial cancer. 53 cases (71.6%) showed increased proliferation of the Ki-67 marker. These results are acceptable since it is well known that increased proliferation is responsible for tumor growth. In addition this may explain the significant correlation between Her-2/neu over expression and higher level of Ki-67 since P-value, (0.040). **Conclusion:** We concluded that there was moderate association between Her2 /neu and Ki-67, whereas no other significant association detected between her 2/ neu and the other biomarker.

KEYWORD: HER-2/neu, p53, estrogen, progesterone receptor, Ki67

INTRODUCTION

The human HER -2/ neu (c-erbB-2) gene product is a transmembrane receptor with an intracellular tyrosine kinase that plays an important role in coordinating the endometrial growth factor receptor signaling network. Her-2/neu is a proto-oncogene associated with poor prognosis in women with breast and ovarian carcinoma. The significance of Her-2/neu in endometrial carcinoma is less clearly established.[1]

Recently various immunohistochemically detected prognostic factors in endometrial carcinomas, such as Her-2/neu, p53, estrogen and progesterone receptor (ER, PR) cathepesin D, and laminin. Immunohistochemical assessment of hormone receptors expression is measured semi-quantitatively with score system. This study was conducted to find out the association between Her2/neu oncoprotein and p53, oesrogen, progesterone receptor (ER, PR) and Ki67 biomarkers.[2]

MATERIALS AND METHODS

This is a retrospective cohort study that carried out during the period from October 2010 to December 2013. This study was performed at both El Hassan lab for Histopathology, Khartoum, Sudan and King Khalid University Hospital (KKUH), Saudi Arabia. Archival pre-exist formalin fixed paraffin embedded blocks referred to the lab in the period from 2007 to 2013 were used in this study. Ninety one samples were included in this study. From this number 64 were previously diagnosed as endometrial adenocarcinoma, 16 as endometrial Sarcoma and 7 as ovarian cystadenocarcinoma. Four cases were treated as missed cases. Demographical data (age and sex) in addition to histopathology data were collected from the El Hassan Lab records.

Ethical consideration: An ethical permission was obtained from Al-Zaeim Al Azhari ethical committee.

Tissue microarray (TMA) blocks preparation: Target area/s from origin block was identified on the H& E ready stained sections using permanent marker so that the corresponding area on the tissue block can be sampled. Origin block was then subjected to 2mm skin punch (Miltex biopsy punch, Germany) and tissue was carefully punched. The selected core was then brought in to a recipient paraffin block. TMA blocks were then placed upside down onto a glass slide and placed into an oven at 37-40°C overnight. TMA blocks were then placed in refrigerator until cooling. Glass slide was then detached and the block was ready for cutting.

Controls: Studied cases and controls in this research were treated similarly in all techniques and methodologies. All tissue samples were having their own controls located in the same slides of patient samples. A known Her-2/neu positive Breast adenocarcinoma tissue was used to compare the expression and/or amplification of any amount of the oncogene that found in the endometrial tissues of the patients. However; internal tissues were used as negative control.

Haematoxylin and Eosin staining: Slides cut from the TMA blocks were firstly stained with H&E stain as described.[3]

Immunohistochemistry: Next day after oven drying, sections on coated slides were subjected to Xylene and then to decrease graded of alcohols for dehydration. This was done as described by Bancroft.[3]

Epitope retrieval: This was performed in Benchmark XT machine after suitable retrieving protocol selection. Cell conditioning 1 (CC1) was carefully determined, and pH of 8.41 was selected for buffer,while time of 8 min was adjusted.

Method and procedure of the BenchMark XT Automated Slide IHC Stainer: Slides were subjected to barcode label that corresponds to the primary antibody protocol to be performed. Following slide labeling, the primary antibody, appropriate detection kit dispensers and required accessory reagents were loaded onto the reagent tray and the reagent tray was placed on the automated slide stainer. Bulk fluids and waste were checked. The slides were loaded onto the automated slide stainer. After that the run was started. At the completion of the run, the slides were removed from the automated slide stainer, washed in a mild dishwashing detergent to remove the coverslip solution; dehydrated, cleared, and coversliped with permanent mounting media in the usual manner.

