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

Safaa Fawzy[1*], Karima Metwaly[2], Mahmoud N. El-Rouby[1], Abeer Bahnassy[3]
[1] Department of Cancer Biology, Virology and Immunology Unit, National Cancer Institute, Cairo University, Egypt.
[2] Department of parasitology Faculty of science, Al Azhar university (Girls branch) Cairo, Egypt.
[3] Molecular Pathology, National Cancer Institute, Cairo University, Egypt.
***Corresponding author:** safaa.hafez.59@azhar.edu.eg

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 ($p=0.001$each).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 *Survinin* in patients with invasive breast cancer patients. It also aims at defining the effect of *BRCA1-IRIS, Oct4, Cyclin D1* and *Survinin* 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, Survinin* and *β-actin.* The sequence of primers that were used illustrated in Table 1.

Table1. Sequences of the primers

Gene name	Forward	Reverse
BRCA1-IRIS	5'-GTCTGAGTGACAAGGAATTGGTTT-3'	5'-TTAACTATACTTGGAAATTTGTAAAATGTG-3'
OCT4	5'-GATGGCGTACTGTGGGCCC-3'	5'-TGGGACTCCTCCGGGTTTTG-3'
β-actin	5'-ACAGAGCCTCGCCTTTGC-3'	5′-GCGGCGATATCATCATCC-3'
cyclin D1 (CCND1)	5'-CTGGGTGTCCTACAAATG-3'	5′-AGCGGTCCAGGT AGTTCAT-3'
Survivin	5′-TCCACTGCCCCACTGAGAAC-3′	5′-TGGCTCCCAGCCTTCCA-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.**

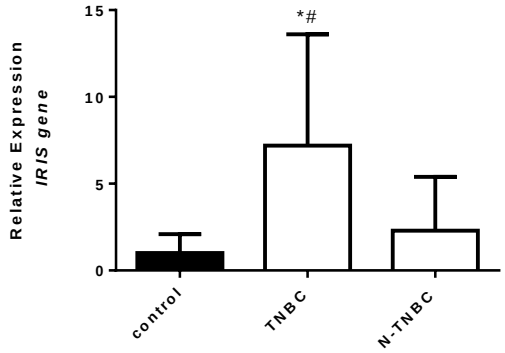

Figure 1: The mean fold relative expression level of *IRIS* gene in TNBC and non-TNBC groups. *# indicate that significant difference from control and N-TNBC groups respectively.

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). Morovere, *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)	53.7±13.0	50.8±11.7	0.239
≤ 50	8 (42.1%)	11 (57.9%)	0.389
>50	43 (53.1%)	38 (46.9%)	
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 >40years (p=0.891). with tumor size ≤5cm, *BRCA1-IRIS* was positive in 26(70%) and in 9(69.2%) with tumor size >5cm (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%)	
> 50	11/36 (30.6%)	25/36 (69.4%)	0.891
Tumor size (cm)			
≤ 5	11/37 (29.7%)	26/37 (70.3%)	
> 5	4/13 (30.8%)	9/13 (69.2%)	0.944
Family History			
Negative	15 (30.6%)	34 (69.4%)	
Positive	0 (0.0%)	1 (100.0%)	1.000
Menopause			
Premenopausal	8/23 (34.8%)	15/23 (65.2%)	
Postmenopausal	7/27 (25.9%)	20/27 (74.1%)	0.496
Grade			
I-II	10/36 (27.8%)	26/36 (72.2%)	
III-IV	5/14 (35.7%)	9/14 (64.3%)	0.582
Stage			
Early	9/30 (30.0%)	21/30 (70.0%)	
Late	6/20 (30.0%)	14/20 (70.0%)	1.000
Lymph node			
Negative	0/7 (0.0%)	7/7 (100.0%)	
Positive	15/43 (34.9%)	28 /43(65.1%)	0.087

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 patients (28%) and36 (72%) were negative. There was non-significant relation between any of the clinicopathological features and *BRCA1-IRIS* expression in this NTNBC 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%)	
> 50 (45)	32/45 (71.1%)	13/45 (28.9%)	1.000
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%)	
Positive	12/18 (66.7%)	6/18 (33.3%)	0.529

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 the 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).The mean expression level of *oct4* gene was significantly high ($p= 0.001$) in *PR* positive patients compared to *PR* negative patients and control one. There were no statistically significant differences observed in the *ER* and *Her2* positive or negative patients (Figure 3A).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 (Figure 3 B &C).

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%) ($p<0.001$, $p= 0.001$and $p= 0.002$; 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%) ($p=0.7$, $p= 0.07$and $p= 0.38$; 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%) **($p=0.001$,$p=0.001$and $p= 0.41$and**; 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.

