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Immunohistochemical Expression Of ROS-1 In Mammary Ductal Carcinoma In Correlation With Hormonal Receptor Status And Her2Neu Expression

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ABSTRACT

ROS-1 protein is a transmembrane protein with a tyrosine kinase activity act as a growth or differentiation factor can derived cell proliferation acting as a proto-oncogen. It is expressed in the kidney and gastrointestinal tract and, in rare cases, in several other organs as well. Genetic rearrangements of ROS1 have been found in non-small cell lung cancer (NSCLC) and in other tumors as well like gastric carcinoma, ovarian carcinoma and others. This protein became of clinical interest because of the era of tyrosin kinase inhibitors role in treating cancer like crizotinib which have been approved for NSCLC with ROS1 alterations.

In this study we studied the immunohistochemical expression of ROS 1in mammary carcinoma in correlation with Hormonal receptor status (ER and PR) and Her2Neu expression.

Paraffin blocks of thirty cases of mammary ductal carcinoma were selelcted and studied by immunohistochemical staining for ER, PR, Her2Neu and ROS 1. Then the findings were analyzed statistically

Thirty-three percent of studied cases showed expression of ROS 1 which was assessed by stained cell proportion and cell staining intensity.

Neither stained cell proportion nor the staining intensity of ER and PR showed significant statistical correlation with ROS 1 expression . However a significant correlation was noted between ROS 1 expression and Her2Neu expression as 70% of ROS 1 positive cases were also positive for Her2Neu. This suggest a negative prognostic role of this protein in mammary carcinoma.

The finding of this relatively significant number of ROS 1 positive cases in mammary carcinoma can open the way for more understanding of mammary carcinoma pathogenesis and can open the way for the use of certain tyrosine kinase inhibitors in the most common type of female cancer.

Keywords: Immunohistochemical Expression Of ROS-1, Mammary Ductal Carcinoma In Correlation, Hormonal Receptor Status

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BACKGROUND

ROS-1 protein is a transmembrane protein with a tyrosine kinase activity act as a growth or differentiation factor coded by *ROS-1* gene (proto-oncogene) located on chromosome 6 (6q22.1) also known as MCF 3 or c-ros-1 first isolated from human glioblastoma cell line[1,2,3].

In animals and humans, ROS1 is expressed in the kidney and gastrointestinal tract and, in rare cases, in several other organs as well including the lungs, epididymis , breast , heart, and liver [4]. Genetic rearrangements of *ROS1* have been found in non-small cell lung cancer (NSCLC). *ROS 1* gene rearrangement have been found in other tumors as well such as gastric adenocarcinoma, ovarian cancer, cholangiocarcinoma, inflammatory myofibroblastic tumor, angiosarcoma, colorectal cancer and epithelioid hemangioendothelioma [5]. The fusion protein resulted for this gene rearrangement (ROS 1) has a constitutively active kinase activity and drives cellular proliferation. In the era of the use of kinase inhibitors in treating cancer this point has been studies thoroughly in different tumor types. Crizotinib is a small molecule inhibitor that is showing promise as an effective therapy in patients with Non Small Cell Lung Cancer (NSCLC) whose cells show alterations in *ROS 1* gene [5, 6].

Studying of this gene rearrangement may also give an insight to the pathogenesis of different tumor types including the mammary carcinoma as in this study.

FISH assays that utilize a break-apart probe spanning the common break point region (including exons 32, 34, and 35) in the *ROS1* gene is the most relied on test for this issue to date. The presence of a *ROS1* rearrangement result is a split signal in the majority of cases, or sometimes the loss of 5' probe signal in the case of the *FIG1-ROS1* translocation [7-9].

Because the FISH is an expensive and cumbersome assay to support in many laboratories, that requires specialized microscopy equipment and technical expertise, new test have been developed by which immunohistochemistry is performed in many pathology laboratories and can be readily interpreted by surgical pathologists in the course of clinical diagnostic practice. A ROS1 immunohistochemical antibody has recently been described that used to detect ROS 1-fusion proteins in NSCLC [8, 9].

Mammary carcinoma is the most common cancer in female in almost all regions in the world that shows different pathogenetic pathways during the tumor progression .

The tyrosine kinase activation pathway have been studied in breast cancer such as the activation of

epidermal growth factor molecules and C-Src molecules[10].

In this study we evaluated the immunohistochemical expression of ROS-1 protein, a tyrosine kinase receptor molecule, in mammary ductal carcinoma (the most common type of mammary carcinoma) and we studied the correlation of the result with hormonal receptor status (estrogen and progesterone receptors) and Her2Neu expression , another molecule of epidermal growth factor receptors family that also has a tyrosine kinase receptor activity [11].

These correlations may have an impact on mammary carcinoma prognosis as these tumors stratified according to the expression of hormonal receptors and Her2Neu expression.

MATERIAL AND METHODE

This study was performed in the pathology department in the college of Medicine/ Baghdad university. Thirty cases of mammary ductal carcinoma were selected from Teaching Laboratory of Baghdad medical city, the teaching hospital of Baghdad college of medicine.

Paraffin embedded sections of surgical and tru-cut needle biopsies were studied by immunohistochemistry using antibodies for Estrogen and Progesterone receptors and Her2Neu (All by DAKO-Agilent antibodies and Envision Flex detection system-USA) and correlated with immunohistochemical expression of ROS-1 (by BioSB-BSB3626-USA).

