THE CORRELATION OF THE BRCA-IRIS GENE EXPRESSION AND BIOMARKER GENES IN BREAST CANCER PATIENTS: CORRELATION WITH TUMOR PROGRESSION AND RESPONSE TO TREATMENT

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Abstract

Background: Triple-negative breast cancer (TNBC) is an aggressive phenotype with bad prognosis and poor survival. The prognostic and predictive values of BRCA1-Iris, octamer-binding transcription factor 4 (oct 4), cyclin D1 and survivin were assessed in TNBC patients compared with N-TNBC patients.

Method: RNA expression levels of BRCA1-IRIS, Oct4, Cyclin D1 and Survivin gene were tested in for 100 breast cancer patients by qRT-PCR.

Result: overexpression of BRCA1-IRIS RNA was found in 49 % of breast cancer patients [(35(71 %) of TNBC cases vs 14(28.6% of N-TNBC] (p<0.001). There was significantly correlated between the BRCA1-IRIS and tumor stage, ER and PR (p=0.036, p<0.001 and p=0.006; respectively) in breast cancer patients. In TNBC or N-TNBC, no statistically significance between BRCA1-IRIS and any clinicopathological features of patients. There was significant association between BRCA1-IRIS positive and the expression of oct4, cyclin D1 and survivin gene in TNBC patients (p<0.001, p=0.001 and p=0.002; respectively). There was a strong significant correlation between the expression of oct4 and the BRCA1-IRIS negative (p<0.001) in TNBC group. No significant association between BRCA1-IRIS positive in N-TNBC and any of the gene expression. BRCA1-IRIS negative was correlated significantly with oct4 and cyclin D1 expression (p=0.001each).No significant correlation between the expression RNA level of oct4, cyclin D1 and survivin gene and the expression RNA level of BRCA1-IRIS except lymphnode with survivin gene expression (p=0.05). No significant relation between the expression level of oct4, cyclin D1 and survivin gene and the relevant clinicopathological features with or without BRCA1-IRIS expression. Expression level of survivin and cyclin D1 was significantly correlation with the response to treatment (p=0.001 and p<0.001). No significant different between overall survival and BRCA1-IRIS expression (p=0.291 long rang). However, disease-free survival was increased in BRCA1-IRIS positive cases (p = 0.052 long rang)

Keywords: Triple negative breast cancer, Non- Triple negative breast cancer, BRCA1-IRIS gene, Oct4 gene, cyclin D1 gene, Survivin gene
1. Introduction

Breast cancer is the most type of cancer in women in either developed or less developed countries. It is a count of 21% of all cancers. WHO (2015). In Egypt, the ratio of female breast cancer reaching 38% of all newly diagnosed cancer cases in the country Azim HA and Ibrahim AS. (2014) and Salhia B. et al., (2011). Breast cancer is a heterogeneous disease of different biological subtypes, which identified by gene expression profiling using DNA and RNA microarrays Perou CM. et al., (2000). Moreover, these biological subtypes have different clinicopathological and molecular features that impact differently on the prognosis and treatment outcome Onitilo AA. et al., (2009). Triple negative breast cancer (TNBC) is defined by aggressive tumors, diagnosed in the younger age group, with shorter disease-free survival (DFS) Dunnwald LK. Et al., (2007) and Rakha EA. Et al., (2007).

BRCA1-IRIS is an oncogene, it is overexpressed in breast cancer, especially the TNBCs Shimizu Y. et al., (2012) and Blanchard Z. et al., (2015). These expressions will upregulate the expression of basal biomarkers Blanchard Z. et al., (2015) and enhances the epithelial- mesenchymal transition in cancer cells Blanchard Z. et al., (2015). BRCA1-IRIS overexpression drives the formation of TNBCs, correlate with lack of BRCA1 expression in the tumors Shimizu Y. et al., (2012). BRCA1-IRIS overexpression enhances the tumor-initiating phenotype in breast cancer cells Sinha et al., (2017).

Cyclin D1 plays an important role in cell cycle progression through the associating with CDK4 and CDK6, which phosphorylate and inactivate the retinoblastoma protein (pRb) leading to the expression of a subset of proliferation-associated E2F target genes (Inoue and Fry, 2015). Some studies demonstrated that cyclin D1 a cell cycle regulatory gene. It is an oncogene that is directly related to the carcinogenesis. Overexpression of cyclin D1 gene was observed in many tumor tissues (Choi et al., 2018). The co-overexpression of cyclin D1 and BRCA1-IRIS in breast cancer cells coupled with increased proliferation. The BRCA1-IRIS complex with steroid receptor co-activators was targeted to the cyclin D1 promoter pre-bound by the c-Jun/AP1 and activated its transcription (Hao and ElShamy, 2007). The survivin gene locus encodes multiple genetic splice variants with unique properties and functions. It has many isoforms, in malignant cells, all these isoforms are expressed at a very high rate compared to normal tissues. Survivin has a dual function, involved in cell death regulation as well as in mitotic progression (Li et al., 2017b). Survivin expression has also been associated with p53 expression, which may be induced by cell damage (Boullosa et al., 2018). The octamer-binding transcription factor 4 (oCT4) is a transcription factor known as POU (Liu et al., 2013). OCT4 is expressed with high rate in the cells of many cancers either solid or hematological (Shen et al., 2014, Guzel et al., 2014 and Poursani et al., 2016). A study has shown that the presence of an internal ribosomal entry site (IRES) for oct4B, can generate three isoforms by alternative translation initiation. Another variant of oct (oct4B1) is localized in both the cytoplasm and nucleus of undifferentiated and pluripotent cells. However, oct4B1 is not considered a stemness marker (Shen et al., 2014).
The aim of this work: to determine the prevalence of *BRCA1-IRIS*, *Oct4*, *Cyclin D1* and *Survivin* in patients with invasive breast cancer patients. It also aims at defining the effect of *BRCA1-IRIS*, *Oct4*, *Cyclin D1* and *Survivin* overexpression on the biological behavior of TNBCs compared to the non-TNBCs cases.

2. Material and Methods

Patients

The study involved one hundred formalin fixed paraffin embedded tissue (FFPE) samples of breast cancer diagnosed as invasive duct carcinoma. Normal breast cancer tissue (20 FFPE) was obtained as control group. All patients attend to National Cancer Institute (NCI), Cairo University. The samples were divided into two groups (TNBC and N-TNBC). The age of patients ranged from 18 to 65 years. The Tumor tissue samples was histologically stablished as graded and invasive duct carcinoma award to WHO classification. The tumor stage confirmed by American Joint Committee on Cancer’s staging Manual, 7th edition *(Edge & Compton, 2010 and Lakhani et al., 2012)*.

RNA extraction.

RNA was extracted from tumors samples and non-malignant breast tissues using RNAeasy Mini Kit (Qiagen, Milan, Italy). Reverse transcription was done using Script TM cDNA Synthesis Kit (Bio-Rad, Milano, Italy) according to manufacturer's instructions. In brief, seven paraffin sections (5µm each) cut into a plastic, sterile, 2ml Eppendorf tube and were used for RNA extraction. QRT-PCR used to demonstrate the expression levels of *BRCA1-IRIS*, *Oct4*, *Cyclin D1*, *Survivin* and β-actin. The sequence of primers that were used illustrated in Table 1.