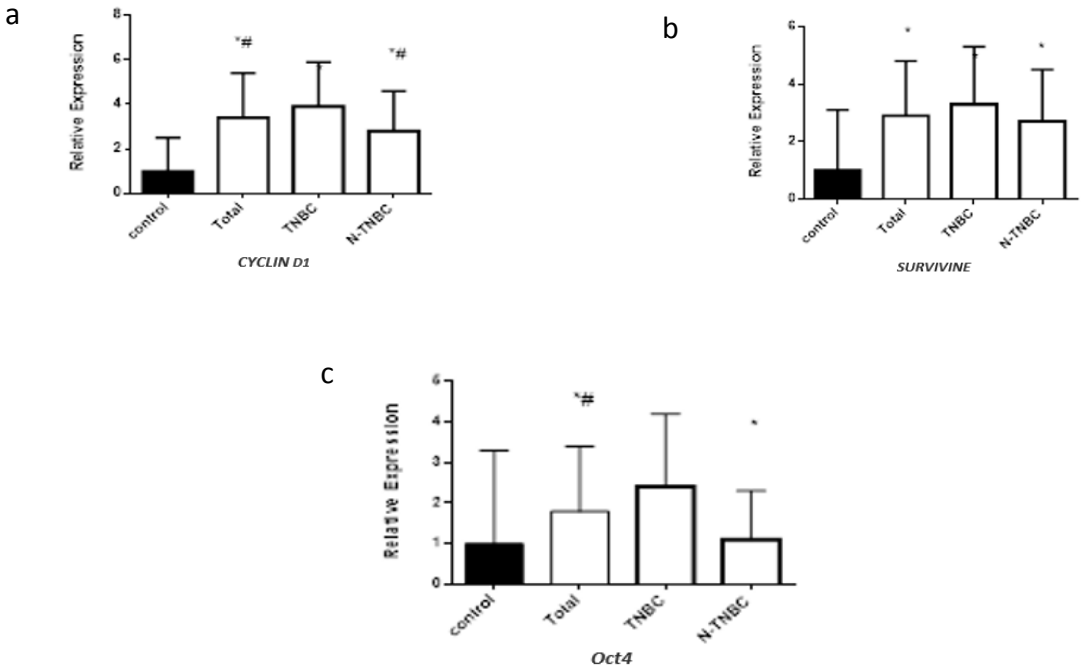

Figure 2 a, b, c: The mean fold relative gene expression of *Oct4, CyclinD1 and Survivin* in *BRCA1-IRIS* positive patients (*Statistically significant from the control, #Statistically significant from the N-TNBC

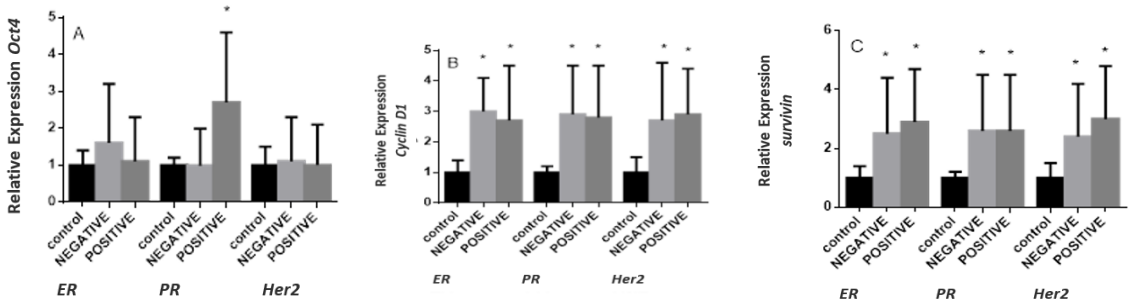

Figure 3: Gene expression levels of Oct4, Cyclin D1 and survivin in N-TBC patients in relation to the absence/presence of *ER*, *PR* and *Her2/Neu*. * indicate significant difference from control.