First, all cases were reviewed by routine histological examination confirming the presence of invasive mammary ductal carcinoma in the studied sections then immunohistochemical staining was performed by the assist of autostainer including the following steps

1. deparaffinization by heating method for 1 hour in 60ºC.
2. Complete deparffinization was performed by Xylene.
3. Sections were rehydrated by graded alcohol.
4. Antigen retrieving using the recommended solution for each antibody by heating method in a water bath.
5. Protein blocking by hydrogen peroxide for 5 minutes.
6. Antibody application in a 60 minutes.
7. Application of the recommended linkers to bind with secondary antibodies.
8. Application the secondary antibody for 30 minutes
9. Immunoperoxidase staining was developed using 3,3'diaminobenzidine (DAB) chromogen (Dako) for 5 min
10. counter staining by Meyer's hematoxilin and the slides covered by aqueous mounting media and visualized under microscope

Washing by the recommended buffer solution was done between the above steps which were performed in automized humid environment

Allred scoring system was used to analyze the immunohistochemical expression of estrogen and progesterone receptors expression which assess the proportion and intensity of staining as follows [12]:

The proportion of cell stained
No cell stained : 0
1% of cells stained : 1
2-10% of cell stained : 2
11-30% of cell stained :3
31-66% of cell stained :4
67-100% of cell stained :5

The intensity of cell staining:
No staining : 0
weak staining : 1
moderate staining : 2
strong staining : 3

Her2Neu expression was assessed by score from 0 to (+++)-(0: No staining, +:weak staining regarded as negative, ++: equivocal staining and +++: (positive)strong complete staining in more than 10% of tumor cells [13]

Quantitative evaluation of immunohistochemical expression of ROS-1 was assessed as follows [14]:
No staining : 0
less than 25% staining : 1
26-50% straining: 2
more than 50% staining : 3

Intensity of ROS-1 staining was assessed as follows:
No staining: 0
weak staining: 1
moderate staining: 2
strong staining: 3

Statistical analysis: Statistical analysis was performed with the SPSS 23 statistical software program. Univariate data were summarized using standard descriptive statistics, tabulation of categorical variables and histograms of numerical variables. Associations between categorical variables was assessed via cross tabulation and chi-square. Exact tests were used to calculate the p value. In all statistical analyses, a p value < 0.05 was considered significant.

Ethical consideration: There were minimal ethical implications and issues since it is a retrospective study. Patient identity and confidentiality were protected by assigning each patient a specific serial number. Moreover, no one except the investigating research team accessed the patients' records. We obtained prior approval from the Institutional Board of Baghdad Medical city since a consent form was not applicable to our study. The study is registered in the college of Medicine/Baghdad university.

RESULTS

Immunohistochemical staining for Estrogen receptors:

All cases were invasive ductal carcinoma, the most common type of mammary carcinoma. Seventeen out of the total 30 cases (56.67%) were positive. Of them 7/17 (41.18%) were of total Allred score 8/8, 1/17 (5.89%) was of total Allred score of 7/8, 2/17 (11.76%) were with Allred score of 5/8, 3/17 (17.65%) were with Allred score of 4/8 and 4/17 (23.52%) were of score 3/8.

Immunohistochemical staining for progesterone receptors:

Of all cases 15/30 (50%) cases were positive. Of them 6/15 (40%) were of Allred score of 8/8, 3/15 (20%) were of score 7/8, 3/15 (20%) were of score 4/8 and 3/15 (20%) were with Allred score of 3/8.

Immunohistochemical expression of Her2Neu:

Eleven of the total 30 cases (36.67%) cases were positive for Her2Neu, 6/30 (20%) were equivocal, while 13/30 (43.33%) were negative

Immunohistochemical staining of ROS-1:

Ten of all cases (33.33%) showed ROS-1 expression. Regarding stained cell proportion 4/10 (40%) cases were of score 1, 1/10 (10%) was of score 2, and 5/10(50%) were of score 3 (Figure 1).

