

COMPARISON BETWEEN HAPTOGLOBIN AND CA125 TO PREDICT NATURE OF OVARIAN MASS

By

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ABSTRACT

Background: Ovarian cancer has been named "the silent killer" because it frequently causes non-specific symptoms which contribute to diagnostic delay. Despite of the improved surgical techniques and effective chemotherapeutic regimens available for management of ovarian cancer, there is no improvement in its early detection. Thus, ovarian cancer remains a leading cause of death from gynecological malignancies with an overall poor prognosis. The identification of novel cancer biomarker opens the possibility for early detection, and better monitoring of tumor progression. Haptoglobin is a novel biomarker which was used in diagnosis in ovarian cancer.

Objectives: To assess the performance of haptoglobin and CA125 to increase the sensitivity and specificity in diagnosis of nature of ovarian mass.

Patients and Methods: This study was carried out on 90 women attending at Al-Hussein and Bab El-Sha'aria University Hospitals. A prospective study was held on patients with ovarian mass prepared for surgery. It was a case control study. It incorporated on 60 patients with ovarian masses (30 women with benign ovarian masses and 30 women with malignant ovarian masses), and 30 women without masses (control group). Those patients were subjected to careful history taking, clinical examination, ultrasound examination and preoperative assessment of serum levels of Haptoglobin and CA125.

Results: the prediction of CA-125 was better than that of haptoglobin but the combination of both markers was better than the prediction of one of them in diagnosis of ovarian cancer.

Conclusion: Haptoglobin has an important role in the prediction of ovarian cancer. It increased significantly in malignant ovarian tumors than in benign ovarian tumors. So, it can be used as a diagnostic tumor marker in cases of ovarian neoplasia.

Key words: Haptoglobin, CA125, Ovarian mass.

INTRODUCTION

Ovarian cancer is a lethal gynecologic malignancy with greater than 70% of women presenting with advanced stage disease. Despite new treatments, long term outcomes have not significantly changed in the past 30 years with the five-year overall survival remaining between 20% and 40% for stage III and IV disease. In contrast patients with stage I disease

have a greater than 90% five-year overall survival. Detection of ovarian cancer at an early stage would likely have significant impact on mortality rate (Cohen et al., 2014).

Given our knowledge about the steep decrease in survival rates relative to the stage at which the disease is diagnosed, it is reasonable to suggest that early detection remains the most promising approach with which to improve the long-

term survival of ovarian cancer patients. Therefore, considerable efforts have been focused on the identification of diagnostic biomarkers for early detection of ovarian cancer (Donach *et al.*, 2010 and Hensley, 2010).

Haptoglobin is an acute phase protein that binds hemoglobin, produced primarily by hepatocytes (Boettger *et al.*, 2016).

The basic haptoglobin molecule is a tetrameric protein with α/β dimmers. β chains are identical in all haptoglobin types and polymorphisms of haptoglobin rise from differences in α chain (Kasvosve *et al.*, 2010). In a manner similar to other acute-phase proteins, an elevation of this peptide could be observed in infections, inflammations, and various malignant diseases, including lung and bladder cancer, leukemia, breast cancer, malignant lymphoma and urogenital tumors (Abdullah *et al.*, 2009).

The present work aimed to assess the performance of haptoglobin and CA125 to increase the sensitivity and specificity in diagnosis of nature of ovarian mass.

PATIENTS AND METHODS

This study was carried out on 90 women attending at Al-Hussein, Bab El-Sha'aria University Hospital and outpatient Gynecological Clinic. After informed consents were obtained, those patients were subjected to careful history taking, clinical examination, ultrasound examination and preoperative assessment of serum levels of Haptoglobin and CA125.

Enrollment of study group in our study and their division into patients having either benign or malignant masses done

according to postoperative histopathological examination, patient with krukensberg tumors and those having metastatic tumors were excluded from our study. Preoperative assessment of serum levels of Haptoglobin and CA125 was done in all cases.

A. Study Group: Consists of 60 women with ovarian mass scheduled for surgery.

It was subdivided into two groups:

Subgroup I includes 30 cases diagnosed as having malignant ovarian tumors.

Subgroup II includes 30 cases diagnosed as having benign ovarian tumors.

B. Control group: Consists of 30 healthy non pregnant women don't have ovarian mass.

All the removed specimens were sent for histo-pathological diagnosis using paraffin sections and stained with haemtoxylin and eosin stain.