Table 7: The relationship between *BRCA1-IRIS* RNA level and the studied genes

Marker	Positive *BRCA* (n=49)		Marker	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)*		(*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)			$\chi2$=0.35		$\chi2$=3.7		$\chi2$=0.9		$\chi2$=0.7		$\chi2$=0.34		$\chi2$=0.19
< 50 (n= 23)	IRIS +ve	14 (78)	P= 0.55	7 (54)	P= 0.06	11 (61)	6	13 (62)	P= 0.4	11 (65)	P= 0.56	13 (62)	P= 0.66
	IRIS -ve	4 (22)		6 (46)		7 (39)	P= 0.33	8 (38)		6 (35)		8 (38)	
≥ 50 (n= 27)	IRIS +ve	11 (69)		13 (87)		13 (76)		12 (75)		14 (74)		15 (68)	
	IRIS -ve	5 (31)		2 (23)		4 (24)		4 (25)		5 (26)		7 (32)	
Menopause					$\chi2$=1.2		$\chi2$=0.0				$\chi2$=1.2		$\chi2$=0.04
Pre (n= 23)	IRIS +ve	12 (75)	$\chi2$=0.03	8 (62)	P= 0.28	12 (67)	6	12 (63)	$\chi2$=0.35	11 (61)	P= 0.28	14 (64)	P= 0.8
	IRIS -ve	4 (25)	P= 0.86	5 (38)		6 (33)	P= 0.8	7 (37)	P= 0.56	7 (39)		8 (36)	
Post (n= 27)	IRIS +ve	13 (72)		12 (80)		12 (70.5)		13 (72)		14 (78)		14 (67)	
	IRIS -ve	5 (28)		3 (20)		5 (29.5)		5 (28)		4 (22)		7 (33)	
Tumor size (cm)			$\chi2$=0.02		$\chi2$=1.2		$\chi2$=0.2		$\chi2$=0.33		$\chi2$=0.00		$\chi2$=0.22
≤ 3 (n= 23)	IRIS +ve	9 (75)	P= 0.89	12 (80)	P= 0.28	11 (73)	8	10 (62.5)	P= 0.57	11 (69)	7	11 (61)	P= 0.64
	IRIS -ve	3 (25)		3 (20)		4 (27)	P= 0.6	6 (37.5)		5 (31)	P= 0.28	7 (39)	
> 3 (n= 27)	IRIS +ve	16 (73)		8 (62)		13 (65)		15 (71)		14 (70)		17 (68)	
	IRIS -ve	6 (27)		5 (38)		7 (35)		6 (29)		6 (30)		8 (32)	
Tumor stage			$\chi2$=0.3		$\chi2$=0.02		$\chi2$=0.0		$\chi2$=0.02		$\chi2$=0.07		$\chi2$=0.03
Early (n= 30)	IRIS +ve	14 (70)	P= 0.58	13 (72)	P= 0.9	13 (68)	0	14 (67)	P= 0.89	17 (71)	P= 0.8	16 (64)	P= 0.86
	IRIS -ve	6 (30)		5 (28)		6 (32)	P= 0.98	7 (33)		7 (29)		9 (36)	
Late (n= 20)	IRIS +ve	11 (79)		7 (70)		11 (69)		11 (69)		8 (67)		12 (67)	
	IRIS -ve	3 (21)		3 (30)		5 (31)		5 (31)		4 (33)		6 (33)	
Tumor Grade			$\chi2$=0.01		$\chi2$=1.7		$\chi2$=0.4		$\chi2$=1.2		$\chi2$=0.23		$\chi2$=0.11
1-2(n= 36)	IRIS +ve	19 (73)	P= 0.9	17 (77)	P= 0.19	18 (72)	8	19 (73)	P= 0.27	20 (71)	P= 0.63	20 (67)	P= 0.75
	IRIS -ve	7 (27)		5 (23)		7 (28)	P= 0.49	7 (27)		8 (29)		10 (33)	
3 (n= 14)	IRIS +ve	6 (75)		3 (50)		6 (60)		6 (54.5)		5 (62.5)		8 (61.5)	
	IRIS -ve	2 (25)		3 (50)		4 (40)		5 (45.5)		3 (37.5)		5 (38.5)	
LN status			$\chi2$=3.2		$\chi2$=1.9		$\chi2$=1.5		$\chi2$=3.4		$\chi2$=1.9		$\chi2$=3.7
Positive (n= 43)	IRIS +ve	18 (67)	P= 0.08	16 (67)	P= 0.17	21 (66)	P= 0.22	19 (61)	P= 0.06	21 (66)	P= 0.17	22 (59)	**P= 0.05**
	IRIS -ve	9 (33)		8 (33)		11 (34)		12 (39)		11 (34)		15 (41)	
Negative (n= 7)	IRIS +ve	7 (100)		4 (100)		3 (100)		6 (100)		4 (100)		6 (100)	
	IRIS -ve	0 (0)_		0 (0)		0 (0)		0 (0)		0 (0)		0 (0)	
Metastasis			$\chi2$=0.05		$\chi2$=0.7		$\chi2$=0.0		$\chi2$=2.3		$\chi2$=0.00		$\chi2$=1.15
M0 (n= 26)	IRIS +ve	10 (71)	P= 0.82	9 (75)	P= 0.13	10 (67)	4	10 (56)	P= 0.13	11 (69)	7	12 (57)	P= 0.28
	IRIS -ve	4 (29)		3 (25)		5 (37)	P= 0.83	8 (44)		5 (31)	P= 0.94	9 (43)	
M1 (n=24)	IRIS +ve	15 (75)		11 (69)		14 (70)		15 (79)		14 (70)		16 (73)	
	IRIS -ve	5 (25)		5 (31)		6 (30)		4 (21)		6 (30)		6 (27)	
Metastatic Site			$\chi2$= 1.12		$\chi2$=0.4		$\chi2$=0.0		$\chi2$= 2.3		$\chi2$=0.16		$\chi2$=1.3
Single (n=10)	IRIS +ve	7 (87.5)	P= 0.57	3 (60)	P= 0.82	5 (71)	5	8 (80)	P= 0.3	6 (75)	P= 0.92	7 (78)	P= 0.5
	IRIS -ve	1 (12.5)		2 (40)		2 (29)	P= 0.97	2 (20)		2 (25)		2 (22)	
Multiple (n=14)	IRIS +ve	8 (67)		8 (73)		9 (69)		7 (78)		8 (67)		9 (69)	
	IRIS -ve	4 (33)		3 (27)		4 (31)		2 (22)		4 (33)		4 (31)	