Table 1. Sequences of the primers

| Gene name       | Forward                     | Reverse                      |
|-----------------|-----------------------------|------------------------------|
| *BRCA1-IRIS*    | 5’-GTCTGAGTGACAAGGAATTG GCC-3’ | 5’-TTAAGCTACTCTTTGGAATTTGTAA AATGTG-3’ |
| *OCT4*          | 5’-GATGGGGACTTCTGAGGGCC-3’  | 5’-GGGACTCCTCCGGGTTTTG-3’   |
| β-actin         | 5’-CGGCGATATCATATCCATCC-3’  | 5’-GCGGCGATATCATCCATCC-3’   |
| *cyclin D1 (CCND1)* | 5’-CTGGGTGTCCTACAAATG-3’     | 5’-AGCGGTCCAGGGATGTC-3’     |
| *Survivin*      | 5’-TCCACTGGGGCCTGAGAAAC-3’  | 5’-TGGGCTCAGCTTCCA-3’       |

Calculation of RNA expression level
The mRNA levels were calculated in triplicates by sued Syber Green Inc., Foster City, CA, USA), normalized to β-actin as a house keeping gene and expressed in relation to a calibrator sample. The final volume used is 25 μl. The mean Ct for each sample was calculated to detect the ΔCt for this sample: ΔCT = Ct for the gene of interest - Ct of the internal control gene (β-actin). Then the ΔΔCT was calculated as follows: ΔΔCT = [(Ct for the gene of interest - Ct of the internal control gene (β-actin) for sample A - (Ct for the gene of interest - Ct of the internal control gene (β-actin) for sample B], where sample B is the calibrator. For statistical analysis, the ΔΔCT and not the raw Ct data were used in the analysis (Livak KJ & Schmittgen TD, 2001).

Statistical method:

Mann-Whitney test (non-parametric t-test) used to comparison Quantitative data between two groups. Survival functions were calculated using the Kaplan-Meier method, and the log-rank test was used to compare the survival curves. Prognostic factors of overall survival and disease-free survival were analyzed by the Cox proportional hazards model, and the hazard ratios (HRs) were calculated with a 95% confidence interval (CI). All tests were two-tailed. A p-value <0.05 was considered significant.

Results

The study included one hundred breast cancer (invasive duct carcinoma). The patients were divided into two groups (TNBC and N-TNBC). The expression of BRCA1-IRIS, Oct4, Cyclin D1 and Survivin was assessed in the two studied groups and the relations with the clinic-pathological features of patients as well as to demonstrate the response to treatment.

1. BRCA1-IRIS gene expression in breast cancer patients.

Out of 100 patients BRCA1-IRIS were upregulated in 49 (49%) and downregulate in 51(51%). BRCA1-IRIS gene was expressed in 35 (71.4%) in TNBC patients compared to 14(28.6%) patients in N-TNBC. BRCA1-IRIS was not expressed in 15 (29.4%) of TNBC patients compared to 36 (70.6 %) patients who had N-TNBC tumor. BRCA1-IRIS gene expression was significantly higher in TNBC group compared to N-TNBC one (p<0.001) (Table2). By qRT-PCR, the mean fold expression level of BRCA1-IRIS gene in TNBC patients was higher (2.7fold) than in N-TNBC (2.3fold) (figure 1).

Table 2. The BRCA1-IRIS gene expression in TNBC and N-TNBC groups

| BRCA1-IRIS       | Breast cancer patients n=100 | TNBC Group n=50 | Non-TNBC Group n=50 | p value |
|------------------|------------------------------|-----------------|----------------------|--------|
| Downregulated    | 51                           | 15 (29.4%)      | 36 (70.6%)           | <0.001 |
| Upregulated      | 49                           | 35 (71.4%)      | 14 (28.6%)           |        |
overexpressing with cutoff defined as expression ≥ 2-fold compared to normal samples. ≠ Downregulated with cutoff defined as expression < 2-fold compared to normal samples.

Figure 1: The mean fold relative expression level of IRIS gene in TNBC and non-TNBC groups. *# indicate that significant difference from control and N-TNBC groups respectively.

2. Clinico-pathological features of patients in relation to BRCA1-IRIS RNA expression

Table 3: No significant difference shown between the whole group and the BRCA1-IRIS gene expression except for late stage, ER and PR. BRCA1-IRIS was positive in 35(43.8%) of the patients who have early tumor stage and 14(70%) of the patients who have late tumor stage (p=0.036). BRCA1-IRIS was positive in 41 (65.1%) cases that negative ER in 8(21.6%) with positive ER receptor (p<0.001). Regard to BRCA1-IRIS positive in 41(67.7%) with negative PR receptor, it was in 8/29(27.6%) with positive PR receptor (p=0.006). BRCA1-IRIS was positive in 11(57.9%) with the patients of the age ≤50 years old (mean SD =50.8 ±11.7) and in 38(46.9%) with the patients of the age > 50 years old (mean SD =50.8 ±11.7) (p= 0.389). BRCA1-IRIS was positive in 38(48.7%) with tumor size ≤5cm and 11(50%) with tumor size >5cm(p=1.000). As for family history, BRCA1-IRIS was positive in 44(49.4%) patients who had negative family history, but it was positive in 5(45.5%) patients that had positive family history (p=1.000). BRCA1-IRIS was positive in 23(50%) in the premenopause status and in 26(48.1%) of postmenopause cases (p=1.000). BRCA1-IRIS was expressed in 37(45.7%) of patients who had grade I &II and found in 12(63.2%) of patients who had grade III &IV(p=0.170). Morover, BRCA1-IRIS was expressed in 12(44.4%) of the patients who had no lymphnode and 37(50.7%) of the patients who have positive lymphnode (p=0.279). BRCA1-IRIS was positive in 41(47.1%) patients who had free margin of tumor while it was positive in 6(85.7%) of patients who had positive margin of tumor (p=0.111). Out of 82 patients, 43 (52%) who had negative Her-2/neu receptor, BRCA1-IRIS was positive. When the Her-2/neu receptor was positive, the BRCA1-IRIS was positive in 6(33.3%) (p=0.194).
Table 3. Relation of BRCA1-IRIS expression with the clinic-pathological features of breast cancer

| Patients features | BRCA1-IRIS | P value |
|------------------|------------|---------|
|                  | Negative n=51 | Positive n=49 |
| Age (years)      |             |         |
| ≤ 50             | 53.7±13.0   | 50.8±11.7 | 0.239 |
| >50              | 8 (42.1%)   | 11 (57.9%) | 0.389 |
| Tumor size (cm)  |             |         |
| ≤ 5              | 40 (51.3%)  | 38 (48.7%) | 1.000 |
| >5               | 11 (50.0%)  | 11 (50.0%) |         |
| Family History   |             |         |
| Negative         | 45 (50.6%)  | 44 (49.4%) | 1.000 |
| Positive         | 6 (54.5%)   | 5 (45.5%)  |         |
| Menopause        |             |         |
| Premenopausal    | 23 (50%)    | 23 (50%)  | 1.000 |
| Postmenopausal   | 28 (51.9%)  | 26 (48.1%) |         |
| Grade            |             |         |
| I-II             | 44 (54.3%)  | 37 (45.7%) | 0.170 |
| III-IV           | 7 (36.8%)   | 12 (63.2%) |         |
| Stage            |             |         |
| Early            | 45 (56.2%)  | 35 (43.8%) | 0.036 |
| Late             | 6 (30%)     | 14 (70%)  |         |
| Lymph node       |             |         |
| Negative         | 15 (55.6%)  | 12 (44.4%) | 0.279 |
| Positive         | 36 (49.3%)  | 37 (50.7%) |         |
| Margin           |             |         |
| Free             | 46 (52.9%)  | 41 (47.1%) | 0.111 |
| Positive         | 1 (14.3%)   | 6 (85.7%)  |         |
| ER               |             |         |
| Negative         | 22 (34.9%)  | 41 (65.1%) | < 0.001 |
| Positive         | 29 (78.4%)  | 8 (21.6%)  |         |
| PR               |             |         |
| Negative         | 30 (42.3%)  | 41 (57.7%) | 0.006 |
| Positive         | 21 (72.4%)  | 8 (27.6%)  |         |
| Her-2/neu        |             |         |
| Negative         | 39 (47.6%)  | 43 (52.4%) | 0.194 |
| Positive         | 12 (66.7%)  | 6 (33.3%)  |         |