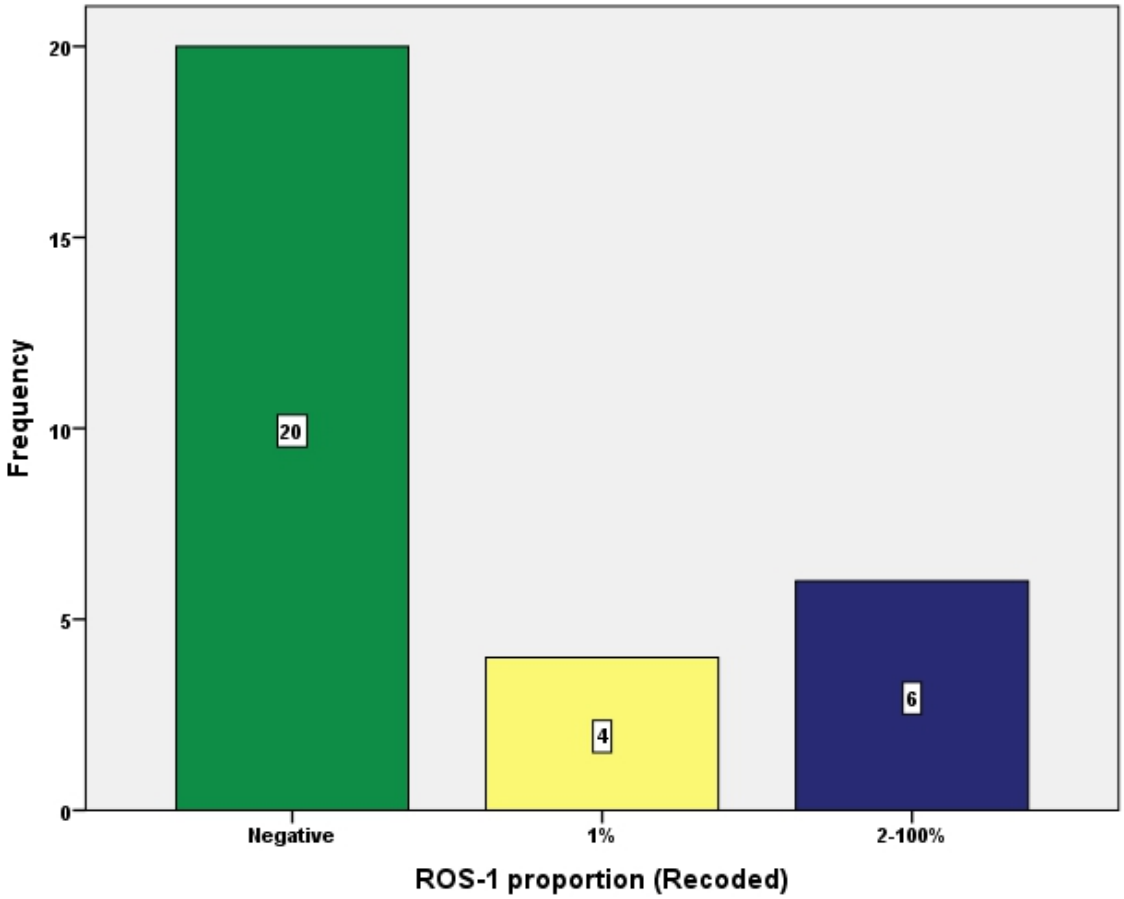

Figure 1: The ROS- positive cell proportion score of studied cases of mammary ductal carcinoma.

While for staining intensity(Figure 3), 3/10 (30%) were of score 1, 5/10 (50%)

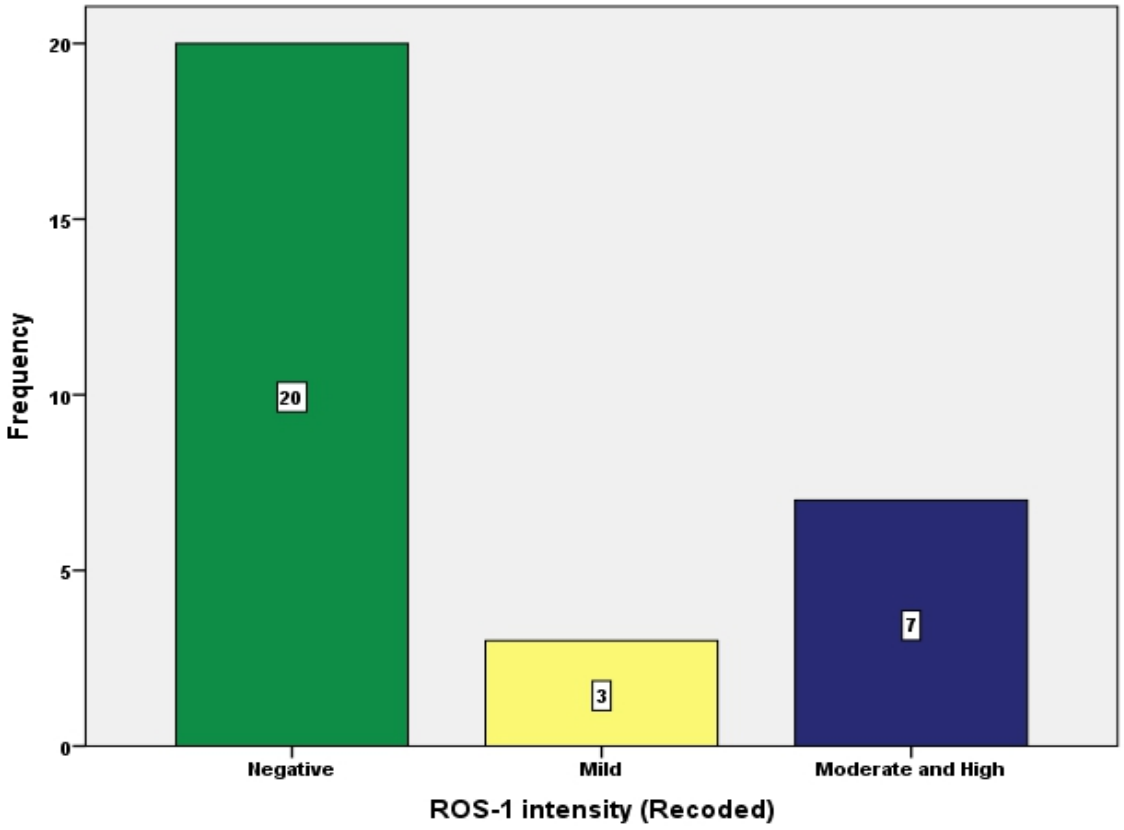

Figure 2: The ROS- positive cell intensity score of studied cases of mammary ductal carcinoma.