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 D 1* 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)			$\chi2$=063		$\chi2$=0.69		$\chi2$=2.2		$\chi2$=0.24		$\chi2$=1.6		$\chi2$=2.9
< 50 (n=22)	IRIS +ve	3 (75)	P= 0.43	4 (36)	P= 0.41	4 (50)	P= 0.14	5 (45)	P= 0.63	4 (57)	P= 0.2	1	P= 0.09
	IRIS -ve	1 (25)		7 (64)		4 (50)		6 (55)		3 (43)		1	
≥ 50 (n=28)	IRIS +ve	3 (50)		2 (20)		3		2 (33)		2 (25)		0	
	IRIS -ve	3 (50)		8 (80)		12		4 (67)		6 (75)		5	
Menopause					$\chi2$=0.3		$\chi2$=3.2				$\chi2$=1.7		$\chi2$=1.6
Pre (n= 23)	IRIS +ve	4 (80)	$\chi2$= 1.68	4 (33)	P= 0.58	5 (50)	P= 0.07	5 (45)	$\chi2$=0.24	4 (57)	P= 0.2	1	P= 0.2
	IRIS -ve	1 (20)	P= 0.2	8 (67)		5 (50)		6 (55)	P= 0.63	3 (43)		2	
Post (n= 27)	IRIS +ve	2		2 (22)		2 (15)		2 (33)		2 (25)		0	
	IRIS -ve	3		7 (78)		11 (85)		4 (67)		6 (75)		4	
Tumor size (cm)			$\chi2$=6.7		$\chi2$=1.05		$\chi2$=0.35		$\chi2$=0.24		$\chi2$=0.4		$\chi2$=0.88
	IRIS +ve	5 (100)	P= 0.01	3 (21)	P= 0.31	3 (25)	P= 0.55	5 (45)	P= 0.63	3 (33)	P= 0.52	1	P= 0.35
≤ 5 (n= 29)	IRIS -ve	0 (0)		11 (79)		9 (75)		6 (55)		6 (67)		3	
	IRIS +ve	1 (20)		3 (43)		4 (36)		2 (33)		3 (50)		0	
> 5 (n= 21)	IRIS -ve	4 (80)		4 (57)		7 (64)		4 (67)		3 (50)		3	
Tumor stage			NA		NA		NA		NA		NA		NA
Early(0,I&II)	IRIS +ve	6 (60)		6 (29)		7 (30)		7 (41)		6 (40)		1	
(n=50)	IRIS -ve	4 (40)		15 (71)		16 (70)		10 (59)		9 (60_		6	
	IRIS +ve	0		0		0		0		0		0	
Late (III) (n=0)	IRIS -ve	0		0		0		0		0		0	

Tumor Grade			χ^2=0.74		χ^2=0.89		χ^2=0.4		χ^2=0.07		χ^2=0.7		NA
1-2 (n= 45)	IRIS	5	P= 0.38	6 (32)	P= 0.35	6 (29)	P= 0.53	6 (40)	P= 0.79	6 (43)	P= 0.4	1	
	+ve	4		13 (68)		15 (71)		9 (60)		8 (67)		6	
3 (n= 5)	IRIS -ve	1 (100)		0 (0)		1 (50)		1 (50)		0 (0)		0	
	IRIS	0 (0)		2 (100)		1 (50)		1 (50)		1 (100)		0	
	+ve												
	IRIS -ve												
LN status			χ^2=0.1		χ^2=1.2		χ^2=		χ^2=1.6		χ^2=0.00		χ^2=1.6
Positive (n= 30)	IRIS	5 (62.5)	P= 0.75	4 (40)	P= 0.27	5 (36)	0.47	2 (25)	P= 0.2	4 (40)	P= 1	1	P= 0.2
	+ve	3 (37.5)		6 (60)		9 (64)	P= 0.49	6 (75)		6 (60)		2	
Negative (n=	IRIS -ve	1 (50)		2 (18)		2 (22)		5 (56)		2 (40)		0	
20)	IRIS	1 (50)		9 (82)		7 (78)		4 (44)		3 (60)		4	
	+ve												
	IRIS -ve												
Metastasis			χ^2=0.07		χ^2=0.88		χ^2=5		χ^2=0.09		χ^2=0.7		NA
M0 (n= 45)	IRIS	4	P= 0.8	6 (32)	P= 0.35	5 (24)	**P= 0.03**	6 (43)	P= 0.76	6 (43)	P= 0.4	1 (14)	
	+ve	3		13 (68)		16 (76)		8 (57)		8 (57)		6 (86)	
M1 (n= 5)	IRIS -ve	2 (33.3)		0 (0)		2 (100)		1 (33)		0 (0)		0	
	IRIS	1 (66.7)		2 (100)		0 (0)		2 (67)		1 (100)		0	
	+ve												
	IRIS -ve												
Ungrouped Response			χ^2=2.2 P= 0.53		χ^2= 1.7 P= 0.63		χ^2=5.5 P= 0.14		χ^2=1.67P = 0.43		χ^2=1.1 P= 0.77		χ^2= 2.9 P= 0.2
CR (n= 33)	IRIS	3		2 (22)		2 (14)		2 (25)		2 (33.3)		0 (0)	
	+ve	2		7 (78)		12 (86)		6 (75)		4 (67.7)		4 (100)	
PR (n=3)	IRIS -ve	0 (0)		1 (50)		1 (50)		0		1 (50)		0 (0)	
	IRIS	1(100)		1 (50)		1 (50)		0		1 (50)		1 (100)	
SD (n=3)	+ve	1 (100)		0 (0)		1 (100)		1 (50)		0 (0)		0 (0)	
	IRIS –	0 (0)		2 (100)		0 (0)		1 (50)		1 (100)		0 (0)	
PD (n= 11)	ve	2 (33)		3 (37.5)		3 (50)		4 (57)		3 (50)		1 (50)	
	IRIS	1 (67)		5 (62.5)		3 (50)		3 (43)		3 (50)		1 (50)	
	+ve												
	IRIS -ve												
	IRIS												
	+ve												
	IRIS -ve												

