

### **Immunohistochemical Expression of 40S Ribosomal Protein SA and Fibronectin-1 in Breast Cancer Tissues from Sudanese Patients**

#### **ABSTRACT**

**Laminin-1 receptor** (40S ribosomal protein SA) and fibronectin-1 belong to the extracellular matrix (ECM) proteins which are involved in cell adhesion, migration and growth. This study was implemented for the purpose of investigating the expression of 40S ribosomal protein SA and fibronectin-1 in breast cancer tissues from Sudanese patients. One hundred and eight cancerous breast tissues and 42 normal breast tissues were involved in this study from the Histopathology laboratory of the Radiotherapy and Isotopes Center in Khartoum (RICK). The expression of 40S ribosomal protein SA and fibronectin-1 was investigated using the immunohistochemistry (IHC) Technique. The 40S ribosomal protein SA was positively expressed in 101 cancerous tissues (93.5%) compared to 15 normal breast tissues (35.7%). The fibronectin-1 staining was positive in 92 cancerous tissues (85.2%) compared to 14 normal breast tissues (33.3%). The expression of the laminin-1 receptor and fibronectin-1 together was positive in 88 cancerous tissues (81.5%) and the two were negative in 3 cancerous tissues (2.8%). The 40S ribosomal protein SA and fibronectin-1 together were positive in 6 normal breast tissues (14.3%) compared to 18 negative normal tissues (42.9%). When the mean ranks of the 40S ribosomal protein SA and the fibronectin-1 in the cancerous tissues were compared to their mean ranks in the normal tissues using the Mann-Whitney test, their differences were significant ( $p \leq 0.000$ ). The collective expression of fibronectin-1 and 40S ribosomal protein SA may be used as targets in trial for the treatment of breast cancer since their expression was positive in 81.5% of the cancerous tissues compared 14.3% of the normal tissues.

**Keywords:** *Cancerous breast tissues, , IHC, ECM, 40S ribosomal protein SA, Fibronectin*

#### **INTRODUCTION**

Extracellular Matrix (ECM) is a group of proteins and polysaccharides, which fill the intercellular spaces. They connect cells to cells and tissues to tissues. The Functions of the Extracellular Matrix include binding of cells and tissues to each others, cell migration, adhesion, differentiation and proliferation [1, 2].

The Extracellular matrix has variable structure and composition between people. The proteins of the ECM are classified to binding proteins and their receptors, ECM modifying enzymes, ECM binding growth factors and other ECM associated proteins [3].

40S ribosomal protein SA (Laminin- 1 receptor) is a one polypeptide glycoprotein with a molecular weight of 67 kDa and with high affinity for laminin -1 glycoprotein of basement membrane. It is expressed on the surface of different cell types. It is registered that laminin-1 receptor and its binding protein are highly expressed in some cases of cancer where they may be associated with tumor metastasis and invasion [4, 5, 6].

Fibronectin-1 is a glycoprotein found in the plasma, cellular surface and ECM. It is composed of two polypeptides with a molecular weight of 220 kDa. It plays a major role in cell adhesion and migration in different vital process like immunity, blood clotting, wound healing, angiogenesis and metastasis [7, 8, 9, 10].

The aim of this article is to investigate the expression of 40S ribosomal protein SA (laminin-1 receptor) and fibronectin-1 in cancerous and normal breast tissues from Sudanese patients.

## **MATERIAL AND METHODS**

### **Study Population**

Hundred and eight formalin fixed cancerous breast tissues from Sudanese patients were involved in this study compared to forty two formalin fixed normal breast tissues. The formalin fixed tissues were obtained from the Radiotherapy and Isotopes Center in Khartoum (RICK) during 2014 and 2015.

### **Ethical License**

This study was implemented after academic and ethical approval from the Sudan Academy of Sciences. A license was obtained from the RICK authorities to use the samples for the conduct of this study.

### **Study Design**

This study can be classified as quantitative, descriptive, laboratory based and case control research design.