Ki-67 immunostaining: Cell conditioning was selected in Ventana XT autostainer program, a mild cell conditioner # 1 short time for 8 min was chosen followed by mild cell conditioning 1 for 30 min. Tissue sections on slides were subjected to Antibody incubation and temperature was adjusted to 37 ^{0}C for I hour. One drop of Ki-67 (Ventana 30-9) antibody was applied and incubated for 0 Hr 24 Min. After that, one drop of Hematoxylin was applied for 4 min as a counter stain. Liquid coversliped was applied and incubated for 4 min. Finally one drop of bluing reagent (post counterstain) was applied and slides were coversliped.

P53 immunostaining: Cell conditioning was selected in Ventan XT autostainer program, a mild cell conditioner # 1 short time for 8 min was chosen followed by mild cell conditioning 1 for 30 min and standard cell conditioning for 60 min. Tissue sections on slides were subjected to antibody incubation and temperature was disabled. One drop of P53 (Ventana 30-9) Antibody was applied and incubated for 0 Hr 40 Min. After that, one drop of Hematoxylin was applied for 4 min as a counter stain. Liquid coversliped was applied and incubated for 4 min. Finally one drop of bluing reagent (post counterstain) was applied and slides were coversliped.

Her-2/neu: Cell conditioning was selected in Ventan XT autostainer program, a mild cell conditioner # 1 short time for 8 min was chosen followed by mild cell conditioning 1 for 30 min.Tissue sections on slides were subjected to Antibody incubation and temperature was adjusted to 37 ^{0}C for I hour. One drop of PATWAY HER2 4B5 antibody was applied and incubated for 0 Hr 24 Min. After that Ultraview detection system including DAB v3 was employed followed by one drop of Hematoxylin was applied for 4 min as a counter stain. Liquid coversliped was applied and incubated for 4 min. Finally one drop of bluing reagent (post counterstain) was applied and slides were coversliped.

ER and PR: This was performed as previously described with minor differences concerning temperature or antibody incubation time.ER 37 ^{0}C for 1 hr and PR 37^{0}C for 1 hr.

Scoring system for her-2/neu: Membrane staining was interpreted as HER-2/neu oncoprotein expression. The amount of staining was scored by Aperio Scan Scope System (digital Slide Scanner System).

Statistical analysis: SPSS (Statistical Package for Social Sciences) for windows 19.0 software was used for the statistical analysis. For the comparison of quantitative data, Chi-square and cross tabulation tests were used. Results were analyzed at $p < 0.05$ significance and 95% confidence interval.

RESULTS

Ninety one archival blocks were included in this study. The mean age of patients was 56.56 (+ 15.6, range) was 15-83, median 60 year and standard deviation SD= 15.155. The frequency, mean and standard deviation of different ages of the patients were shown in Figure1 and the percentage of the ages were classified into groups were shown in Figure 2.The comparison between age and different endometrial diagnoses were shown in

Table 1, while Her-2/neu IHC table revealed the percentage of Her-2/neu oncogen positive cases in Sudanese community. Different immunohistochemical scores (+1,+2 and +3) was found to be 13(15.9%).Her2/neu negative cases were 69 (84.1%) Table 2. Prevalence of Her-2/neuover expression as shown by IHC in Endometrial carcinoma were demonstrated in Table 3. The relation between Her-2/neu, ER, PR, P53 and Ki-67 using Chi-Square tests shown Table 4. Spearman correlation coefficient of different markers for the patients group diagnosed as endometrial adenocarcioma were shownin Table 5. Figure 3 illustrates tissue microarray cores as it has been demonstrated by traditional H&E and immunohistochemistry Her-2/neu. PR, Ki67, P53 and ER demonstrated by IHC were shown in Figure 4. Score 3 Her-2/neu IHC gene has been analyzed by aperio scanscope image analysis system were illustrated in Figure 5. Tissue microarray cores location in the original block were determined from the top corner view square Figure 3.