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) (Table10).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;respictevly).

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 %)	0.349
Positive=49	17(34.7 %)	32(65.3 %)	
Oct4-RNA			
High=44	11(25 %)	33(75 %)	0.077
Low=56	19(33.9 %)	37(66 %)	
Survivin-RNA			
High=58	12(20.7 %)	46(79.3 %)	0.001
Low=42	18(42.8 %)	24(57 %)	
Cyclin D1-RNA			
High=49	5(10.2 %)	44(89.8 %)	<0.001
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)

(Figure 4). 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 proportion	49.5%	41.6%	0.291
Disease-free Survival proportion	47.0%	35.9%	0.052

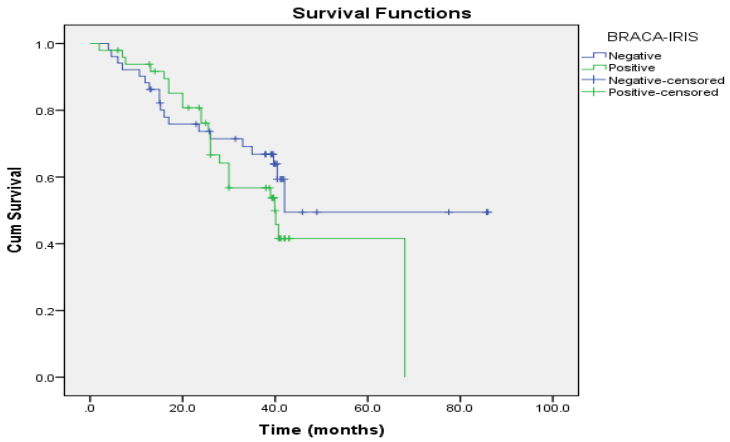

Figure 4: Overall survival in relation to *BRCA1-IRIS* expression

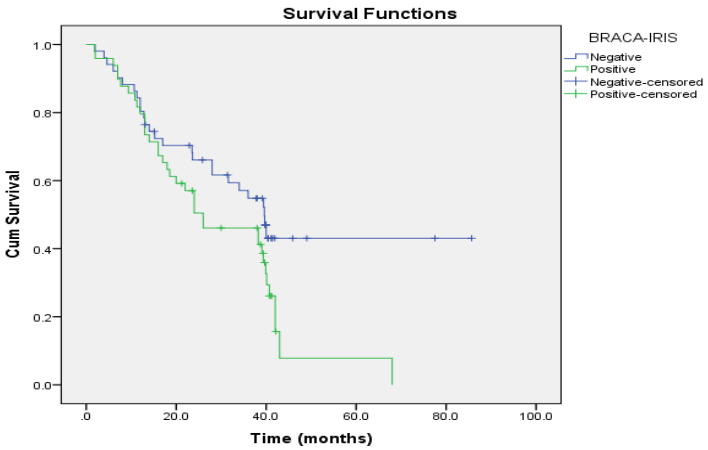

Figure 5. Disease free survival in relation to *BRCA1-IRIS* expression

Discussion

Breast cancer is one of the most common disease 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 12.3years; 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%) in 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. **Plevova 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 ($p=0.7$, $p=0.7$and 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.

