Significance of BRAF and Nrf2 in evaluation of serous ovarian tumors

Amira E. Soliman[a], Ghada A. Abd El-Fattah[a], Taghreed A. Abd Elaziz[a], Magda H. Nasreldin[b], Ahmed M. Khalil[c], Hala A. Agina[a]

[a]Department of Pathology, Faculty of Medicine, Benha University, [b]Department of Pathology, Faculty of Medicine, Ain Shams University, [c]Department of Obstetrics and Gynecology, Faculty of Medicine, Benha University, Egypt

Correspondence to Amira E. Soliman, MSc, Department of Pathology, Benha University, Benha, Qualiobyia, 13518, Egypt.
E-mail: am_path@yahoo.com

Background

Ovarian carcinoma is the second leading cause of cancer-related deaths among women. Many treatment strategies have been developed; however, the prognosis is still poor. This study aims to evaluate the immunohistochemical expression of the serine/threonine-protein kinase B-raf (BRAF) and nuclear factor erythroid 2-related factor 2 (Nrf2) in serous ovarian tumors and to correlate their expression with clinicopathological features of the cases.

Materials and methods

This uncontrolled retrospective study was performed on 50 cases: benign ($n=10$), borderline ($n=12$), and malignant serous ovarian neoplasm ($n=28$). The malignant serous tumors included nine low-grade cases and 19 high-grade cases. The immunohistochemical expression of Nrf2 and BRAF was correlated with clinicopathological features of prognostic importance.

Results

The mean of BRAF expression is increased in borderline but it is almost equal for low-grade and high-grade carcinoma cases, which was statistically significant ($P<0.05$). Nrf2 was significantly expressed in the studied borderline and malignant tumors when compared with benign tumors ($P<0.01$). The mean of Nrf2 expression is increased from benign to borderline to ovarian carcinoma (low grade and high grade). This was highly statistically significant ($P<0.01$). A significant association was found between Nrf2 and BRAF expression and between each of them and mean age and laterality of the studied cases ($P<0.01$). A significant association was found between Nrf2 expression and peritoneal metastasis of the cases ($P<0.05$). A nonsignificant association was found between either of BRAF or Nrf2 and lymphovascular invasion, lymph node metastasis, distant metastasis, and TNM stage.

Conclusion

BRAF and Nrf2 could play a significant role in the step of carcinogenesis and then in progression of serous ovarian tumors.

Keywords:

BRAF, immunohistochemistry, nuclear factor erythroid 2-related factor 2, serous ovarian tumors

Introduction

Ovarian carcinoma is the second leading cause of cancer-related deaths among women. It is a rapidly developing disease with bad prognosis. In spite of its gradual development, cases are often diagnosed at a late stage (Siegel *et al.*, 2016; Torre *et al.*, 2018).

In Egypt, primary malignant ovarian neoplasms constituted 1.82% of all primary malignant neoplasms and 32.58% of malignant neoplasms of female genital system at National Cancer Institute. Benign neoplasms represented 16.45% of all ovarian lesions, borderline tumors represented 13.41%, and malignant epithelial neoplasms represented 49.16% (Mokhtar *et al.*, 2016).

The serine/threonine-protein kinase B-raf (BRAF) gene, located on chromosome 7, encodes the BRAF protein. This protein participates in regulation of variable important cellular functions, including growth, differentiation, proliferation, and apoptosis. Mutations of BRAF lead to activation of target proteins in the nucleus and cytoplasm (Chinnaiyan *et al.*, 2017; Quan *et al.*, 2017).

Previous studies revealed the role of oxidative stress in tumorigenesis and progression and also in occurrence of metastasis. Recently, oxidative stress was proved to have a close relation to malignant biological behavior in ovarian carcinoma (Wu *et al.*, 2018).

The nuclear factor erythroid 2-related factor 2 (Nrf2) plays an important role to keep the cells sensitive and adaptive to oxidative and chemical stresses. Nrf2 is regulated by another protein, Keap1. Oxidative stress interrupts the interaction between Nrf2 and Keap1, resulting in accumulation of Nrf2 inside the nucleus, a process that influences the behavior of the cell (Yamamoto *et al.*, 2018).

This study aims to evaluate the immunohistochemical expression of BRAF and Nrf2 in serous ovarian tumors and to correlate their expression with clinicopathological data.

Materials and methods

This is an uncontrolled retrospective study performed on selected cases of 50 different serous ovarian lesions designated as 10 cases of benign serous neoplasms, 12 cases of borderline serous neoplasms, nine cases of low-grade serous carcinoma, and 19 cases of high-grade serous carcinoma. The study was approved by the Ethical committee of Faculty of Medicine, Benha University. The material included archival, formalin-fixed, paraffin-embedded blocks, processed from January 2016 to December 2016, collected from Department of Pathology and Early Cancer Detection Unit, Faculty of Medicine, Benha University, and Pathology Lab of Early Cancer Detection Unit/Maternity Hospital, Faculty of Medicine, Ain Shams University. Clinicopathological data including the patient's age, tumors laterality, lymphovascular invasion, nodal metastasis, distant metastasis, peritoneal metastasis, and TNM stage, were obtained by reviewing the patients' files. Staging was applied according to Prat and FIGO Committee on Gynecologic Oncology (2015).

