

Research Article

BRCA1 Immunohistochemical Study of Serous Ovarian Cancer

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Abstract

BRCA1 is the first gene reported to be responsible for breast cancer inheritance, located on chromosome 17q21. Some mutations were found to be correlated with increased susceptibility to breast and ovarian cancer and described the protein transcript BRCA1. The life time risk of developing OvCa in patients carry mutations in BRCA1 is 30-60%. High-grade sporadic epithelial OvCa might show dysfunction of the BRCA1 pathway. IHC assessment of BRCA1 is found to be a valuable screening method, as well as for management of cases without a strong family history and with sporadic cases. **Aim:** Our aim was to study both the BRCA1-MS12 and BRCA1-GLK3 clones' expression in 19 serous ovarian cancer cases and correlated the expression with the tumor grade, stage and overall survival. **Materials and Methods:** Serous ovarian 19 cases with different stages and grades were subjected to immunohistochemical staining using both BRCA1-MS12 and BRCA1-GLK3 monoclonal antibodies. Statistical analysis was performed using SPSS16. **Results:** Positive expression for BRCA1-MS12 detected in the nucleus and cytoplasm or in the cytoplasm only without nuclear staining of malignant cells. Our studied cases revealed that, (42.1%) were negative for BRCA1-MS12 and (57.9%) showed positive expression. Spearman Correlation between the staining intensity and serous carcinoma grade, was found to be inversely correlated significantly (P-value <0.05). The higher the grade of the cancer is, the weaker the staining intensity of the OvCa by BRCA1-MS12. The spearman correlation between the staining intensity with the different stages of the cases studied it was found to be inversely correlated with a nearly significant value (p-value <0.08). The higher the stage is, the weaker the staining intensity of the OvCa. The overall survival is higher among the cases with negative expression than those with positive expression of the same antibody. The Spearman correlation is inverse non-significant (p-value>0.05). Negative expression for BRCA1-GLK3 was found in (78.9%) of cases, and only (21.1%) show positivity. Our positive cases are 100% cytoplasmic with no nuclear expression detected either in normal or cancer cells. Staining pattern indicate exon 11 mutation of BRCA1 gene. The positive cases, the biggest percentage was for the low grade cases (70%) followed by the high grade group (30%), (P-value >0.05). The spearman correlation between the BRCA1-GLK3 and the tumor grade is weak and inverse, with non-significant p-value (>0.05). The spearman correlation between BRCA1-GLK3 cytoplasmic expression and the tumor staging was weak direct, the higher the expression the higher the stage, with non-significant p-value (>0.05). The negative group has better survival than the positive group. These results means that mutations in exon 11 detected by the positive BRCA1-GLK3 cytoplasmic staining is associated with poor prognosis as indicated by the low overall survival of the positive cases. **Conclusion:** BRCA1 expression was associated with poor prognosis parameters and considered loss of BRCA1 activity as a marker of tumor aggressiveness with worse prognosis These results suggest that IHC using combined assay of BRCA1-MS12 and BRCA1-GLK3 is a reliable and useful technique for BRCA1 mutations at least in predicting the status of mutation; upstream or downstream of exon 11.

Keywords: BRCA1, BRCA1-MS12, BRCA1-GLK3, Ovarian Cancer, Serous Ovarian Cancer,

Epithelial Ovarian Cancer.

Ovarian cancer (OvCa) is a major public health problem, as there are more than 190,000 new In United States (USA), OvCa is the second most common gynecologic malignancy, accounting for 3% of total malignancies among females, with total number of cases of OvCa estimated at the end of 2011 was 22,280, while the total deaths from OvCa was 10,000 cases of OvCa every year all over the world (Siegel et al., 2012). OvCa has the highest death-to-incidence ratio because the ineffective screening methods available and the absence of early specific symptoms for OvCa (Jemal et al., 2008). In Egypt, there is no total national cancer registry but instead, there are many important regional registries including; Gharbia Population Based Cancer Registry (GPBCR), the average estimated new cases are 70 OvCa per year accounting for 3.5% of all newly diagnosed female cancers (Abd El-bar, 2007). Another important regional registry in Egypt is Aswan regional registry in which 30 OvCa cases were registered over the year 2008 representing 0.6% of all female cancers (Egypt National Cancer Registry, Aswan Profile-2008). In Minia Governorate, the latest published records of the National Cancer Institute indicated that the incidence of OvCa represents 3.7% of all female cancers with a mean age 59.6 years (Ibrahim et al., 2011). There are neither effective biomarkers to identify early-stage disease, nor reliable prognostic markers to predict clinical response and to guide treatment regimes. (Lawrenson et al., 2009). Prognosis of OvCa depends mostly on the stage of the neoplasm when first discovered, as early localized stages has 50 year survival rate, reaching up to 90%, while it drops to be less than 30% in advanced stages (Horner et al., 2009). Breast Cancer Type1 Susceptibility gene (BRCA1) is the first gene reported to be responsible for breast cancer inheritance, located on chromosome 17q21, identified by (Hall et al., 1990). Later, the gene was cloned by (Miki et al., 1994), who detected some mutations that increase the susceptibility to breast and ovarian cancer and described the protein transcript BRCA1. The life time risk of developing OvCa in patients carry mutations in

