Role of Human Kallikrein 6 as a Serum Biomarker in Early Diagnosis of Ovarian Carcinoma

Original Article

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ABSTRACT

Objective : Serum levels of hk6 for patients with benign ovarian masses, malignant ovarian masses, and normal healthy controls are to be measured and compared.

Methods : Two groups of women were included in the study : Group 1:ninety-nine women who had ovarian swellings that required laparotomy or laparoscopic surgery after being detected clinically and radiologically Group 2:Seven women who appeared to be in good health served as controls (women attending to Gynecological outpatient clinic for family planning consultation from).

Results : Groups with benign neoplasms and those with malignant ovarian masses had greater blood levels of hk6, with the former group having a higherlevel than the latter. A CA125 level of 31.51 or above yielded 73% sensitivity, 80% specificity, and 59% positive predictive value; an 88% negative predictive value was the optimal cut off point. Each biomarker's diagnostic sensitivity and accuracy have risen when ca125 and hk6 are combined.

Conclusion: Serum hk6 concentration is an easy-to-use and dependable immunoassay for ovarian cancer patient management. When it comes to ovarian cancer diagnostic confirmation, CA-125 remains the gold standard for confirmation of diagnosis ofovarian cancer. CA125 is clinically approved for following the response to treatment and predicting prognosis after treatment but the addition of hk6 may increase sensitivity and this should be taken into consideration.

Key Words: Diagnosis, human kallikrein 6, ovarian carcinoma.

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INTRODUCTION

Of all the gynecological cancers, ovarian cancer (OC) is the most difficult to treat; 70 percent of cases are discovered at an advanced stage, and only 35 percent of patients survive after five years, with a death rate that has remained consistently high for many years[1]. Ninety percent of ovarian cancers that are malignant start as superficial epithelial tumors. These tumors resemble peritoneal mesothelial cells, but they are different in that they can become malignant due to repeated assaults from growth factors and hormones, such as ovulation[2]. As 70% of women with OC are identified with advanced stage illness, part of the reason for the high fatality-to-case ratio linked to the condition is that there is no discernible pattern of symptoms in the early stages. An early diagnosis (stage II or earlier) of this disease offers an 85% 5-year survival rate is 85%, However, in women presenting with stage III or IV cancer, survival drops to fewer than 20%[3]. It is obvious that the creation of novel techniques for early ovarian cancer detection would probably lead to better patient outcomes.

About 20 years ago, the first well-validated tumor marker for ovarian cancer was found: CA-125. The clinical use of CA-125 for illness monitoring includes its usage as a tool for early relapse diagnosis, response to therapy evaluation, and assessment. Additionally, CA-125 can help diagnose diseases and has some prognostic value[4]. There is optimism that new cancer biomarkers may be found shortly as a result of the sequencing of the human genome. Through the use of whole-genome mining techniques, researchers have discovered several potential indicators for the diagnosis and prognosis of ovarian cancer[5].

The biggest cluster of contiguous protease genes in the human genome, chromosome 19q13.4, colocalizes with 15 structurally identical steroid hormone-regulated genes (kallikreins.KLK), which encode tissue kallikreins (hk). Numerous typical physiological processes, including blood pressure regulation, electrolyte balance, tissue remodeling, prohormone processing, brain plasticity, and skin desquamation, are associated with high expression of hKs in a variety of tissues[6].

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Numerous kallikreins have been found to be promising indicators for diagnosis and/or prognosis for ovarian, breast, and prostate cancers, among other forms of cancer. Additionally, new research indicates that hKs may have a causal role in the development of cancer, namely in the invasion and metastasis of tumors. As a result, they may make appealing therapeutic targets[6].

AIM OF THE STUDY

In order to evaluate human kallikrein 6's potential use as a biomarker for the diagnosis of ovarian cancer, we assessed the blood level of the protein in women with ovarian swellings and correlated it with the conclusive histology.

MATERIALS AND METHODS

Duration of study:

Study was done in Benha university hospitals and Ain shams university hospitals in the duration from April 2024 to October 2024.

This prospective conducted on 97 women.

Two groups of women were included in the study and were classified as follows:

Group 1: ninety-nine women who had ovarian swellings that required laparotomy or laparoscopic surgery after being detected clinically and radiologically

Group 2: Seven women who appeared to be in good health served as controls (women attending to Gynecological outpatient clinic for family planning consultation from).