Immunohistochemical study

For immunohistochemical analysis, streptavidin-biotin complex technique was used following the manufacturer's instructions. Target retrieval (citrate pH=6) was used. Sections were incubated with the primary rabbit polyclonal antibody for BRAF (1 : 100) (Chongqing, YPA1475, China) and Nrf2 (1 : 100) (Chongqing, YPA1342, China) overnight. Standard labeled streptavidin-biotin system was applied (Genemed, South San Francisco, California, USA). Freshly prepared chromogen diaminobenzidine was used. Mayer's hematoxylin was used as a counterstain. Prostatic adenocarcinoma was used as positive control for BRAF. Pancreatic adenocarcinoma was used as positive control for Nrf2. For negative controls, we omitted the step of primary antibody.

Immunohistochemical interpretation

Five random fields ×400 were selected and analyzed. Percentage of positively staining area was calculated for each marker.

BRAF expression was detected as cytoplasmic brown coloration and was interpreted according to Hayashi *et al.* (2014). Nrf2 expression was evaluated according to Peng *et al.* (2016); its expression was detected as cytoplasmic and/or nuclear brown coloration.

Statistical analysis

Results were analyzed by using SPSS (version 16) Statistical Package for Microsoft windows (SPSS Inc., Chicago, Illinois, USA). Categorical data were presented as number and percentages, whereas quantitative data were expressed as mean and SD. Receiver operating characteristic (ROC) curve was used to determine cutoff value of Nrf2 and BRAF with optimum sensitivity and specificity in the diagnosis of different serous ovarian tumors. The accepted level of significance in this work was stated at 0.05 ($P<0.05$ was considered significant). Quantitative variables were presented as mean±SD using standard *t*-test.

Results

The age of all the examined cases ranged between 24 and 63 years, with mean±SD of 45.46±1.13 years. Minimum age was in the third decade, and maximum age was in the seventh decade.

Regarding the histopathological type, it showed significant positive statistical relations with patient's age groups ($P<0.01$), peritoneal metastasis ($P<0.01$), and TNM stage ($P<0.01$), and no significant statistical relations were found with laterality of ovarian neoplasm ($P>0.05$), lymphovascular invasion ($P>0.05$), nodal metastasis ($P>0.05$), nor distant metastasis ($P>0.05$).

Immunohistochemical staining results of BRAF

Immunohistochemical staining of BRAF on serous ovarian tumors revealed 36 cases (72%) with positivity for BRAF expression and 14 cases (28%) were negative.

There was a highly statistically significant relation between histopathological results and BRAF expression ($P<0.01$) (Table 1).

Table 1 Relation between BRAF expression and histopathological types

BRAF expression	Histopathology [n (%)]				P value
	Benign serous neoplasm	Borderline serous neoplasm	Low-grade serous carcinoma	High-grade serous carcinoma	
Negative	10 (71.4)	1 (7.1)	1 (7.1)	2 (14.3)	<0.01 (HS)
Positive	0	11 (30.6)	8 (22.2)	17 (47.2)	
Total	10 (20)	12 (24)	9 (18)	19 (38)	

BRAF, the serine/threonine-protein kinase B-raf; HS, highly significant.

Graph 1
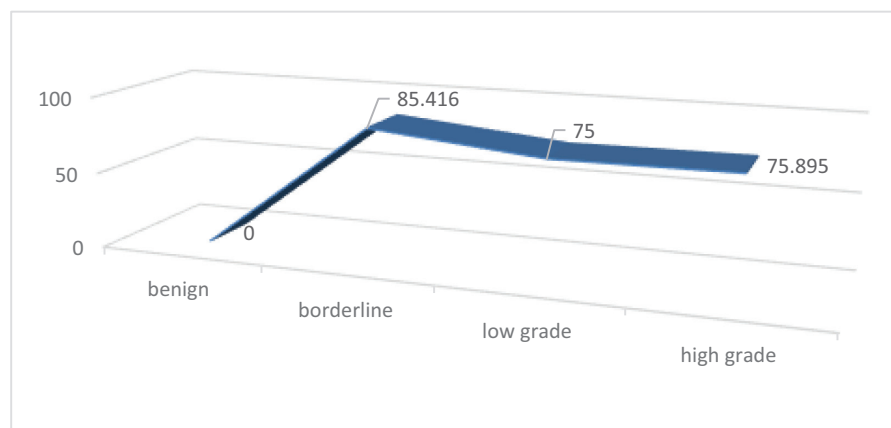
The relation between mean of BRAF expression and histopathological types. BRAF, the serine/threonine-protein kinase B-raf.

Figure 1
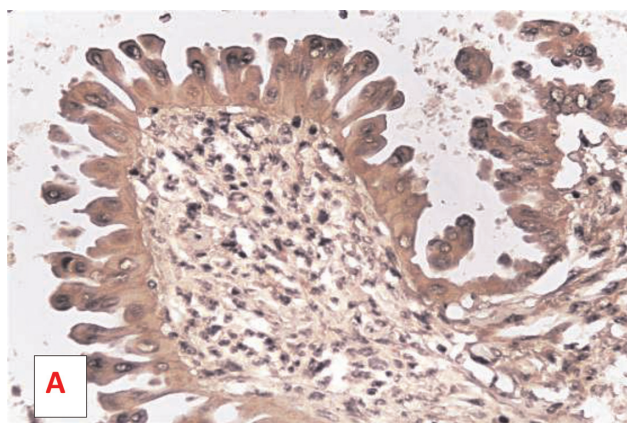
Borderline serous neoplasm, showing positive cytoplasmic staining (>50%) of cells for BRAF (ABC, ×400). ABC, avidin biotin complex; BRAF, the serine/threonine-protein kinase B-raf.

