http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS

Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2020.1332.1337

Research Article
Concordance Between Immunohistochemistry (IHC) and Silver Situ Hybridization (SISH) in Endometrial Carcinoma Diagnosis: Using HER-2/neu

[1]Osman Mohammed Elmahi and [2]Hisham Ali Waggiallah

[1]Department of Histopathology, Medical Laboratory Sciences college, Karari University, Khartoum, Sudan
[2]Department of Medical Laboratory Science, Prince Sattam Bin Abdul Aziz, Alkharj, Alriyadh, Saudi Arabia

Abstract

Background and Objective: Human epidermal growth factor receptor 2 (Her-2/neu) has been demonstrated as biomarker in Endometrial Carcinoma (EC). This study was conducted to assess the concordance between Sudanese women between immunohistochemistry (IHC) and Silver Situ Hybridization (SISH) for EC diagnosis using HER-2/neu. **Materials and Methods:** This was a retrospective cross sectional study performed in the state of Khartoum, Sudan. This research used eighty-eight samples of embedded blocks of formalin fixed paraffin referred to the histopathology lab from 2007-2013. The technique of tissue microarray (TMA) was used in which paraffin blocks were prepared before IHC and SISH were subjected. **Results:** In this analysis, 88 specimens were previously diagnosed as endometrial adenocarcinoma. The number of Her-2/new oncogene positive cases among Sudanese women by using IHC were (15.9%), however Her-2/neu amplification in EC were 11.5% by using SISH analysis and strong correlation between Her-2/neu IHC and Her-2/neu SISH is existed, since p-value is 0.,000. **Conclusion:** SISH is a reliable technique that can be used for detecting Her-2/neu oncoprotein and it has many advantages over other methods, also SISH can be used as an alternative to FISH technique.

Key words: Immunohistochemistry, silver situ hybridization, HER-2/neu, endometrial carcinoma, oncogene, c-erb-B2

Citation: Osman Mohammed Elmahi and Hisham Ali Waggiallah, 2020. Concordance between immunohistochemistry (IHC) and silver situ hybridization (SISH) in endometrial carcinoma diagnosis: Using HER-2/neu. Pak. J. Biol. Sci., 23: 1332-1337.

Corresponding Author: Hisham Ali Waggiallah, Department of Medical Laboratory Science, Prince Sattam Bin Abdul Aziz, Alkharj, Alriyadh, Saudia Arabia Tel: 00966558675733

Copyright: © 2020 Osman Mohammed Elmahi and Hisham Ali Waggiallah. This is an open access article distributed under the terms of the creative commons attribution License, which permits unrestricted use, distribution and reproduction in any medium, provided the original author and source are credited.

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

HER-2/neu (c-erbB-2) and certain factors under investigation currently include estrogen and progesterone receptor status, p53 status, ploidy cytometric flow review, S-phase portion[1]. Prognosis, phase, lymph-node metastasis and myometrial invasion are strongly associated with histological level. Level is one of the prognostic factors that are applied in treatment decisions. The most commonly used rating standards are those of the International Gynecology and Obstetrics Federation (FIGO) and the World Health Organization (WHO), which include both architectural and nuclear characteristics[2]. In recent years, EC's molecular research has detected anomalies in the expression, structure or behavior of oncogenic products that may contribute to the development and maintenance of the malignant phenotype such as HER/2 NEU[3]. For women with breast and ovarian carcinoma, HER-2/neu is a proto-oncogene associated with poor prognosis. The role of HER-2/neu is less clearly established in endometrial carcinoma[4]. HER-2, also known as c-erb-B2, is a HER-2/new encoded 185 kd receptor protein[5] that is located on chromosome17. Together with HER-1, HER-3 and HER-4, HER-2 is a member of the epidermal growth factor receptor family with tyrosine kinase activity. In a small number of studies, HER-2 overexpression in uterine serous papillary carcinoma and other EC histotypes were evaluated.[6]

It has not been introduced in Sudan to use HER-2/neu as a marker for diagnosing EC and neither has the comparison been made between SISH and IHC to know the most accurate and precise technique, therefore the aim of this study was to evaluate the concordance between immunohistochemistry (IHC) and Silver situ hybridization (SISH) in endometrial cancer diagnosis using HER-2/neu.

