

HE4 and Ovarian Cancers: New Era of Diagnosis

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Abstract

Introduction: Ovarian cancer is the 5th worldwide leading cause of death of women due to cancer. In more than 70% of cases, it is diagnosed at late stages. The prognosis for ovarian cancer is poor, with 46% 5-year survival rate. Human Epididymis Protein 4 (HE4) is a new biomarker which has been currently evaluated for diagnosing ovarian malignant tumors.

Aim of the work: To study the prevalence and clinical significance of HE4 expression and its association with clinic pathological features of ovarian carcinoma in Tobruk-Libya

Patients, Materials and Methods: The study group included 23 selected cases of ovarian cancers, diagnosed at Pathology department of Tobruk Medical Center, Libya, between 2016 and 2019. All patients were surgically treated and underwent total hysterectomy with bilateral salpingo-oophorectomy. The selection process was based on the clinical, radiological and histological criteria for diagnosis of ovarian cancers. Other clinic pathological data (age, tumor size, laterality and lymph node metastasis) were extracted from medical files. Serum samples were kept frozen until used to measure HE4.

Results: The mean age of the patients at initial surgery was 49.5 years (range, 31–68 years). 12 cases of the tumours were papillary serous cystadenocarcinoma (52.2%); 7 cases were endometrioid carcinoma (30.4%) and 4 cases were mucinous cystadenocarcinoma (17.4%). Tumor size ranged between 8 and 17 cm, with a mean of 12.5 cm. Bilaterality was present in 9 cases (39.1%) while 14 cases (60.9%) were unilateral. Lymph nodes metastasis were present in 6 cases (26.1%) and 17 cases (73.9%) shows no lymph nodes metastasis. Analysis of HE4 expression was done in all cases. Ten cases of papillary serous cystadenocarcinoma (83.3%) showed high HE4 serum level while only 2 cases (16.7%) showed normal HE4 serum level. Six cases of endometrioid carcinoma (85.7%) showed high HE4 serum level while only 1 case (14.3%) showed normal HE4 serum level. Only one case of Mucinous cystadenocarcinoma (25%) showed high HE4 serum level while 3 cases (75%) showed normal HE4 serum level.

Conclusion: HE4 can be used as diagnostic and prognostic marker useful for ovarian carcinoma with the well-known clinicopathological prognostic factors.

Keywords: HE4; tumor marker; ovarian cancer

1. INTRODUCTION

Cancer is a global health problem associated with increasing mortality rates, in spite of advances in diagnostic and therapeutic approaches [1]. Several pathological parameters and specific blood tumor markers have been proposed as predictive prognostic factors in cancer [2, 3]. However, the high incidence of cancer-related deaths indicates a need for reliable and efficient biomarkers for patient stratification and treatment selection [4].

Ovarian cancer is the 5th worldwide leading cause of death of women due to cancer [5]. In more than 70% of cases, it is diagnosed at late stages. The prognosis for ovarian cancer is poor, with 46% 5-year survival rate [6]. The prognosis is closely related to the stage at diagnosis: survival rate of > 70% after 5 years for stage I or II, survival rates between 20 and 40% for stage III or IV [7, 8].

Concerning laboratory exams, several tumor biomarkers have been evaluated. The

Carbohydrate Antigen 125(CA125) was first described in the early 1980's [9].CA 125 is a widely used tumor marker for diagnosis and monitoring of ovarian cancer, but is not increased in some histological types of ovarian cancer [10]. It also has a high false positive rate in benign gynecological diseases such as ovarian cysts and uterine myomas [11].Therefore, CA 125 alone is not sufficient for screening and differential diagnosis of ovarian cancer [12]. Given these circumstances, many studies have introduced human epididymis protein 4 (HE4) as a new tumor marker to help diagnose ovarian cancer [13-15].

Human Epididymis Protein 4 (HE4) is a new biomarker which has been currently evaluated for diagnosing ovarian malignant tumors [16]. It is a glycoprotein belonging to the family of whey acidic four-disulfide core proteins, accounting for its alternative name of WFDC2 and the larger protein family called "WAP" for whey acidic proteins. The main genes coding for the WAP proteins are mainly located on chromosome 20q12–13.1 [17].Present in whey, these proteins are called WAP, which is composed of around 50 amino acids, and its biological function has not yet been completely identified [18].HE4 has been approved by the US Food and Drug Administration as a new tumor marker for the diagnosis of early stage ovarian cancer [19].