BRCA1 is 30-60%. High-grade sporadic epithelial OvCa might show dysfunction of the BRCA1 pathway (Bast et al., 2009). BRCA1 consists of 24 exons, encoding 1863 amino acids (Miki et al., 1994). Subsequently, more detailed studies of BRCA genes and their association with the risk of breast and ovarian cancers (Evans et al., 2008). The protein encoded by BRCA1 is mainly nuclear and has 3 highly conserved domains; Ring domain in the N-terminus, with E3 ubiquitin ligase activity (Hashizume et al., 2001) and two BRCT motifs at the C-terminus, which mediates the phosphorylated DNA repair factors and share in the protein damage response. The Ring domain is activated by BARD1 (Huen et al., 2001). In the present study, we selected BRCA1-MS13 antibody (Ab-2) against the N-terminal 305 amino acids which is highly specific for BRCA1 D11b splice variant (Fraser et al., 2003). BRCA1-MS13 is a monoclonal antibody against the N-terminal 305 amino acids of BRCA1 and detects the BRCA1 D11b splice variant in a highly specific manner. The D11b splice variant lacks the majority of exon 11, resulting in the 110kDa protein lacking the nuclear expression and accumulates in the cytoplasm, but some translocation of the D11b splice variant to the nucleus could exist leading to some faint expression (Farser et al., 2003). Detection of the cytoplasmic BRCA1-GLK2 protein is considered as an abnormal location and indicates for BRCA1 mutation at least in exon 11 (Kashima et al., 2000). The BRCA1 C-terminal (BRCT) domain mutations lead to altered p53 function are associated with breast and/or ovarian cancers (Rodriguez et al., 2004), (Gough et al., 2007) & (Leung & Glover, 2011). The exon 11 region is frequently a target of cancer-associated mutations (Venkitaraman, 2009) & (Kashima et al., 2000). Nuclear localization signals (NLSs) exist in exon 11, and that a BRCA1 splice variant lacking exon 11 is localized in the cytoplasm (Thakur et al., 1997).

Mutations of BRCA1 at these 3 conserved domains have been found to be related to cancer

(Shakya et al., 2011). Immunohistochemistry (IHC) assessment of BRCA1 is found to be a valuable screening method, as well as for management of cases without a strong family history and with sporadic cases (Skyttei et al., 2011). It was found that cases with low BRCA1 protein expression in sporadic ovarian cancers are associated with better prognosis (Swisher et al., 2009).

Materials and methods

The current work studies BRCA1 immunohistochemical staining in 19 Serous OvCa cases of different stages as well as different grades. The histologic type of ovarian cancer according to WHO classification according to (AJCC, 2002) into: 19 cases of serous carcinoma; 11 cases were of high grade and 8 cases were of low grade carcinoma according to the 2-tier grading system. They also represent different stages 15 of them were stage III, one case of stage II, and 3 cases of stage I. Immunohistochemical study of two different clones of BRCA1 antibodies expression utilizing the Streptavidin-Biotin-Peroxidase method. For both BRCA1 clones breast cancer is used as the positive control tissue. Positive staining was detected when cytoplasmic and/or nuclear staining of the cells turns brown. Unstained slides were subjected to two changes of fresh xylene to remove paraffin followed by rehydrated using descending grades of alcohol. Endogenous peroxidase activity was blocked using methanol-hydrogen peroxide in a ratio of 1:1. The slides were immersed inside antigen retrieval solution (citrate buffer, pH6) in a container and placed in microwave for 30 minutes from the boiling point. One drop of serum blocking solution were put and incubated for 20-30 minutes. One drop of the primary antibody is placed on each slide. Incubation time was 60 minutes in the humidity chamber. One drop of biotinylated second antibody is placed on each slide followed by the enzyme conjugate reagent. One drop of DAB chromogen mixed with another drop of concentrated hydrogen peroxide were applied to every slide and incubating them for 5 minutes. Counter staining was done using Mayer's Hematoxylin (Hx). Slides were dehydrated in ascending grades of alcohol. The slides were cleared in xylene, mounted by DPX and covered

by cover slip. Examining slides under light microscope for detection of brown expression and to be scored according to H-score. In between the different steps, the slides were put in phosphate buffer solution for 5 minutes. Assessment of the IHC staining of BRCA1 for MS13 clone with the regard of both the staining intensity and percentage of stained cells was done to create H-score according to (Abd El-Rehim et al., 2000) & (Vilmar et al., 2012). H-score is calculated by multiplying the staining intensity by the percentage of stained cells. The staining intensity is scored as follow: 0=No expression, 1=weak, 2=moderate & 3=strong expression. The percentage of positive cells is scored as follow: 0=0%, 1=1-9%, 2=10-49% & 3=50% or more. The cut-off point was chosen a priori as the median value of the H-scores to separate biomarker-positive (H-score > median) tumours from biomarker-negative (H-score ≤ median) ones. The median value of H-score is 1, considering the cases with H-score > 1 are positive, while cases with H-score ≤ 1 are negative. The data were analyzed using the Statistical Package for Social Sciences (SPSS version 16.0) software. The association between negative immunohistochemistry and clinical data was analyzed using the two-tailed Fisher's exact test and normal chi square tests. The p-values less than (0.05) were considered as significant.

Results

We examined the subcellular localization of BRCA1 proteins using immunohistochemical staining of 19 sporadic OvCa cases with N-terminal (BRCA1-MS13 or Ab-2) antibody and C-terminal (BRCA1-GLK2) antibody.

1- BRCA1-MS13:

Immunohistochemical staining of OvCa cases, using BRCA1-MS13 clone show both cytoplasmic and nuclear expression, among which the preserved nuclear expression indicates preserved function of BRCA1 gene, and the intensity of the cytoplasmic staining is correlated directly with the activity of BRCA1 gene. Current work revealed that 8 cases were negative for BRCA1-MS13 (42.1%) and 11 cases showed positive expression representing (57.9%) of cases representing the bigger group.