MATERIALS AND METHODS

This is a retrospective cohort study conducted between October, 2010 and December, 2013. This study was conducted at the Histopathology Laboratory in El Hassan, Khartoum, Sudan. This research used pre-existing formalin-fixed paraffin embedded blocks referred to the laboratory during the period 2007-2013. In this analysis, 88 specimens were previously diagnosed as endometrial adenocarcinoma and Demographic data (age) were obtained from the El Hassan Lab records in addition to histopathology data.

Ethical consideration: An ethical permission has been received from Karary University ethical committee (No: KU/CM/EC.1-5/21/2010).

Preparations of tissue microarray (TMA) blocks: Goal areas from the initial block was marked using a permanent marker on the H and E stained parts so that the corresponding region can be sampled on the tissue block. Initial block was subjected to a skin punch of 2 mm and carefully punched tissue. The selected core was then passed to a group of paraffin recipients. Then TMA blocks are placed on a glass slide upside down and dropped in an oven overnight at 37-40°C. TMA blocks were then put in the refrigerator before cooling. Glass slide was then removed and the block was prepared for cutting.

Controls: In all methods and methodologies, cases and controls examined in this study have been treated similarly. Both tissue samples had their own controls in the same patient test slides.

Haematoxylin and eosin staining: First of all, slides cut from the TMA blocks are stained with H and E stain as mentioned by Bancroft *et al.*[7].

Immunohistochemistry: After the drying of the oven, parts on coated slides are subjected to xylene and then decreased alcohol gradation for dehydration. This was done in the manner mentioned by Bancroft *et al.*[7].

Epitope retrieval: This was achieved in the Benchmark XT system after proper protocol collection had been recovered. Cell conditioning 1 (CC1) was carefully calculated and the buffer pH of 8.41 was chosen and the time changed was 8 min.

BenchMark XT automated slide IHC stainer process and procedure: Slides have been exposed to a barcode tag referring to the primary antibody protocol to be performed (Ventan XT autostainer program, Roche). The primary antibody, suitable detection kit dispensers and appropriate accessory reagents were loaded onto the reagent tray after slide labeling and the reagent tray was put on the automatic slide stainer. The slides were mounted onto the stainer for the automatic display. The run started after that. Upon completion of the process, the slides were removed from the automatic slide stainer

and washed to eliminate the coverslip solution in a mild dishwashing detergent; dehydrated, cleaned and covered in the usual manner with permanent mounting paper.

Scoring system for Her-2/neu: Membrane staining has been described as an expression of HER-2/new oncoprotein. Aperio Scan Scope System (Digital Slide Scanner System) scored the amount of staining.

Inform her2 dual ISH DNA probe cocktail assay

Slide scoring: Ventana quantitative scoring algorithm was used (manual method) (Aperio Scan Scope System, Leica Biosystems, USA). An adequate target area was identified; the HER2 and Chr17 scores were recorded for copy numbers present in 20 representative nuclei. When the resulting HER2/Chr17 ratio decreased between1.8-2.2, an additional 20 nuclei would have scored and the resulting ratio from the maximum 40 nuclei was determined. HER2 gene status was reported as non-amplified (Her2/Chr17<2.0) or amplified (HER2/Chr17>2.0).

Statistical analysis: The statistical analysis was based on the Statistical Package for Social Sciences (SPSS) for Windows 19.0 technology. Chi-square and cross tabulation tests have been used to compare quantitative data. Reports are evaluated at a significance of $p<0.05$ and a confidence interval of 95%.

RESULTS

Age group EC patients: Eighty eight samples were included in this study. Table 1 shows the age that classified in to seven categories. The highest number of cases falls between 61 and 70 age group this indicate that the majority of specimens collected for post-menopausal women.

Her-2/neu expression in EC by using IHC and SISH: The percentage of Her-2/neu oncogen positive cases in Sudanese community for +1,+2 and +3 collectively were 13(15.9%), meanwhile negative cases were 69 (78.4%) as revealed in Table 2. There are 4 cases (11.5%) which amplified by SISH analysis. 3 (8.6%) of this are amplified with low level, while 1 (2.9%) was strongly amplified. About 31 out of 35 were not amplified, as shown in Table 3.