Corroboration of the histopathology with serum haptoglobin and CA125 was done to assess the value of these pre-operative investigations for discrimination between benign and malignant tumors.

Statistical analysis of the data

Data were fed to the computer using IBM SPSS software package version 20.0.

Qualitative data were described using number and percent. Comparison between different groups regarding categorical variables was tested using Chi-square test.

Quantitative data were described using mean and standard deviation for normally distributed data between two independent populations using independent t-test, while more than two populations were analyzed using F-test (ANOVA).

Correlations between two quantitative variables were assessed using Pearson coefficient.

Significance test results were quoted as two-tailed probabilities, and the obtained results were judged at the 5% level.

RESULTS

The data which were obtained from history, symptoms, clinical examination, ultrasonic assessment and tumor markers measurement, were compared with the

operative findings and the final histopathological findings of the masses in order to assess the diagnostic role of Haptoglobin biomarker in ovarian cancer.

In this study, out of the 97 subjects primarily enrolled, 60 patients with ovarian mass (30 case as benign and 30 case as malignant) and 30 cases as a control, 7 cases were excluded, as they were found to have secondary ovarian tumors (5 cases of krukenberg tumors and 2 cases of endometrial clear cell tumor).

Table (1): Comparison between the three studied groups regarding serum Ca 125 (U/ml) and haptoglobin (mg/ml).

Parameters	Control group (No.= 30)		Benign group (No.= 30)		Malignant group (No.= 30)	
	Ca 125 (U/ml).	Haptoglobin (mg/ml)	Ca 125 (U/ml).	Haptoglobin (mg/ml)	Ca 125 (U/ml).	Haptoglobin (mg/ml)
Range	10.50 - 32.00	0.20 - 1.30	20.00 - 80.00	1.00 -1.80	88.00 - 463.00	0.50 - 10.50
Mean	18.75	0.67	50.37	1.50	231.93	5.94
S.D.	5.79	0.33	13.49	0.22	115.17	2.77
p	0.0001					

On analysis of both serum CA-125 and haptoglobin, it was found that there was a significant difference between the three studied groups with p value =0.0001(Table 1).

and malignant tumor.

Variables	Values
Area under the ROC curve (AUC)	0.69
Cut off value (U/ml)	>36.0
Sensitivity %	90.0
Specificity %	92.0
PPV %	88.0
NPV %	91.0
P value	0.002

Table (2): The sensitivity and specificity of serum Ca 125 level to predict benign

Analysis of CA-125 as a single biomarker using calculated cut off value >36 U/ml showed that the sensitivity of CA-125 was 90.0%, specificity 92.0%,

positive predictive value 88.0%, and negative predictive value 91.0% in the diagnosis of malignant ovarian tumors with p value = 0.002 (Table 2).

Analysis of serum haptoglobin using calculated cut off value >1.9 mg /ml showed that the sensitivity of haptoglobin was 88.0%, specificity 85.0% and positive predictive value 84.0% in the diagnosis of malignant ovarian tumors with p value = 0.0016 (Table 3).

Table (3): The sensitivity and specificity of Serum haptoglobin level to predict benign and malignant tumor.

Variables	Values
Area under the ROC curve (AUC)	0.62
Cut off value (mg/ml)	>1.9
Sensitivity %	88.0
Specificity %	85.0
PPV %	84.0
NPV %	83.0
P value	0.016

Analysis of both serum CA-125 and haptoglobin biomarkers using calculated cut off value >36 U/ml and >1.9 mg/ml respectively showed that the sensitivity of both was 94.0%, specificity 93.0%, positive predictive value 95.0%, and negative predictive value 92.0% in the diagnosis of malignant ovarian tumors with p value = 0.001 (Table 4).

Table (4): The sensitivity and specificity of combined use of serum Ca 125 and haptoglobin level to predict benign and malignant tumor.

Variables	Values
Area under the ROC curve (AUC)	0.81
Sensitivity %	94.0
Specificity %	93.0
PPV %	95.0
NPV %	92.0
P value	0.001

DISCUSSION

In the present study, CA-125 at a cut off 36 U/ml was a significant predictor of malignancy in the studied cases. The result was in agreement with **Kim et al. (2009)** who found significant differences between median CA-125 levels between benign and malignant ovarian masses. They observed that the mean serum CA-125 in stage III ovarian cancer patients was significantly higher than in the control women. Similarly, **Chen et al. (2008)** observed that serum CA-125 levels were significantly higher in malignant ovarian tumors compared to non-malignant ovarian tumors and normal subjects. It was also significantly higher in non-malignant ovarian tumors compared to normal subject. The initial findings of serum CA-125 levels greater than 35U/ml in approximately 83% of patients with epithelial ovarian cancer lead to use of serum CA-125 as a biomarker for ovarian cancer (**Chen et al., 2008; Van Calster et al., 2015 and Bast et al., 2016**).