Table (1): Comparison between the BRCA1-MS13 staining intensity to the grade of OvCa

	Low Grade	High Grade	Total	P-Value
Negative BRCA1-MS13	1 (12.0%)	7 (87.0%)	8 (100%)	< 0.05
Positive BRCA1-MS13	7 (63.6%)	4 (36.4%)	11 (100%)	< 0.05
Total	8	11	19	-
Spearman Correlation	-0.5			< 0.05

Most of the low grade cases (63.6%) are of positive expression. However, among the high grade cases, no staining was found in (87.0%) and only (36.4%) show positive BRCA1-MS13 staining. P-Value was significant for both states

(< 0.05). Regarding the spearman correlation, it was found that the staining intensity was inversely correlated significantly (p-value < 0.05) to the grade.

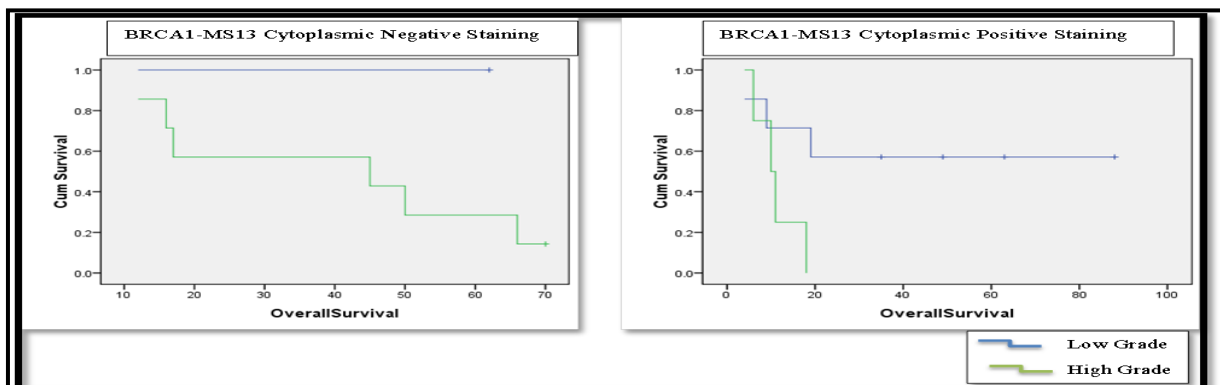
Table (2): Comparison between the BRCA1 (MS13) clone staining intensity to the stage of OvCa.

	Stage I	Stage II	Stage III	Stage IV	Total	P-Value
Negative BRCA1-MS13	1 (14.3%)	0	6 (85.7%)	0	7 (100%)	> 0.05
Positive BRCA1-MS13	4 (33.3%)	1 (8.3%)	7 (58.3%)	0	12 (100%)	> 0.05
Total	5 (26.3%)	1 (5.3%)	13 (68.4%)	0	19 (100%)	
Spearman Correlation	-0.3					> 0.05

Most of negative cases, (85.7%) were of stage III, while only (14.3%) was of stage I. Among positive (33.3%) were of stage I, (8.3%) of stage II and (58.3%) of positive cases were of stage III (p-value > 0.05). Regarding the spearman correlation between the cytoplasmic staining

intensity with the different stages of the cases studied it was found to be inversely correlated with a nearly significant value (p-value = 0.08). The higher the stage is, the weaker the staining intensity of the OvCa.

Kaplan Meir Survival Curves of different Grades of OvCa classified according to the IHC expression state of BRCA1-MS13 antibody:

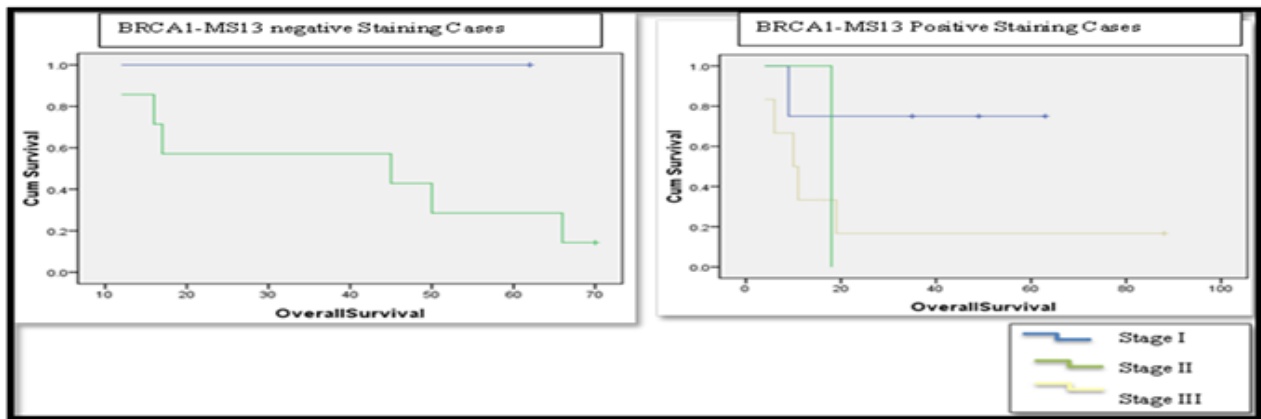


Kaplan Meir Survival curves of the overall survival of our cancer cases divided according to grades (low grade and high grade cancers) and represented in 2 the different expression's states groups; positive and negative BRCA1-MS13 according to the H-score. The X-axis represents the duration's of overall survival in months, while the Y-axis represents the percentage of cumulative survival across our cases. The left curve represents the negative group and the right curve for the positive group of cases.