3. The relation between BRCA1-IRIS expression and clinic-pathological features in TNBC group.

BRCA1-IRIS expression was found in 35(70%) patients and it was negative expression in 15(30%). No significant relation was present between any relevant clinic-pathological features and BRCA1 IRIS expression in the TNBC group. BRCA1-IRIS was positive in 10(71.4%) with age ≤ 40 years compared with 25(69.4%) with age >40 years (p=0.891). with tumor size ≤ 5 cm, BRCA1-IRIS was positive in 26(70%) and in 9(69.2%) with tumor size >5 cm (p=0.944). As for patients who had negative family...
history, BRCA1-IRIS was positive in 34(69%) and patients who had positive family history, BRCA1-IRIS was positive in 1(100%) (p=1.00). Moreover, BRCA1-IRIS was positive in 26(72.2%) with grade I-II and in 9(64.3%) with grade III-IV (p=0.582). Regarding to tumor stage, BRCA1-IRIS was positive in 21(70%) with early tumor stage and in 14(70%) with late stage (p=1.00). Lymphnode status was positive when BRCA1-IRIS was expressed in 28(65.1%) and in 7(100%) with negative lymphnode (p=0.087). Finally, the expression of BRCA1-IRIS was found in 15(65.2%) premenopausal status and in 10(74.1%) postmenopausal (p=0.469) (Table4).

Table 4: BRCA1-IRIS expression related to the clinic-pathological features of the TNBC group

| Patients features   | BRCA1-IRIS                  | p value |
|---------------------|-----------------------------|---------|
|                     | Negative n=15 | Positive n=35 |         |
| Age (years)         |               |               |         |
| ≤ 50                | 4/14 (28.6%) | 10/14 (71.4%) | 0.891   |
| > 50                | 11/36 (30.6%) | 25/36 (69.4%) |         |
| Tumor size (cm)     |               |               |         |
| ≤ 5                 | 11/37 (29.7%) | 26/37 (70.3%) | 0.944   |
| > 5                 | 4/13 (30.8%)  | 9/13 (69.2%)  |         |
| Family History      |               |               |         |
| Negative            | 15 (30.6%)    | 34 (69.4%)    | 1.000   |
| Positive            | 0 (0.0%)      | 1 (100.0%)    |         |
| Menopause           |               |               |         |
| Premenopausal       | 8/23 (34.8%)  | 15/23 (65.2%) | 0.496   |
| Postmenopausal      | 7/27 (25.9%)  | 20/27 (74.1%) |         |
| Grade               |               |               |         |
| I-II                | 10/36 (27.8%) | 26/36 (72.2%) | 0.582   |
| III-IV              | 5/14 (35.7%)  | 9/14 (64.3%)  |         |
| Stage               |               |               |         |
| Early               | 9/30 (30.0%)  | 21/30 (70.0%) | 1.000   |
| Late                | 6/20 (30.0%)  | 14/20 (70.0%) |         |
| Lymph node          |               |               |         |
| Negative            | 0/7 (0.0%)    | 7/7 (100.0%)  | 0.087   |
| Positive            | 15/43 (34.9%) | 28/43 (65.1%) |         |

4. The relation between BRCA1-IRIS and clinic-pathological features of the patients in N-TNBC group.

In the N-TNBC group, BRCA1-IRIS expression was positive in 14 (28%) and 36 (72%) were negative. There was non-significant relation between any of the clinicopathological features and BRCA1-IRIS expression in this NTN BC group (Table5).
Table 5: The patients characteristics of the N-TNBC patients in relation to BRCA1-IRIS expression

| Patients Characteristics | BRCA1-IRIS | p value |
|--------------------------|------------|---------|
|                         | Negative n=36 | Positive n=14 |
| Age (years)             |             |         |
| ≤ 50 (5)                | 4/5 (80.0%) | 1/5 (20.0%) | 1.000 |
| > 50 (45)               | 32/45 (71.1%) | 13/45 (28.9%) |
| Tumor size (cm)         |             |         |
| ≤ 5                     | 29/41 (70.7%) | 12/41 (29.3%) | 0.670 |
| > 5                     | 7/9 (77.8%) | 2/9 (22.2%) |
| Family History          |             |         |
| Negative                | 30/40 (75.0%) | 10/40 (25.0%) | 0.345 |
| Positive                | 6/10 (60.0%) | 4/10 (40.0%) |
| Menopause               |             |         |
| Premenopausal           | 15/23 (65.2%) | 8/23 (34.8%) | 0.324 |
| Postmenopausal          | 21/27 (77.8%) | 6/27 (22.2%) |
| Grade                   |             |         |
| I-II                    | 34/45 (75.6%) | 11/45 (24.4%) | 0.126 |
| III-IV                  | 2/5 (40.0%) | 3/5 (60.0%) |
| Lymph node              |             |         |
| Negative                | 15/20 (75.0%) | 5/20 (25.0%) | 0.700 |
| Positive                | 21/30 (70.0%) | 9/30 (30.0%) |
| ER                      |             |         |
| Negative                | 7/13 (53.8%) | 6/13 (46.2%) | 0.090 |
| Positive                | 29/37 (78.4%) | 8/37 (21.6%) |
| PR                      |             |         |
| Negative                | 15/21 (71.4%) | 6/21 (28.6%) | 0.939 |
| Positive                | 21/29 (72.4%) | 8/29 (27.6%) |
| Her-2/neu               |             |         |
| Negative                | 24/32 (75.0%) | 8/32 (25.0%) | 0.529 |
| Positive                | 12/18 (66.7%) | 6/18 (33.3%) |

5. mRNA expression levels of all studied markers (Oct4, Survivin and Cyclin D1) in breast cancer patients. The mean expression level of Oct4, Cyclin D1 and Survivin gene in all studied group was 26 ± 8.0, 28 ± 8.0 and 26.3± 9.0fold; respectively. However, the mean expression level of Oct4, Cyclin D1 and Survivin gene in N-TNBC group were 28.4±8.9, 29.8±7.7 and 27.9±9.6fold; respectively compared to 24.5±6.9, 25.9±7.2 and 24.7±8.1 fold expression change in TNBC (p=0.015, p=0.011 and p= 0.073; respectively) (Table 6).