Relationship between Her-2/neu IHC and Her-2/neu SISH: Strong correlation between Her-2/neu IHC and Her-2/neu SISH is existed, since p-value is 0.000 ($p\leq 0.05$) (strongly significant) Table 4. the measure of agreement between Her-2/neu IHC and Her-2/neu SISH after non amplified and low amplification cases being combined in one group and also (+1,+2) combined in another group, the following matrix was obtained. There is also strong direct correlation between her-2/neu IHC and Her-2/neu since p-value is 0.000 ($p\leq 0.05$).

The prevalence of the endometrial adenocarcinoma grades in Sudanese women by using IHC technique in various stages of adenocarcinoma. +3 means positive and complete membrane staining of Her2/neu gene composed 33.3% of well and moderately differentiated types of EC, while +1, +2 mean negative and incomplete membrane staining of Her2/neu gene) appeared in late stage of EC poorly differentiated as 57.0 and 66.7%, respectively that means Her2/neu gene was more sensitiva in early stages of endometrial carcinoma as exhibited in Table 5.

Table 1: Distribution of patients age

Age per year	Frequency (%)
15-20	3 (3.4)
21-30	2 (2.3)
31-40	8 (9.1)
41-50	17 (19.3)
51-60	22 (25.0)
61-70	26 (29.5)
71-highest	10 (11.3)
Total	88 (100.0)

Table 2: Prevalence of Her-2/neu expression by IHC in EC

Her-2/neu IHC	Frequency (%)
Negative	69 (78.4)
+1	7 (8.0)
+2	3 (3.4)
+3	3 (3.4)
Missing	6 (6.8)
Total	88 (100.0)

Negative: Incomplete membranous staining is detected, +2: Complete membrane staining is observed, +3: Positive, complete membrane staining

Table 3: Prevalence of Her-2/neu gene by using SISH in EC

Her-2/neu SISH	Frequency (%)
Not amplified	31 (35.2)
Low level	3 (8.6)
Amplified	1 (2.9)
Total	35 (39.8)
Missing	53 (60.2)
Total	88 (100.0)

Table 4: Correlation between Her2 IHC and Her2 SISH

	Her 2 SISH					
		Not amplified	Low level	Amplified	Total	p-value
Her2IHC	Negative	27	2	0	29	0.000
	+1	2	1	0	3	
	+3	0	0	1	1	
Total		29	3	1	33	

p<0.5

Table 5: Distribution of Her2/neu in different endometrial adenocarcinoma grades

Grades	Well differentiated	Moderately differentiated	Poorly differentiated	Not graded	p-value
Her2 IHC					
0	21 (30.4%)	10 (14.4%)	18 (26.0%)	20 (30.0%)	
+1	2 (28.6%)	1 (14.2%)	4 (57.1%)	0 (0.0%)	
+2	0 (0.0%)	0 (0.0%)	2 (66.7%)	1 (33.3%)	0.476^{NS}
+3	1 (33.3%)	1 (33.3%)	0 (0.0%)	1 (33.3%)	

0: No staining is observed, or membrane staining, +1: Negative, incomplete membranous staining is detected, +2: Complete membrane staining is observed, +3: Positive, complete membrane staining, p<0.5, NS: Not significant

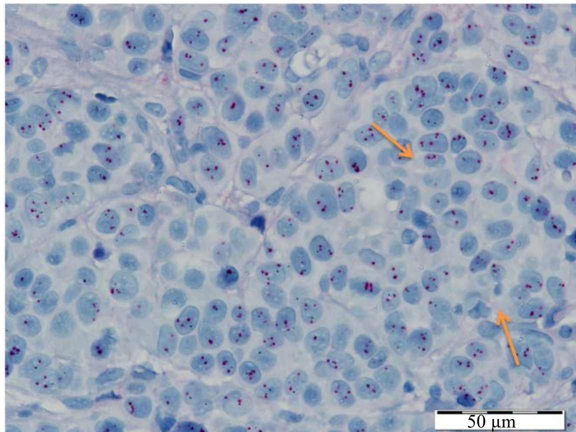

Fig. 1: Her-2 gene against chromosome 17 using SISH
Her-2 neu gene is observed amplified as black dots rated against chromosome 17 red dots, orange arrows in every single nucleus demonstrated by SISH assay