BRAF expression was negative in benign serous neoplasms, whereas in borderline serous neoplasms, the mean BRAF expression was 85.416±16.014. In cases of carcinoma, the mean of low-grade cases was 75.00±29.15, whereas in high-grade cases, it was 75.89±27.50 (Graph 1 and Fig. 1).

BRAF expression showed high statistically significant relation with the mean age of the studied cases ($P<0.01$) and laterality of the neoplasm ($P<0.01$). On the contrary, it showed insignificant statistical relation with lymphovascular invasion, nodal metastasis, distant metastasis, peritoneal metastasis, and TNM stage of studied groups ($P>0.05$ for each).

Diagnostic accuracy
The diagnostic accuracy of BRAF expression was determined by using ROC plots. These plots show the specificity (true negative fraction) and sensitivity (true positive fraction) of the test for all possible thresholds. The accuracy of the test is given by the area under the curve. Performance of BRAF in different serous ovarian tumors is shown in Table 2.

Immunohistochemical staining results of Nrf2
Immunohistochemical staining of Nrf2 on serous ovarian tumors (benign, borderline and malignant cases) revealed nine cases (18%) with score 0, two cases (4%) with score +1, two cases (4%) with score +2, nine cases (18%) with score +3, and 28 cases (56%) with score +4. Positive Nrf2 immunostaining appeared as a brown color, with nuclear and/or cytoplasmic expression (Figs 2 and 3).

Nuclear expression of Nrf2 was detected in two cases (22.2%) of low-grade serous carcinoma and 3 cases

(15.79%) of high-grade carcinoma. However, cytoplasmic expression of Nrf2 was detected in all cases (100%) of benign serous tumors and borderline serous tumors, nine cases (77.7%) low-grade carcinoma, and 16 cases (84.21%) of high-grade carcinoma. As number of cases with nuclear expression was so few, it was not possible for statistical analysis. However, nuclear expression is increased from borderline serous ovarian tumors group to serous ovarian carcinoma group.
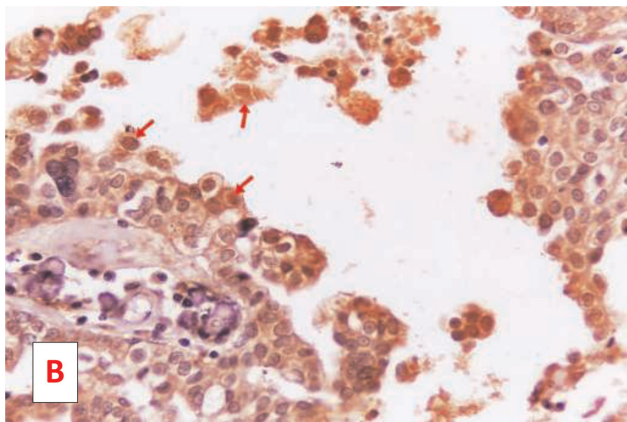
A highly statistically significant relation was found between Nrf2 expression and histopathological type of the cases included in the study ($P<0.01$) (Table 3).

Relation between mean of Nrf2 expression and histopathological types
A linear progression of mean Nrf2 expression from normal to borderline and malignant lesions was found. The difference among these groups was highly statistically significant ($P<0.01$). Mean value of Nrf2 expression was found to increase as the nature of the lesion changed from benign (9±28.46) to high-grade serous carcinoma cases (88.1±10.46) (Graph 2 and Fig. 2).

A highly statistically significant relation between the Nrf2 score of expression and the mean age of the cases studied and laterality of tumor was found ($P<0.01$ for each). Moreover, a statistically significant relation was noted between Nrf2 score

Table 2 Performance of BRAF in diagnosis of serous ovarian neoplasms

	Borderline serous tumors	Low-grade carcinoma	High-grade carcinoma
Sensitivity (%)	91.6	88.8	98.4
Specificity (%)	100	100	100
PPV (%)	100	100	100
NPV (%)	90.9	90.9	83.3
Accuracy (%)	95.4	94.7	93.1
AUC	0.95 (95% CI=0.86–1.00)	0.94 (95% CI=0.81–1.00)	0.94 (95% CI=0.86–1.00)
P value	<0.001 (HS)	0.001 (HS)	<0.001 (HS)

AUC, area under the curve; BRAF, the serine/threonine-protein kinase B-raf; CI, confidence interval; HS, highly significant; NPV, negative predictive value; PPV, positive predictive value.
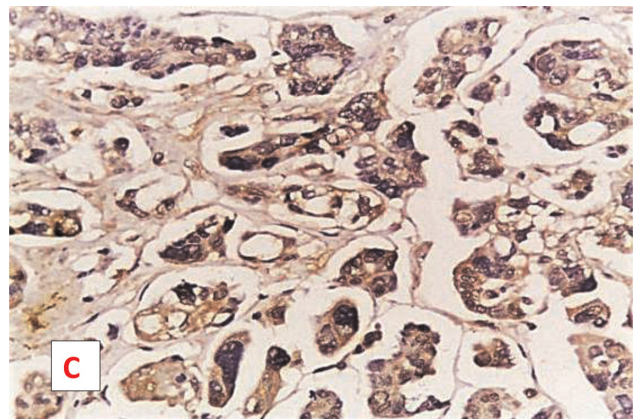
Figure 2

High-grade serous carcinoma showing nuclear (arrowed) and cytoplasmic staining, in 100% of cells, score (4+) for Nrf2 (ABC, ×400). Nrf2, nuclear factor erythroid 2-related factor 2. ABC, avidin biotin complex.

