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ORIGINAL ARTICLE

Immunohistochemical expression of CD117 in borderline, low- and high-grade ovarian surface epithelial tumours: A clinicopathological study

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Abstract

Introduction: Ovarian cancer is one of leading causes of cancer related death in gynecology. CD117 is a tyrosine kinase receptor that plays an important role in regulation of apoptosis, cell proliferation and adhesion by binding to its ligand-stem cell factor. Recent studies demonstrated its aberrant overexpression in various malignancies and concluded that it may play a pivotal role in carcinogenesis. Aim: To evaluate CD117 expression in ovarian surface epithelial tumours. *Materials and Methods:* This retrospective study included 30 ovarian epithelial borderline, low and highly malignant tumours' formalin-fixed paraffin-blocks (FFPE) tissue blocks. Tissue sections were subjected to the routine haematoxylin-eosin stain and with the anti-CD117 immunohistochemically. *Results:* There is a high significant difference in CD117 expression between borderline and malignant groups (P = 0.001). Additionally, there was significant difference in expression in relation to histopathological type (serous versus non-serous) in low-grade and the high-grade ovarian surface epithelial tumours (p=0.04, p=0.035 respectively). Tumour grade and stage strongly correlates with CD117 expression (p=0.014, p=0.019 respectively). *Conclusion:* We concluded that CD117 expression was significantly correlated with higher ovarian tumour grade and stage.

Keywords: Epithelial ovarian cancer, serous tumours, CD117, C-Kit, immunohistochemistry

INTRODUCTION

Ovarian cancers are still considered the most lethal malignancy of the female reproductive system. Worldwide, epithelial ovarian cancer (EOC) is regarded as a common gynecologic malignancy. In Iraq, ovarian tumours rank the 7th commonest cancer among females and constituted 3.98% according to the latest published Iraqi Cancer Board Registry in 2017.[1–3] Approximately two-thirds of primary ovarian tumours are of epithelial type. Most epithelial tumours are derived from the surface epithelium of the ovary that develops from the mesothelium that covers the embryonic gonad. This embryonic proximity is reflected in the various directions of Müllerian differentiation exhibited by the ovarian surface epithelium when it undergoes neoplasia. Ovarian epithelial tumours are classified according to tumour cell morphology into many types (serous, endometrioid, clear cell, and mucinous). Further subdivision is used depending on the biological behavior of the tumour into benign (cystadenomas), frankly malignant (carcinomas), and those that have features intermediate between these two, tumours of ("low malignant potential" or tumours of "borderline" malignancy).[4,5]

Depending on recent histopathological and molecular studies that integrate the morphological features, immunohistochemistry and molecular genetics facilities, a dualistic model has been proposed to clarify the pathogenesis of ovarian epithelial cancer. Type I, indolent and slowly growing tumours that are relatively genetically stable that involve clear cells, Brenner carcinoma, low-grade serous, mucinous and endometrioid. They lack TP53 mutations and exhibit a shared lineage with the corresponding benign cystic

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neoplasm often through borderline tumour step. In contrast, second group of tumours, designated as type II, that composed of high-grade serous, high-grade endometrioid and undifferentiated carcinomas, these tumours are rapidly evolving, lack of morphological precursor, more than 80% of cases carrying TP53 mutations and showing early metastatic spread. However, it rarely has mutations that characterise most type I tumours such as KRAS, BRAF, ERBB2 and PTEN.[6,7]

Cancer stem cells (CSCs) or tumour initiating cells (TIC) are a distinct subpopulation of cancer cells, their possession for several unique features, like self-renewal with unlimited capacity and ability to differentiate into different cell lineages making them play a pivotal role in tumour initiation, progression and metastasis. Therefore, they play a crucial role in cancer recurrence and chemotherapy resistance.[8,9] Based on its biological behaviour and clinical course, ovarian carcinoma is regarded as a typical example of CSC-driven disease. Heterogeneity within tumour cells may affect disease course and its response to treatment. There is a highly potent subpopulation of ovarian CSCs with a property of treatment resistance and the ability to repopulate again and cause tumour relapse, leading to even more aggressive, drug-resistant disease.[10]

Many studies revealed that CSC-induced malignant characteristics may be conditioned by the activation of oncogenic signaling pathways or the epithelial–mesenchymal transition pathway and specific cell surface markers, like CD117 that have been commonly used to identify CSCs among EOC cells.[1]