### **Sampling**

108 Cancerous tissues form formalin fixed tissues were cut and embedded in paraffin to be ready for the immunohistochemistry. From the hundred and eight formalin fixed tissues, only thirty two samples contained normal tissues, 10 normal breast tissues from benign tumors were added to the control samples to increase their total number. The forty two samples were also embedded in paraffin.

### **Methods**

The immunohistochemical staining for laminin-1 receptor was done according to the manufacturer instructions (Thermo Scientific, Rev 120502J). Regarding the staining of fibronectin-1 in the paraffin embedded tissues, the USBiological Life Sciences kit number 030235 was used.

The results were statistically analysed by Mann-Whitney test using SPSS software (version 20).

## **RESULTS AND DISCUSSION**

### **Results**

This study involved 108 cancerous breast tissues, 32 of cancerous tissues were with cancer free margins. The total number of the control tissues were 42; 32 of them were from the margins of the cancerous tissues and 10 were normal tissues from benign tumors.

The majority of the cancerous tissues were classified as ductal carcinoma (94), lobular carcinoma (6) and others (8). The other histological types were multicentric tubular carcinoma, mucinous carcinoma, metaplastic carcinoma, terminal carcinoma and papillary carcinoma. The grades and their numbers of the cancer tissues were grade I (13), II (33) and III (61). However, one of the cancerous tissues grades was missing, so the sum of the three grades is 107 rather than 108. The ages of the study subjects were located under three age groups (20- 40; 39, 41- 64; 51 and  $\leq$  65; 18).

Regarding the staining results of 40S ribosomal protein SA of the cancerous and control (normal) tissues; it was positive in 101 of the cancerous tissues (93.5%) and in 15 of the normal tissues (35.7%). The negative tissues were 7 from the cancerous tissues (6.5%) and 27 from the normal tissues (64%) (Fig.1, Fig.2 and Fig.3). It can be concluded that laminin- 1 receptor was positive in the majority of the cancerous tissues while it was positive in one third of the normal tissues (Fig.1, Fig.2 and Fig.3).

The fibronectin-1 staining showed that 92 (85.2%) of the cancerous tissues were positive compared to 14 of the normal tissues (33.3%). The conclusion of the fibronectin-1 results is that the fibronectin-1 was positive in the majority of the cancerous tissues compared to one third of the normal tissues (Fig.1, Fig.2 and Fig.3).

The collective staining results of the 40S ribosomal protein SA and Fibronectin-1 showed that 81.5% (88) of the cancerous tissues were positive for both the 40S ribosomal protein SA and the fibronectin-1 compared to 14.3% (6) of the normal tissues. The negative results of the cancerous tissues were 3 (2.8%) while 42.9% (18) of the normal tissues were negative (Table.1).

The majority of the diagnosed breast cancer were classified as ductal carcinoma (94, 87%). 27 of the 94 ductal carcinoma tissues contained cancer free margins (control tissues). The staining results showed that 40S ribosomal protein SA was positive in 89 (94.7%) of the ductal carcinoma tissues compared 8 (29.6%) of the normal margin tissues. The fibronectin-1 positive cases in the cancerous and normal tissues were 82 (87.2%) and 9 (33.3%) respectively. Both laminin-1 receptor and fibronectin-1 were positive in 79 (84%) of the ductal carcinoma tissues and one normal tissue (3.7%) was positive for both of the analytes. The collective results in the ductal carcinoma tissues showed that combining the results of 40S ribosomal protein SA and fibronectin-1 gave a useful result since 79 samples of the cancerous tissues were positive compared only 1 normal tissue (Table.2).

With regard to the IHC staining of the different breast cancer grades, it was clear that only one tissue in each grade was negative for both of the parameters. The highest percentage of the both positive results was seen in Grade II (Table.3).

The non parametric Mann-Whitney test was used to compare between the expression of the 40S ribosomal protein SA and fibronectin-1 in the cancerous and normal breast tissues. Regarding the expression of the laminin-1 receptor (40S ribosomal protein SA), the mean ranks in the cancerous and normal tissues were 88.14 and 43 respectively, the Mann-Whitney U was 903 and the 2-tailed significance was  $\leq 0.000$  (Table.4). The fibronectin-1 expression in the cancerous and normal tissues showed that the mean ranks were 86.39 and 47.50 respectively, Mann-Whitney U was 1092 and the significance was  $\leq 0.000$  (Table.4). We can conclude that the expression of the 40S ribosomal protein SA and fibronectin-1 was significantly increased in the cancerous breast tissues compared to their expression in the normal breast tissues.