Table 6: mRNA expression levels of all studied markers (Oct4, Survivin and Cyclin D1) expression in breast cancer patients

| Type of breast cancer | Oct4 Fold change | Cyclin D1 Fold change | Survivin Fold change |
|-----------------------|------------------|-----------------------|---------------------|
| Total (n=100) Mean±SD | 26 ± 8           | 28 ± 8                | 26.3± 9             |
| N-TNBC (n=50) Mean±SD | 28.4±8.9         | 29.8±7.7              | 27.9±9.6            |
| TNBC (n=50) Mean±SD   | 24.5±6.9         | 25.9±7.2              | 24.7±8.1            |
| P value               | 0.015            | 0.011                 | 0.073               |

P value ≤0.05 is statically significant, analysis done by independent t test
6. The RNA expression level of all studied markers (Oct4, Survivin and Cyclin D1) expression in correlation to BRCA1-IRIS status:

BRCA1-IRIS was positive in 49 cases and negative in 51 cases of breast cancer. The oct4 genes expression was significantly high in the TNBC IRIS-positive group compared to both the N-TNBC IRIS-positive group (p<0.001) and control. However, the significant high expression level of oct4 gene was observed in N-TNBC IRIS-positive group compared to control group (Figure 2a). The survivin gene expression was significantly high in the both TNBC and N-TNBC IRIS-positive groups compared to control group. However, insignificant high expression level of survivin gene was observed in TNBC IRIS-positive group compared to N-TNBC (Figure 2b). The cyclin D1 gene expression was significantly high in both TNBC and N-TNBC IRIS-positive groups compared to control group. However, significant high expression level of cyclin D1 gene was observed in TNBC IRIS-positive group compared to N-TNBC (Figure 2c).

In TNBC group, BRCA1-IRIS, positive was in 35/49(71.4%) patients. The oct4 was positive in 25 (71%) cases, cyclin D1 was positive in 20 (57%) and survivin was positive in 24 (69%) (p<0.001, p= 0.001and p= 0.002; respectively). BRCA1-IRIS was negative in fifteen(29.4%) TNBC cases (i.e. expressing levels similar to that observed in normal samples), The oct4 was positive in 9 (60%) cases (p<0.001), cyclin D1 was positive in 8 (53%) (p= 0.4) and survivin was positive in 11 (73%) (p<0.001 ,p= 0.4 and p= 0.6; respectively) (Table 7).

In N-TNBC group, BRCA1-IRIS positive in 14/49(28.6%) patients. The oct4 showed positivity in 6 (43%) cases, cyclin D1 was positive in 6 (43%) and survivin was positive in 7 (50%) (p=0.7, p= 0.07and p= 0.38; respectively). In contrary, 36/51(70.6%) N-TNBC cases were BRCA1-IRIS negative, The Oct4 showed positivity in 4 (11%) cases, cyclin D1 was positive in 15 (42%) and survivin was positive in 16 (44%) (p=0.001,p=0.001and p= 0.41and; respectively).

There was significant moderate to strong correlation between BRCA1-IRIS-positive and oct4, cyclin D1 and survivin in TNBC patients (P< 0.001, P= 0.001 and P= 0.002; respectively). Moreover, there was a significant correlation between BRCA1-IRIS-negative and oct4 expression in N-TNBC and TNBC group (p<0.001). In addition, there was a significant association between BRCA1-IRIS-negative and cyclin D1 expression in N-TNBC group only (p<0.001) (Table 7).

By qRT-PCR, among TNBC BRCA1-IRIS positive cases, in TNBC, all the 25 positive for oct4, 20 positive for cyclin D1 showed high showed gene overexpression (with cutoff defined as expression ≥2 fold compared to normal samples), also all the 24 cases positive for survivin mean mRNA fold expression level of 2.5fold change.
Figure 2 a, b, c: The mean fold relative gene expression of *Oct4*, *CyclinD1* and *Survivin* in *BRCA1-IRIS* positive patients (*Statistically significant from the control, #Statistically significant from the N-TNBC*).

Figure 3: Gene expression levels of *Oct4*, *Cyclin D1* and *survivin* in N-TBC patients in relation to the absence/presence of *ER, PR* and *Her2/Neu*. * indicate significant difference from control.
Table 7: The relationship between *BRCA1-IRIS* RNA level and the studied genes

| Marker | Positive *BRCA* (n=49) | Negative *BRCA* (n=51) |
|--------|------------------------|------------------------|
|        | N-TNBC (n=14)          | TNBC (n=35)            | N-TNBC (n=36) | TNBC (n=15) |
| **Oct4** |                        |                        | **Oct4** |                        |                        |
| High n (31) | 6 (43%)                | 25 (71%)              | High n (13) | 4 (11%)                | 9 (60%)                |
| Low n (18) | 8 (57%)                | 10 (29%)              | Low n (38) | 32 (89%)              | 6 (40%) (P<0.001)      |
|          | (P=0.7)               | (P<0.001)            |          |                        |                        |
| **CyclinD1** |                      |                        | **CyclinD1** |                      |                        |
| High n (26) | 6 (43%)                | 20 (57%)              | High n (23) | 15 (42%)              | 8 (53%)                |
| Low n (23) | 8 (57%)                | 15 (43%)              | Low n (28) | 21 (58%)              | 7 (47%)                |
|          | (P=0.7)               | (P=0.001)            |          | (P=0.001)            | (P=0.4)                |
| **Survivin** |                      |                        | **Survivin** |                      |                        |
| High n (31) | 7 (50%)                | 24 (69%)              | High n (27) | 16 (44%)              | 11 (73%)                |
| Low n (18) | 7 (50%)                | 11 (31%)              | Low n (24) | 20 (56%)              | 4 (27%)                |
|          | (P=0.38)              | (P=0.002)            |          | (P=0.41)              | (P=0.6)                |

7. Relationship between markers expression and tumor characteristics in TNBC patients in regarding to *BRCA1-IRIS* expression

Table 8 showed the relation between the RNA level of *oct4*, *cyclin D1* and *survivin* gene and characteristics features of patients with or without *BRCA1-IRIS* expression in TNBC group. No significant correlation between the expression RNA level of *oct4*, *cyclin D1* and *survivin* gene and the expression RNA level of *BRCA1-IRIS* except lymphnode with *survivin* gene expression (p=0.05).
### Table 8: Relationship between markers expression and tumor characteristics in TNBC patients in regarding to BRCA1-IRIS expression