C-KIT (CD117) is a transmembrane tyrosine kinase receptor, encoded by C-KIT protooncogene (in chromosome 4). It is normally activated by phosphorylation and binding to its ligand stem cell factor (SCF), and it is expressed in the surface of haematopoietic stem cells, epithelial cells of breast, germ cells, and other cell types. It has been also found that overexpression or *de novo* expression of C-KIT (CD117) is observed in some tumours, such as gastrointestinal stromal tumours (GISTs), renal, prostate, ovarian cancer, endometrial cancer, osteosarcoma, and others, suggesting the role of C-KIT receptor as a widely used marker for recognition of cancer stem cells in different types of tumours, which in turn reflect its possible role in carcinogenesis of these tumours.[11,12]

In ovarian carcinoma, Bapat and colleagues found in 2005 that CD117 expression was identified in two clones which possessed stem cell-like characteristics that isolated from patient ascites with capability for serial propagation in mice. Zhang *et al*. reported in 2008 a non-adherent, self-renewing ovarian cancer spheres showing a high expression of CD117. In 2011, Choi *et al*. demonstrated a close relation of the side population phenotype in heterogenous human ovarian cancer clones with CD117.[13]

In this retrospective study the immuno-histochemical expression of cancer stem cell marker (CD117) was assessed in low and high grade ovarian epithelial tumours (borderline and malignant) and the relation of this marker to clinicopathological parameters including (age of patient, tumour type, and tumour grade and tumour stage) was investigated.

MATERIALS AND METHODS

The study was approved by the scientific and the ethical committee in Al-Nahrain College of Medicine. This study included a total of 30 ovarian FFPE tissue blocks (ovarian epithelial borderline and malignant tumours) that was collected from Ghazi Al-Harriri Hospital for Medical Specialties and private laboratories throughout September to November 2019. Clinico-pathological parameters were recorded and these included (age of the patients, tumour histopathological type, grade and stage).

From each tissue block 2 sections were taken, each of 5 μm thickness. One section was stained with the routine haematoxylin and eosin stain and the slides were revised by 2 independent pathologists to determine the histopathological diagnosis, tumour histological type and grade according to FIGO system.[14] Tumours that show epithelial proliferation higher than that seen in their benign counterparts, variable nuclear atypia, and no destructive stromal invasion is considered Borderline Ovarian Epithelial tumours. In contrast the Epithelial Ovarian carcinomas show a high proliferation, higher atypia, and destructive stromal invasion. The slides were revised by 2 independent pathologists to determine the histopathological diagnosis. This has been added to the methods section.[2]

The other tissue section was deparaffinised and rehydrated at room temperature; then antigen retrieval was carried out using citrate buffers PH 6.0 (S1699 DAKO, Denmark) in a microwave 1x10 min. Slides were then allowed to cool for 20 minutes. Immunohistochemical staining was done over two days, in the first day primary anti-CD117 polyclonal rabbit

anti-human antibody (A4502, c-kit, DAKO, Denmark ((dilution 1:200) was added with overnight incubation.[15] The second day includes completion of the run by adding anti-mouse secondary antibody and the visualisation of the reaction was through specific HRP/DAB detection IHC kit (ab80436) (Abcam, UK) per manufacturer's instructions. Counterstaining of the sections by Mayer's Haematoxylin stain for 20-30 seconds then followed by mounting of the sections by using Roti®-Mount Aqua (ROTH, Germany). All procedures performed in the current study were approved by The National Research Ethics committee (reference number: IRB-2019-BA8524. Date: 5th/Feb/2019) in accordance with the 1964 Helsinki declaration and its later amendments.

Evaluation of immunostaining and quality control for CD117

Membranous and cytoplasmic pattern of staining in the ovarian epithelial tumour cells was considered as positive.[16–18] A section of seminoma tissue was taken as a positive quality control tissue, while omitting of primary antibody was used as the technical negative control. The immunohistochemical expression of CD117 positivity was analysed in a semi-quantitative scheme score. According to this scoring system, percentage of positive staining of cells and the staining intensity were evaluated. Intensity of the staining was scored as the following; 0 (no staining), 1 (weak staining), 2 (moderate staining), while strong staining was regarded as 3. The percentage of the positive stained cells was scored as follows: 0 (no staining), 1 (less than 10 %), 2 (10-50%), 3 (51-80%), and if more than 80 % was given score 4. For each case, the final score was obtained by multiplication of the value of the two calculated parameters (percentage of the positive cells and predominant intensity). The final score ranged from 0 to 12, in which the scores > 0 were considered as positive.[16]

Statistical analysis

All data were presented as the mean ± standard error of the mean (SEM) unless otherwise specified. The p-value was calculated using Microsoft Office, and GraphPad Prism 6 software with a p-value of < 0.05 considered statistically significant at the 95% confidence level. Unpaired t-tests were used as appropriate for the comparison between data types unless otherwise specified.