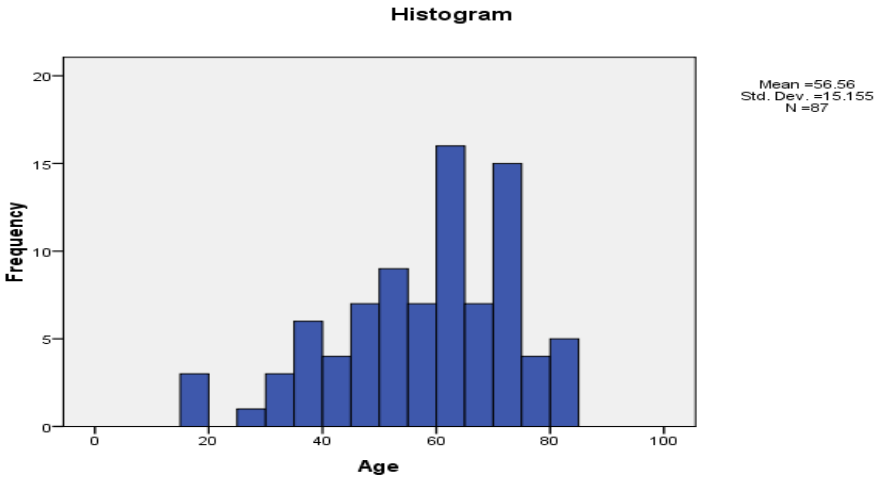


Figure1: This histogram shows the frequency, mean and standard deviation of different ages of the patients under study.

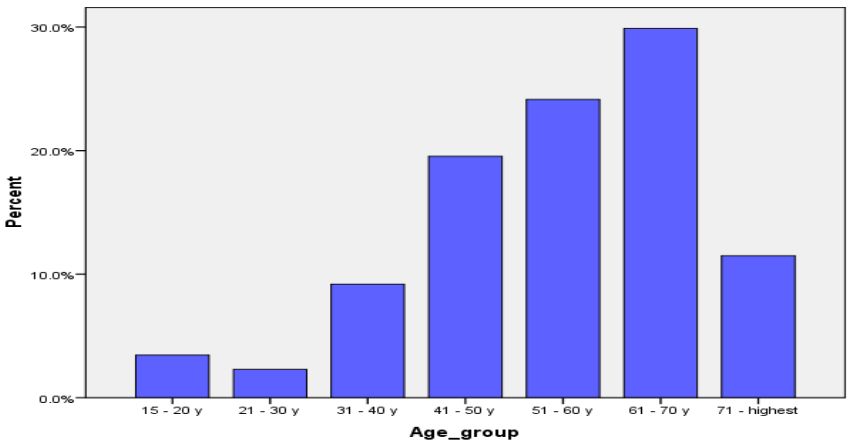


Figure 2 : This histogram shows the percentage of the age classified into groups for the patients used in the study.

Table 1: Comparison between age and different endometrial diagnoses

Age	N	Mean	Std. Deviation	95% Confidence Interval for Mean		P-value
				Lower Bound	Upper Bound	
Endometrial adenocarcinoma	64	56.88	14.477	53.26	60.49	0.418
Sarcoma	16	53.00	19.558	42.58	63.42	
Ovariancystadenocarcinoma	7	61.86	8.214	54.26	69.45	
Total	87	56.56	15.155	53.33	59.79	

*P ≤ 0.05 **P ≤ 0.01

Table 2: Her-2/neu IHC The table reveals the percentage of Her-2/neu oncogen positive cases in Sudanese community for (+1,+2 and +3) is 13(15.9%), meanwhile negative cases are 69 (84.1%).

Score	Frequency	Percent
Negative	69	78.4
+1	7	8.0
+2	3	3.4
+3	3	3.4
Total	82	93.2
Missing	6	6.8
Total	88	100.0

Table 3: Shows Prevalence of Her-2/neu expression by IHC in Endometrial carcinoma

IHC score	No.(%) of cases (N=88)
0	69 (84.1%)
1+	7(8.5%)
2+	3(3.7%)
3+	3(3.7%)

Table 4: This table shows the relation between Her-2/neu, ER, PR, P53 and Ki-67 using Chi-Square Tests:

Pearson Chi-Square	Value	Df	Asymp. Sig. (2-sided)
Her2IHC* ER	3.682	3	0.298
Her2IHC* PR		2.968	3.0
Her2IHC* P53	5.187	3	0.159
Her2IHC*Ki67	6.006	3	0.111

*P ≤ 0.05 **P ≤ 0.01

Table 5: Spearman correlation coefficient for different markers for patients group diagnosed as endometrial adenocarcioma (n =65)

		Her2IHC	ER	PR	P53	Ki67	Grade
Her2IHC	Correlation Coefficient	1.000					
	Sig. (1-tailed)	.					
	N	59					
ER	Correlation Coefficient	.198	1.000				
	Sig. (1-tailed)	.068	.				
	N	58	60				
PR	Correlation Coefficient	.142	.566**	1.000			
	Sig. (1-tailed)	.149	.000	.			
	N	56	58	59			
P53	Correlation Coefficient	.198	.381**	.085	1.000		
	Sig. (1-tailed)	.072	.002	.272	.		
	N	56	56	54	59		
Ki67	Correlation Coefficient	.307*	.285*	.176	.178	1.000	
	Sig. (1-tailed)	.010	.014	.096	.097	.	
	N	57	59	57	55	59	
Grade	Correlation Coefficient	.189	-.066	.074	.168	.101	1.000
	Sig. (1-tailed)	.076	.307	.289	.101	.223	.
	N	59	60	59	59	59	65