| Parameters          | Positive for OCT4 (n=34) N (%) | P value | Positive for cyclin D1 (n=28) N (%) | P value | Positive for survivin (n=35) N (%) | P value | High OCT4 (n=37) N (%) | P value | High cyclin D1 (n=36) N (%) | P value | High survivin (n=43) N (%) | P value |
|---------------------|--------------------------------|---------|------------------------------------|---------|-----------------------------------|---------|------------------------|---------|--------------------------|---------|--------------------------|---------|
| **Age (yr)**        |                                |         |                                    |         |                                   |         |                        |         |                          |         |                          |         |
| < 50 (n=23)         | IRIS +ve                        | 14 (78) | χ² = 0.35 P = 0.55                 |         | 7 (54)                            |         | 11 (61)                |         | 13 (62)                 |         | 11 (65)                  |         |
| ≥ 50 (n=27)         | IRIS +ve                        | 5 (31)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 4 (22)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS +ve                        | 11 (69) |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 2 (23)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
| **Menopause**       |                                |         |                                    |         |                                   |         |                        |         |                          |         |                          |         |
| Pre (n=23)          | IRIS +ve                        | 12 (75) | χ² = 0.03 P = 0.86                 |         | 8 (62)                            |         | 12 (67)                |         | 16 (62.5)               |         | 11 (66)                  |         |
| Post (n=27)         | IRIS +ve                        | 5 (28)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 4 (25)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS +ve                        | 13 (72) |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 3 (20)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
| **Tumor size (cm)** |                                |         |                                    |         |                                   |         |                        |         |                          |         |                          |         |
| ≤ 3 (n=23)          | IRIS +ve                        | 9 (75)  | χ² = 0.02 P = 0.89                 |         | 12 (80)                           |         | 11 (73)                |         | 10 (62.5)               |         | 11 (69)                  |         |
| > 3 (n=27)          | IRIS +ve                        | 16 (73) |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 6 (27)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS +ve                        | 14 (70) |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 6 (30)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS +ve                        | 11 (79) |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 3 (21)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
| **Tumor stage**     |                                |         |                                    |         |                                   |         |                        |         |                          |         |                          |         |
| Early (n=30)        | IRIS +ve                        | 19 (73) | χ² = 0.01 P = 0.9                  |         | 17 (77)                           |         | 18 (72)                |         | 19 (73)                 |         | 20 (71)                  |         |
| Late (n=20)         | IRIS +ve                        | 3 (21)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 6 (75)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS +ve                        | 7 (27)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 2 (25)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
| **Tumor Grade**     |                                |         |                                    |         |                                   |         |                        |         |                          |         |                          |         |
| 1-2 (n=16)          | IRIS +ve                        | 18 (67) | χ² = 1.3 P = 0.22                 |         | 21 (66)                           |         | 19 (61)                |         | 20 (71)                 |         | 22 (59)                  |         |
| 3 (n=14)            | IRIS +ve                        | 9 (33)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 7 (100) |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS +ve                        | 6 (100) |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 0 (0)   |                                    |         |                                    |         |                        |         |                          |         |                          |         |
| **LN status**       |                                |         |                                    |         |                                   |         |                        |         |                          |         |                          |         |
| Positive (n=43)     | IRIS +ve                        | 18 (67) | χ² = 3.1 P = 0.08                 |         | 16 (67)                           |         | 21 (66)                |         | 19 (61)                 |         | 21 (66)                  |         |
| Negative (n=7)      | IRIS +ve                        | 9 (33)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 7 (100) |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS +ve                        | 3 (100) |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 0 (0)   |                                    |         |                                    |         |                        |         |                          |         |                          |         |
| **Metastasis**      |                                |         |                                    |         |                                   |         |                        |         |                          |         |                          |         |
| M0 (n=26)           | IRIS +ve                        | 10 (71) | χ² = 0.05 P = 0.82                 |         | 9 (75)                            |         | 10 (67)                |         | 10 (56)                 |         | 12 (57)                  |         |
| M1 (n=24)           | IRIS +ve                        | 15 (75) |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 5 (25)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS +ve                        | 8 (67)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 4 (33)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
| **Metastatic Site** |                                |         |                                    |         |                                   |         |                        |         |                          |         |                          |         |
| Single (n=10)       | IRIS +ve                        | 7 (87.5)| χ² = 1.12 P = 0.57                |         | 3 (60)                            |         | 5 (71)                 |         | 8 (80)                  |         | 7 (78)                   |         |
| Multiple (n=14)     | IRIS +ve                        | 8 (67)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |
|                     | IRIS -ve                        | 4 (33)  |                                    |         |                                    |         |                        |         |                          |         |                          |         |

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8. Relationship between markers expression and tumor characteristics in N-TNBC patients in regarding to BRCA1-IRIS expression

Table 9: showed the relation between the RNA level of oct4, cyclin D1 and survivin gene and the features of patients with or without BRCA1-IRIS expression in N-TNBC group. No significant correlation between the expression RNA level of oct4, cyclin D1 and survivin gene and the relevant clinicopathological features regarding to the expression RNA level of BRCA1-IRIS except the expression level of oct4 and tumor size (p=0.01). Also, there was a significant correlation between metastasis status and the expression level of cyclin D1 gene (p=0.03).
Table 9: Relationship between *oct4*, *cyclin D1* and *survivin* and tumor characteristics in non-TNBC patients in regarding to *BRCA1-IRIS* expression

| Parameters                      | Positive for OCT4 (n=10) N (%) | P value | Positive for cyclin D1 (n=21) N (%) | P value | Positive for survivin (n=23) N (%) | P value | High OCT4 (n=17) N (%) | P value | High cyclin D1 (n=15) N (%) | P value | High survivin (n=7) N (%) | P value |
|---------------------------------|--------------------------------|---------|-------------------------------------|---------|------------------------------------|---------|------------------------|---------|------------------------|---------|------------------------|---------|
| **Age (yr)**                    |                                |         |                                     |         |                                    |         |                        |         |                        |         |                        |         |
| < 50 (n=22)                     | IRIS +ve                       | 3 (75)  | 4 (36)                              | P= 0.43 | 4 (50)                             | P= 0.14 | 5 (45)                 | P= 0.63 | 4 (57)                 | P= 0.2  | 1                      | 0.09    |
|                                | IRIS -ve                       | 1 (25)  | 7 (64)                              |         | 4 (50)                             |         | 6 (55)                 |         | 3 (43)                 |         | 1.6                    |         |
| ≥ 50 (n=28)                     | IRIS +ve                       | 3 (50)  | 2 (20)                              |         | 3                                  |         | 2 (33)                 |         | 6 (25)                 |         | 0                      |         |
|                                | IRIS -ve                       | 3 (50)  | 8 (80)                              |         | 12                                 |         | 4 (67)                 |         | 6 (75)                 |         | 5                      |         |

| **Menopause**                   |                                |         |                                     |         |                                    |         |                        |         |                        |         |                        |         |
| Pre (n= 23)                     | IRIS +ve                       | 4 (80)  | 4 (33)                              | P= 0.2  | 5 (50)                             | P= 0.07 | 5 (45)                 | P= 0.63 | 4 (57)                 | P= 0.2  | 1                      | 0.2     |
|                                | IRIS -ve                       | 1 (20)  | 8 (67)                              |         | 5 (50)                             |         | 6 (55)                 |         | 3 (43)                 |         | 1.7                    |         |
|                                 | IRIS +ve                       | 2       | 2 (22)                              |         | 2 (15)                             |         | 2 (33)                 |         | 2 (25)                 |         | 0                      |         |
|                                 | IRIS -ve                       | 3       | 7 (78)                              |         | 11 (85)                            |         | 4 (67)                 |         | 6 (75)                 |         | 0                      |         |
| Post (n= 27)                    | IRIS +ve                       | 4 (80)  | 4 (33)                              | P= 0.2  | 5 (50)                             | P= 0.07 | 5 (45)                 | P= 0.63 | 4 (57)                 | P= 0.2  | 1                      | 0.2     |
|                                | IRIS -ve                       | 1 (20)  | 8 (67)                              |         | 5 (50)                             |         | 6 (55)                 |         | 3 (43)                 |         | 1.7                    |         |
|                                 | IRIS +ve                       | 2       | 2 (22)                              |         | 2 (15)                             |         | 2 (33)                 |         | 2 (25)                 |         | 0                      |         |
|                                 | IRIS -ve                       | 3       | 7 (78)                              |         | 11 (85)                            |         | 4 (67)                 |         | 6 (75)                 |         | 0                      |         |