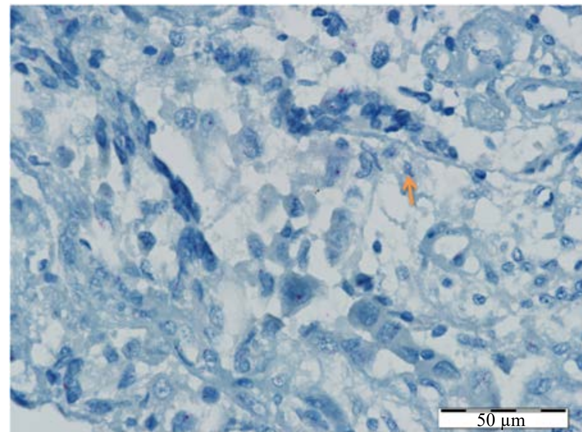

Fig. 2: Her-2/neu gene appearance using SISH
Her-2/neu gene is not amplified appeared as black dots rated against chromosome, 17 red dots (orange arrow) in every single nucleus, demonstrated by SISH assay

Appearance of amplified Her-2 neu gene: Her-2 neu gene is observed amplified as existed gene (Fig. 1) appeared in black dots rated against chromosome 17 red dots in every single nucleus as it has been demonstrated by HER2 SISH assay.

Not amplified Her-2/neu gene manifestation: Figure 2 illustrates Her-2/neu gene black dots rated against chromosome 17 red dots in every single nucleus as it has been demonstrated by HER2 SISH Assay. No observed amplification, that mean no gene existed as negative result.

DISCUSSION

HER-2/neu molecular studies were limited in breast cancer and the prognostic utility of these alterations; there were no previous studies using HER-2/neu as a marker for diagnosing EC, this study was considered as the first one in Sudan.

No major correlation was found in present research between endometrial cancer and patient age or endometrial cancer and endometrial diagnosis types. There were 13 (15.9%) EC patients over expression indicating Her-2/neu. These are accepted with the findings of the lower range

obtained by other researchers who consider this above expression[8] in a percentage between 9 and 60%. It is also confirmed with other results obtained by 21% of other groups in the European country[9]. In comparison, these findings are not in accordance with Raspollini *et al*.[10], Sawada *et al*.[11] and Santin *et al*.[12], who demonstrated Her-2/new expression in 32.1, 56, 55.2, 71 and 80% in endometrial carcinosarcoma, respectively.

Among 35 cases that have been subjected to SISH analysis, four cases (11.5%) were amplified by SISH, from these 4 cases, 3 cases (8.6%) were amplified with low level, while 1 case (2.9%) was strongly amplified. This is in contrast with what Ramier *et al*.[13] found in their analysis in Italy, 28% of score +2 showed positive gene amplification using the SISH process. Although Bae *et al*. found in Korea that 62.2% of the score +2 showed SISH gene amplification[14]. Since HER2/new gene amplification is directly related to protein expression rates in breast cancers, IHC should substitute each other for HER2/new protein expression and ISH for HER2/new gene amplification. DNA is usually less affected by the tissue-processing artifacts and experimental errors occur less frequently during ISH HER2/new gene evaluation. The findings of IHC can be influenced by many important factors such as tissue processing (preanalytical factors), antigen retrieval methods, form of reagents and primary antibodies used (analytical factors) and subjective interpretation of staining findings (post analytic factors)[15].

According to the joint HER2/new Guidelines Recommendations of the American Society of Clinical Oncology and the College of American Pathologists (ASCO/CAP), a score of 0 or +1 is regarded as a negative test result, +2 is equivocal and +3 is regarded positive for HER2[16]. A modified HER2-testing algorithm was proposed to provide ISH bright-field retesting for specimens with equivocal (+2) immunohistochemical scores[17]. Two negative cases with score (+1) HER2/neu showed positive SISH results on Ventana digital imaging in the current report, accounting for 20% of the score +1 and 10% of the overall negative cases. Young *et al*.[14] found that Bae (1.7%) cases out of 756 with negative IHC tests showed gene amplification by SISH.