REFERENCE

Azim HA and Ibrahim AS., 2014. Breast cancer in Egypt, China and Chinese: statistics and beyond. **J Thorac Dis, 6(7) :864-866.**

Blanchard Z, Paul BT, Craft B, ElShamy WM., 2015. BRCA1-IRIS inactivation overcomes paclitaxel resistance in triple negative breast cancers. ***Breast Cancer Res*, 17:5.**

Bogan, D., Meile, L., El Bastawisy, A., Yousef, H.F., Zekri, A.N., Bahnassy, A.A. and ElShamy, W.M., 2017. The role of BRCA1-IRIS in the development and progression of triple negative breast cancers in Egypt: possible link to disease early lesion. **BMC Cancer 17, 329.**

Boullosa, L.F., Savaliya, P., Bonney, S., Orchard, L., Wickenden, H., Lee, C., Smits, E., Banham, A.H., Mills, K.I., Orchard, K. and Guinn, B.A., 2018. Identification of survivin as a promising target for the immunotherapy of adult B-cell acute lymphoblastic leukemia. **Oncotarget 9, 3853-3866.**

Chang CJ, Chien Y, Lu KH, Chang SC, Chou YC, Huang CS, Chang CH, Chen KH, Chang YL, Tseng LM *et al*., 2011. Oct4-related cytokine effects regulate tumorigenic properties of colorectal cancer cells. **Biochem Biophys Res Commun 415(2):245-251.**

Chen X, Duan N, Zhang C and Zhang W., 2016. Survivin and Tumorigenesis: Molecular Mechanisms and Therapeutic Strategies. *J* **Cancer 7(3):314-323.**

Choi, E.K., Lim, J.A., Kim, J.K., Jang, M.S., Kim, S.E., Baek, H.J., Park, E.J., Kim, T.H., Deng, C.X., Wang, R.H. and Kim, S.S., 2018. Cyclin B1 stability is increased by interaction with BRCA1, and its overexpression suppresses the progression of BRCA1-associated mammary tumors. **Exp Mol Med 50, 136.**

Dunnwald LK, Rossing MA, Li CI., 2007. Hormone receptor status, tumor characteristics, and prognosis: a prospective cohort of breast cancer patients. **Breast Cancer Res, 9(1): R6.**

Edge SB and Compton CC., 2010. The American joint committee on cancer: the 7thedition of the AJCC cancer staging manual and the future of TNM. **Ann Surg Oncol.17(6):1471–4.**

Eisenberg, ALA, Pinto, IV, Koifman, S.,2013. Triple-negative breast cancer in Brazilian women without metastasis to axillary lymph nodes: ten-year survival and prognostic factors. **Br J Med Med Res. 3:880–896.**

Ezeh, U.I., Turek, P.J., Reijo, R.A. and Clark, A.T., 2005. Human embryonic stem cell genes OCT4, NANOG, STELLAR, and GDF3 are expressed in both seminoma and breast carcinoma. **Cancer 104, 2255-2265.**

Goncalves, H., Jr., Guerra, M.R., Duarte Cintra, J.R., Fayer, V.A., Brum, I.V. and Bustamante Teixeira, M.T., 2018. Survival Study of Triple-Negative and Non-Triple-Negative Breast Cancer in a Brazilian Cohort. **Clin Med Insights Oncol 12.**

Guzel, E., Karatas, O.F., Duz, M.B., Solak, M., Ittmann, M. and Ozen, M., 2014. Differential expression of stem cell markers and ABCG2 in recurrent prostate cancer. **Prostate 74, 1498-1505.**

Hackett, C.H. and Fortier, L.A., 2011. Embryonic stem cells and iPS cells: sources and characteristics. **Vet Clin North Am Equine Pract 27, 233-242.**

Hall, M.J., Obeid, E.I., Schwartz, S.C., Mantia-Smaldone, G., Forman, A.D.and Daly, M.B., 2016. Genetic testing for hereditary cancer predisposition: BRCA1/2, Lynch syndrome, and beyond. **Gynecol Oncol 140, 565-574.**

Hao, L. and ElShamy, W.M., 2007. BRCA1-IRIS activates cyclin D1 expression in breast cancer cells by downregulating the JNK phosphatase DUSP3/VHR. **Int J Cancer 121, 39-46.**

Hernandez-Aya, LF, Chavez-MacGregor, M, Lei, X., 2011. Nodal status and clinical outcomes in a large cohort of patients with triple-negative breast cancer. **J Clin Oncol. 29:2628–2634.**

Houssami, N. and Cho, N., 2018. Screening women with a personal history of breast cancer: overview of the evidence on breast imaging surveillance. **Ultrasonography 37, 277-287.**

Inoue, K. and Fry, E.A., 2015. Aberrant expression of cyclin D1 in cancer. **Sign Transduct Insights 4, 1-13.**

Kerri L. Chock, Jamie M.S. Allison, Yoshiko Shimizu and Wael M. ElShamy. 2010. BRCA1-IRIS Overexpression Promotes Cisplatin Resistance in Ovarian Cancer Cells. **Therapeutics, Targets, and Chemical Biology.**

Lakhani, S.R., Ellis, I.O.. Schnitt,S.J., Tan, P.H., van de Vijver, M.J. (Eds), 2012. WHO classification of Breast Tumours. **IARC WHO Classifiaction of Tumours No 4.**