RESULTS

CD117 is upregulated in malignant ovarian surface epithelial tumours

CD117 was expressed in 13 out of 30 (44.33%) cases. Statistical analysis of CD117 immunohistochemical expression has shown that the expression of this marker at the protein level was significantly higher (p=0.025) in the malignant tumours group compared to the borderline group in terms of frequency of cases with positive expression; as shown in the frequency Table 1 and in Figure 1. The majority of cases showing positive staining (66.67%) were in the malignant group compared to only (20%) in the borderline group.

This was further consolidated by assessing the immunohistochemical expression of CD117 using the semi-quantitative scoring system. IHC scoring showed a highly significant (p=0.001) ten-fold increase in the expression of CD117 in the malignant group in comparison to the borderline group; with a mean score of (0.46 ± 0.29, n=15) in the borderline group compared to (4.73 ± 1.16, n=15) in the malignant group (see Table 2, Figure 2).

Serous ovarian lesions have overexpression of CD117

More extensive analysis was carried out and the collected samples were categorised according to the histopathological type into two major entities within each group, namely serous and non-serous. Serous tumours accounted for the majority of malignant tumours (60%), and (40%) of the borderline ones. CD117 expression was assessed (using the semi-quantitative scoring system) in each entity to reveal that the level of expression was significantly higher in the serous entity compared to the non-serous (endometrioid and mucinous) regardless of the group being borderline or malignant. As there was a one-fold increase in CD117 expression in the serous entity in the borderline group (p=0.044) and a 3.5-fold increase in CD117 expression in the serous entity in the malignant group (p=0.035) compared to the non-serous entity in each group (see Table 3).

Not only CD117 was more expressed in the serous entity within the individual groups themselves, but it was also significantly higher in the intergroup comparison of the entities (malignant versus borderline). As the level of expression of CD117 was significantly higher (p=0.014) in the malignant serous entity

Table 1: Frequency table of CD117 IHC expression in borderline and malignant ovarian surface epithelial tumours

	Negative		Positive		Total	p value (Chi^2)
Borderline	12	(80%)	3	(20%)	15	**0.025**
Malignant	5	(33.33%)	10	(66.67%)	15	
Total	17		13		30	

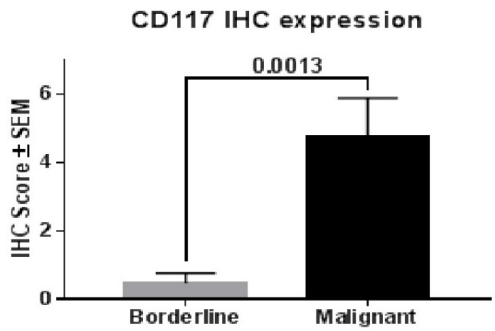

FIG. 1: CD117 expression in surface epithelial ovarian tumours. Data represent mean ± standard error of the mean (SEM).

compared to the serous entity in the borderline group; with a (5.7) fold difference being higher in the malignant group (see Figure 3, Table 3).

Albeit CD117 expression was much lower in the non-serous entity compared to the serous cases in both groups (malignant and borderline), the level of expression in the non-serous entity was nonetheless significantly higher (p=0.047) in the malignant non-serous entity compared to the borderline one (see Figure 3, Table 3).