Moreover; the concordance between SISH and IHC assays was studied in order to evaluate and determine the status of HER2 gene amplification in endometrial carcinoma. The result revealed strong correlation between her-2/neu IHC and Her-2/neu SISH since p-value was 0.000 (less than 0.05). The Her-2/neu rate over expression found in the current study (15.9%) may therefore not be indicative of an unselected population of endometrial cancer patients, what can be said that it was strongly significant result. Kappa measurement has also been calculated to measure the agreement between Her-2/neu IHC and Her-2/neu SISH. When the data base was searched there was only little information estimating Her-2/neu proto-oncogene using Silver dual ISH and tissue microarray in endometrial carcinoma. In comparison, these results are consistent with Garcia *et al*.[18] the SISH is a fairly recent tool for the identification of the HER2 gene amplification. Numerous studies have reported a strong concordance with other methods of in situ hybridisation, such as CISH and FISH. ISH shows a higher sensitivity and precision compared to the IHC and a higher reproducibility. This argument can be verified by comparing on our methods the concordance of amplified events. Results showed a very strong agreement between the two approaches (100.0%; $\kappa = 1$). In addition, the ISH is less sensitive than IHC due to a greater stability of DNA compared to tissue fixation and processing discrepancies[18].

In fact this is the first study concerning the application of Silver Dual ISH to endometrial carcinoma in Sudan. This is become certainly after tissue microarray parameter has been conjugated to this study. In present study have slight remarks to be taken into account when analyzing the information. First, it is necessary to consider the potential for choice and knowledge bias inherent in most retrospective investigations. Secondly, the requirement to include available and discarded pathological content (tissue blocks) created additional potential for selection bias, so that case studies were not handled successively.

Therefore the small number of cases in the sample prevented multivariate analysis to determine whether the clinicopathological features correlated with Her-2/neu over expression are independent or confused by other variables. However, another disadvantage of the analysis is the fact that Silver Dual In situ hybridization (SISH) has not been performed on some tissue samples. Regarding these limitations, current data indicate that if a larger sample size was used, it would have detect more over expression of Her-2/new proto-oncogene in Sudanese women. This is most likely due to the small number of cases being analyzed by SISH.

CONCLUSION

Based on the results obtained in this study we drown the following conclusions; Silver In Situ Hybridization is a reliable technique that can be used for detecting Her-2/neu oncoprotein and it has many advantages over other methods. SISH can be used as an alternative to FISH technique in the field of endometrial carcinoma and other approaches used to evaluate HER2/neu status.

SIGNIFICANCE STATEMENT

This study discover SISH technique has many advantages such as accuracy and precision that can be beneficial for diagnosis of endometrial carcinoma. This study will help the researcher to uncover the critical areas of approaches used to evaluate HER2/neu, that many researchers were not able to explore. Thus a new theory of using SISH technique instead of FISH technique may be arrived.

ACKNOWLEDGMENT

This Publication was supported by the Deanship of Scientific Research at Prince Sattam bin Abdulaziz University. We show our respect and express our gratitude to the El Hassan Lab staff for their help and who participation in our study.