Li, P., Zhou, L., Zhao, T., Liu, X., Zhang, P., Liu, Y., Zheng, X. and Li, Q., 2017b. Caspase-9: structure, mechanisms and clinical application. **Oncotarget 8, 23996-24008.**

Liu, A., Yu, X. and Liu, S., 2013. Pluripotency transcription factors and cancer stem cells: small genes make a big difference. **Chin J Cancer 32, 483-487.**

Liu, T., Sun, B., Zhao, X., Li, Y., Gu, Q., Dong, X. and Liu, F., 2014. OCT4 expression and vasculogenic mimicry formation positively correlate with poor prognosis in human breast cancer. **Int J Mol Sci 15, 19634-19649.**

Livak, K.J. and Schmittgen, T.D., 2001. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. **Methods 25, 402-408.**

Onitilo AA, Engel JM, Greenlee RT, Mukesh BN., 2009. Breast cancer subtypes based on ER/PR and Her2 expression: comparison of clinicopathologic features and survival. **Clin Med Res, 7(1-2):4-13.**

Paul, B.T., Blanchard, Z., Ridgway, M. and ElShamy, W.M., 2015. BRCA1-IRIS inactivation sensitizes ovarian tumors to cisplatin. **Oncogene 34, 3036-3052.**

Perou CM, Sorlie T, Eisen MB, van de Rijn M, Jeffrey SS, Rees CA, Pollack JR, Ross DT, Johnsen H, Akslen LA et al., 2000. Molecular portraits of human breast tumours. ***Nature*, 406(6797):747-752.**

Plevova P, Cerna D, B alcar A, Foretova L, Zapletalova J, Silhanova E, Curik R, Dvorackova J, Ccnd and Znf. 2010. 217 gene amplification is equally frequent in BRCA1 and BRCA2 associated and non-BRCA breast cancer. **Neoplasma 57, 4**

Poursani, E.M., Mohammad Soltani, B., Mowla, S.J., 2016. Differential Expression of OCT4 Pseudogenes in Pluripotent and Tumor Cell Lines. **Cell J 18, 28-36.**

Rakha EA, Tan DS, Foulkes WD, Ellis IO, Tutt A, Nielsen TO, Reis-Filho JS., 2007. Are triple-negative tumours and basal-like breast cancer synonymous?. **Breast Cancer Res, 9(6):404; author reply 405.**

Ruan, Z., Yang, X. and Cheng, W., 2019. OCT4 accelerates tumorigenesis through activating JAK/STAT signaling in ovarian cancer side population cells. **Cancer Manag Res 11, 389-399.**

Saigusa, S., Tanaka, K., Toiyama, Y., Yokoe, T., Okugawa, Y., Ioue, Y., Miki, C. and Kusunoki, M., 2009. Correlation of CD133, OCT4, and SOX2 in rectal cancer and their association with distant recurrence after chemoradiotherapy. **Ann Surg Oncol 16, 3488-3498.**

Salhia B, Tapia C, Ishak EA, Gaber S, Berghuis B, Hussain KH, DuQuette RA, Resau J, Carpten J., 2011. Molecular subtype analysis determines the association of advanced breast cancer in Egypt with favorable biology. **BMC Womens Health, 11:44.**

Shao, M., Bi, T., Ding, W., Yu, C., Jiang, C., Yang, H., Sun, X. and Yang, M., 2018. OCT4 Potentiates Radio-Resistance and Migration Activity of Rectal Cancer Cells by Improving Epithelial-Mesenchymal Transition in a ZEB1 Dependent Manner. **Biomed Res Int 2018.**

Shen, L., Qin, K., Wang, D., Zhang, Y., Bai, N., Yang, S., Luo, Y., Xiang, R. and Tan, X., 2014. Overexpression of Oct4 suppresses the metastatic potential of breast cancer cells via Rnd1 downregulation. **Biochimica et biophysica acta 1842, 2087-2095.**

Shimizu Y, Luk H, Horio D, Miron P, Griswold M, Iglehart D, Hernandez B, Killeen J, ElShamy WM., 2012. BRCA1-IRIS overexpression promotes formation of aggressive breast cancers. **PLoS One, 7(4):e34102.**

Sinha A, Paul BT, Sullivan LM, Sims H, El Bastawisy A, Yousef HF, Zekri AN, Bahnassy AA and ElShamy WM., 2017. BRCA1-IRIS overexpression promotes and maintains the tumor initiating phenotype: implications for triple negative breast cancer early lesions. **Oncotarget 8(6):10114-10135.**

Wang, G., Zhou, H., Gu, Z., Gao, Q. and Shen, G., 2018. Oct4 promotes cancer cell proliferation and migration and leads to poor prognosis associated with the survivin/STAT3 pathway in hepatocellular carcinoma. **Oncol Rep 40, 979-987.**

World Health Organization [homepage on the Internet], 2015. The global burden of disease: 2004 update. WHO; [Accessed June 8, 2015].