Each and every Group endured:

A thorough investigation and careful discussion of the past

The following was measured using PCR to determine the serum kallikrein 6 level:

Types of Systems:

Complete splenocyte populations from C57BL6/J or PAR1 defective mice were used to investigate the impact of KLK6 on immune cell survival. Mice lacking in PAR1TM1 /− (B6.129S4-F2rtm1Ajc/J). Adherence to the NIH Guidelines for animal care and safety was strictly followed during all animal research.

Cell Culture

After lysing red blood cells with ammonium chloride buffer and homogenizing spleens in RPMI-1640, splenocytes were grown in tissue culture. 100% air and 5% CO_2 were used to keep all of the cells at 37°C. Within a particular experiment, each culture condition was evaluated in triplicate, and each experiment was conducted at least twice.

Substances:

With the use of an insect/baculovirus system, recombinant KLK1 and KLK6 were produced, purified, and activated. Homo sapiens KLK6 and Rattus norvegicus KLK6 are identified as clear orthologs by phylogenetic analysis. No amino acid insertions or deletions were found in a pairwise analysis of the KLK6 proteins from these species. The length of the mature proteins in each instance is 223 amino acids. There has been a direct comparison between the distinctive preference for arginine over lysine in the substrate P1 position uses the same tripeptide substrates to directly compare the mature KLK6 proteins of Rattus norvegicus and Homo sapiens, and the results show that these species orthologs have a high degree of conservation. This report's main finding is that KLK6 increases the survival of human Jurkat T cells and murine splenocytes. This finding was made in experiments using both the Rattus norvegicus and Homo sapiens forms of KLK6, with the Rattus norvegicus form of KLK6 being used in all tests presented. KLK1 from Homo sapiens recombinant form was used in all KLK1-related investigations.

At doses between 1 and 10 µg/ml (40 and 400 nM), which are equivalent to those at which our earlier research has demonstrated to elicit intracellular signaling, the functional activity of recombinant KLKs was seen.

KLK6-Over Expression:

RNA STAT-60 (Tel-Test, Inc. Friendswood, TX) was used to extract total RNA from G418-selected Jurkat cells transduced with KLK6-CMV or vector alone. 0.5 µg of total RNA was then subjected to RT-PCR using the Light Cycler-RNA Amplification Kit SYBR Green I and an i-Cycler IQ Real-Time PCR apparatus. The expression levels of KLK6 were compared to those of glyceraldehyde phosphate 3-dehydrogenase (GAPDH). The primers for Homo sapiens KLK6 were 5′-TGCCAGGGTGATTCTGGG-3′ and 5′-TGCAGACGTTGGTGTAGACT-3′, respectively, whereas the primers for GAPDH were 5′-ACCACCATGGAGAAGGC-3′ and Reverse 5′-GGCATGGACTGTGGTCATGA-3′. Every case's expression levels were measured in relation to standard curves created by amplifying KLK6 or GAPDH nucleic acid templates.

Flow Cytometry:

Cells were taken out and stained using combinations of antibodies that recognized CD45, CD3, and B220 after experimental incubation times that varied from 4

to 72 hours. The FACSCalibur flow cytometer was used to evaluate the cells using flow cytometry. The common leukocyte marker CD45 was used to identify live immune cells, although PI was not present in them. By labeling cells with carboxyfluorescein succinimidyl ester before plating, possible impacts on cell proliferation were investigated. Then, the FlowJo proliferation platform was used for analysis.

Western Blot Analysis:

Whole splenocyte cell cultures' protein lysates were separated on SDS-polyacrylaminde gels before being transferred to nitrocellulose membranes. Cell Signaling Technology provided the antibodies needed to identify Bcl-XL and Bim. By re-probing blots for β-Actin, equal loading was confirmed. Supersignal chemiluminescence was used in each instance to identify proteins of interest on film. Films and photos were digitized in order to quantify. The percentage that the 89-kDa fragment represented of the total PARP (116+89 kDa fragments) found allowed researchers to calculate the percent PARP cleavage. Every Western blot was performed at least twice with comparable outcomes utilizing different cell culture preparations.

Women with ovarian tumors (Group 1) were subjected to:

Routine laboratory investigations (complete blood picture, coagulation and bleeding profile: bleeding time, coagulation time, prothrombin time, urine analysis, fasting blood sugar, renal function tests, liver function tests, and ECG).