REFERENCES

1. Uharček, P., 2008. Prognostic factors in endometrial carcinoma. J. Obstetrics Gynecology Res., 34: 776-783.
2. Crawford, S.C., L. De Caestecker, C.R. Gillis, D. Hole, J.A. Davis, G. Penney and N.A. Siddiqui, 2002. Staging quality is related to the survival of women with endometrial cancer: A Scottish population based study. Deficient surgical staging and omission of adjuvant radiotherapy is associated with poorer survival of women diagnosed with endometrial cancer in Scotland during 1996 and 1997. Br. J. Cancer, 86: 1837-1842.
3. Benevolo, M., A. Vocaturo, F. Novelli, L. Mariani and G. Vocaturo *et al*., 2007. Prognostic value of HER2 and progesterone receptor expression in endometrial carcinoma with positive peritoneal washing. Anticancer Res., 27: 2839-2844.
4. Rolitsky, C.D., K.S. Theil, V.R. McGaughy, L.J. Copeland and T.H. Niemann, 1999. HER-2/neu amplification and overexpression in endometrial carcinoma. Int. J. Gynecological Pathol., 18: 138-143.
5. Selvarajan, S., B.H. Bay, M.J. Chng and P.H. Tan, 2004. The Hercep Test and routine C-erbB2 immunohistochemistry in breast cancer: any difference? Ann. Acad. Med. Singapore, 33: 473-476.
6. Macwhinnie, N. and H. Monaghan, 2004. The use of P53, PTEN, and C-erbB-2 to differentiate uterine serous papillary carcinoma from endometrioid endometrial carcinoma. Int. J. Gynecological Cancer, 14: 938-946.
7. Bancroft, J.D. and M. Gamble, 2008. Theory and Practice of Histological Techniques. 6th Edn., Elsevier Health Sciences, Philadelphia, PA., ISBN-13: 9780443102790, Pages: 725.
8. Backe, J., A.M. Gassel, S. Krebs, T. Müller and H. Caffier, 1997. Immunohistochemically detected HER/neu-expression and prognosis in endometrial carcinoma. Arch. Gynecol. Obstet., 259: 189-195.
9. Kohlberger, P., A. Loesch, H. Koelbl, G. Breitenecker, C. Kainz and G. Gitsch, 1996. Prognostic value of immunohistochemically detected HER-2/neu oncoprotein in endometrial cancer. Cancer Lett., 98: 151-155.
10. Raspollini, M.R., T. Susini, G. Amunni, M. Paglierani and F. Casttiglione, 2006. Expression and amplification of HER-2/neu oncogene in uterine carcinosarcomas: A marker for potential molecularly targeted treatment? Int. J. Gynecol. Cancer, 16: 416-421.
11. Sawada, M., H. Tsuda, M. Kimura, S. Okamoto and T. Kita *et al*., 2005. Different expression patterns of KIT, EGFR, and HER-2 (c-erbB-2) oncoproteins between epithelial and mesenchymal components in uterine carcinosarcoma. Cancer Sci., 94: 986-991.
12. Santin, A.D., S. Bellone, M. Gokden, M. Palmieri and D. Dunn, 2002. Overexpression of HER-2/neu in uterine serous papillary cancer. Clin. Cancer Res., 8: 1271-1279.
13. Ramieri, M.T., R. Murari, C. Botti, E. Pica, G. Zotti and P.L. Alo, 2010. Detection of HER2 amplification using the SISH technique in breast, colon, prostate, lung and ovarian carcinoma. Anticancer Res., 30: 1287-1292.
14. Bae, Y.K., G. Gong, J. Kang, A. Lee and E.Y. Cho *et al*., 2012. HER2 status by standardized immunohistochemistry and Silver-enhancedIn situ hybridization in Korean breast cancer. J. Breast Cancer, 15: 381-387.
15. Sauter, G., J. Lee, J.M.S. Bartlett, D.J. Slamon and M.F. Press, 2009. Guidelines for human epidermal growth factor receptor 2 testing: biologic and methodologic considerations. J. Clin. Oncol., 27: 1323-1333.
16. Wolff A.C., M.E. Hammond, J.N. Schwartz, K.L. Hagerty and D.C. Allred, 2007. American Society of Clinical Oncology/College of American Pathologists guideline recommendations for human epidermal growth factor receptor 2 testing in breast cancer. Arch. Pathol. Lab. Med., 131: 18-43.
17. Wolff, A.C., M.E.H. Hammond, D.G. Hicks, M. Dowsett and L.M. McShane *et al*., 2013. Recommendations for human epidermal growth factor receptor 2 testing in breast cancer: American Society of Clinical Oncology/College of American Pathologists Clinical Practice Guideline Update. J. Clin. Oncol., 31: 3997-4013.
18. García-García, E., C. Gómez-Martín, B. Angulo, E. Conde, A. Suárez-Gauthier *et al*., 2011. Hybridization for human epidermal growth factor receptor 2 testing in gastric carcinoma: a comparison of fluorescence in-situ hybridization with a novel fully automated dual-colour silver in-situ hybridization method. Histopathology, 59: 8-17.