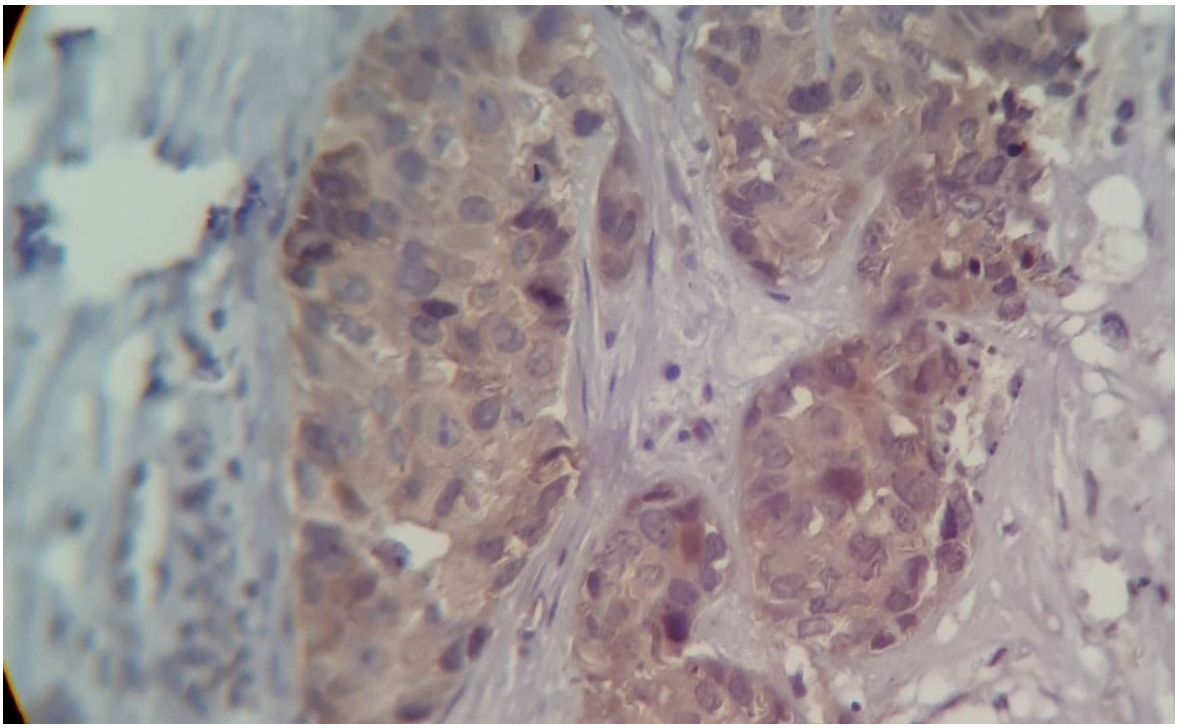

Figure 3: Moderately intense staining for ROS 1 in mammary carcinoma (score 2) (X400)

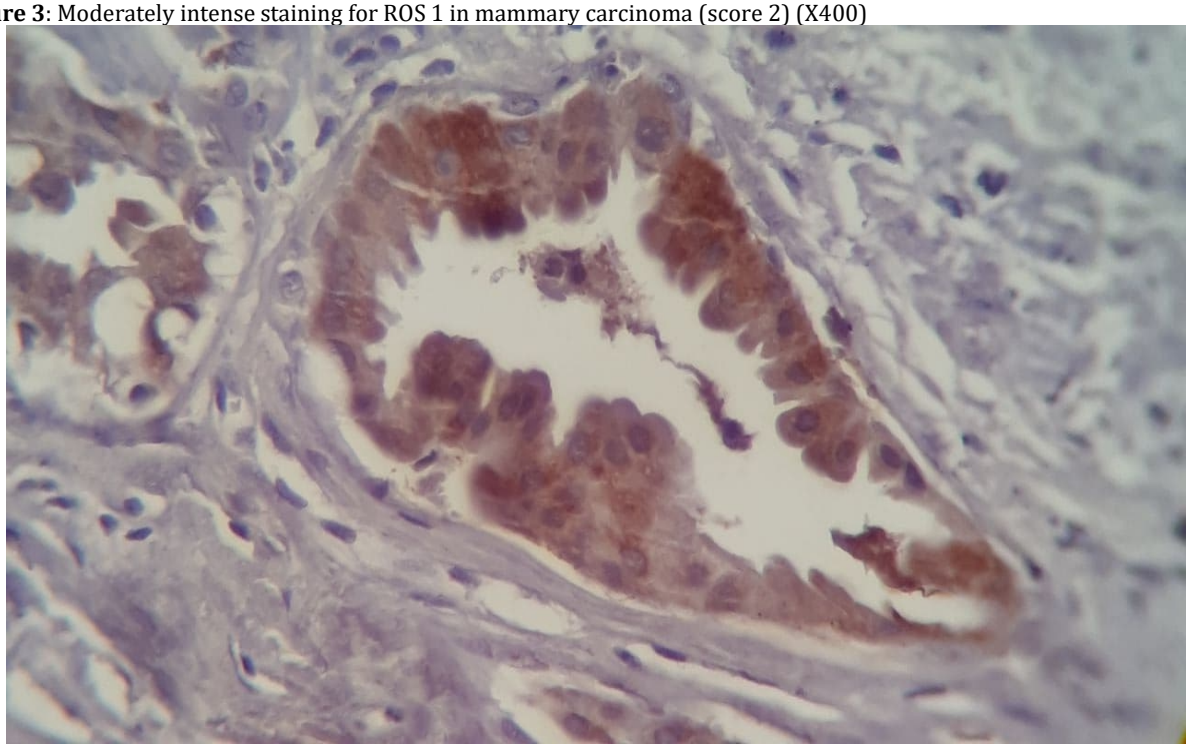

Figure : Intense staining for ROS 1 in mammary ductal carcinoma (score 3 intensity) (X400)