Pelviabdominal ultrasound (abdominal or transvaginal).

Serum level of tumor marker CA-125.

CT and MRI on pelvis and abdomen when relevant.

CA19-9, and Alpha-fetoprotein, carcinoembryonic antigen (CEA) when relevant.

Chest X-ray for malignant cases.

Intravenous pyelogram, barium meal or gastroscopy, barium enema or endoscopy when relevant.

Endometrial biopsy for cases of vaginal bleeding when relevant.

Operative notes: extent of surgical procedure (Quality of debulking either optimal or suboptimal)

Its pathological nature: Benign (neoplastic and non neoplastic) –border line –malignant (stage, grade).

For all patients and controls the aim of the study was well explained and a verbal consent was obtained before collection the blood sample.

All patients had been subjected to exploratory laparotomy and were properly staged according to the International Federation of Gynecology and Obstetrics

After the final pathology of patients included in the study is divided as follows:

1-Group of Benign ovarian mass (N=65)

2-Group of Malignant ovarian mass (n=25)

Statistical Analysis:

The data were coded, entered and processed on an computer using SPSS version 24.

RESULT

There was no statistical significant difference between the studied groups as regards the distribution of age groups, parity and contraceptive methods (P>0.05). Table (1). The most common symptom is abdominal pain (50) (55.56%) then vaginal bleeding (17) (18.89%), and gastrointestinal (GIT) symptom in the form of (constipation, nausea, bloating) 15(16.67%). Table (2) . Diagnostic and operative laparoscopies were done on 15 patients (23.08%) and laparotomy to 50 patients (76.92%) with benign ovarian neoplasm, Table (3). There was statistical significant difference between malignant and benign ovarian tumors in Serum levels of CA125, hk6 in various groups. (P<0.05) The serum level of hk6 increase in groups with benign neoplasm and groups with malignant ovarian mass but higher in malignant than benign groups and there was area of overlapping between malignant group and benign group. Table (4). CA125level of 31.51 or greater provided a sensitivity of 73%, a specificity of 80% and a positive predictive value of 59%, negative predictive value of 88% was the best cut off point.hk6 level of 3.14 or greater provided a sensitivity of 64%, a specificity of 57% and a positive predictive value of 46%, negative predictive value of 73% was the best cut off point. CA 125 is the more reliable predictive test; hk6 had a weak predictive ability. The combination of ca125 and hk6 have increased the diagnostic sensitivity and accuracy of each biomarker Table (5). (Figures 1-3)

Table 1: Comparison between the studied groups as regard age, contraceptive methods and parity:

		Normal healthy control	Malignant ovarian mass	Benign ovarian mass	*P*-value
Age (years)		40.29±7.3	43.64±9.5	36.25±11.2	0.26
parity	Para <4	3(42.9%)	11(44%)	39(60%)	0.67
	Para> 4	4(57.1)	10(40%)	13(20.2%)	
contraceptive methods					
not used		0(0%)	21(84 %)	40(61.5%)	0.89
IUD		1(14.2%)	1(4 %)	10(15.3.9%)	
OCP		3(42.9%)	2(8 %)	9(13.8%)	
Injections		3(42.9%)	1(4 %)	6(9.2%)	

Table 2: Comparison between the studied groups as regard clinical presentation (symptom):

Types / Symptom	Malignant ovarian mass *n*=25	Benign ovarian mass *n*=65	Total *n*=90
vaginal bleeding	6 (24%)	11(16.92%)	17(18.89%)
abdominal pain	10 (40%)	40(61.54%)	50(55.56%)
GIT symptom	5 (20%)	10(15.38%)	15(16.67%)
abdominal Swelling	4(16%)	4(6.15%)	8(8.89%)

Table 3: Comparison between the studied groups as regard operative intervention (procedures):

Types / Operative Procedures	Malignant ovarian mass *n*=25	Benign ovarian mass *n*=65	Total *n*=90
U/S guided biopsy	0(0%)	0(0)	0(0%)
Laparoscopy (Diagnostic, operative)	0(0%)	15(23.08%)	15(16.67%)
Laparotomy.	25(100%)	50(76.92%)	75(83.33%)