CD117 correlation is strongly associated with tumour grade and stage

Analysis of the collected clinical data showed that the vast majority of malignant serous tumours were of grade III (77.78%), while the rest of

Table 2: Immunohistochemical expression of CD117 according to the semiquantitive scoring system in borderline and malignant groups

Marker	Borderline (n= 15) Mean ± SEM	Malignant (n= 15) Mean ± SEM	p value
CD117	0.47 ± 0.29	4.73 ± 1.16	**0.0013**

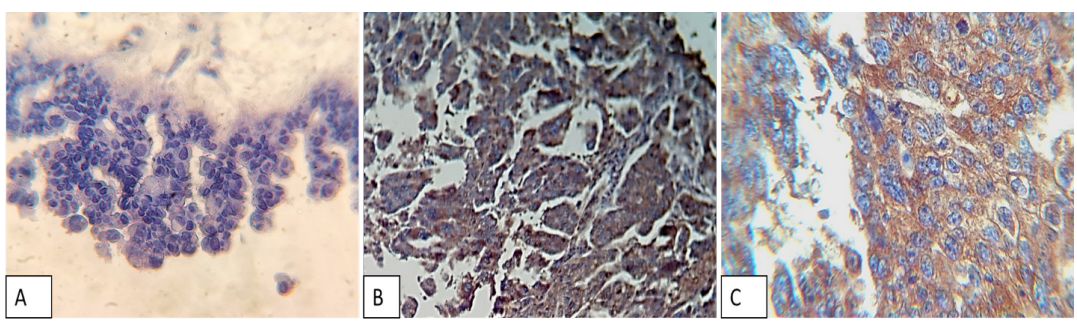

FIG. 2: Tissue sections from ovarian tumours. A), Immunohistochemical staining of Borderline serous tumour tissue section showing negative expression of CD117 (40X). B) Serous cystadenocarcinoma (FIGO grade III) tissue section showing a diffuse brown cytoplasmic and membranous positive immunohistochemical expression of CD117 of > 75% of cells with strong intensity and a score of 12 (10X). C) (40X).

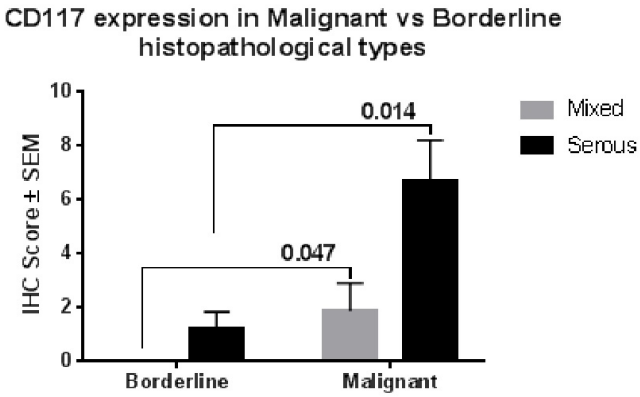

FIG. 3: CD117 expression in the histopathological types of border line and malignant surface epithelial ovarian tumours. Data represent mean ± standard error of the mean (SEM)

Table 3: Score of the immunohistochemical expression of CD117 in relation to histopathological type of ovarian tumours

Histopathological Type	Borderline Mean ± SEM	Malignant Mean ± SEM	p value
Serous	1.17 ± 0.65 (n=6)	6.67 ± 1.52 (n=9)	**0.014**
Non-serous	0 (n=9)	1.83 ± 1.04 (n=6)	**0.047**
p value	**0.044**	**0.035**	

the malignant serous tumours were of grade II (22.23%). Additionally, the majority of the malignant serous tumours had a tumour stage of equal or more than II (55.56%) (see Table 4, Table 5).

In order to assess the strength of the relationship between the level of CD117 expression and the clinicopathological parameters in our study,

Table 4: Histological grading of the malignant tumours

FIGO Grade	Serous		Non-serous		Total
	Number	%	Number	%	
Grade I	0	55.57	5	83.33	10
Grade II	2	22.22	1	16.67	3
Grade III	7	77.78	0	0.00	2
Total	9	100	6	100	15

Table 5: FIGO staging of malignant tumours according to the histological type

FIGO Stage	Serous		Non-serous		Total
	Number	%	Number	%	
Stage I	4	44.44	6	100	10
Stage II	3	33.34	0	0.00	3
Stage III	2	22.22	0	0.00	2
Total	9	100	6	100	15

Table 6: Correlation of CD117 score with age, tumour grade and stage

Variables \ Score	CD117 IHC score	
	Pearson	
	r value	p value
Age	0.205	0.461
Tumour grade	0.617	**0.014**
Tumour stage	0.595	**0.019**

further statistical analysis was performed using correlation coefficient statistical measure.

This analysis revealed a significant positive correlation between CD117 level of expression and the increment in tumour grade and stage, with a p value of (0.014) and (0.019) respectively (see table 6); reflecting some role of CD117 in the tumour progression. There was no significant correlation between CD117 and the age of the patients (p=0.46).