The previous Kaplan-Meier's curves show the overall survival among different grades of OvCa cases stratified according to the staining expression of BRCA1-MS13 in 2 different curves. From the first curve of the negative group; we find that the overall survival in low grade cases is 100%. While, the overall survival decreases gradually with time in the high grade

cases. Among the positive group, the high grade cases show marked drop in the overall survival in a large proportion of cases, while the low grade cases have high overall survival. The overall survival is better among the cases with negative BRCA1-MS13 expression than those with positive expression of the same antibody.

Kaplan-Meier's Survival Curves of different Grades of OvCa classified according to the IHC expression state of BRCA1-MS13 antibody:



Kaplan Meir Survival curves of the overall survival of our cancer cases divided according to stages and represented in 2 the different expression's states groups; positive and negative BRCA1-MS13 of the staining according to H-scoring system. The X-axis represents the duration of overall survival in months, while the Y-axis represents the percentage of cumulative survival across our cases. The left curve represents the negative group and the right curve for the positive group of cases.

The previous curves show the overall survival of different stages of our studied OvCa cases stratified according to the staining expression of BRCA1-MS13. From the negative group; we find that the overall survival in stage I cases is 100%, however, the overall survival decreasing gradually with time in the stage II cases. Among the positive group, a small proportion of cases show decrease in the survival with time but still a better survival than both stages II and III.

2- BRCA1-GLK2

IHC staining of OvCa cases using BRCA1 clone (GLK2), only cytoplasmic expression of malignant cells that have exon11 mutation of BRCA. The frequency of the staining intensity among our samples showed that (58.9%) are negative and only (21.1%) with positive expression.

Table (3): Comparison between the staining intensity of BRCA1-GLK2 and the grades of OvCa cases

	Low Grade	High Grade	Total	P-Value
Negative BRCA1-GLK2	0 (33.3%)	10 (66.7%)	10 (100%)	> 0.05
Positive BRCA1-GLK2	3 (75%)	1 (25%)	4 (100%)	> 0.05
Total	3	11	14	
Spearman correlation	-0.3			> 0.05

The comparison between the cytoplasmic staining intensity of BRCA1-GLK2 and the grade of the tumors showed that (66.7%) among the negative cases were of the high grade group, and only (33.3%) were of low grade cases. Among the positive cases, the biggest percentage was for the low grade cases (50%)

followed by the high grade group (50%), (P-value > 0.05). The spearman correlation between the BRCA1-GLK2 and the tumor grade shows inverse correlation between the expression and the tumor grading, with non-significant p-value (> 0.05).

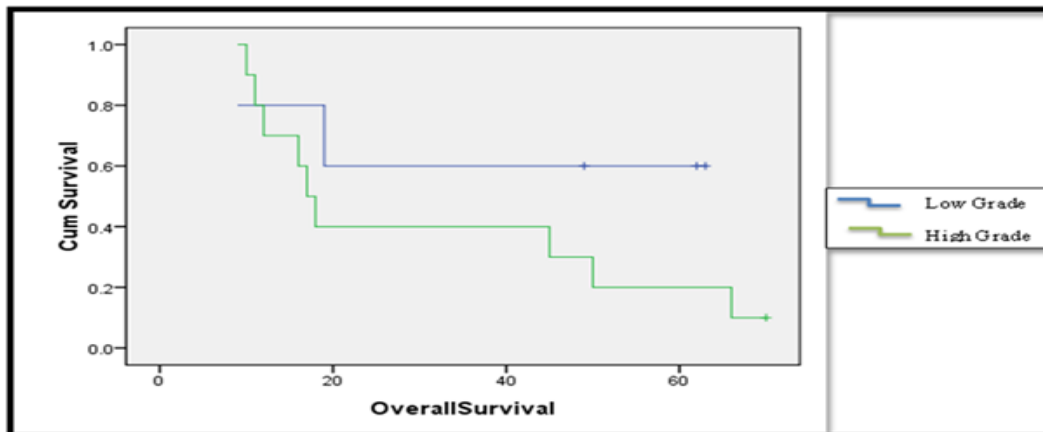
Table (4): Comparison between the staining intensity of BRCA1-GLK2 and the stage of OvCa cases

	Stage I	Stage II	Stage III	Total	P-Value
Negative BRCA1-GLK2	4 (26.7%)	1 (6.7%)	10 (66.7%)	15 (100%)	> 0.05
Positive BRCA1-GLK2	1 (50%)	0	3 (50%)	4 (100%)	> 0.05
Total	5	1	13	19 (100%)	
Spearman correlation	-0.6				> 0.05

The comparison table showed that most of the cases studied for BRCA1-GLK2 expression, 15 out of 19 cases (78.9%) were negative and only 4 cases out of 19 show positivity for the same antibody. Among the negative cases, most of them were of stage III 10 out of 15 cases (66.7%), only one case out of 15 (6.7%) was of stage II, and 4 cases out of 15 (26.7%) were of

stage I. Among the positive cases, 3 out of 4 cases (75%) were of stage III, while only one case out of 4 (25%) of the positive cases was of stage I. (P-Value > 0.05). The spearman correlation between BRCA1-GLK2 cytoplasmic expression and the tumor staging was weak direct, the higher the expression the higher the stage, with non-significant p-value (> 0.05).