Table 4: Serum levels of CA125, hk6 in various groups:

types		CA125	hK6
Normal healthy control	Mean	---	2.39
	±SD	---	1.05
Malignant ovarian mass	Mean	507.69*	5.31*
	±SD	881.54	4.67
Benign	Mean	81.78	3.10
	±SD	384.60	1.41

Table 5: Comparison between Sensitivity, specificity, PPV, NPV and diagnostic accuracy of CA125 alone, its combination with hk6 and hk6 alone:

	Sensitivity	Specificity	PPV	NPV	Diagnostic Accuracy
CA125 + hK6	86.36	42.86	44.19	85.71	57.81
hk6	64.00	56.82	45.71	73.53	59.42
CA125	72.73	80.36	59.26	88.24	78.21

ROC Curve:

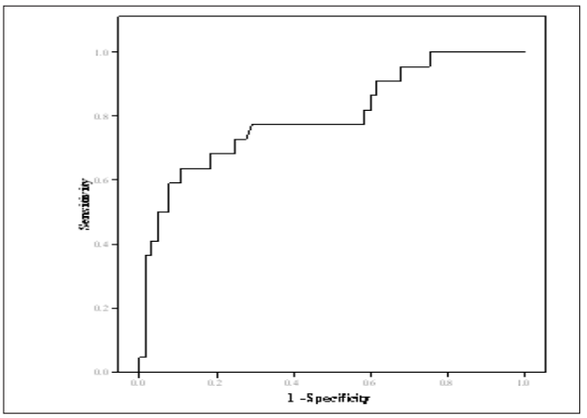

Fig. 1: Receiver Operating Characteristic (ROC) curve for cutoff levels of CA125 (31.55) in the diagnosis of ovarian tumor.

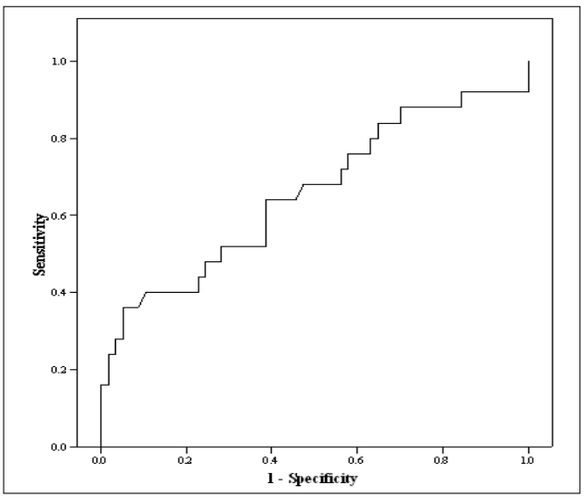

Fig. 2: Receiver Operating Characteristic (ROC) curve for cutoff levels of hK6 (3.14) in the diagnosis of ovarian tumor.

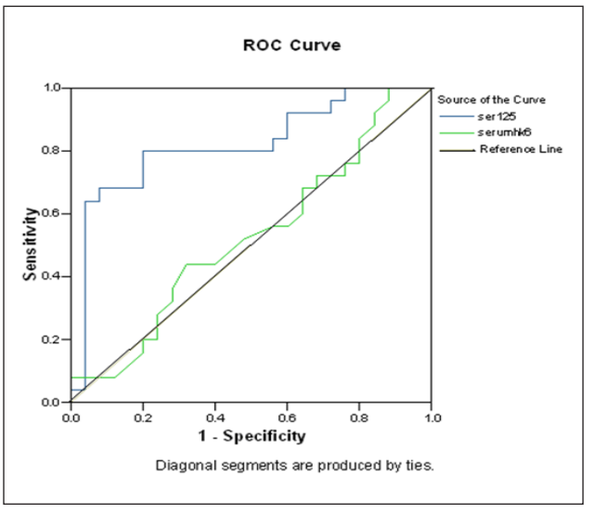

Fig. 3: The combination of ca125 and hk6 have increased the diagnostic sensitivity and accuracy of each biomarker:

DISCUSSION

Given the high mortality rate and heterogeneity of ovarian cancer, better clinical outcomes may result from the identification of novel biomarkers for the illness that may be used for early detection, tracking, and therapy response prediction. The only well recognized biomarker for ovarian cancer is CA-125. Although several additional putative indicators for ovarian cancer have been found, it is unknown what effect they will have in practice[7]. More than half of patients with early-stage ovarian cancer do not have raised CA 125 levels during the period when several benign diseases, such as endometriosis, certain ovarian tumors, hepatic cirrhosis, and peritonitis, may induce increased blood levels of CA 125. Additionally, a subset of women with ovarian epithelial cancers—mostly those with mucinous tumors—never have CA 125 levelsincreased. There is a pressing need for novel markers which are specific and sensitive and can improve diagnosis when used in combination with cA 125[8].