In agreement with **Ong et al. (2013)** found that mean serum CA-125 was significantly higher in patients with cancer

ovary compared to normal subjects, patients with benign tumors and patients with other gynecological cancers.

In the current study, at cut off value of 36U/ml, the predictive value of serum CA-125 in diagnosis of malignant ovarian tumors showed sensitivity (90%), specificity (92%), positive predictive value (88%), and negative predictive value (91%).

These figures were close to **Kim et al. (2009)** who found that at cut off of 35 U/ml, CA-125 had a sensitivity of 84% but low specificity 35.2%. Also, **Gadducci et al. (2009)** reported that diagnostic value of serum CA-125 in distinguishing a benign from malignant ovarian mass has been demonstrated with a sensitivity ranging from 56%-100% and a specificity ranging from 60%-92%.

In other study, **Su et al. (2007)** and **Biskup et al. (2014)** found that the evaluation of serum CA-125 as a marker in detection of ovarian carcinoma was sensitivity of 50-62%, specificity of 30%, positive predictive value of 57% and negative predictive value of 70.6%.

In the current study, the serum CA-125 ranged from 88-463 with a mean value of 231.93 in malignant group. It was significantly lower in benign compared to malignant group.

Kim et al. (2009) and **Garibay-Cerdenares et al. (2014)** observed that the mean serum haptoglobin expression in stage III ovarian cancer patients was four folds higher than in the control subjects. They carried their study on women with high grade ovarian cancer and compared to a control group of healthy women. Similarly, **Kim et al. (2009)** found that

serum haptoglobin was significantly higher in cancer ovary. Also, **Zhao et al. (2007)** observed that mean serum haptoglobin was significantly higher in malignant ovarian tumors even those with early stage tumor, compared to those with benign conditions.

The same results were obtained by **Chen et al. (2008)** who studied serum haptoglobin levels in malignant and nonmalignant tumors of the ovary. They found that serum haptoglobin levels was significantly higher in malignant ovarian tumors compared to non-malignant tumors and normal subjects. This indicated that haptoglobin plays an important role in the evaluation of the cancer ovary. Other investigations revealed that haptoglobin is a natural inhibitor of collagen degradation and is locally expressed by fibroblasts in arterial walls (**Smeets et al., 2013**).

The importance of haptoglobin in extra cellular matrix degradation and cell migration suggests a role for this polypeptide in cancer, and also provides an evidence supporting haptoglobin as a molecular target for therapy in epithelial ovarian carcinoma (**Kim et al., 2009** and **Miyamoto et al., 2016**).

In the current study, at cut off value of 1.9 mg/ml, the accuracy value of serum haptoglobin in diagnosis of malignant ovarian tumors showed sensitivity was 88%, specificity (85%), positive predictive value (84%) and negative predictive value (83%).

In the current study, the serum haptoglobin ranged from 0.5-10.5 mg/ml with a mean of 5.94 in malignant group. It was significantly different from control group.

The serum haptoglobin in benign group ranged from 1-1.8 mg/ml with a mean 1.50 which was significantly different from than control group. It was found that serum level of haptoglobin in malignant and benign group was significantly different from control group, while serum level of haptoglobin in malignant cases were more than that in the benign cases.

These results were in agreement with **Kim et al. (2009)** who found that haptoglobin in malignant cases were significant difference from normal controls, benign ovarian tumors and other gynecological cancers respectively, and agreed with **Zhao et al. (2007)** who found that haptoglobin concentration were significantly higher in late stage ovarian cancer than benign and healthy controls, and there was a significant difference between early stage cancer and healthy controls.

This was in agreement with **Weiz et al. (2016)** who found that, in the group of ovarian cancer, haptoglobin level was more than three times than control groups. In the group of nonmalignant tumors, the haptoglobin level was twice as high as in the control group.