Kaplan-Meir's Survival Curves of different Grades of OvCa classified according to the IHC expression state of BRCA1-GLK2 antibody:



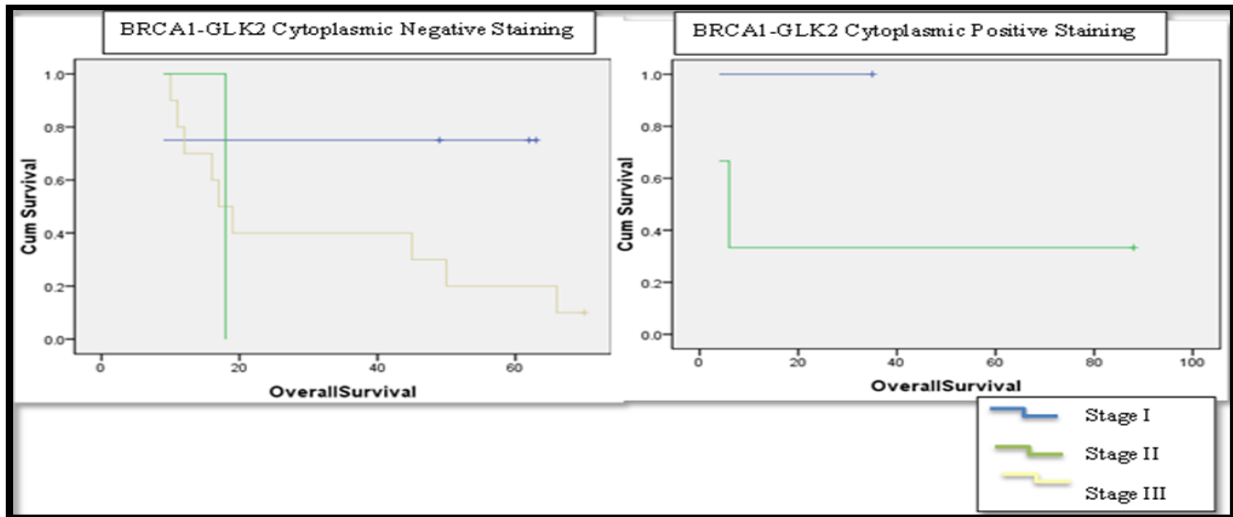
Kaplan Meir Survival curves of the overall survival of our cancer cases divided according to grades (low grade and high grade cancers) and represented in 2 the different expression's states groups; positive and negative BRCA1-GLK2 according to the H-score. The X-axis represents the duration of overall survival in months, while the Y-axis represents the percentage of cumulative survival across our cases. The left curve represents the negative group and the right curve for the positive group of cases.

The previous curve show the survival among the OvCa cases stratified according to the staining expression of BRCA1-GLK2 antibody to find

differences in the overall survival among different BRCA1- GLK2 expressions states of the cytoplasm of cancer cells. From the curve

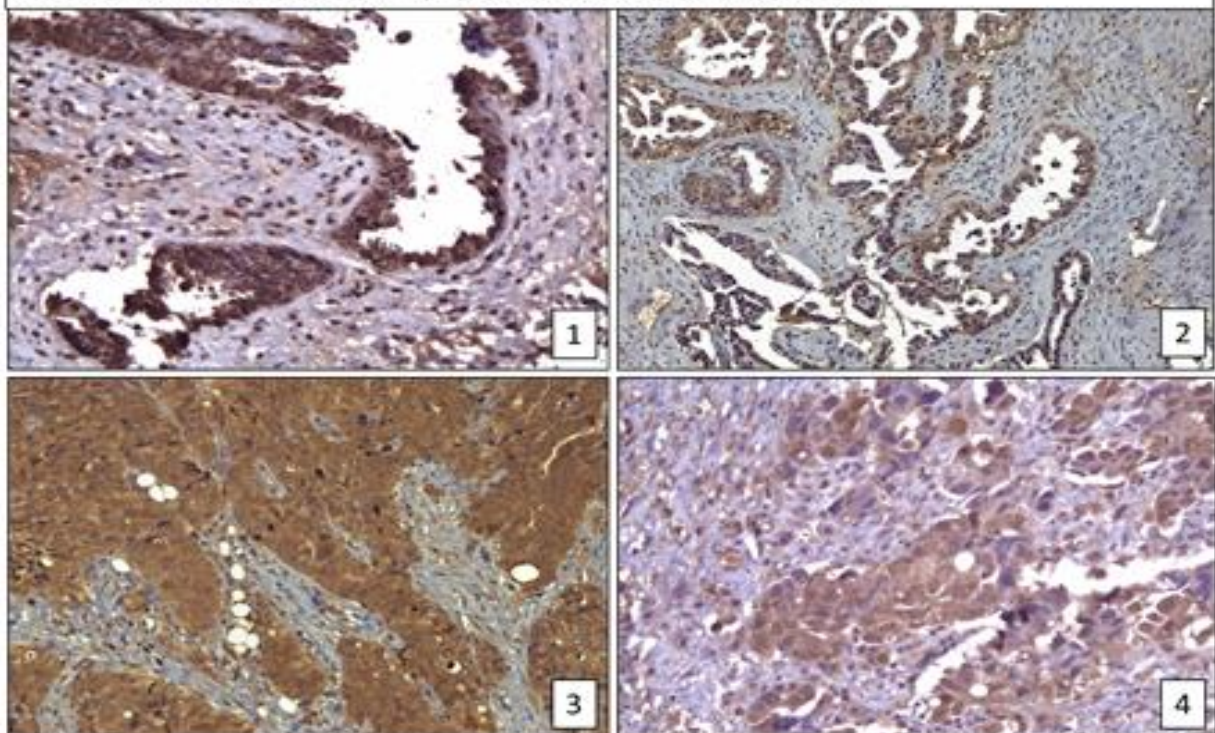
we can find no much difference in the overall survival among both groups of cases (i.e. positive and negative expression). The overall Kaplan-Meir's Survival Curves of different stages of OvCa classified according to the IHC cytoplasmic expression of BRCA1-GLK2:

survival decreases gradually with time in both groups.