| **Tumor size (cm)**            |                                |         |                                     |         |                                    |         |                        |         |                        |         |                        |         |
| ≤ 5 (n= 29)                    | IRIS +ve                       | 5 (100) | 3 (21)                              | P= 0.01 | 3 (25)                             | P= 0.55 | 5 (45)                 | P= 0.63 | 3 (33)                 | P= 0.52 | 1                      | 0.35    |
|                                | IRIS -ve                       | 1 (20)  | 11 (79)                             |         | 9 (75)                             |         | 6 (55)                 |         | 6 (67)                 |         | 3                      |         |
|                                | IRIS +ve                       | 4 (80)  | 3 (43)                              |         | 4 (36)                             |         | 2 (33)                 |         | 3 (50)                 |         | 3                      |         |
|                                | IRIS -ve                       | 4 (80)  | 4 (57)                              |         | 7 (64)                             |         | 4 (67)                 |         | 3 (50)                 |         | 3                      |         |
| > 5 (n= 21)                    | IRIS +ve                       | 5 (100) | 3 (21)                              | P= 0.01 | 3 (25)                             | P= 0.55 | 5 (45)                 | P= 0.63 | 3 (33)                 | P= 0.52 | 1                      | 0.35    |
|                                | IRIS -ve                       | 1 (20)  | 11 (79)                             |         | 9 (75)                             |         | 6 (55)                 |         | 6 (67)                 |         | 3                      |         |
|                                | IRIS +ve                       | 4 (80)  | 3 (43)                              |         | 4 (36)                             |         | 2 (33)                 |         | 3 (50)                 |         | 3                      |         |
|                                | IRIS -ve                       | 4 (80)  | 4 (57)                              |         | 7 (64)                             |         | 4 (67)                 |         | 3 (50)                 |         | 3                      |         |

| **Tumor stage**                |                                |         |                                     |         |                                    |         |                        |         |                        |         |                        |         |
| Early(0,I&II) (n=50)           | IRIS +ve                       | 6 (60)  | 6 (29)                              | NA      | 7 (30)                             | NA      | 7 (41)                 | NA      | 6 (40)                 | NA      | 1                      | 0       |
|                                | IRIS -ve                       | 4 (40)  | 15 (71)                             | 0       | 16 (70)                            | 0       | 10 (59)                | 0       | 9 (60)                 | 0       | 6                      | 0       |
| Late (III) (n=0)               | IRIS +ve                       | 0       | 0                                   | NA      | 0                                  | NA      | 0                      | NA      | 0                      | NA      | NA                     | NA      |
| Tumor Grade | IRIS +ve | IRIS -ve | χ² | P | IRIS +ve | IRIS -ve | χ² | P | IRIS +ve | IRIS -ve | χ² | P | IRIS +ve | IRIS -ve | χ² | P | IRIS +ve | IRIS -ve | χ² | P | IRIS +ve | IRIS -ve | χ² | P | IRIS +ve | IRIS -ve | χ² | P | IRIS +ve | IRIS -ve | χ² | P |
|-------------|---------|---------|----|---|---------|---------|----|---|---------|---------|----|---|---------|---------|----|---|---------|---------|----|---|---------|---------|----|---|---------|---------|----|---|---------|---------|----|---|---------|---------|----|---|
| 1-2 (n= 45) | 5       | 4       | 6  | 32 | 14      | 68      | 15 | 71 | 9        | 60      | 6   | 40 | 1        | 50      | 1   | 50 | 1        | 50      | 6   | 40 | 1        | 50      | 1   | 50 | 1        | 50      | 6   | 40 | 1        | 50      | 1   | 50 |
| 3 (n= 5)    | 1 (100) | 0 (0)   | 2  | 100| 0 (0)   | 2 (100) | 1  | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 |
| LN status   |         |         |    |    |         |         |    |    |         |         |    |    |         |         |    |    |         |         |    |    |         |         |    |    |         |         |    |    |
| Positive (n= 30) | IRIS +ve | IRIS -ve | 5  | 62.5| 4        | 40      | 2   | 60 | 2        | 18      | 2   | 18 | 9        | 82      | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 |
| Negative (n= 20) | IRIS +ve | IRIS -ve | 1  | 50  | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 |
| Metastasis  |         |         |    |    |         |         |    |    |         |         |    |    |         |         |    |    |         |         |    |    |         |         |    |    |         |         |    |    |
| M0 (n= 45)  | IRIS +ve | IRIS -ve | 4  | 3   | 3        | 37.5    | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 |
| M1 (n= 5)   | IRIS +ve | IRIS -ve | 2  | 33.3| 1 (66.7) | 1 (66.7)| 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 |
| Ungrouped Response | IRIS +ve | IRIS -ve | 3  | 2   | 2        | 22      | 2   | 14 | 2 (14)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 | 1 (50)   | 1 (50)  | 1   | 50 |
| CR (n= 33)  | IRIS +ve | IRIS -ve | 0  | 0   | 1 (100)  | 1 (100) | 0   | 0  | 2 (100)  | 1 (100)| 0   | 0  | 2 (100)  | 1 (100)| 0   | 0  | 2 (100)  | 1 (100)| 0   | 0  | 2 (100)  | 1 (100)| 0   | 0  |
| PR (n=3)    | IRIS +ve | IRIS -ve | 1  | 100 | 0 (0)    | 0 (0)   | 0   | 0  | 1 (50)   | 1 (50)  | 0   | 0  | 1 (50)   | 1 (50)  | 0   | 0  | 1 (50)   | 1 (50)  | 0   | 0  | 1 (50)   | 1 (50)  | 0   | 0  |
| SD (n=3)    | IRIS +ve | IRIS -ve | 2  | 33  | 1 (67)   | 1 (67)  | 3   | 37.5| 3 (37.5) | 3 (50)  | 3   | 50 | 3 (50)   | 3 (50)  | 3   | 50 | 3 (50)   | 3 (50)  | 3   | 50 | 3 (50)   | 3 (50)  | 3   | 50 |
| PD (n= 11)  | IRIS +ve | IRIS -ve | 2  | 33  | 1 (67)   | 1 (67)  | 3   | 37.5| 3 (37.5) | 3 (50)  | 3   | 50 | 3 (50)   | 3 (50)  | 3   | 50 | 3 (50)   | 3 (50)  | 3   | 50 | 3 (50)   | 3 (50)  | 3   | 50 |

CR=complete response, PR=partial response, SD=stationary disease, PD=progressive disease
9. Response to treatment

Out of 100 breast cancer patients, 30(30%) were responded to treatment and 70(70%) were not responded to treatment. The patients with negative BRCA1-IRIS, 13(25.5%) were responders and 38(74.5%) non-responders. While the patients with positive BRCA1-IRIS, 17(34.7%) were responders and 32(65.3%) non-responders (p=0.349). As for high expression of oct4, 11(25%) were responded to treatment and 33(75%) were not responded to treatment. With low expression of oct4 gene, 19(33.9%) were responded to treatment and 37(66%) were not responded to treatment (p=0.077). Moreover, 12(20.7%) of patients who had high expression of survivin gene were responders and 46(79.3%) non-responders. On the other hand, 18(42.8%) of patients who had low expression of survivin gene were responders compared to 24(57%) non responders (p=0.001). Regarding to high expression of cyclin D1, 5(10.2%) were responded to treatment and 44(89.8%) were not responded to treatment. At low expression of cyclin D1, 25(49%) were responded to treatment and 26(50%) were not responded to treatment(p<0.001) (Table 10). Only, there were a significant association between the response to treatment and the expression RNA level of survivin and cyclin D1(p=0.001 and p<0.001,respectively).