DISCUSSION

Ovarian cancer being the 4th common cause in European women, and the 7th most common malignancy in Iraqi women has attracted a lot of researchers' attention in an attempt to understand the factors responsible for the pathogenesis, progression and prognosis; especially with its reputation of having a high recurrence rate within 2-5 years and poor prognosis due to advanced stage at presentation.[3,18,19]

One of the factors blamed to play a part in ovarian cancer is CD117. It is a transmembrane tyrosine kinase receptor encoded by a proto-oncogene. It is involved in the development of several cell types as well as in the progression of several malignancies. One of these tumours is the ovarian surface epithelial tumours.[20]

In the current study, CD117 was expressed in (44.33%) of cases (overall expression) compared to another study by Conic *et al*. that showed CD117 positive expression in only (27.5%) of cases. The detailed analysis of our results showed that (66.67%) of serous tumours had CD117 expression compared to only (32.6%) of serous cases by Conic *et al*.; nevertheless, both studies were statistically significant with a p value of (0.025) and (< 0.05) respectively.[18]

Furthermore, CD117 immunohistochemical expression was assessed and investigated in ovarian surface epithelial tumours subtypes (in both borderline and malignant tumours). Our analysis showed that CD117 was expressed in about two thirds of the malignant tumours (66.67%) compared to only (20%) of the borderline tumours. This difference between malignant and borderline groups was statistically significant (p=0.025) and concurring with a study by Schmandt *et al*. regarding having significant difference, but the percentage of cases expressing CD117 was higher in our study (66.67%) compared to only (26%) in their study.[19] Worth noting that previous studies have shown low expression of CD117 in normal ovarian tissue (10%) and benign ovarian tumours (20%) that was statistically not significant.[2-21]

Thorough analysis of CD117 IHC score of the collected cases showed that serous tumours had the highest level of expression compared to non-serous (mucinous and endometrioid). This high level of expression was statistically most significant when comparing serous tumours in the borderline to malignant groups (p=0.014), being higher in malignant serous (5.7-fold difference). When we compared the overall CD117 IHC score for serous tumours to the overall score of non-serous (non-serous) tumours, the level of significance was 0.006. Ozer *et al*. showed similar significant higher level of expression of CD117 in serous tumours compared to non-serous tumours (p<0.05); but opposite to our study they failed to show any significant difference between the malignant and borderline groups.[16]

Huang *et al*. showed a statistically significant higher level of CD117 expression in (19.6%) of serous tumours with a p value of (0.01), and their percentage was lower than our percentage with CD117 positivity in serous tumours (66.67%).[12]

Analysis of the clinicopathological data is mandatory in order to have a proper evaluation; and to investigate any possible correlation of the CD117 IHC expression with the collected clinicopathological data; also, it was necessary to test and have a better understanding of the presented hypothesis. As it was mentioned earlier, in this study the majority of the malignant cases were of serous type; and all of them

were of high grade (77.82% were of grade III and 22.23% were of grade II). CD117 was strongly and significantly correlated with the tumour grade (p=0.014). Several studies were in conformity with our results. Schmandt *et al*. showed significant positive correlation with the grade of tumours (p=0.01).[19] Lassus *et al*. and Brustmann also showed highly significant CD117 correlation with tumour grade (p<0.0001 and 0.0008 respectively).[22,23]

In regards to the tumour stage (FIGO system), we found that the more advanced stage the higher the IHC score of CD117 expression, and therefore there was a significant positive correlation between CD117 and disease progression (p=0.019). This is in parallel with the findings of several papers that link CD117 with disease progression whether it was ovarian tumours (Brustmann, p=0.02)[23], or other malignant tumours like prostatic carcinoma (Foroozan *et al*., p=0.001).[24]

In summary, the current study showed that CD117 expression was significantly higher in malignant tumours and specifically in serous subtypes and it was closely correlated with tumour grade and stage reflecting a role of CD117 in tumour cell proliferation and progression of the ovarian surface epithelial tumours. Thus, its expression has the potential to be an effective therapeutic target using available anti tyrosine kinase treatment.

Limitation

Limitation of the current study is the small sample size but this was due to the lack of the complete clinical registry that led to exclusion of some of the cases. This can be overcome in future prospective studies.

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