*P ≤ 0.05 **P ≤ 0.01

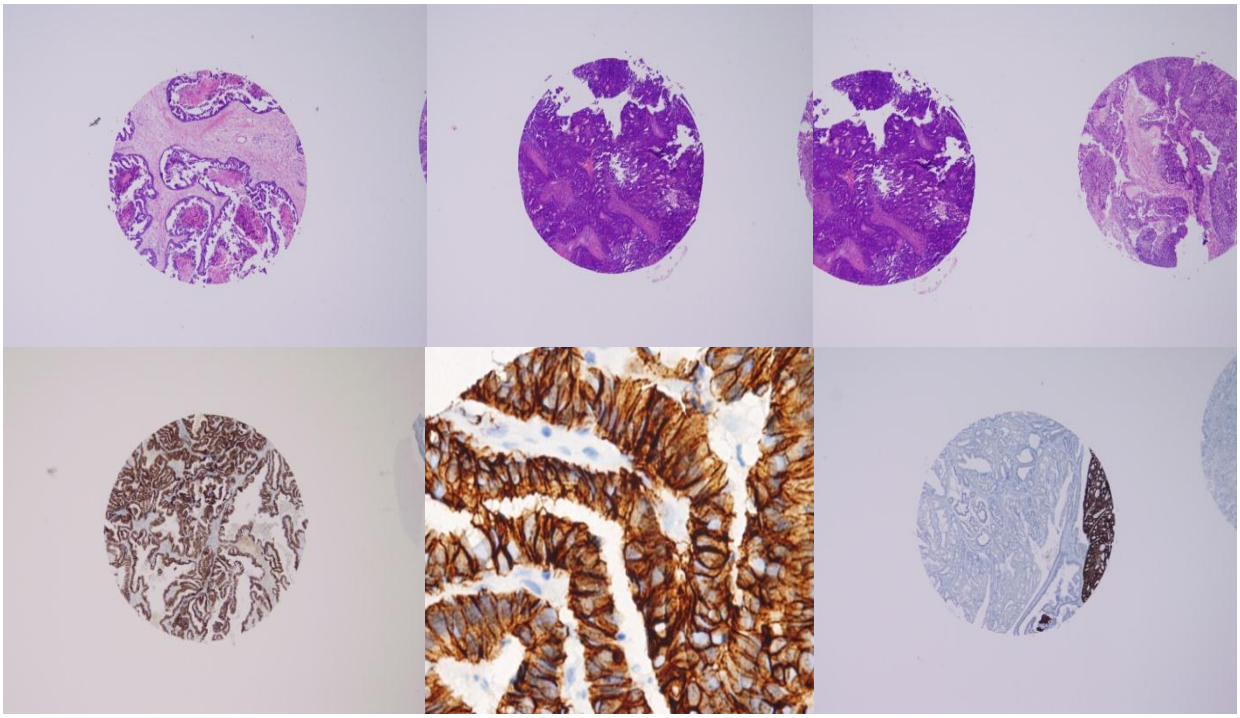
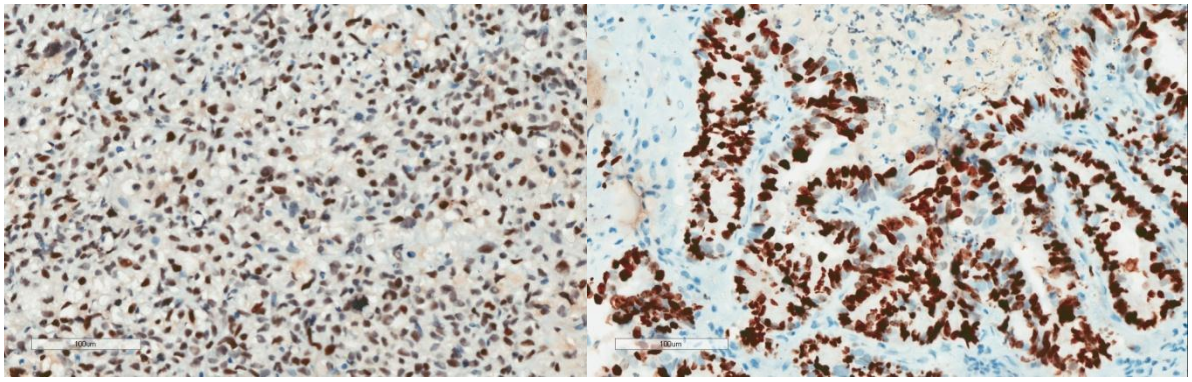
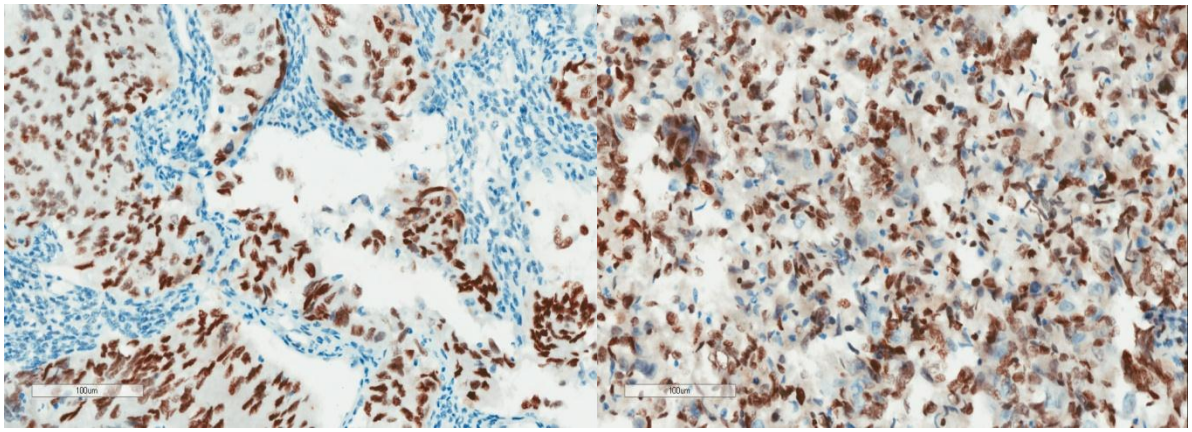


Figure 3: This figure illustrates tissue microarray small cores as it has been demonstrated by traditional H&E (top 1st row) and immunohistochemistry Her-2/neu (bottom 2nd row).



PR IHC **Ki67 IHC**



P53 IHC **ER IHC**

Figure 4: This figure shows PR, Ki67, P53 and ER demonstrated by IHC.