Figure 3

High grade serous carcinoma showing cytoplasmic staining, score (4+) for Nrf2 (ABC, ×400). Nrf2, nuclear factor erythroid 2-related factor 2. ABC, avidin biotin complex.

Table 3 Relation between Nrf2 expression and histopathological types

| Nrf2	Histopathology [*n* (%)]				*P* value
	Benign serous neoplasm	Borderline serous neoplasm	Low-grade carcinoma	High-grade carcinoma	
Score 0	9 (100)	0	0	0	<0.001 (HS)
Score +1	0	1 (50)	1 (50)	0	
Score +2	0	2 (100)	0	0	
Score+3	0	4 (44.4)	3 (33.3)	2 (22.2)	
Score +4	1 (3.6)	5 (17.9)	5 (17.9)	17 (60.7)	
Total	10 (20)	12 (24.0)	9 (18.0)	19 (38.0)	

HS, highly significant; Nrf2, nuclear factor erythroid 2-related factor 2.

Graph 2
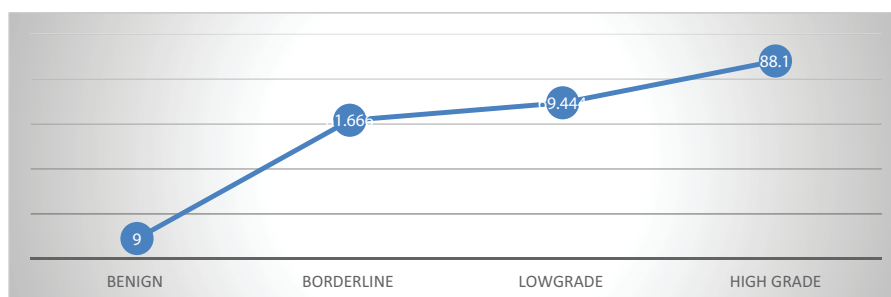
Relation between mean of Nrf2 and study groups. Nrf2, nuclear factor erythroid 2-related factor 2.

Graph 3
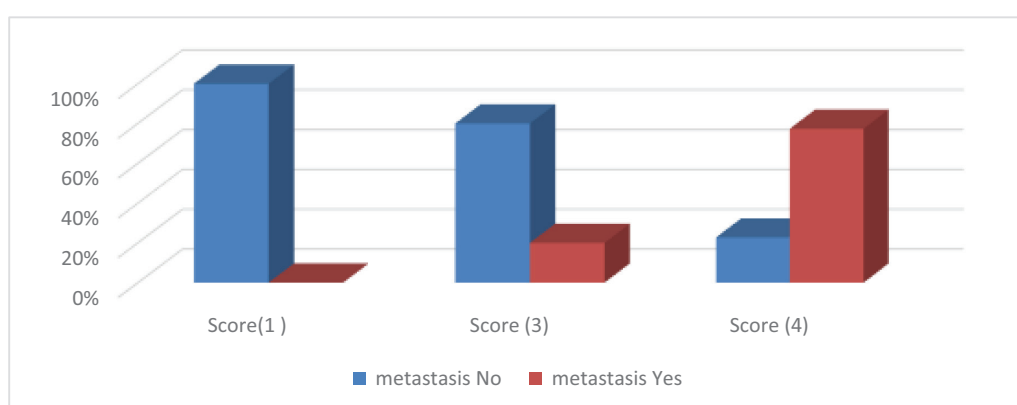
Relation Nrf2 expression in cases of ovarian carcinoma and peritoneal metastasis. Nrf2, nuclear factor erythroid 2-related factor 2.

Table 4 Performance of Nrf2 in serous ovarian tumors

	Borderline serous tumors	Low-grade carcinoma	High-grade carcinoma
Cut off (%)	20	35	67.5
Sensitivity (%)	91	89	95
Specificity (%)	90	90	90
PPV (%)	91	89	89
NPV (%)	90	90	90
Accuracy (%)	91	89	93
AUC	0.90 (95% CI=0.72–1.00)	0.91 (95% CI=0.74–1.00)	0.94 (95% CI=0.84–1.00)
P value	0.001 (HS)	0.003 (HS)	<0.001 (HS)

AUC, area under the curve; CI, confidence interval; HS, highly significant; NPV, negative predictive value; Nrf2, nuclear factor erythroid 2-related factor 2; PPV, positive predictive value.

of expression and peritoneal metastasis ($P<0.05$) (Graph 3).

There was an insignificant statistical relation between Nrf2 scores of expression and lymphovascular invasion, nodal metastasis, distant metastasis, or TNM stage of studied cases ($P>0.05$ for each).

Diagnostic accuracy

The diagnostic accuracy of Nrf2 expression was determined by using ROC plots. These plots show the specificity (true negative fraction) and sensitivity (true positive fraction) of the test for all possible thresholds. The accuracy of the test is given by the area under the curve. Performance of Nrf2 in different serous ovarian tumors is shown in Table 4.