ROS-1 expression , the stained cell proportion and staining intensity did not showed significant statistical correlation with estrogen and progesterone receptor status (expression, stained cell proportion and staining intensity) (ALL *p* Values were more than 0.05) as explained in tables 1-10.

		ER expression		Total
		Negative	Positive	
ROS-1 expression	Negative	9	11	20
		45.0%	55.0%	100.0%
	Positive	4	6	10
		40.0%	60.0%	100.0%
Total		13	17	30
		43.3%	56.7%	100.0%

p = 1.0 (N.S)

Table 1: Association between the overall ROS 1 expression and ER expression in mammary ductal carcinoma

		PR expression		Total
		Negative	Positive	
ROS-1 expression	Negative	9	11	20
		45.0%	55.0%	100.0%
	Positive	6	4	10
		60.0%	40.0%	100.0%
Total		15	15	30
		50.0%	50.0%	100.0%

$p = 0.7$ (N.S)

Table 2: Association between the overall ROS 1 expression and PR expression in mammary ductal carcinoma

		ER proportion (Recoded)			Total
		Negative	1-30%(score 1-3)	31-100%(score4-5	
ROS-1 proportion (Recoded)	Negative	9	5	6	20
		45.0%	25.0%	30.0%	100.0%
	Score 1	2	2	0	4
		50.0%	50.0%	0.0%	100.0%
	Score 2-3	2	2	2	6
		33.3%	33.3%	33.3%	100.0%
Total		13	9	8	30
		43.3%	30.0%	26.7%	100.0%

$p = 0.81$ (N.S)

Table 3: Association between ROS 1-positive cell proportion and ER-positive cell proportion in mammary ductal carcinoma.

		PR proportion (Recoded)			Total
		Negative	Score 1-3	Score 4-5	
ROS-1 proportion (Recoded)	Negative	9	5	6	20
		45.0%	25.0%	30.0%	100.0%
	Score 1	4	0	0	4
		100.0%	0.0%	0.0%	100.0%
	Score 2-3	2	1	3	6
		33.3%	16.7%	50.0%	100.0%
Total		15	6	9	30
		50.0%	20.0%	30.0%	100.0%

$p = 0.32$ (N.S)

Table 4: Association between ROS 1-positive cell proportion score and PR-positive cell proportion score in mammary ductal carcinoma.

		ER intensity (Recoded)			Total
		Negative	Mild	Moderate and High	
ROS-1 proportion (Recoded)	Negative	9	1	10	20
		45.0%	5.0%	50.0%	100.0%
	Score 1	2	0	2	4
		50.0%	0.0%	50.0%	100.0%
	Score 2-3	2	1	3	6
		33.3%	16.7%	50.0%	100.0%
Total		13	2	15	30
		43.3%	6.7%	50.0%	100.0%

$p = 0.84$ (N.S)

Table 5: Association between ROS 1-positive cell proportion score and ER-positive cell intensity score in mammary ductal carcinoma.

		PR intensity (Recoded)		Total
		Negative	Moderate and High	
ROS-1 proportion (Recoded)	Negative	9	11	20
		45.0%	55.0%	100.0%
	Score 1	4	0	4
		100.0%	0.0%	100.0%
	Score 2-3	2	4	6
		33.3%	66.7%	100.0%
Total		15	15	30
		50.0%	50.0%	100.0%

$p = 0.14$ (N.S)

Table 6: Association between ROS 1-positive cell proportion score and PR-positive cell intensity score in mammary ductal carcinoma.

		ER proportion (Recoded)			Total
		Negative	Score 1-3	Score 4-5	
ROS-1 intensity (Recoded)	Negative	9	5	6	20
		45.0%	25.0%	30.0%	100.0%
	Mild	2	1	0	3
		66.7%	33.3%	0.0%	100.0%
	Moderate and High	2	3	2	7
		28.6%	42.9%	28.6%	100.0%
Total		13	9	8	30
		43.3%	30.0%	26.7%	100.0%

p = 0.83 (N.S)

Table 7: Association between ROS 1-positive cell intensity score and ER-positive cell proportion score in mammary ductal carcinoma.

		PR proportion (Recoded)			Total
		Negative	Score 1-3	Score 4-5)	
ROS-1 intensity (Recoded)	Negative	9	5	6	20
		45.0%	25.0%	30.0%	100.0%
	Mild	2	1	0	3
		66.7%	33.3%	0.0%	100.0%
	Moderate and High	4	0	3	7
		57.1%	0.0%	42.9%	100.0%
Total		15	6	9	30
		50.0%	20.0%	30.0%	100.0%

p = 0.48 (N.S)

Table 8: Association between ROS 1-positive cell intensity score and PR-positive cell proportion score in mammary ductal carcinoma.