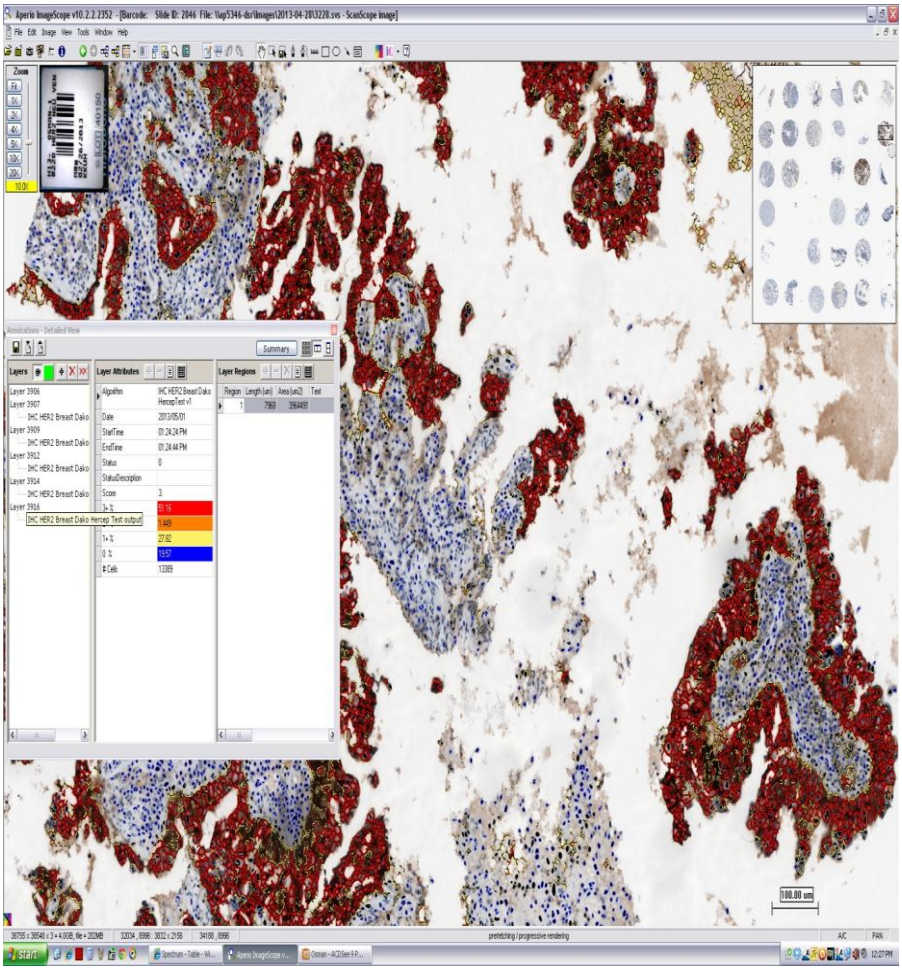


Figure 5: This figure illustrates score 3 Her-2/neu IHC gene as it has been analyzed by aperio scanscope image analysis system. Core location in original block is determined from the top corner view square

DISCUSSION

In endometrial carcinoma, immunohistochemical or molecular studies addressing the HER-2/neu protein or gene status, and the prognostic utility of these alterations are limited.

In the present study no significant association between endometrial cancer and patient age or between endometrial cancer and types of endometrial diagnosis was observed.

In this study 13 (15.9%) patients with EC showed Her-2/neu over expression. These are agreed with the lower range results obtained by other authors who found this over expression in a percentage between 9% and 60%.[4] It is also agreed with other results obtained by other groups in European country which are 21%.[5] In contrast these results are not in agreement with Raspollini et al, Sawada et al and Santin et al[6, 7, 8] who demonstrated Her- 2/neu expression in 32.1%, 56%, 55.2, 71% and 80% respectively in endometrial carcinosarcoma. These differences may be explained by the high reproducibility and consistency obtained in the current study due to the use of aperio scanscope digital analysis system to score Her-2/neu genes. Another reason may be attributed to genetic factors as other studies were performed on European patients.