The combined ability of both markers (serum CA-125 and serum haptoglobin) to detect ovarian malignant tumors was tested in the current study at the cut off value of 36 U/ml for serum CA-125 and 1.9 mg/ml for serum haptoglobin. The predictive values of both markers together in diagnosis of malignant ovarian tumor showed sensitivity (94%), specificity (93%), positive predictive value (95%) and negative predictive value (92%).

The results of the present study were similar to that of **Kim et al. (2009)** who

found that the combined ability of both markers (serum CA-125 and serum haptoglobin) showed better results with 91% sensitivity and 95% specificity in the whole study groups.

CONCLUSION

Haptoglobin has an important role in the prediction of ovarian cancer and its serum level increased in early stages of cancer ovary. It increased significantly in malignant ovarian tumors than in benign ovarian tumors. So, it can be used as a diagnostic tumor marker in cases of ovarian neoplasia. So, haptoglobin was a novel marker for ovarian cancer with accuracy near to that of CA-125. The combination of haptoglobin and CA-125 showed better prediction than haptoglobin or CA-125 alone.

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مقارنة بين الهابتوجلوبين و سى ايه - ١٢٥ كدلائل للأورام فى الكشف عن طبيعة أورام المبيض

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خلفية البحث : سرطان المبيض هو أكثر أورام أمراض النساء المميتة بسبب طبيعة المرض الصامتة ونقص الأعراض خصوصاً فى المراحل المبكرة من المرض . ويلقب سرطان المبيض بالتهديد الصامت حيث أن معظم المريضات تأتيين بمراحل متقدمة من المرض مع إحصائية الحياة لمدة ٥ سنوات فى حوالى ٣٠% من الحالات وذلك بسبب عدم القدرة على التشخيص المبكر للمرض. ومع تطور إستخدام دلالات الأورام فى الكشف عن سرطان المبيض، نشأت فكرة هذا البحث على أورام المبايض المحتمل كونها سرطانية ، وذلك لتقييم دور معامل الهابتوجلوبين الذى يستخدم فى الكشف عن سرطان المبيض والذى هو موضوع دراساتنا الحالية .

الهدف من البحث: مقارنة أداء سى ايه-١٢٥ والهابتوجلوبين لزيادة حساسية التشخيص ،ولزيادة دقة التشخيص لمعرفة طبيعة الورم على المبيض هل هو حميد أم خبيث .

المريضات وطرق الدراسة: تمت الدراسة على ٩٠ سيدة من مستشفى الحسين الجامعى ومستشفى باب الشعرية .

وقد قسمت الحالات قيد الدراسة إلى مجموعتين:

- المجموعة الأولى: وتشمل ٣٠ سيدة قد تم تشخيصهن بأنهن مصابات بورم خبيث بالمبيض.
- المجموعة الثانية: وتشمل ٣٠ سيدة قد تم تشخيصهن بأنهن مصابات بورم حميد على المبيض.

هذا بالإضافة إلى المجموعة الضابطة وهى مكونة من ٣٠ سيدة ليس بهن ورم على المبيض ولسن
حوامل.

بعد أخذ الموافقة من جميع السيدات المشتركات فى الدراسة البحثية، تم أخذ عينات من دم الحالات لقياس نسبة الهابتوجلوبين ونسبة سى ايه-١٢٥ فى الدم قبل إجراء العمليات الجراحية لهن.

النتائج: قيمة سى ايه-١٢٥ (٣٦ ميكروجرام/ملل) هى القيمة ذات الحساسية الأعلى فى تحديد أورام خبيثة للمبيض بنسبة ٩٠% ، والدقة الأكثر بنسبة ٩٢% فى تحديد الأورام الخبيثة للمبيض. وقد وجد أن قيمة الهابتوجلوبين (١,٩ ملي جرام/ملل) هى القيمة ذات الحساسية الأعلى فى تحديد الأورام الخبيثة للمبيض بنسبة ٨٨% ، والدقة الأكثر بنسبة ٨٥% فى تحديد الأورام الخبيثة للمبيض.

الاستنتاج: الهابتوجلوبين من دلائل الأورام التى يمكن إستخدامها فى تشخيص الأورام الخبيثة للمبيض، ولكنه أقل حساسية من سى ايه-١٢٥ فى تشخيص الأورام الخبيثة للمبيض ، إلا أن حساسية ودقة كلاهما معا كانت اعلى من حساسية ودقة كل واحد منهما على حدة فى تشخيص الأورام الخبيثة للمبيض.