		ER intensity (Recoded)			Total
		Negative	Mild	Moderate and High	
ROS-1 intensity (Recoded)	Negative	9	1	10	20
		45.0%	5.0%	50.0%	100.0%
	Mild	2	1	0	3
		66.7%	33.3%	0.0%	100.0%
	Moderate and High	2	0	5	7
		28.6%	0.0%	71.4%	100.0%
Total		13	2	15	30
		43.3%	6.7%	50.0%	100.0%

p = 0.18 (N.S)

Table 9: Association between ROS 1-positive cell intensity score and ER-positive cell intensity score in mammary ductal carcinoma.

		PR intensity (Recoded)		Total
		Negative	Moderate and High	
ROS-1 intensity (Recoded)	Negative	9	11	20
		45.0%	55.0%	100.0%
	Mild	2	1	3
		66.7%	33.3%	100.0%
	Moderate and High	4	3	7
		57.1%	42.9%	100.0%
Total		15	15	30
		50.0%	50.0%	100.0%

p = 0.75 (N.S)

Table 10: Association between ROS 1-positive cell intensity score and PR-positive cell intensity score in mammary ductal carcinoma.

A statistically significant correlation was noted between Ros-1 and Her2Neu expression (p Value is <0.05) as shown in table 11. Seven out of 10 positively stained cases for Ros-1 (70%) were also positive for Her2Neu. While 2/10(20%) were equivocal and 1/10 (10%) was negative for Her2Neu.

		Her-2 Neu expression			Total
		Negative	Equivocal	Positive	
ROS-1 expression	Negative	12	4	4	20
		60.0%	20.0%	20.0%	100.0%
	Positive	1	2	7	10

			10.0%	20.0%	70.0%	100.0%
Total			13	6	11	30
			43.3%	20.0%	36.7%	100.0%

***p* = 0.01**

Table 11: Association between ROS 1 expression and Her2Neu expression in mammary ductal carcinoma.

DISCUSSION

Beside its expression in glioblastoma multiforme and to lesser extent in meningioma, ROS-1 expression was proved to be associated with stomach cancer and in hepatoma cell line in rat [15]. ROS 1 mutated gene product was assumed to contribute to malignant progression by activation of tyrosine kinase pathway.

In the era of targeted therapy used to hit cancer progression by targeting tyrosine kinase pathway, drugs like entrectinib and crizotinib have been developed to hit ros 1 pathway in lung adenocarcinoma [16].

These facts evoked a work to study the role of ROS 1 pathway in different human cancers including ovarian carcinoma, sarcoma, cholangiocarcinoma and other [5,17].

Regarding breast lesions, ROS 1 was proved to be expressed at levels more than two folds in fibroadenoma than in normal breast tissue [18]. Ros1 mutation detection is not routinely assessed in breast cancer, yet Benjamin et al proved exceptional responses to crizotinib in patients with breast cancer in whom MET and ROS 1 mutation were detected by next generation tumor sequencing (NGS) [19,20] highlighting its proposed oncogenic role in mammary carcinoma.

Although Ros1 mutation was first characterized by FISH and Tissue Micro Array (TMA) assays, recently developed antibodies were proved to be valuable in assessing mutated ros1 protein by immunohistochemistry [21].

Classifying breast cancer into molecular subtypes is an important aspect of treatment decision making. Classical immunohistochemical assessment of ER, PR and Her2Neu of breast cancer play a crucial role in mammary carcinoma molecular classification and considered an important clinicopathological factor by itself to be correlated with other tumor aspect[22, 23]. In this study we correlated this aspect with the immunohistochemical expression of ROS 1 protein. In this way we can guess the prognostic value of ROS 1 in mammary ductal carcinoma.

In this study we did not found statistically significant correlation between expression of ROS 1 and hormonal receptor status (ER and PR) assessed by Allred scoring system. Neither stained cell proportion nor staining intensity for both receptors was correlated with ROS1 expression (assessed by stained cell proportion and staining intensity).

Minseob Eom, et al found that 70% of cases positive for Ros-1 were positive for ER while 30% were negative for ER [14], yet neither the staining intensity nor stained cell proportion was explained in their study. In somewhat similar finding in this study 6/10 (60%) of ROS1 positive cases were also positive for ER while 4/10 (40%) were negative for ER while the opposite was found regarding PR expression as 4/10 (40%) of cases were positive for PR and 6/10(60%) were negative for PR.

In this study ROS1 expression was significantly correlated with Her2 Neu expression (*p value* was less than 0.05) as 7/10 (70%) were also positive for Her2Neu, 2/10 (20%) were equivocal and 1/10(10%) was negative. As Her2Neu expression is considered a poor prognostic factor by itself [24] we can suggest that ROS 1 expression could also represent also a poor prognostic factor. When Minseob Eom et al used Kaplan Meier analysis in their study they found that higher ROS 1 expression was associated with lymph node metastasis which is a poor prognostic factor opposite to their final conclusion that ROS 1 expression is associated with good prognosis when they correlate it with grade, mitotic index and Ki67 proliferative index [14].