Due to increased incidence of ovarian cancers in Tobrouk, Libya, we were able to collect a cohort of 23 ovarian cancers with follow up information. In this project we utilized this patient collection to investigate prevalence and clinical significance of HE4 expression in serum of patients with ovarian cancers.

2. PATIENTS, MATERIALS AND METHODS

The present study is a retrospective study.

The study group included 23 selected cases of ovarian cancers, diagnosed at Pathology department of Tobrouk Medical Center, Libya, between 2016 and 2019. All patients were surgically treated and underwent total hysterectomy with bilateral salpingo-oophorectomy. The selection process was based on the clinical, radiological and histological criteria for diagnosis of ovarian cancers.

Other clinic pathological data (age, tumor size, laterality and lymph node metastasis) were extracted from medical files.

One sample was taken during diagnosis and before surgery. Two more samples were taken from patients with diagnosed ovarian cancers

who underwent surgery. The second sample was drawn one week and the third sample was drawn one month postoperatively.

Serum samples were kept frozen until used to measure HE4. This tumor marker was measured in all patients with ovarian cancers for diagnosis and after surgery and treatment for follow up, using Cobas e411 (Roche).

2.1. Processing Procedures

Five mL blood was collected from all females and transferred to the laboratory. After the clotting, the blood samples were centrifuged. The serum was transferred to another tube and stored at -20°C until use. HE4 was measured using Cobas e411 (Roche). Values ≥ 95 pmol/l were considered abnormal.

2.2. Statistical Analysis

- The collected data were coded then entered and analyzed using the SPSS version 22 (Statistical package for social science).
- Descriptive statistics was done for categorical variables by frequency and percentage, and for numerical variables in the form of mean and standard deviation (mean \pm SD).
- Suitable statistical tests of significance were used:
- Chi-Square (χ^2) test for categorical data
- P-values equal to or less than 0.05 were considered statistically significant.

3. RESULTS

3.1. Clinicopathological Features

The details of 23 patients selected for analyses are as follows. The mean age of the patients at initial surgery was 49.5 years (range, 31–68 years). 12 cases of the tumours were papillary serous cystadenocarcinoma(52.2%); 7 cases were endometriosis carcinoma (30.4%) and 4 cases were mucinous cystadenocarcinoma (17.4%).Tumor size ranged between 8 and17cm, with a mean of 12.5cm. Bilaterality was present in 9 cases (39.1%) while 14 cases (60.9%) were unilateral. Lymph nodes metastasis were present in 6 cases (26.1%) and 17 cases (73.9%) shows no lymph nodes metastasis.

3.2. HE4 Expression

Analysis of HE4 expression was done in all cases. Ten cases of papillary serous cystadenocarcinoma (83.3%) showed high HE4serum level while only 2 cases (16.7%) showed normal HE4serum level. Six cases of endometrioid carcinoma (85.7%) showed high

HE4serum level while only 1 case (14.3%) showed normal HE4serum level. Only one case of Mucinous cystadenocarcinoma (25%) showed

high HE4serum level while 3 cases (75%) showed normal HE4serum level (Table 1).

Table1: HE4 expression in different subtypes of ovarian carcinomas.

Histological subtypes	HE4 expression		Total
	Elevated	Normal	
Papillary serous cystadenocarcinoma	10	2	12
Endometrioid carcinoma	6	1	7
Mucinous cystadenocarcinoma	1	3	4
Total	17	6	23

3.3. Correlations between HE4 Expression and Clinicopathological Features

Univariate analysis revealed significant differences between HE4 expression (elevated versus normal) and histopathological subtype

(Table 2). No significant correlation was found between HE4 expression and age, tumor size, tumor laterality, as well as lymph node metastases (Table 2).

Table2: Relationship between HE4 expression and clinicopathological features.