## Discussion

This study registered that 81.5% of the cancerous tissues were positive for both 40S ribosomal protein SA and fibronectin-1 compared 14.3% of the normal tissues. However, their expression was significantly increased in the cancerous breast tissues compared to their expression in the normal breast tissues (2-tailed significance  $\leq 0.000$ ). The ductal carcinoma staining results were comparable to the results of the all types since 87% of the ductal carcinoma samples were positive for the two parameters compared to 3.7% of the normal tissues. However, the 40S ribosomal protein SA and fibronectin-1 expression are better to be used in treatment trials of ductal carcinoma patients since only 3.7% of the normal tissues were positive for both of them.

The normal tissues positives percentages were 35.7% for 40S ribosomal protein SA and 33.3% for fibronectin-1. The positive normal tissues for both laminin-1 receptor and fibronectin-1 were 3.7% i.e. the collective results of 40S ribosomal protein SA and fibronectin-1 are better for the prediction of prognosis and they can targeted to treat breast cancer and fight metastasis.

Grade wise, grade II was characterized by the highest expression of both of the parameters in all the samples and in the ductal carcinoma tissues.

We have noted that Reidar Albrechtsen and his colleagues in 1981 concluded that malignant breast cells were strongly positive for laminin and that the epithelial cells of the ducts of the normal breast tissues were slightly positive for laminin [11]. However, our results showed that only 14.3% of the normal tissues were positive for laminin-1 receptor (40S ribosomal protein SA). Jonathan Scheiman and colleagues stated that Laminin Receptor was over expressed in many cancers and expressed the possibility of using it as a target for gene therapy [12]. Our study registered that laminin receptor was positive in (93.5%) of the study populations and in (94.7%) of the ductal carcinoma tissues which indicate the possibility of using it as a target for any type of cancer therapy or in vaccination trials. Lourdes A. Marques research group studied the prognostic value of laminin receptor and 49.8% of their study samples were positive for laminin receptor [13]. In a comparison of integrative pathway analysis of Inegrins, Laminin and EGFR in pre menopause and post menopause breast cancer, Serena Liao concluded that pre menopause breast cancer is characterized by elevated level of integrin\ laminin and EGFR signaling [14].

Regarding the Axillary Node Negative (ANN) breast carcinoma, Yun NIU and his colleagues in 2002 mentioned that Laminin receptor together with EGFR may be used as biological markers to predict metastasis in ANN breast cancer [15].

Lise Christensen study group found that 55% of invasive breast carcinoma cases were positive for fibronectin-1 [8], however, we have registered that fibronectin was positive in 85.2% of all of our study subjects and in 87.2% of the ductal carcinoma cases.

We have found that Bae YK in 2013 studied the expression of epithelial and stromal fibronectins in invasive breast cancer samples and he found that the epithelial form of fibronectin was moderate or highly positive in 76.1% of his study subjects compared to 85.2% positive for fibronectin-1 of our study subjects [16]. In another study Takei H registered 65.7% positivity of fibronectin in invasive breast carcinomas compared to 34.3% negative tissues [17].

A French study revealed that oncofetal fibronectin was positive in 60% of adenocarcinoma cases and it was not expressed in the normal glands and benign tumors while similar to our findings normal fibronectin was expressed in the normal, benign and carcinoma tissues [18].

Concerning the ductal carcinoma results Lawrence F. Brown et al in 1999 stated that 35 out 43 (81.4%) of invasive ducatal carcinoma tissues were strongly positive for fibronectin compared to low and moderate expression in 19 samples out of 28 (67.9%) normal tissues [19], while we registered 82 out 94 (87.2%) positive ductal carcinoma tissues for fibronectin-1 and 9 out of 32 (28.1%) of the normal tissues were positive for fibrnectin-1.