What also suggest that ROS1 alterations are associated with poor prognosis are the findings of Jeremy Force et al who found that these alterations are significantly associated with metastatic breast cancer spread to the brain and lymph nodes [25]. These findings call for studying ROS 1 expression prognostic significance individually with following up patients clinically for a significant period.

Apart from its prognostic significance, ROS 1 expression could open the way for the use of targeted therapy (tyrosine kinase inhibitors) in breast cancer as the work of Benjamin M. et al showed [19] by inducing growth inhibition and cell death [26].

We recommend that ROS 1 alterations need to be studied more in mammary carcinoma and to be studied individually as a prognostic factor particularly if the use of tyrosine kinase inhibitors is considered in cases showed alterations of ROS1.

REFERENCES

1. Birchmeier C, O'Neill K, Riggs M, Wigler M. Characterization of ROS1 cDNA from a human glioblastoma cell line. *Proc Natl Acad Sci* U S A. 1990;87:4799–4803. [PMC free article] [PubMed] [Google Scholar]
2. Lin JJ, Shaw AT. Recent Advances in Targeting ROS1 in Lung Cancer. *J Thorac Oncol*. 2017;12(11):1611-1625. doi:10.1016/j.jtho.2017.08.002
3. Nagarajan L, Louie E, Tsujimoto Y, Balduzzi PC, Huebner K, Croce CM. The human c-ros gene (ROS) is located at chromosome region 6q16----6q22. *Proc Natl Acad Sci U S A*. 1986 Sep;83(17):6568-72. doi: 10.1073/pnas.83.17.6568. PMID: 3529088; PMCID: PMC386545.
4. El-Deeb IM, Yoo KH, Lee SH. ROS receptor tyrosine kinase: a new potential target for anticancer drugs. *Med Res Rev*. 2011 Sep;31(5):794-818. doi: 10.1002/med.20206. Epub 2010 Aug 4. PMID: 20687158.
5. Davies KD, Doebele RC. Molecular pathways: ROS1 fusion proteins in cancer. *Clin Cancer Res*. 2013 Aug 1;19(15):4040-5. doi: 10.1158/1078-0432.CCR-12-2851. Epub 2013 May 29. PMID: 23719267; PMCID: PMC3732549.
6. Sholl LM, Sun H, Butaney M, Zhang C, Lee C, Jänne PA, Rodig SJ. ROS1 immunohistochemistry for detection of ROS1-rearranged lung adenocarcinomas. *Am J Surg Pathol*. 2013 Sep;37(9):1441-9. doi: 10.1097/PAS.0b013e3182960fa7. PMID: 23887156; PMCID: PMC3831351.
7. Takeuchi K, Soda M, Togashi Y, Suzuki R, Sakata S, Hatano S, Asaka R, Hamanaka W, Ninomiya H, Uehara H, Lim Choi Y, Satoh Y, Okumura S, Nakagawa K, Mano H, Ishikawa Y. RET, ROS1 and ALK fusions in

lung cancer. *Nat Med*. 2012 Feb 12;18(3):378-81. doi: 10.1038/nm.2658. PMID: 22327623.