Table 10: Relation between the response to treatment and the NA level of the studied genes and BRCA1-IRIS

| The RNA level of gene | Response | P value |
|------------------------|----------|---------|
|                        | Respond (n=30) | Not Respond (n=70) |
| **BRCA1-IRIS**         |           |         |
| Negative=51            | 13(25.5 %) | 38(74.5 %) |
| Positive=49            | 17(34.7 %) | 32(65.3 %) |
| **Oct4-RNA**           |           |         |
| High=44                | 11(25 %)  | 33(75 %)  |
| Low=56                 | 19(33.9 %) | 37(66 %)  |
| **Survivin-RNA**       |           |         |
| High=58                | 12(20.7 %) | 46(79.3 %) |
| Low=42                 | 18(42.8 %) | 24(57 %)  |
| **Cyclin D1-RNA**      |           |         |
| High=49                | 5(10.2 %)  | 44(89.8 %) |
| Low=51                 | 25(49 %)  | 26(50 %)  |

10. Survival analysis

BRCA1-IRIS was expressed in 49% of breast cancer patients and did not expressed in 51% of patients. The average follow-up period was 43 months. The median overall survival of the 100 breast cancer patients was 43 months (range, 2–68 months), and the median time of disease-free interval was 42 months (range, 6–43 months) (Table 11). The overall survival was 49.5% of the BRCA1-IRIS negative and 41.6% of the BRCA1-IRIS positive. Moreover, the Disease-free survival was 47% in BRCA1-IRIS negative and 35.9% in BRCA1-IRIS positive. By Kaplan-meier, there was no significant different between BRCA1-IRIS expression and overall survival (p=0.291 long rang)
However, disease-free survival was apparently worse in BRCA1-IRIS positive cases (p = 0.052 long rang) (Figure 5).

Table 11. Survival proportion of the whole studied group in relation to BRCA1-IRIS expression

|                  | BRCA1-IRIS | p value |
|------------------|------------|---------|
|                  | Negative n=51 | Positive n=49 |
| Overall Survival | 49.5%       | 41.6%   | 0.291 |
| Disease-free Survival | 47.0%       | 35.9%   | 0.052 |

Figure 4: Overall survival in relation to BRCA1-IRIS expression

Figure 5. Disease free survival in relation to BRCA1-IRIS expression

Discussion
Breast cancer is one of the most common diseases affecting women in the whole world. It is represented 32% of the diagnostic cases in female worldwide and it is also considered the second cause of cancer related death. Houssami and Cho, (2018). Some studies considered breast cancer as an inherited disease. BRCA1 and BRCA2 genes has been identified to be linked with the breast cancer (Hall et al., 2016). BRCA1-IRIS is an oncogene related to the BRCA1 and BRCA2 genes. It works as anti-apoptotic, cell cycle enhancer and metastasis-related gene. BRCA1-IRIS overexpression is associated with aggressive phenotypes and resistant to the target therapy (Chen X et al., 2016).

Some studies demonstrated that the IRIS overexpression in cancer cells correlated with chemotherapy resistance in animal cancer models (Blanchard et al., 2015 and Paul et al., 2015). BRCA1-IRIS overexpression helpful for the formation of an aggressive breast cancer, and that in patients with HER2+ or TN/BL subtypes (Shimizu et al., 2012). Goncalves et al.,(2018), reported that the mean age of breast cancer patients was 57 years whereas in the current study, the TNBC and N-TNBC groups were comparable in regarding the age of patient (mean±SD is 51.2±12.5 years and 53.3±12.3 years; respectively) which may be reflect late diagnosis or difficult access to health care services.

In the current study, BRCA1-IRIS was upregulated or expressed in 49/100 (49%) cases. IRIS gene expression was significantly higher in the TNBC group compared to the N-TNBC, since IRIS was overexpressed in 35/49 (71.4%) of TNBC vs 14/49 (28.6%) in N-TNBC patients. By RT-PCR, the expression level was over 2-fold compared to normal samples. The BRCA1-IRIS was down regulated in 51% (i.e. expression levels like that observed in normal samples) of all patients. IRIS was expressed in 15/51 (29.4%) of TNBC vs 36/51 (70.6%) of N-TNBC patients. The mean fold expression level of the IRIS gene (7.2-fold) in TNBC was higher than in N-TNBC (2.3-fold). At the tumor size, in the TNBC/ IRIS-positive 9/13 (69.2%) cases were ≥5cm compared to 2/9 (22.2%) in the N-TNBC. Regard to tumor grade, out of 14 patients with TNBC, 9 (64.2%) who were positive for IRIS with high tumor grade (III-IV) compared to 3 (60%) out of 5 patients in the N-TNBC cases. Lymph node metastasis was found in 28/43 (65.1%) TNBC cases who were IRIS-positive compared to 9/30 (30%) in the N-TNBC cases.

Bogan et al., (2017) studied ninety-six breast cancer patients diagnosed as invasive ductal carcinomas. From the ninety-six- breast cancer, 45% were TNBC and 55% were non-TNBC. No significant difference between age, menopausal status and TNBC as well as with non-TNBC groups except after BRCA1-IRIS expression was factored in. In the TNBC group, ≤ 5 cm tumors were BRCA1-IRIS-overexpressing, whereas in the non-TNBC group they were BRCA1-IRIS-negative (p=0.00007). BRCA1-IRIS-overexpressing in most of the TNBC patients diagnosed with grade I or II, while in non-TNBCs IRIS was negative (p=0.00035). No statistical significance between the two groups and grade III. There was a significant difference between N-TNBC and TNBCs and tumor stage regard to BRCA1-IRIS-overexpression. Positive lymph node metastases were associated with BRCA1-IRIS overexpression in TNBC group, and with BRCA1-IRIS-negative status in the non-TNBC patients (p=0.00009). The relapse after chemotherapy (p<0.00001) and local recurrence/distant metastasis after surgery (p=0.0028) were more declared in TNBC patients with positive
expression of BRCA1-IRIS compared to non-TNBC patients. Finally, disease-free survival was decreased in TNBC/BRCA1-IRIS-overexpressing patients compared to TNBC/BRCA1-IRIS-negative patients and decreased overall survival in TNBC as well as non-TNBC patients was driven by BRCA1-IRIS overexpression. This study is agreed with the present study only in tumor stage status, lymphnode and humor grade III.

Many studies aimed to explain a link between oct4 and the malignant potential of cancer cells and the role of oct4 in tumor metastasis (Shao et al., 2018; Wang et al., 2018; Ruan et al., 2019).

In the current study, the oct4 gene expression was significantly high in the TNBC IRIS-positive group compared to the N-TNBC IRIS-positive group (p<0.001). oct4 genes expression was high in TNBC and N-TNBC IRIS-positive group compared to control group. oct4 expression had poor diagnosis and outcome of patients. Ruan et al.,(2019) reported that the oct4 gene has been expressed in some human tumor cells but not in normal somatic tissues. Cancer cells have been characterized as having many phenotypic features like that in an undifferentiated embryonic cells Hackett and Fortier, (2011).