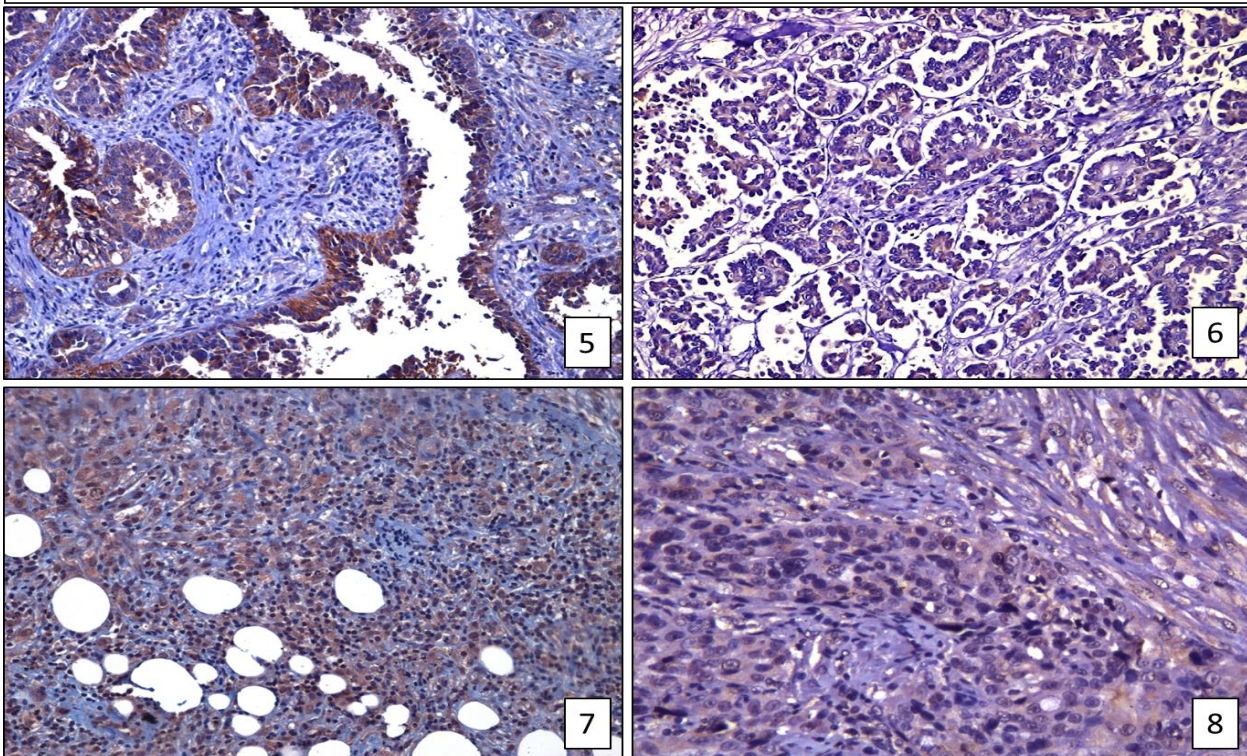


Kaplan Meir Survival curves of the overall survival of our cancer cases divided according to stages and represented in the different expression's states groups; positive and negative BRCA1-GLK2 of the cytoplasmic staining. The X-axis represents the duration of overall survival in months, while the Y-axis represents the percentage of cumulative survival across our cases. The left curve represents the negative group and the right curve for the positive group of cases.

(Fig1) Nuclear staining with strong cytoplasmic expression of BRCA1 (MS-13) in low grade serous carcinoma case (20x). (Fig2) Moderate expression (nuclear and cytoplasmic) of BRCA1 (MS-13) in low grade serous carcinoma (10x). (Fig3) Nuclear staining with strong cytoplasmic expression of BRCA1 (MS-13) in high grade serous carcinoma case (40x). (Fig4) Nuclear staining with moderate cytoplasmic expression of BRCA1 (MS-13) in high grade serous carcinoma case (40x)



(Fig5) BRCA1-GLK2 Moderate cytoplasmic expression in low grade serous carcinoma (10x) .(Fig6) Strong cytoplasmic expression of BRCA1-GLK2 in high grade serous carcinoma (10x). (Fig7) Weak cytoplasmic expression of BRCA1-GLK2 in low grade serous carcinoma (10x). (Fig8) Weak cytoplasmic expression of BRCA1-GLK2 in high grade serous carcinoma (20x)



The previous Kaplan-Meir's curves show the overall survival among different stages of our studied OvCa cases stratified according to the cytoplasmic staining expression results of BRCA1-GLK2 antibody in 2 different curves. From the negative group; we find the overall survival in stage I cases is 100%, however, it decreases gradually with time in the stage III cases. Stage II cases among this group have the lowest overall survival. Among the positive group, the stage I cases show no decrease in the overall survival with time but the overall survival of stages II is low.

Discussion

Understanding OvCa mechanisms on the molecular level combined with high throughput technology will lead to improvement in translational research of advanced OvCa. Adding to this, the great problem of high mortality in patients with OvCa contributes to diminish the efforts to improve outcome through antineoplastic treatment. The primary objective of the present study is to ascertain the somatic involvement of BRCA1 gene in the

pathogenesis of sporadic OvCa by analyzing its protein expression by IHC and correlate the expression with the grades and stages of cases, in addition to correlation to the overall survival. Researches of OvCa reported that, 10% of epithelial OvCa are associated with germline mutations of (BRCA1; 17q12-21) and (BRCA2; 12q13-14) genes and account for 90% of hereditary OvCa (Risch et al., 2001). Studies of familial cases of breast cancer and OvCa found that BRCA1 mutation carriers may have a lifetime risk of OvCa up to 44%. (Clark et al., 2012).