8. Rimkunas VM, Crosby KE, Li D, Hu Y, Kelly ME, Gu TL, Mack JS, Silver MR, Zhou X, Haack H. Analysis of receptor tyrosine kinase ROS1-positive tumors in non-small cell lung cancer: identification of a FIG-ROS1 fusion. *Clin Cancer Res*. 2012 Aug 15;18(16):4449-57. doi: 10.1158/1078-0432.CCR-11-3351. Epub 2012 Jun 1. PMID: 22661537.
9. Bergethon K, Shaw AT, Ou SH, Katayama R, Lovly CM, McDonald NT, Massion PP, Siwak-Tapp C, Gonzalez A, Fang R, Mark EJ, Batten JM, Chen H, Wilner KD, Kwak EL, Clark JW, Carbone DP, Ji H, Engelman JA, Mino-Kenudson M, Pao W, Iafrate AJ. ROS1 rearrangements define a unique molecular class of lung cancers. *J Clin Oncol*. 2012 Mar 10;30(8):863-70. doi: 10.1200/JCO.2011.35.6345. Epub 2012 Jan 3. PMID: 22215748; PMCID: PMC3295572.
10. Biscardi JS, Ishizawar RC, Silva CM, Parsons SJ. Tyrosine kinase signalling in breast cancer: epidermal growth factor receptor and c-Src interactions in breast cancer. *Breast Cancer Res.* 2000;2(3):203-10. doi: 10.1186/bcr55. Epub 2000 Mar 7. PMID: 11250711; PMCID: PMC138776.
11. Andrechek ER, Muller WJ. Tyrosine kinase signalling in breast cancer: tyrosine kinase-mediated signal transduction in transgenic mouse models of human breast cancer. *Breast Cancer Res*. 2000;2(3):211-6. doi: 10.1186/bcr56. Epub 2000 Apr 12. PMID: 11250712; PMCID: PMC138777.
12. Qureshi A, Pervez S. Allred scoring for ER reporting and it's impact in clearly distinguishing ER negative from ER positive breast cancers. *J Pak Med Assoc*. 2010 May;60(5):350-3. PMID: 20527604.
13. Goud KI, Dayakar S, Vijayalaxmi K, Babu SJ, Reddy PV. Evaluation of HER-2/neu status in breast cancer specimens using immunohistochemistry (IHC) & fluorescence in-situ hybridization (FISH) assay. *Indian J Med Res*. 2012 Mar;135(3):312-7. PMID: 22561616; PMCID: PMC3361866.
14. Eom M, Lkhagvadorj S, Oh SS, Han A, Park KH. ROS1 expression in invasive ductal carcinoma of the breast related to proliferation activity. *Yonsei Med J*. 2013 May 1;54(3):650-7. doi: 10.3349/ymj.2013.54.3.650. PMID: 23549810; PMCID: PMC3635654.
15. Yovchev MI, Grozdanov PN, Joseph B, Gupta S, Dabeva MD. Novel hepatic progenitor cell surface markers in the adult rat liver. *Hepatology.* 2007 Jan;45(1):139-49. doi: 10.1002/hep.21448. PMID: 17187413.
16. Facchinetti F, Friboulet L. Profile of entrectinib and its potential in the treatment of ROS1-positive NSCLC: evidence to date. *Lung Cancer (Auckl).* 2019 Sep 9;10:87-94. doi: 10.2147/LCTT.S190786. PMID: 31572036; PMCID: PMC6747675.
17. Lovly CM, Gupta A, Lipson D, Otto G, Brennan T, Chung CT, Borinstein SC, Ross JS, Stephens PJ, Miller VA, Coffin CM. Inflammatory myofibroblastic tumors harbor multiple potentially actionable kinase fusions. *Cancer Discov*. 2014 Aug;4(8):889-95. doi: 10.1158/2159-8290.CD-14-0377. Epub 2014 May 29. PMID: 24875859; PMCID: PMC4125481.
18. Eom M, Han A, Yi SY, Shin JJ, Cui Y, Park KH. RHEB expression in fibroadenomas of the breast. *Pathol Int*. 2008 Apr;58(4):226-32. doi: 10.1111/j.1440-1827.2008.02215.x. PMID: 18324915.
19. Benjamin M. Parsons, David R. Meier, Sreeja Sreekumar, Craig S. Richmond, Kyle J. Ernzen, Kristopher A. Lofgren, Jake A. Deviley, Grzegorz T. Gurda and Paraic A. Kenny. Exceptional responses to crizotinib in breast cancer patients with somatic MET and ROS1 alterations. *AACR Annual Meeting 2019*; March 29-April 3, 2019; Atlanta, GA. DOI: 10.1158/1538-7445.SABCS18-1317
20. Ayoub NM, Al-Shami KM, Alqudah MA, Mhaidat NM. Crizotinib, a MET inhibitor, inhibits growth, migration, and invasion of breast cancer cells in vitro and synergizes with chemotherapeutic agents. *Onco Targets Ther.* 2017 Oct 5;10:4869-4883. doi: 10.2147/OTT.S148604. PMID: 29042798; PMCID: PMC5634371.
21. Yoshida A, Tsuta K, Wakai S, Arai Y, Asamura H, Shibata T, Furuta K, Kohno T, Kushima R. Immunohistochemical detection of ROS1 is useful for identifying ROS1 rearrangements in lung cancers. *Mod Pathol.* 2014 May;27(5):711-20. doi: 10.1038/modpathol.2013.192. Epub 2013 Nov 1. PMID: 24186139.
22. Viale G. The current state of breast cancer classification. *Ann Oncol. 2012* Sep;23 Suppl 10:x207-10. doi: 10.1093/annonc/mds326. PMID: 22987963.
23. Goldhirsch A, Wood WC, Coates AS, Gelber RD, Thürlimann B, Senn HJ; Panel members. Strategies for subtypes--dealing with the diversity of breast cancer: highlights of the St. Gallen International Expert Consensus on the Primary Therapy of Early Breast Cancer 2011. *Ann Oncol*. 2011 Aug;22(8):1736-47. doi: 10.1093/annonc/mdr304. Epub 2011 Jun 27. PMID: 21709140; PMCID: PMC3144634.
24. Eggemann H, Ignatov T, Burger E, Kantelhardt EJ, Fettke F, Thomssen C, Costa SD, Ignatov A. Moderate HER2 expression as a prognostic factor in hormone receptor positive breast cancer. *Endocr Relat Cancer. 2015* Oct;22(5):725-33. doi: 10.1530/ERC-15-0335. Epub 2015 Jul 17. PMID: 26187126.
25. Jeremy Force, Ethan S Sokol, Mary Love Taylor, Dale Huang, Paul K Marcom, Monika Davare and Jeffrey Marks. Abstract P3-06-09: Incidence of ROS1 genomic alterations in breast cancer. *Cancer Res* February 15 2020 (80) (4 Supplement) P3-06-09; **DOI:** 10.1158/1538-7445.SABCS19-P3-06-09
26. Gu TL, Deng X, Huang F, Tucker M, Crosby K, Rimkunas V, Wang Y, Deng G, Zhu L, Tan Z, Hu Y, Wu C, Nardone J, MacNeill J, Ren J, Reeves C, Innocenti G, Norris B, Yuan J, Yu J, Haack H, Shen B, Peng C, Li H, Zhou X, Liu X, Rush J, Comb MJ. Survey of tyrosine kinase signaling reveals ROS kinase fusions in human cholangiocarcinoma. *PLoS One*. 2011 Jan 6;6(1):e15640. doi: 10.1371/journal.pone.0015640. PMID: 21253578; PMCID: PMC3017127.