In the present study, over expression of P53 was seen in 39 cases(50.6%), this is comparable with Ioffe et al[9] who found P53 was positive in 61% of the tumors. In previous series of endometrial carcinoma, the reported rate is quite variable (15–77%), which may reflect the lack of consensus of the scoring methods among the studies.[10, 11]

Concerning Ki- 67 expression, although data on the prognostic relevance of Ki-67 in endometrial carcinoma are variable, the expression in the current study was observed in 57 cases (70.4 %) which comprised more than half number of studied cases. This finding almost always agreed with Salvesen et al [11] who observed reactivity for Ki-67 in 50 % of cases. These results are acceptable since it is well known that increased proliferation is responsible for tumor growth. In addition this may explain the significant correlation between Her-2/neu over expression and higher level of Ki-67 since P-value, (0.010). In fact this finding was in line with Peiro et al [12] who also found frequent high levels of Ki-67 and accumulated P53.

A weak correlation, but strong significance between Her-2/neu IHC and ki67 were observed among patients diagnosed as endometrial adenocarcinoma, since correlation coefficient was 0.307 and a significant P-value (0.010).

The results obtained in this study for ER and PR were positive in 58 (70.7%) and 55 (70.5%) respectively. This finding agreed with data from literature which indicates the positivity for ER and PR in 35–90% [13, 14] of the endometrial carcinomas also this is almost always in agreement with Catalina et al [15] who found ER and PR were positive in 86.3% and 81.1% respectively. When ER/PR were correlated with different type of pathological diagnoses, the current result showed that there is a significant correlation with well differentiated endometrial cancer P-value,(0.002), moderately differentiated P-value, (0.001) and poorly differentiated P-value,(0.032).

There were varying degrees of association between ER/PR and well differentiated endometrial carcinoma (P-value, 0.002), moderately differentiated endometrial carcinoma (P-value, 0.011) and poorly differentiated endometrial carcinoma (P-value,0 .032). Similar studies found the correlation of the hormone receptors content (estrogen and progesterone) with several histopathological features especially the tumor differentiation. The well-differentiated tumors are more frequently positive for the estrogen and the progesterone receptors than the poorly differentiated lesions.[16] On the other hand, there are some studies disputed the importance of the ER and PR immune expression, which failed to show direct correlations with the tumor grade or the stage of the differentiation, but only with the myometrial and the vascular invasion.[17] Some authors also suggest a better prognosis impact separately for the ER and PR expression, comparing these with tumor hormonal status viewed as a whole, while others think that only some isoforms ER and PR correlate significantly with the histopathologic parameters that have a prognostic value.[14]

The present study revealed no significant correlation between IHC Her-2/neu and ER/PR, since P-value was 0.298, 0.397 respectively.

A significant association between ER and PR was observed, since P- value for was 0.000. Also the present study compared ER/PR expression with various indicators of hormone dependency in endometrial cancer, including histological grade and subtype, but the comparison came out with no significant association except for Ki67 since correlation coefficient was 0.307 and P-value 0.010.

All these findings supporting the notion that Her-2/neu over expression may be a major prognostic factor and Her-2/neu IHC could be incorporated as a prognostic variable in patients with endometrial carcinoma. The findings obtained in this study suggested that Her-2/neu may represent a crucial molecular-genetic prognostic factor responsible for the highly aggressive biologic behavior of endometrial carcinoma harboring Her-2/neu over expression.

CONCLUSION

We concluded that there was moderate association between Her2 /neu and Ki-67, whereas no other significant association detected between her 2/ neu and the other biomarkers.