Relation between BRAF and Nrf2 expressions

The more positivity with respect to BRAF expression, the higher the expression of Nrf2, so there was a highly statistically significant relation between BRAF expression and Nrf2 expression of cases ($P<0.01$) (Table 5).

Discussion

Ovarian cancer is one of the most malignant gynecological tumors in the world, with a high fatality rate and resistance to chemotherapy (Wu *et al.*, 2018).

In this work, the older the age of the patient, the higher the percentage of high –grade neoplasm, which was

Table 5 Relation between BRAF expression and Nrf2 expression of cases.

Histopathology	BRAF expression [n (%)]		Nrf2 expression [n (%)]			
	Positive	Negative	Positive	Negative	Total	
Benign serous tumors	0	10 (100)	1 (10)	9 (90)	10 (100)	$P<0.01$ (HS)
Borderline serous tumors	11 (91.7)	1 (8.3)	12 (100)	0	12 (100)	
Low-grade serous carcinoma	8 (88.9)	1 (11.1)	9 (100)	0	9 (100)	
High-grade serous carcinoma	17 (89.5)	2 (10.5)	19 (100)	0	19 (100)	
Total	(36) 72	14 (24)	41 (82)	9 (18)	50 (100)	

BRAF, the serine/threonine-protein kinase B-raf; HS, highly significant; Nrf2, nuclear factor erythroid 2-related factor 2.

highly statistically significant ($P<0.01$). This was in agreement with El-Kady et al. (2018) and Torre et al. (2018), who reported that incidence of ovarian carcinoma among women younger than 65 years has generally declined. These results were attributed to the prolonged oral contraceptives usage, which may decrease ovarian serous tumor risk.

Regarding peritoneal metastasis, cases of high-grade carcinoma showed more peritoneal metastasis than low-grade carcinoma cases ($P<0.01$). Lau et al. (2017) had similar results and explained this relation by the activity of cancer-associated fibroblasts in the stroma which could promote occurrence of peritoneal metastasis from ovarian carcinoma in vivo via activation of many growth factors found in the tumor microenvironment.

Another significant statistical correlation was noted between histopathological type of the studied cases and TNM staging ($P<0.01$), as 88.9% of cases were of low grade. These results agreed with Nasioudis et al. (2018) who found 63.1% of their included cases of low-grade carcinoma and 37.6% of high-grade carcinomas belonged to stage I.

However, Song et al. (2018), in their study on high-grade carcinoma, found that the higher percentage of their studied cases were diagnosed as stage III, reflecting the aggressive nature of this tumor. On the contrary, Abdelrahman et al. (2018) attributed the late stage at the time of diagnosis to the lack of symptoms in the early stages and to the late age of disease presentation. The difference in results may be attributed to low number of cases in the current study and to difference in constitutional factors of the tumors.

BRAF is a member of an extracellular protein kinase pathway that controls cell responses to growth signals, in which, RAS oncogenes play an important role in tumorigenesis (Chinnaiyan et al., 2017).

In this study, BRAF expression showed rising levels from borderline tumors (91.7%) followed by high-grade serous carcinoma (89.5%) then low-grade serous carcinoma (88.9%) of studied cases, which was statistically highly significant ($P<0.01$). These results agreed with Preusser et al. (2013) in their genetic studies, who found that BRAF mutation was exclusive to serous low malignant potential and low-grade serous carcinoma.

These results could be explained by the idea that cases of high-grade carcinoma positive for BRAF may be belonged to type I ovarian carcinoma, which were progressed from low-grade cases. On the contrary, high-grade carcinoma cases that were negative for BRAF belonged to type II pathway, which developed malignancy from the start.

In this thesis, The mean of BRAF expression is increased from benign (negative) to borderline (85.416±16.014). It is almost equal for low grade (75.00±29.15) and high-grade carcinoma (75.89 ±27.50) which was statistically significant (P value <0.05). So BRAF could be considered as a diagnostic marker for borderline serous neoplasms from benign serous tumors, and this was in agreement with Hayashi et al. (2014).

Positive expression of BRAF was detected in patients with mean age of 47.91 years, which was statistically significant ($P<0.01$). Similarly, Xu et al. (2017) found that more expression of BRAF was detected in greater than 45 years old.

This is in contrast with Nakayama et al. (2008), who stated an absence of statistically significant relation between expression of BRAF and age of cases ($P=0.293$). This conflict may be attributed to the different group classification of age (<60 and >60).

In this work, an insignificant relation was found between BRAF expression and peritoneal metastasis ($P>0.05$). However, Schirripa et al. (2015) found that BRAF-mutated patients had a higher incidence of peritoneal metastases ($P<0.01$) in colorectal

carcinoma. They explained their results by the epigenetic loss of mismatch repair genes in association with BRAF mutations in patients with metastatic disease. This difference is attributed to the difference in type of the tissue.

A statistical insignificant relation in this study was reported between BRAF expression and tumor stage in studied cases ($P>0.05$). Similarly, Preusser *et al.* (2013), Grisham *et al.* (2013), and McLachlan *et al.* (2016) stated that positivity of BRAF mutation in low-grade serous neoplasms was related to cases with early stages and good prognosis.