Many mutations of BRCA1 genes have been identified to be linked to increased cancer risk, due to the impairment of the gene function. Up to 700 different mutation points are detected in the BRCA1 with no hot spots areas. Mutations can be small involving single base pair or very large DNA rearrangement (Esmail et al., 2012). Due to the high frequency of BRCA1 mutations associated with invasive ovarian cancers, it has been recommended that genetic counseling should be applied in these cases (Simons &

Petrucci, 2009). Although, new methodologies for genetic testing have been developed, they are still laborious and expensive because the BRCA1 gene is very large and carry wide diversity of mutation possibilities to be tested (Simons & Petrucci, 2009).

Our work revealed that, positive expression for BRCA1-MS12 detected among our cases was found in the nucleus and cytoplasm or in the cytoplasm only without nuclear staining of malignant cells. Our results were not similar to a previous research (Chen et al., 1990) which reported that expression is nuclear in normal but cytoplasmic in breast and ovarian carcinoma cells. Another research done by Wilson and his group (Wilson et al., 1999) described BRCA1-MS12 positive expression as being only nuclear with no cytoplasmic staining opposite to ours. However, our results go with the many works (Kashima et al., 2000), (Fraser et al., 2003) & (Al-Mulla et al., 2000) who found that BRCA1-MS12 is of cytoplasmic expression with some expression in the nucleus explained by the ability of MS12 to detect both the D11b splice variant in the cytoplasm as well as the full length BRCA1 in the nucleus. These contradictory results about the location of the BRCA1 protein probably result from the quality of the archival paraffin embedded breast cancer tissues affecting protein detection and the quality of the antibodies (Yoshikawa et al., 1999).

Our studied cases revealed that, (42.1%) were negative for BRCA1-MS12 and (57.9%) showed positive expression. These results are nearly similar to (Dinesh et al., 2006) who detected down regulation of BRCA1 protein in 54% of cancer cases using IHC. Regarding the nuclear expression of our cases, we found that staining is lost in (47.4%) and is preserved in (52.6%). These results are similar to other research done by (Zheng et al., 2000) who found that BRCA1 expression has been shown to be much reduced in sporadic epithelial OvCa and the IHC nuclear expression is detected in only (49%) of epithelial OvCa.

Different grades of our OvCa cases show different cytoplasmic expression intensities. The low grade cases show lost expression in (12.0%)

low grade cases, while, most of the low grade cases (87.0%) are of positive expression. The high grade cases show no staining in cases of the high grade group (72.6%) and only (27.4%) show positive BRCA1-MS12 staining (P-value < 0.05). The spearman correlation was found to be inversely correlated significantly (P-value < 0.05). The higher the grade of the tumor is, the weaker the staining intensity of the OvCa.

Our results are similar to the results detected by (Zheng et al., 2000) & (Tung et al., 2010) who found that loss of BRCA1-MS12 expression was significantly correlated with the high grade tumors. In contrast to our results, no significant correlation was observed by (Fraser et al., 2003) with any of the biological or pathological markers studied (nodal status, tumor size, tumor grade), suggesting that increased levels of D11b splice variant are an independent marker of poor prognosis.

Studying of the different stages of OvCa cases show different expression patterns. Among negative cases, only (14.3%) was of stage I, while (85.7%) of negative cases were of stage III. Among positive cases (33.3%) were of stage I (8.3%) of stage II and (25.0%) of positive cases were of stage III (p-value > 0.05). The spearman correlation between the staining intensity with the different stages of the cases studied it was found to be inversely correlated with a nearly significant value (p-value < 0.08). The higher the stage is, the weaker the staining intensity of the OvCa.

Going with our results, (Dinesh et al., 2006) & (Clark et al., 2012) reported near significant inverse correlation ($P < 0.063$) between BRCA1 protein expression and clinico-pathological parameters including the tumor stages and they confirmed that the results revealed in this study support the involvement of BRCA1 in the pathogenesis of sporadic breast cancers studied.

The overall survival of our studied OvCa cases for expression of BRCA1-MS12 antibody we find that, the overall survival is higher among the cases with negative expression than those with positive expression of the same antibody. The Spearman correlation is inverse non-

significant ($p\text{-value} > 0.05$). Our results are similar to many studies which reported that BRCA1 mutation associated ovarian cancer cases have a better survival than ovarian cancers without mutations of BRCA1 (Fraser et al., 2003) & (Quinn et al., 2003). In contrast to our results, (Turchetti et al., 2000) reported that, BRCA1 mutation status had no influence on outcome as they found no differences in event-free or overall survival between women with BRCA1 mutations and those without, although the power to detect these variables is limited by the small size of the study done by (Clark et al., 2012).

Controversy between the results may be attributed to the methods of BRCA1-MS13 antibody IHC scoring which may vary between reports. For instance, a reported method does not incorporate staining intensity (Thrall et al., 2006). In addition to the considerable subjectivity between studies in the classification of staining scoring with using the terms of relatively low, intermediate and high BRCA1-expressing tumors, with varying cut-off points in the 'high' group from $>3\%$ to $>7\%$ of positive cells (Swisher et al., 2009).