In the current study, the mean expression levels of oct4, cyclin D1 and survivin genes in the two groups were 26±8.0, 28±8.0 and 26.3±9.0 folds, respectively. On the other hand, the mean expression levels of oct4, cyclin D1 and survivin genes in N-TNBC were 28.4±8.9, 29.8±7.7 and 27.9±9.6folds; respectively vs 24.5±6.9, 25.9±7.2 and 24.7±8.1folds in TNBC. Ezeh et al., (2005) reported that in normal breast tissues oct4 did not express but in breast carcinoma it expressed with late stage along with other stem cell markers. In addition, Chang et al., (2011) found that oct4 promotes tumorigenesis of colorectal cancer cells in both autocrine and paracrine way. Saigusa et al., (2009) reported that oct4 expression is related with the recurrence of rectal cancer after target therapy and Liu et al.(2014) showed that the oct4 expression is associated with breast cancer due to its role in angiogenesis vasculogenic mimicry formation by increasing cancer stem cells subpopulation, thereby potentiating breast cancer metastasis. Oct4 expression was correlated with grade, tumor size, N stage and TNM stage, and it could be served as an independent biomarker to predict worse prognosis in surgical patients with TNBC Zhang et al., (2018). The previous studies were comparable with the current study.

In the present study, the survivin gene expression was significantly high in the both TNBC and N-TNBC IRIS-positive groups compared to control group. However, there was a significant correlation between high expression level of survivin compared to the low expression level in TNBC IRIS-positive group (p=0.002). In contrast, there was not a significant associated between high expression level of survivin compared to the low expression level of survivin in N-TNBC IRIS-negative groups. The cyclin D1 gene expression was significantly high in the both TNBC and N-TNBC IRIS-positive groups compared to control group and a significant high expression level of cyclin D1 gene was observed in TNBC IRIS-positive group compared to N-TNBC. The mean expression levels of oct4 gene was significantly high in PR-positive patients compared to PR-negative patients and controls one. There were no statistically significant differences observed in the ER and Her2 positive or negative patients. In relation to the gene
expression level of cyclin D1 and survivin, the ER, PR and Her2 positive and negative patients were highly significant compared to control group.

Kerri L. Chock et al., (2010) hypothesis that inhibition of the BRCA1-IRIS-AKT-survivin pathway could be enhance the response to treatment with chemotherapy in ovarian tumor. This data was different from the current study. Shimizu Y et al., (2012) reported that BRCA1-IRIS expressed in high level in TNBC tumor. So, the increasing of p-AKT and survivin expression, and lack of BRCA1 expression were present. This result is comparable with the present data. Plevoa P. et al., (2010) detected that out of 40 breast cancer cases, 15 were BRCA1 and 9 were BRCA2 mutation carriers. Patients without mutation (16 patients) as control. By fluorescence in situ hybridization method, eight tumors showed CCND1 amplification and 38 cases showed ZNF217 amplification. No significant difference in CCND1 and ZNF217 amplification with BRCA1, BRCA2 as well as in negative BRCA tumors. CCND1 amplification was correlated with decreased disease-free (P = 0.045) and overall survival (P = 0.015). BRCA1/CCND1 amplification tumors were associated with estrogen receptor negative. There was no significant association between CCND1 and ZNF217 amplification and estrogen receptor, progesterone receptor, and ERBB2 expression and TNM classification. The obtained results showed similarities with the present study.

In the current study, statistically significance associations were found in the expression of oct4, cyclin D1 and survivin at the mRNA levels and BRCA1-IRIS positive in the TNBC group (p<0.001, p=0.001 and p=0.002; respectively). BRCA1-IRIS negative was associated significantly with oct4 expression (p<0.001). In contrary, in the N-TNBC patients, there was no correlation between BRCA1-IRIS positive and the expression of the three genes (p=0.7, p=0.7and p0.38; respectively). BRCA1-IRIS negative was significantly associated with oct4 expression only (p<0.001).

The current results showed decreased overall survival (OS) among TNBC patients compared to non-TNBC patients that strongly correlate to BRCA1-IRIS overexpression thus BRCA1-IRIS drives poor survival outcomes in TNBC patients. The univariate analysis of BRCA1-IRIS positive TNBC patients showed that menopausal status associated significantly with disease-free survival (DFS). Multivariate analysis showed that only menopausal status was an independent risk factor for disease-free survival (p= 0.01). The univariate analysis of BRCA1-IRIS positive N-TNBC patients showed no significant association between overall survival rate and any of the assessed clinic-pathological features of the patients.

TNBC women without lymph node involvement had a survival rate of 69% in 5 years and 61.6% in 10 years in Brazilian cohort. The histological grade, and Ki67 were identified as prognostic and predictive factors. Other Brazilian results, the 5-year survival was 67.8% in TNBC, compared with non-TNBC subtypes (86.4% for luminal A tumors and 91.4% for luminal B tumors) Eisenberg, ALA. Et al., (2013). The lymphnode involvement was a prognostic factor for both mortality and recurrence in the TNBC group, representing a nearly 3 times risk of mortality. The same was observed in an American cohort, in which a 5-year overall survival of 80% was reported for the patients with TNBC without lymph node involvement, compared to 65% in those with up to 3 positive lymph nodes (Hernandez-Aya et al., 2001).
In conclusion, the BRCA1-IRIS-positive overexpression is relatively common in TNBC patients. It could be used as a prognostic and predictive factors of aggressive breast tumors especially the TNBC. The results were confirmed with three genes panel (Oct4, Survivin and Cyclin D1) expression were high in TNBC IRIS-positive compared to IRIS-negative patients. Therefore, this three genes panel could help in predicting the prognosis of the TNBC patients, however this must be confirmed on larger number of patients.

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علاقة التعبير الجيني لجين البراكا-1RIS والجيوات الحيوية بتطور الورم في مرضى سرطان الثدي

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الملخص

يتمثل سرطان الثدي الثلاثي السلبي (TNBC) هو نمط ظاهرى عدىوى ذو تشكُّي سلبي ومواد البقاء على قيد الحياة تكون ضئيلة. وقد تم تقييم القدمة التنوبية والمندرة بعدئض الحمض للجيوات الأثية في حالات سرطان الثدي سواء كان سرطان الثدي الثلاثي السلبي أو غير ثلاثي السلبية.

وفي هذه الدراسة تم استخراج الحاصل النموى الريبوزي لمائة عينة من أورام نسجة مرضى سرطان الثدي BRCA-IRIS, Oct4, Cyclin D1 and Survivin في سرطان الثدى BRCA-IRIS, Oct4, Cyclin D1 and Survivin. وقياس نسبة التعبير الزائد لجيوات ثلاثي السلبية أو غير ثلاثي السلبية باستخدام الqRT-PCR. وكانت النتيجة أن التعبير الزائد لجين BRCA-IRIS, Oct4, Cyclin D1 and Survivin في حالات سرطان الثدى ثلاثي السلبية (14) 28.6% في حالات سرطان الثدى غير ثلاثي السلبية وكان العامل الأحصائي p=0.001. لا يوجد دلالة هجوبية بين التعبير الزائد لجين BRCA-IRIS, Oct4, Cyclin D1 and Survivin في سرطان الثدى ثلاثي السلبية أو غير ثلاثي السلبية. الدراسة استخدمت نتائج التعبير الزائد لجين BRCA-IRIS, Oct4, Cyclin D1 and Survivin في سرطان الثدى ثلاثي السلبية أو غير ثلاثي السلبية.

الكلمات المفتاحية: سرطان الثدي الثلاثي السلبي، سرطان الثدي غير ثلاثي السلبية، جين البراكا-1RIS، جين السيريغين، جين الافكتور، جين السكريات، جين السكيفين.