Clinicopathological features	HE4expression		Chi-square test
	Elevated 17	Normal 6	
Age at initial surgery (median)			
<50 (8 cases)	6 (75%)	2 (25%)	P=0.931
≥ 50 (15 cases)	11 (73.3%)	4 (26.7%)	
Tumor size (median)			
< 12.5 (6 cases)	5 (83.3%)	1 (16.7%)	P=0.541
≥ 12.5 (17 cases)	12 (70.6%)	5 (29.4%)	
Histopathological subtype			
Papillary serous cystadenocarcinoma (12 cases)	10 (83.3%)	2 (16.7%)	P=0.491*
Endometrioid carcinoma(7 cases)	6 (85.7%)	1 (14.3%)	
Mucinous cystadenocarcinoma (4 cases)	1 (75%)	3 (25%)	
Laterality			
Unilateral (14 cases)	10 (71.4%)	4 (28.6%)	P=0.735
Bilateral (9 cases)	7 (77.8%)	2 (22.2%)	
Lymph node metastasis			
+ve (6 cases)	5 (83.3%)	1 (16.7%)	P=0.541
-ve (17 cases)	12 (70.6%)	5(29.4%)	

*p-value <0.05 was considered to be statistically significant.

4. DISCUSSION

Ovarian cancer has a high mortality rate and represents fifth leading cause of cancer-related death in women. In ovarian cancer diagnosis, therefore, all efforts are directed towards finding a new, better biomarker that would be more specific to this disease. In the present practice, the most commonly used ovarian cancer biomarker is CA125. More recently, from all of the potential biomarkers tested, the HE4 had the best results for clinical use [20].

HE4 is a new tumor biomarker, which has been a subject of intense research in recent years. HE4, originally discovered by Kirchoff in the human distal epididymal epithelial cells, [21] is located on chromosome 20 at 20q12-13 and contains fiveexons and four introns [22]. It contains a gene encoding proteindomains that have homology with whey acidic protein, by which the product encoded is mainly protease

inhibitor. As a member of the protease inhibitor family, it has an inhibitory effect on cell proliferation [23].

Researchers found that HE4 is more effective in recognizing serous subtype ovarian carcinoma than endometrial and mucinous subtypes. Given that serousovarian cancer cells are the most common cellular form and are most difficult to diagnose if they are only restricted to ovaries, markers such as HE4 may bring major improvement in the detection of ovarian cancer [24].

This biomarker is weakly expressed in the epithelium tissues of respiratory and reproductive organs, but is over expressed in ovarian tumors, especially in endometriosis ovarian cancer [25]. In addition, it appears that HE4 is not as strongly expressed in clear cell ovarian carcinomas as in other epithelial ovarian cancers [26]. Yanaranop et al. reported a

specificity of 86% for HE4 [27]. These data, in accordance with those reported in a recent Italian multicentre study included 387 patients, showed that HE4 for diagnosing ovarian epithelial cancer appeared more reliable than CA125 [28].

Park et al also reported that the levels of CA 125 and HE4 increased in various benign gynecological diseases, especially in adenomyosis. The increase in CA125 and HE4 concentrations differed according to disease, and an increase in CA 125 was more frequent than an increase in HE4 in benign conditions. Therefore, it is considered that the specificity of HE4 is higher than that of CA 125. Furthermore, the degrees of increase in the levels of these tumor markers also varied according to the pathologic type of ovarian cancer [29, 30]. In this study, the level of HE4 was high in serous carcinoma and endometriosis carcinoma, whereas the level of HE4 was low in mucinous carcinoma.

The clinic pathological characteristics and HE4 expression relationship in our results shows no significant association with age, tumor size, laterality and lymph node metastasis. However, there is significant association with histopathological subtypes. Unfortunately, there is no much studies to discuss with these results.

Limitations of the Study

Our study has some limitations. First, a small sample size was used to identify the value of HE4 expression in ovarian carcinomas because of the short study period. Second, there is no follow-up documents of the patients and this study is designed and performed recently.

5. CONCLUSION

Our results show that HE4 is a useful biomarker in diagnosis and follow up of most of ovarian carcinomas, with the well-known clinic pathological prognostic factors. Definitely, we are attentive that further, larger researches are compulsory to validate HE4 as independent diagnostic, prognostic or predictive factor in ovarian carcinoma. A long-term follow-up study will be necessary to identify the clinical value of HE4 expression in ovarian carcinoma.

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