The second BRCA1 antibody used in this work was BRCA1-GLK2 is monoclonal antibody to the C-terminal region of BRCA1 (Kashima et al., 2000). The frequency of BRCA1-GLK2 IHC expression in our studied cases showed that, most of the cases, (98.9%), were negative and only (1.1%) show positivity. Our positive cases are 100% cytoplasmic with no nuclear expression detected either in normal or cancer cells. Regarding the frequency of expression, (Troudi et al., 2007) found that about (0%) of OvCa cases are positive for BRCA1-GLK2, which is much more than our positive cases. This discrepancy between their results and ours may be attributed to our small sample size. Regarding the pattern of expression, our data go with many studies of breast cancer patients (Kashima et al., 2000), who used the same antibody BRCA1-GLK2 and they attributed that to the accumulation of BRCA1 protein in cytoplasm when the exon 11 is mutated (Kashima et al., 2000). Contrasting to our results, concerning the subcellular localization

of the BRCA1, it has been reported as nuclear in normal but cytoplasmic in breast and ovarian carcinoma cells (Chen et al., 1990), nuclear in all cases (Vaz et al., 2007), or membrane associated on the cytoplasmic aspect of nuclear invaginations (Coene et al., 1997). This debate has been driven by concerns regarding the specificity of the monoclonal antibodies available for BRCA1 detection (Wilson et al., 1996) & (Thakur et al., 1997). Different expression patterns may be explained by the fact that exons 11-13 encodes for two nuclear localization sequences (NLS) (Irwin, 2008). Mutation of the NLS results in altered subcellular localization of BRCA1 that leads to shift toward cytoplasmic localization, and decreasing the tumor suppressor activity of BRCA1. The epitope recognized by this monoclonal antibody might be hidden in the nuclear form of BRCA1, either by its conformation or due to interactions with other nuclear molecules (Clapperton et al., 2004). Significant correlation between the staining pattern and the mutation position of BRCA1 gene; exon 11 mutated gene show cytoplasmic staining, while, mutations in exons other than 11 show absence of staining. Cases of no mutations in BRCA1 are indicated by nuclear staining (Kashima et al., 2000) & (Esmail et al., 2012). Comparison between the cytoplasmic staining intensity of BRCA1-GLK2 and the grade of the tumors showed that negative (66.67%) cases were of the high grade group, and only (33.33%) were of low grade cases. Among the positive cases, the biggest percentage was for the low grade cases (50%) followed by the high grade group (50%), ($P\text{-value} > 0.05$). The spearman correlation between the BRCA1-GLK2 and the tumor grade is weak and inverse between the expression and the tumor grading, with non-significant $p\text{-value} > 0.05$. Our results are similar to those of (Senturk et al., 2010) & (Albederi et al., 2012) who found that most BRCA1 mutation-associated ovarian carcinomas have been reported to be high-grade serous carcinomas, a relatively small number has been diagnosed as undifferentiated, high-grade endometrioid, mixed epithelial, or mucinous. Confirming our results, in many breast cancer studies of the BRCA1 mutations of the C-terminal part, they

found that the majority of positive cases are of high grade invasive ductal carcinomas (Comanescu & Popescu 2009). Contrast to our results, study obtained by (Madjd et al., 2011) & (Albderi et al., 2012) they found no correlation between the BRCA1 expression and breast cancer grade.

The comparison between the different stages and the BRCA1-GLK γ IHC showed that among the negative cases, most of them were of stage III, (76.7%), only (6.7%) was of stage II, and (16.6%) were of stage I. Among the positive cases, (70%) were of stage III, while only (30%) of the positive cases was of stage I, (P-Value > 0.05). The spearman correlation between BRCA1-GLK γ cytoplasmic expression and the tumor staging was weak direct, the higher the expression the higher the stage, with non-significant p-value (>0.05). These results somehow going with (Albderi et al., 2012), as they found that the frequency of positive cases is more in large sized tumors (T 4 and T 5) than that of the small sized tumors (T 1 and T 2), and also they reported significance correlation between the expression of BRCA1 and nodal metastasis (N 1). Our results are opposite to other studies as they reported no significant correlation between cytoplasmic expression of BRCA1 and any prognostic factor including lymph node metastasis, tumor size and vascular invasion (Fraser et al., 2003) & (Thrall et al., 2006).

From the overall survival analysis among the OvCa cases stratified according to the staining expression results of BRCA1-GLK γ antibody scoring of the cytoplasmic expression with H-scoring, we found that the negative group have better survival than the positive group. These results means that mutations in exon 11 detected by the positive BRCA1-GLK γ cytoplasmic staining is associated with poor prognosis as indicated by the low overall survival of the positive cases. These results are going with other studies which reported that mutation positive tumors or altered BRCA1 expression was associated with poor prognosis parameters and considered loss of BRCA1 activity as a marker of tumor aggressiveness with worse prognosis (Trainer et al., 2010) and result in an

accumulation of genetically unstable breast stem cells, providing targets for more carcinogenic events. (Madjd et al., 2011). However, other studies that have contradictory results compared to ours found that the BRCA1 mutated OvCa cases have a better clinical outcome (Yang et al., 2011) and reduced BRCA1 activity is associated with enhanced sensitivity to platinum-based chemotherapy with cisplatin and paclitaxel. Other studies found no differences in disease-free and overall survival between women with BRCA1-associated and sporadic tumors (Troudi et al., 2007). In a study done in Egypt using BRCA1-GLK γ antibody in sporadic colonic carcinoma, they found that 10% of cases that previously known to have BRCA1 non-exon 11 mutations, however, 95% of the cases with exon 11 mutation show cytoplasmic expression (Esmail et al., 2012).

These results suggest that IHC using combined assay of BRCA1-MS13 and BRCA1-GLK γ is a reliable and useful technique for BRCA1 mutations at least in predicting the status of mutation; upstream or downstream of exon 11.

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