This study shows that, 25 patients were diagnosed as malignant ovarian tumors, 65 patients has benign ovarian tumors. The mean age for patients with malignant tumors was 43.64, which was younger than the age recorded in other studies as Diamandis and colleagues, found that at time of diagnosis of ovarian cancer mean age among the group with malignant ovarian tumors was 56 y (range from 28 to 78)[9]. The mean age recorded in the FIGO report (2006) was 57.6 years. It also younger than the age recorded by Yancik who indicated that the greatest number of ovarian cancers are diagnosed between (50) and (59) years. This can be explained by that either ovarian cancer started to shift to a younger age group or, it may be duo to the smaller life expectancy in our locality so that the majority of ovarian cancer cases are diagnosed at younger age[10].

Regarding CA125 in this study the level of CA125 is 31.55u/ml is the best cut off point. It also differ from upper limit of normal value for tumor maker CA125 reported by another study which was 35u/ml[9]. This marked deference between both cut off point, due to increased number of malignant cases (96), advanced age, advanced stage and grade used by Diamandis and colleagues[9].

For the first study, Diamandis and associates demonstrated that a sizable percentage of patients with ovarian cancer had a substantial rise in blood Hk6 levels. The hypothesis put up was that the rise in hk6 concentration in serum seemed to be mostly unique to ovarian cancer, since it did not increase in any way in other cancers (breast, gastrointestinal, prostate, or lung)[9].

Additionally, Diamandis *et al.*'s study revealed that CA125 is not a reliable marker for early detection of ovarian cancer. Raised levels of CA125 are observed in many

benign gynecological disorders, contributing to its limited specificity and low sensitivity for early illness detection. Now, it's well acknowledged that no Searches for hk6 are conducted in the hopes that one cancer biomarker will provide all the information required for the best possible cancer diagnosis and outlook. A novel ovarian cancer biomarker is serum hk6[11].

Regarding malignant ovarian tumors vs nonmalignant ovarian tumors, another study discovered that hk6 is often overexpressed. Compared to tumors from early stages of illness, those from late stages of disease tended to display this overexpression more frequently[12].

In our study, the mean of hk6 in malignant ovarian mass was 5.31± 4.67 versus the mean of hk6 in benign ovarian mass was 3.1± 1.41.There was a statistical significant difference between benign neoplastic group and malignant ovarian mass group as regards the mean value of hk6 (P<0.05).

In this study hk6 had a weak predictive ability compared to the previous study. This may be due to a smaller number of patients included relative to other studies. In other studies number of patients included as patient group with benign ovarian neoplasm was (141), malignant ovarian tumor was (146) and normal healthy control was (97)[11].

In the current study,the cut off point to hk6 is 3.14 ug/L or greater provided a sensitivity of 64%, a specificity of 57% and diagnostic accuracy 59.42, which is less than cut off value detected by Diamandis *et al* which is 4.4 ug/L (95% diagnostic specificity) or 4.2ug/L (90% diagnostic specificity)[11].

There was no discernible relationship between the CA125 and hk6 concentrations, indicating that these two biomarkers could work in tandem to diagnose and treat ovarian cancer[9]. Some individuals had high hk6 levels while having normal CA125 levels. Therefore, by utilizing the developed combination function f(x) = 3.95 log (hk6) + 1.97 log (CA125), it is possible to improve the diagnostic sensitivity of the biomarkers alone by combining CA125 and Hk6[11].

Therefore, we came to the following conclusion: Future research to determine the clinical use of serum hk6 analysis for the management of patients with ovarian cancer will be facilitated by the present availability of a straightforward and accurate immunoassay for assessing serum hk6 concentration.The gold standard for confirming an ovarian cancer diagnosis is still CA-125.

CONFLICT OF INTERESTS

There are no conflct of interests.

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