REFERENCES

1. Int J GynecolPathol. 1999 Apr; 18(2): 138-43.HER-2/neu amplification and overexpression in endometrial carcinoma.Rolitsky CD, Theil KS, McGaughy VR, Copeland LJ, NiemannTH.Department of Pathology, Ohio State University, Columbus, USA.
2. Int J Gynecol Cancer. 2004 Sep-Oct;14(5):938-46.The use of P53, PTEN, and C-erbB-2 to differentiate uterine serous papillary carcinoma from endometrioid endometrial carcinoma.Macwhinnie N, Monaghan H.Department of Laboratory Medicine (Pathology), Royal Infirmary of Edinburgh, Edinburgh EH16 4SA, UK.
3. Bancroft, D., Stevens, A, Turner, R .(2008). Theory and Practice of Histological techniques. 6th edition. Churchill Livingston.
4. Backe, J., Gassel, AM., Krebs, S., Müller, T.,.Caffier, H. Immunohistochemically detected HER/neu-expression and prognosis in endometrial carcinoma. Arch Gynecol Obstet, 1997; 259: 189-95.
5. Kohlberger, P., Loesch, A., Koelbl, H., Breitenecker, G., Kainz, C., Gitsch, G.(1996). Prognostic value of immunohistochemically detected HER-2/neu oncoprotein in endometrial cancer. Cancer Lett, 98(2): 151-5.
6. Raspollini, MR., Susini, T., Amunni, G., Paglierani M., Casttiglione. F., Garbini F., Carriero, C., Scarselli, G and Taddei, GL. (2006): Expression and amplification of her-2/neu oncogene in uterine carcinisarcomas: a marker for potential molecularly targeted treatment? Int J Gynecol Cancer; 16:416.
7. Sawada, M., Tsuda, H., Kimura, M. Different expression patterns of KIT, EGFR and Her-2/neu (c-erbB2) oncoprotiens between epithelial and mesenchymal components in uterine carcinosarcoma. Cancer Sci., 2003; 94: 986.
8. Santin, AD., Bellone, S., Gokden, M. Overexpression of HER-2/neu in uterine serous papillary cancer. Clin Cancer Res, 2002; 8: 1271– 9.
9. Ioffe, OB., Papadimitriou, JC., Drachenberg, CB. Correlation of proliferation indices, apoptosis, and related oncogene expression (bcl-2 and c-erb B2) and p53 in proliferative, hyperplasic, and malignant endometrium. Hum Pathol 1998; 29: 1150–1159.
10. Coronado, PJ., Vidart, JA., Lo´pez-Asenjo, JA. p53 overexpression predicts endometrial carcinoma

recurrence better than HER-2/neu overexpression. Eur J Obstet Gynecol Reprod Biol, 2001; 98: 103–108.
11. Salvesen, HB., Iversen, OE., Akslem, LA. Prognostic significance of angiogenesis and Ki-67, p53, and p21 expression: a population-based endometrial carcinoma study. J Clin Oncol, 1999; 17: 1382–1390.
12. Peiro, G., Mayr, D., Hillemanns, P., Lo, U., Diebold, J. Analysis of HER-2/neu amplification in endometrial carcinoma by chromogenic in situ hybridization. Correlation with fluorescence in situ hybridization, HER-2/neu, p53 and Ki-67 protein expression, and outcome. Modern Pathology., 2004; 17: 277–287.
13. Ferrandina, G., Ranelletti, FO., Larocca, LM., Maggiano, N., Fruscella, E., Legge, F., Santeusanio, G., Bombonati, A., Mancuso, S., Scambia, G. Tamoxifen modulates the expression of Ki67, apoptosis, and microvessel density in cervical cancer, Clin Cancer Res, 2001; 7(9): 2656–2661.
14. Shabani, N., Kuhn, C., Kunze, S., Schulze, S., Mayr, D., Dian, D., Gingelmaier, A., Schindlbeck, C., Willgeroth, F., Sommer, H., Jeschke, U., Friese, K., Mylonas, I. Prognostic significance of oestrogen receptor alpha (ERalpha) and beta (ERbeta), progesterone receptor A (PR-A) and B (PR-B) in endometrial carcinomas, Eur J Cancer, 2007; 43(16): 2434–2444.
15. Catalina, S., Simionescu, C., Margaritescu, CL., Stepan A., Nurciu, M. Endometrial carcinomas: correlation between ER, PR, Ki67 status and histopathological prognostic parameters. Rom J Morphol Embryol, 2011; 52(2): 631–636.
16. Nyholm, HC. Endometrial cancer and steroid hormone receptors, Ugeskr Laeger, 1990; 152(9): 593–597.
17. Wolf, HK., Stober, C., Hohenfellner, R. Prognostic value of p53, p21/WAF1, Bcl-2, Bax, Bak, and Ki-67 immunoreactivityin pT1 G3 urothelial bladder carcinomas. Tumour Biol, 2001; 22: 328–336.