Similar to our findings that laminin Receptor (40S ribosomal protein SA) and fibronectin are both expressed by cancerous and normal breast tissues, Lise Christensen group stated that laminin and fibronectin are expressed by normal and cancerous cultured human breast cells [20].

The conclusion of this study is that the 40S ribosomal protein SA and fibronectin-1, collectively, may be useful as targets for future breast cancer therapies or in vaccination trials since their expression was significantly increased in the cancerous tissues compared to the normal tissues. The laminin-1 receptor and fibronectin-1 were positively expressed in the majority of the cancerous breast tissues (81.5%) while they were expressed in less than 15% of the normal breast tissues.

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**Table.1: Collective Staining Results of the 40S Ribosomal Protein SA and Fibronectin-1**

| IHC staining                                                       | Tumor |      | Normal |      |
|--------------------------------------------------------------------|-------|------|--------|------|
|                                                                    | No    | %    | No     | %    |
| Both positive                                                      | 88    | 81.5 | 6      | 14.3 |
| Both negative                                                      | 3     | 2.8  | 18     | 42.9 |
| 40S Ribosomal Protein SA positive<br>and<br>Fibronectin-1 negative | 13    | 12.0 | 10     | 23.8 |
| 40S Ribosomal Protein SA negative<br>and<br>Fibronectin-1 positive | 4     | 3.7  | 8      | 19.0 |
| Total                                                              | 108   | 100  | 42     | 100  |

The 40S ribosomal protein SA and fibronectin-1 were both positive in the majority of the cancerous tissues (81.5%) while they were positive in 14.3% of the normal tissues. This result reflected the possibility of using both of them as targets for future drugs or in vaccination trials

**Table.2: The Histological Types of the Breast Cancer and their Staining Results**

| Histological type | No  |    | 40S ribosomal protein SA Positive |    | Fibronectin-1 Positive |    | Both positive |   | Both negative |    |
|-------------------|-----|----|-----------------------------------|----|------------------------|----|---------------|---|---------------|----|
|                   | T   | N  | T                                 | N  | T                      | N  | T             | N | T             | N  |
| Ductal carcinoma  | 94  | 27 | 89                                | 8  | 82                     | 9  | 79            | 1 | 2             | 11 |
| Lobular carcinoma | 6   | 2  | 5                                 | 2  | 4                      | 1  | 3             | 1 | 0             | 0  |
| Others            | 8   | 3  | 7                                 | 1  | 6                      | 1  | 6             | 1 | 1             | 2  |
| Totals            | 108 | 32 | 101                               | 11 | 92                     | 11 | 88            | 3 | 3             | 13 |

The 40S ribosomal protein SA and the fibronectin-1 were both positive in 84% of the ductal carcinoma cases while they were positive in one normal tissue (3.7%).

**Table.3: The Staining result of the 40S Ribosomal Protein SA and Fibronectin-1 in the Different Breast Cancer Grades**

| Grade | 40S ribosomal protein SA |      |          |      | Fibronectin-1 |      |          |      | Both results |      |          |     |
|-------|--------------------------|------|----------|------|---------------|------|----------|------|--------------|------|----------|-----|
|       | Positive                 |      | Negative |      | Positive      |      | Negative |      | Positive     |      | Negative |     |
|       | No                       | %    | No       | %    | No            | %    | No       | %    | No           | %    | No       | %   |
| I     | 10                       | 76.9 | 3        | 23.1 | 9             | 69.2 | 4        | 30.8 | 7            | 53.8 | 1        | 7.7 |
| II    | 32                       | 97.0 | 1        | 3.0  | 28            | 84.8 | 5        | 15.2 | 28           | 84.8 | 1        | 3.0 |
| III   | 58                       | 95.1 | 3        | 4.9  | 54            | 88.5 | 7        | 11.5 | 41           | 67.2 | 1        | 1.6 |