Regarding Nrf2, it was reported to be involved in regulating antioxidant processes for cell protection. Its activation helps both normal and tumor cells to overcome oxidative stress. Normally, low levels of Nrf2 are expressed in human organs. Its overexpression may save malignant cells from cytotoxicity of anticancer agents, evolving resistance for radio- or chemotherapy (Lo and Matthews, 2013; Namani *et al.*, 2018).

By immunohistochemistry, we recorded Nuclear expression of Nrf2 was detected in 22.2% of low-grade serous carcinoma and 15.79% high-grade carcinoma. However, cytoplasmic expression of Nrf2 was detected in all cases of benign serous tumors and borderline serous tumors, 77.7% low-grade carcinoma, and 84.21% of high grade carcinoma. These observations agreed with previous studies carried by Chen *et al.* (2010), Osman *et al.* (2015), and Czogalla *et al.* (2019). Others detected Nrf2 expression mainly inside the nucleus (Onodera *et al.*, 2014; Kawasaki *et al.*, 2015; Bao *et al.*, 2017).

Increased cytoplasmic levels of Nrf2 could reveal the ability of the neoplastic cells to overcome the oxidative and chemical stress. High cytoplasmic Nrf2 expression may result from high expression of other regulatory proteins, increasing the stability of Nrf2 through occupying Keap1-binding sites. Other possible mechanisms include Keap1 downregulation, dysregulation of Nrf2 degradation, upregulation of *Nrf2 transcription*, and stabilization of Nrf2 owing to persistent oxidative stress (Lister *et al.*, 2011).

Nuclear localization of Nrf2 requires further increases in oxidative stress. Nuclear expression of Nrf2 leads to production of antioxidants that help protecting malignant cells from stress signals. High nuclear Nrf2 concentrations may lead to increase in the stage of cancer, aggressive tumor behavior, and poor prognosis. So, Copple *et al.* (2010) and Lister *et al.*

(2011) recommended the evaluation of nuclear Nrf2 expression.

In agreement with the current study, Czogalla *et al.* (2019) found that combined nuclear and cytoplasmic Nrf2 staining was different between histological subtypes with high nuclear expression in the mucinous subtype and lower nuclear expression regarding serous, endometrioid, and clear cell subtypes. In comparison, the weakest and strongest cytoplasmic expression of Nrf2 was detected in clear followed by serous cell subtypes. On the contrary, cases with low-grade malignancy had markedly higher cytoplasmic Nrf2 expression.

Czogalla *et al.* (2019) considered the cytoplasmic Nrf2 expression is the inert form of Nrf2 with good prognosis as nuclear Nrf2 expression could protect cancer cells from chemotherapy and leads to chemotherapeutic resistance. It is well established that oxidative stress result from chemotherapy is the primary signal that causes cytoplasmic Nrf2 to accumulate within the nucleus which results in the production of antioxidants that protect cancer cells from reactive oxygen species. So, higher concentration of Nrf2 in the nucleus may reflect aggressive tumor behavior and poor clinical outcome. So, nuclear Nrf2 expression in cancer cells would have a higher malignant potential.

This work showed a statistically high significant cytoplasmic expression of Nrf2 in high-grade tumors ($P<0.01$). Moreover, regarding the mean Nrf2 expression in different serous ovarian lesions, we noticed that the mean cytoplasmic Nrf2 expression was increased from benign to borderline to ovarian carcinoma (low grade and high grade), with high statistically significant value ($P<0.001$). These results agreed with Osman *et al.* (2015) who thought that increased cytoplasmic expression of Nrf2 may results from prolonged exposure to sex hormones, suggesting the possible role of these hormones in development of ovarian cancer through regulating the expression of Nrf2.

Studies carried by Lister *et al.* (2011), Hu *et al.* (2013), and Onodera *et al.* (2014) in pancreatic, gastric, and breast carcinomas, respectively, reported overexpression of Nrf2 in carcinomas than precancerous lesions and normal tissues. So, Nrf2 cytoplasmic expression could be an early molecular event in tumorigenesis in many organs.

In this work, the relation between mean age groups and Nrf2 expression was statistically highly significant

(*P*<0.01). Similarly, Liew *et al.*, 2015 reported significant association between Nrf2 expression and age of carcinoma cases. This is in contrast to Konstantinopoulos *et al.* (2011) and Osman *et al.* (2015), who found no differences between Nrf2 expression and mean age of cases. This difference could be attributed to difference in age groups of cases included in the different studies.

In this work also, another statistically significant relation was found between Nrf2 expression and peritoneal metastasis (*P*<0.05). This is concordance with Kim *et al.* (2018) who explained these results by the ability of ovarian cancer cells to upregulate certain mitochondrial antioxidant enzymes that help cells to overcome matrix detachment induced by oxidative stress.

The current work showed insignificant association between cytoplasmic Nrf2 expression and lymph nodes metastasis, distant metastasis, and TNM stages (*P*>0.05 for all). The same results were reported by Czogalla *et al.* (2019) as in their study most of cases with N0/x and M0/x were positive for cytoplasmic Nrf2. However, in another study by Wang *et al.* (2010) on gallbladder adenocarcinoma, cytoplasmic Nrf2 overexpression was correlated with TNM staging and metastasis. So, the authors concluded that it has an important role in tumorigenesis and tumor progression and contributes to poor prognosis of cancer patients. This work negates the presence of statistically significant relation between cytoplasmic Nrf2 expression and lymphovascular invasion (*P*>0.05). However, they were significantly related in gastric carcinoma, in addition to other significant relations with nodal metastases and clinical stage (Kawasaki *et al.*, 2015). In their study, they considered that the persistent exposure to oxidative stress would lead to overexpression of cytoplasmic Nrf2 and its translocation into the nucleus of malignant cell. Then, it likely prevents the harmful effects of reactive oxygen species on malignant gastric cells. Though, nuclear Nrf2 expression could explain the poor prognosis of gastric carcinomas. This difference may be attributed to difference in tissue type or genetic constitution.