Zhang, J.M., Wei, K. and Jiang, M., 2018. OCT4 but not SOX2 expression correlates with worse prognosis in surgical patients with triple-negative breast cancer. **Breast Cancer 25, 447-455.**

علاقة التعبير الجينى لجين البراكا ايريس والجينات الحيوية بتطور الورم فى مرضى سرطان الثدى

صفاء فوزي*[١] ، كريمة متولي[٢] ، محمود الروبي[٣] ، عبير بهنسي[٤]

[٣,١]قسم الفيروسات والمناعة معهد الاورام القومي جامعة القاهرة ،مصر

[٢]قسم طفيليات بكلية العلوم جامعة الازهر ،القاهرة ،مصر

[٤]قسم باثولوجي جزيئية ،معهد الاورام ،جامعة القاهرة ،مصر

***البريد الالكتروني للباحث الرئيسي :** safaa.hafez.59@azhar.edu.eg

الملخص:

يعتبر سرطان الثدى الثلاثى السلبية (TNBC) هو نمط ظاهرى عدوانى ذو تشخيص سلبى ومددة البقاء على قيد الحياة تكون ضئيلة. وقد تم تقيم القيمة التنبؤية والمنذرة بحدوث المرض للجينات الأتية BRCA-IRIS, Oct4,Cyclin D1 and Survivin فى حالات سرطان الثدى سواء كان سرطان ثدى ثلاثى السلبية او غير ثلاثى السلبية.

وفى هذه الدراسة تم استخراج الحامض النووى الريبوزى لمائة عينة من اورام انسجة مرضى سرطان الثدى وقياس نسبة التعبير الزائد لجينات BRCA-IRIS, Oct4,Cyclin D1 and Survivin فى سرطان الثدى ثلاثى السلبية او غير ثلاثى السلبية باستخدام ال qRT-PCR . وكانت النتيجة ان التعبير الزائد ل جين BRCA-IRIS وجد فى ٤٩% من الحالات (٣٥)٧١%فى حالات سرطان الثدى ثلاثى السلبية و (١٤) ٢٨.٦% فى حالات سرطان الثدى غير ثلاثى السلبية وكان العامل الأحصائى $p<0.001$ أى ان توجد دلالة احصائية بين التعبير الزائد ل جين BRCA-IRIS و سرطان الثدى.ويوجد أيضا ان هناك دلالة احصائية بين التعبير الزائد ل جين BRCA-IRIS و مرحلة الورم ومستقبلات هرمون البروجيستيرون و الأستروجين (ER and PR) وكان العامل الأحصائى $p=0.036$, $p<0.001$ and $p=0.006$ على التوالى. وفى مجموعة سرطان الثدى الثلاثية السلبية لايوجد دلالة احصائية بين التعبير الزائد ل BRCA-IRIS الموجبة و اى من الصفات الأكلينيكية للمرضى و لكن يوجد دلالة احصائية بين BRCA-IRIS الموجبة و التعبير الزائد لكل من Oct4, Cyclin D1 and Survivin gene وكان العامل الأحصائى $p=0.001$, $p=0.002$ and $p<0.001$ على التوالى . ويوجد دلالة احصائية قوية بين BRCA-IRIS السلبية و و التعبير الزائد لجين Oct4 فقط فى مجموعة سرطان الثدى الثلاثية السلبية $p<0.001$ أما فى مجموعة سرطان الثدى الغير ثلاثية السلبية لا ويوجد دلالة احصائية بين IRIS الموجبة و التعبير الزائد لجينات Oct4,Cyclin D1 and Survivin . اما فى عدم وجود التعبير الزائد ل BRCA-IRIS negative فيوجد دلالة احصائية بين BRCA-IRIS negative و التعبير الزائد لكل من جين Oct4,Cyclin D1 وكان العامل الأحصائى $p=0.001$ لكل منهما.و لا توجد دلالة احصائية بين مستوى الحامض النووى الريبوزى فى BRCA-IRIS, Oct4,Cyclin D1 and Survivin مع الصفات الاكلينيكية للمرضى ما عدا فى وجود الغدد الليمفاوية و التعبير الزائد لجين survivin ($p=0.05$). لا توجد دلالة احصائية بين التعبير الزائد للجينات الثلاثة و كل من الصفات الأكلينيكية للمرضى ونجد ان التعبير الزائد ل Cyclin D1 and Survivinيرتبط احصائيا مع الأستجابة للعلاج $P=0.001$ and $p<0.001$ و اخيرا لا توجد اى علاقة بين مدة البقاء على قيد الحياة والتعبير الزائد ل BRCA-IRIS($p=0.291$ long rang) اما بالنسبة للبقاء على قيد الحياة خالى من الأمراض تزيد مع وجود BRCA-IRIS($p=0.052$ long rang)

الكلمات المفتاحية :- سرطان الثدي ثلاثي السلبية ، سرطان الثدي الغير ثلاثي السلبية، جين البراكاأيرس، جين الاكتوفور، جين السيكلين، جين السرفيفين