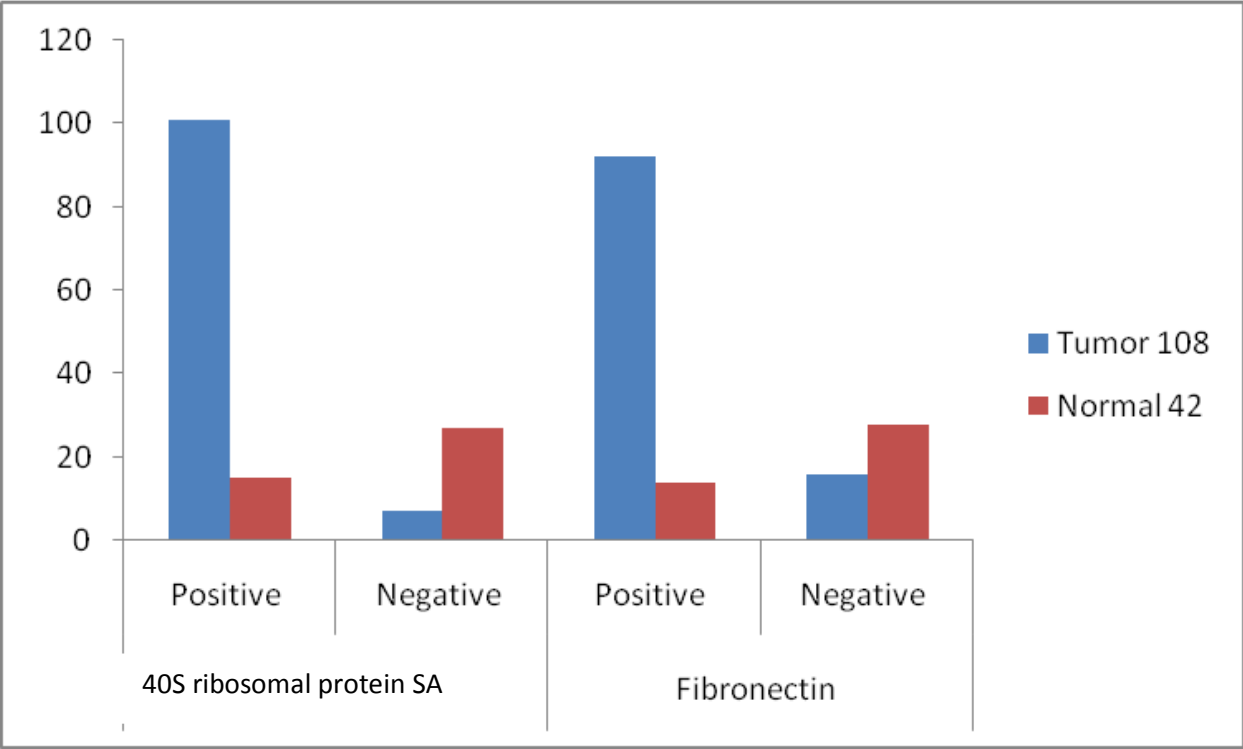
Grade II was associated with the highest expression in 40S ribosomal protein SA and fibronectin-1 collectively. The expression of the 40S ribosomal protein SA was highly positive in grade II compared to the other grades while fibronectin-1 was highly positive in grade III.

**Table.4: Comparison between the Study Groups using the Mann-Whitney U Test**

|                         | 40S ribosomal protein SA |           | Fibronectin-1 |           |
|-------------------------|--------------------------|-----------|---------------|-----------|
|                         | Number                   | Mean rank | Number        | Mean rank |
| Cancerous tissues       | 108                      | 88.14     | 108           | 86.39     |
| Normal tissues          | 42                       | 43        | 42            | 47.50     |
| Mann-Whitney U          | 903                      |           | 1092          |           |
| Significance (2-tailed) | 0.000                    |           | 0.000         |           |