Regarding the association of expression of both BRAF and Nrf2 in studied cases, this thesis reported positive relations between them mainly in carcinomas. This could be attributed to the expression of oncogenic allele of BRAF or KRAS by the cell, which then could be able to activate Nrf2 via the MAPK pathway in mouse embryonic fibroblasts (DeNicola *et al.*, 2011; Yamadori *et al.*, 2012). Similarly, loss of KEAP1 gene regulates the cell response to BRAF, allowing malignant cells to increase their ability to resist treatments and keep proliferating.

Conclusion

In conclusion, BRAF may be a useful diagnostic marker for borderline serous neoplasm. Nrf2 expression may be a principal factor in the development and progression of serous ovarian neoplasms. Nrf2 could be used as a marker for aggressiveness of serous ovarian neoplasm in old age patients.

Usage of both markers could be an advantage for detecting early serous tumors development in old age patients.

Nrf2 could be a potential target to control cancer cell resistance to oxidative stress, chemotherapy, and radiotherapy hoping to use Nrf2 in the development of new strategies for treatment of chemoresistant cases.

Reference

Abdelrahman AE, Fathy A, Elsebai EA, Nawar N, Etman WM (2018). Prognostic impact of Apaf-1, Cyclin D1, and AQP-5 in serous ovarian carcinoma treated with the first-line chemotherapy. Ann Diagn Pathol 35:27–37.

Bao L, Wu J, Dodson M, Rojo de la Vega EM, Ning Y, Zhang Z, *et al.* (2017). ABCF2, an Nrf2 target gene, contributes to cisplatin resistance in ovarian cancer cells. Mol Carcinogenesis 56:1543–1553.

Chen N, Yi X, Abushahin N, Pang S, Zhang D, Kong B, Zheng W (2010). Nrf2 expression in endometrial serous carcinomas and its precancers. Int J Clin Exp Pathol 4:85–96.

Chinnaiyan A, Palanisamy N, Kalyana-Sundaram S (2017). US Patent No. 9, 567, 644. Washington, DC: US Patent and Trademark Office.

Copple IM, Goldring CE, Kitteringham NR, Park BK (2010). The keap1-nrf2 cellular defense pathway: mechanisms of regulation and role in protection against drug-induced toxicity. Handb Exp Pharmacol 233–266.

Czogalla B, Kahaly M, Mayr D, Schmoeckel E, Niesler B, Kolben T, *et al.* (2019). Interaction of ERα and NRF2 impacts survival in ovarian cancer patients. Int J Mol Sci 20:112.

DeNicola GM, Karreth FA, Humpton TJ, Gopinathan A, Wei C, Frese K, *et al.* (2011). Oncogene-induced Nrf2 transcription promotes ROS detoxification and tumorigenesis. Nature 475:106.

El-Kady NM, Mohamed AE, Aiad HA, El-Wahed MMA, Asaad NY, Allam DM (2018). Evaluation of the role of HIF-1α and GLUT-1 in the pathogenesis of ovarian surface epithelial tumors and their prognostic impact. Egypt J Pathol 38:110–119.

Grisham RN, Iyer G, Garg K, Delair D, Hayman DM, Zhou Q, *et al.* (2013) BRAF mutation is associated with early stage disease and improved outcome in patients with low grade serous ovarian cancer. Cancer 119:548–554.

Hayashi Y, Sasaki H, Takeshita S, Nishikawa R, Nishikawa H, Arakawa A, *et al.* (2014). Usefulness of immunohistochemistry for the detection of the BRAF V600E mutation in ovarian serous borderline tumors. Oncol Rep 32:1815–1819.

Hu XF, Yao J, Gao SG, Wang XS, Peng XQ, Yang YT, Feng XS (2013). Nrf2 overexpression predicts prognosis and 5-FU resistance in gastric cancer. Asian Pac J Cancer Prev 14:5231–5235.

Kawasaki Y, Ishigami S, Arigami T, Uenosono Y, Yanagita S, Uchikado Y, *et al.* (2015). Clinicopathological significance of nuclear factor (erythroid-2)-related factor 2 (Nrf2) expression in gastric cancer. BMC Cancer 15:5.

Kim Y, Vallur PG, Jones V, Shin DH, Worley B, Phaeton R, *et al.* (2018). Increased expression and activity of Sod2 promotes transcoelomic metastasis in ovarian cancer. Free Rad Biol Med 128:S68–S69.

Konstantinopoulos PA, Spentzos D, Fountzilas E, Francoeur N, Sanisetty S, Grammatikos AP, *et al.* (2011). Keap1 mutations and Nrf2 pathway activation in epithelial ovarian cancer. Cancer Res 71:5081–5089.

Lau TS, Chan LK, Wong EC, Hui CW, Sneddon K, Cheung TH, Yim SF, Lee JH, *et al.* (2017). A loop of cancer-stroma-cancer interaction promotes peritoneal metastasis of ovarian cancer via TNFα-TGFα-EGFR. Oncogene 36:3576.