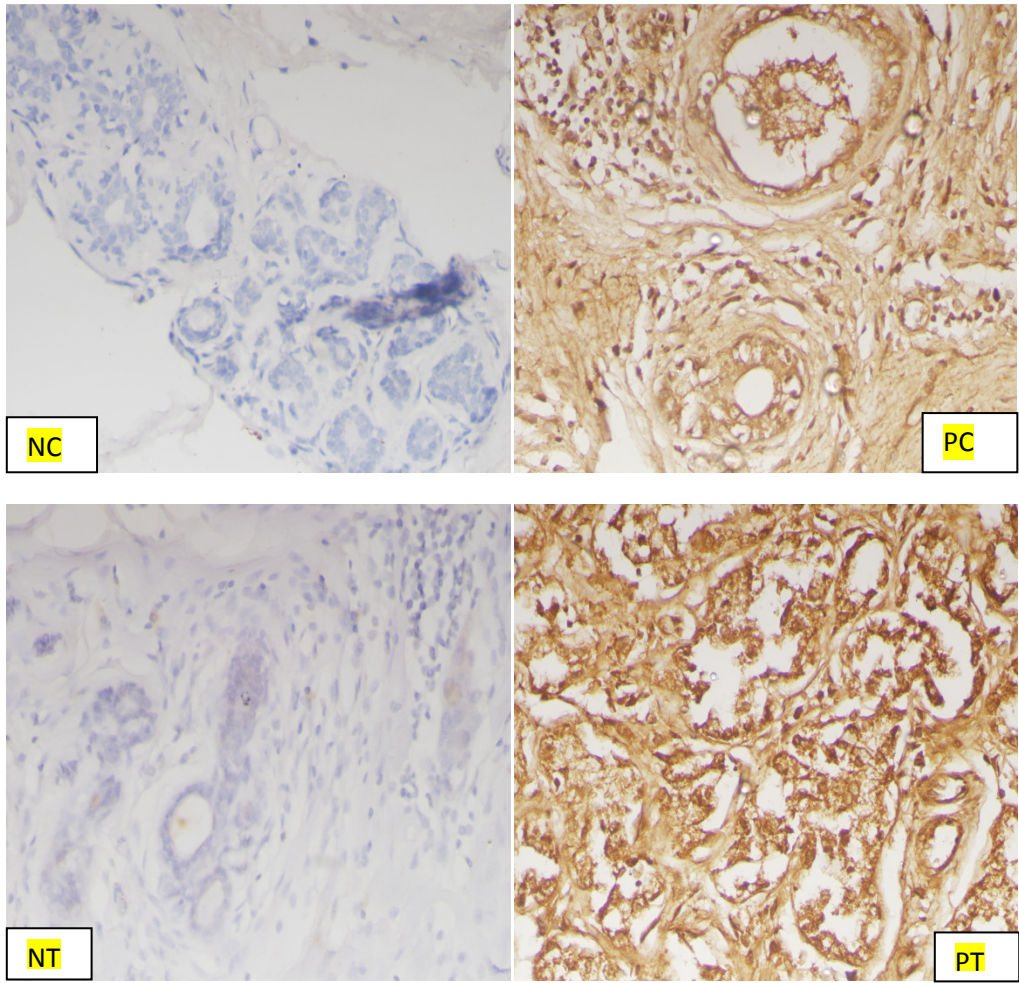
The Mann-Whitney non parametric test showed that there was significant difference between the expression of the study parameters in the cancerous and normal breast tissues

**Fig.1: The Staining Results of 40S Ribosomal Protein SA and Fibronectin-1 in the Study Subjects**



The staining results of the 40S ribosomal protein SA and fibronectin-1 were highly expressed in the majority of the tumor tissues compared to the normal tissues

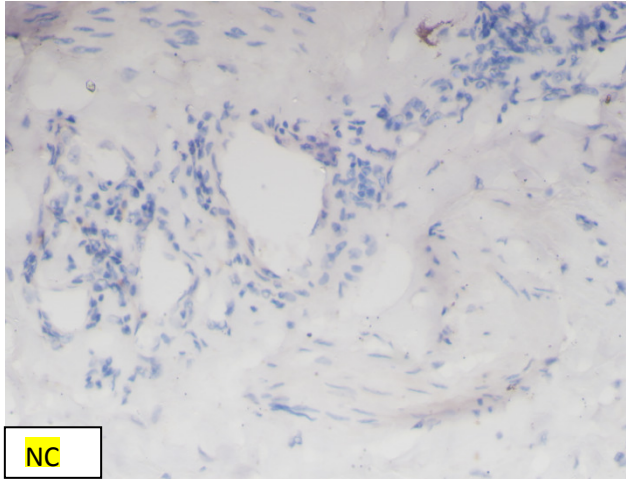
**Fig.2: Representative Staining Results of the 40S Ribosomal Protein SA**



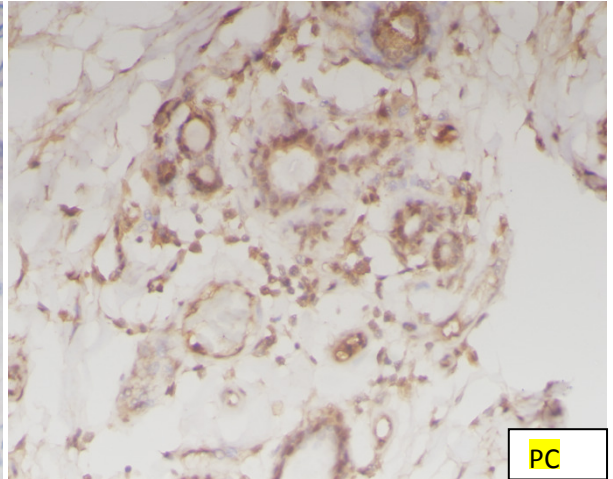
The 40S ribosomal protein SA was positively and negatively stained in both the tumor and the normal breast tissues. The expression of the 40S ribosomal protein SA was positive in 93.5% of the cancerous tissues compared to 35.7% of the normal tissues.

NC; Negative Control , PC; Positive Control, NT; Negative Tumor and PT; Positive Tumor  
Controls are the normal breast tissues

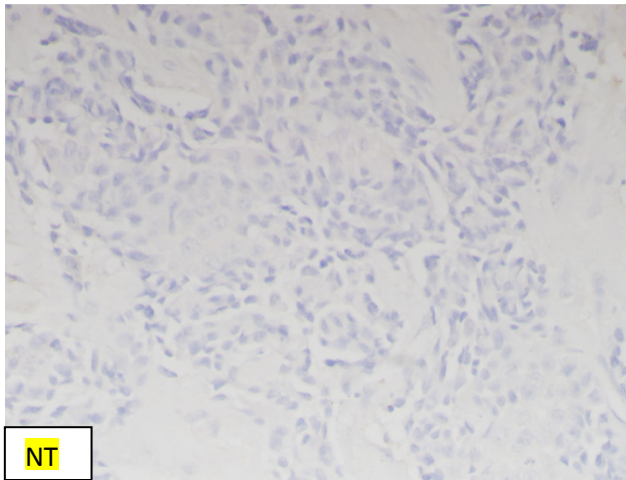
**Fig.3: Staining Results of Fibronectin -1 in the Cancerous and Normal Tissues**



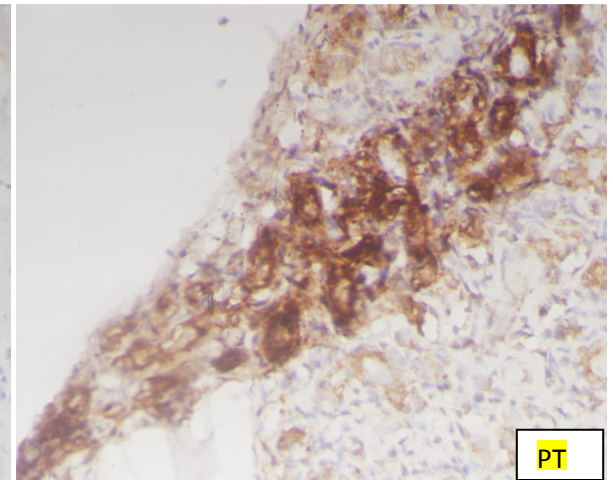
NC



PC



NT



PT

The expression of fibronectin- 1 was positive and negative in the control and tumor breast tissues with different frequencies. It was positive in 85.2% of the cancerous tissues compared to 33.3% of the normal breast tissues.

NC; Negative Control, PC; Positive Control, NT; Negative Tumor and PT; Positive Tumor

Controls are the normal breast tissues