Liew PL, Hsu CS, Liu WM, Lee YC, Lee YC, Chen CL (2015). Prognostic and predictive values of Nrf2, Keap1, p16 and E-cadherin expression in ovarian epithelial carcinoma. Int J Clin Exp Pathol 8:5642.

Lister A, Nedjadi T, Kitteringham N R, Campbell F, Costello E, Lloyd B, *et al.* (2011). Nrf2 is overexpressed in pancreatic cancer: implications for cell proliferation and therapy. Mol Cancer 10:37.

Lo R, Matthews J (2013).The aryl hydrocarbon receptor and estrogen receptor alpha differentially modulate nuclear factor erythroid-2-related factor 2 trans-activation in MCF-7 breast cancer cells. Toxicol Appl Pharmacol 270:139–148.

McLachlan J, Gore M, Banerjee S (2016). Targeting the mitogen-activated protein kinase pathway in low-grade serous carcinoma of the ovary. Pharmacogenomics 17:1353–1363.

Mokhtar N, Gouda I, Adel I (2016). Cancer Pathology Registry 2003-2004 and Time Trend Analysis. Chapter 1; Table 1.4 Ranking of Some Important Malignant Tumor, Pathology Department NCI. Cairo, Egypt: Cairo University.

Nakayama N, Nakayama K, Yeasmin S, Ishibashi M, Katagiri A, Iida K, *et al.* (2008). KRAS or BRAF mutation status is a useful predictor of sensitivity to MEK inhibition in ovarian cancer. Br J Cancer 99:2020–2028.

Namani A, Matiur Rahaman M, Chen M, Tang X (2018). Gene-expression signature regulated by the KEAP1-NRF2-CUL3 axis is associated with a poor prognosis in head and neck squamous cell cancer. BMC Cancer 18:46.

Nasioudis D, Mastroyannis SA, Ko EM, Latif NA (2018). Does tumor grade influence the rate of lymph node metastasis in apparent early stage ovarian cancer?. Arch Gynecol Obstetr 298:179–182.

Onodera Y, Motohashi H, Takagi K, Miki Y, Shibahara Y, Watanabe M, *et al.* (2014). NRF2 immunolocalization in human breast cancer patients as a prognostic factor. Endocr Relat Cancer 21:241–252.

Osman NA, El-Maqsoud NM, El Gelany SA (2015). Correlation of NQO1 and Nrf2 in female genital tract cancer and their precancerous lesions (Cervix, Endometrium and Ovary). World J Oncol 6:364.

Peng L, Duan Y, Zhang Y, Zhao D, Wen Y, Yao J, Da M (2016). Expression of Nrf2 and NQO1 in human gastric cancer and their clinical significance. Int J Clin Exp Pathol 9:1635–1643.

Prat J, FIGO Committee on Gynecologic Oncology. (2015). FIGO's staging classification for cancer of the ovary, fallopian tube, and peritoneum: abridged republication. J Gynecol Oncol, 26:87–89.

Preusser M, Capper D, Berghoff AS, Horvat R, Wrba F, Schindl M, *et al.* (2013). Expression of BRAF V600E mutant protein in epithelial ovarian tumors. Appl Immunohistochem Mol Morphol 21:159–164.

Quan C, Xiao J, Liu L, Duan Q, Yuan P, Zhu F (2017). Protein kinases as tumor biomarkers and therapeutic targets. Curr Pharm Design 23:4209–4225.

Schirripa M, Bergamo F, Cremolini C, Casagrande M, Lonardi S, Aprile G, *et al.* (2015). BRAF and RAS mutations as prognostic factors in metastatic colorectal cancer patients undergoing liver resection. Br J Cancer 112:1921.

Siegel RL, Miller KD, Jemal A. (2016). Cancer statistics, 2016. CA Cancer J Clin 66:7–30.

Song IH, Kim KR, Lim S, Kim SH, Sung CO (2018). Expression and prognostic significance of epithelial-mesenchymal transition-related markers and phenotype in serous ovarian cancer. Pathol Res Pract 214:1564–1571.

Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, *et al.* (2018). Ovarian cancer statistics, 2018. CA Cancer J Clin 68:284–296.

Wang J, Zhang M, Zhang L, *et al.* (2010). Correlation of Nrf2, HO-1, and MRP3 in gallbladder cancer and their relationships to clinicopathologic features and survival. J Surg Res 164:99–105.

Wu X, Han LY, Zhang XX, Wang L (2018). The study of Nrf2 signaling pathway in ovarian cancer. Crit Rev Eukaryot Gene Expr 28: 329–336.

Xu Y, Bi R, Xiao Y, Tu X., Li M, Li A, *et al.* (2017). Low frequency of BRAF and KRAS mutations in Chinese patients with low-grade serous carcinoma of the ovary. Diagn Pathol 12:87.

Yamadori T, Ishii Y, Homma S, Morishima Y, Kurishima K, Itoh K, *et al.* (2012). Molecular mechanisms for the regulation of Nrf2-mediated cell proliferation in non-small-cell lung cancers. Oncogene 31:4768.

Yamamoto M, Kensler TW, Motohashi H (2018). The KEAP1-NRF2 system: a thiol-based sensor-effector apparatus for maintaining redox homeostasis. Physiol Rev 98:1169–1203.