

Human Kallikrein 6 (Zyme/Protease M/Neurosin): A New Serum Biomarker of Ovarian Carcinoma

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Background: There is an urgent need for discovery and validation of new serum biomarkers for ovarian carcinoma. Early diagnosis of ovarian cancer with serologic analysis may improve clinical outcomes through administration of effective treatment. Human kallikrein 6 (hK6, encoded by the KLK6 gene) is a serine protease of the kallikrein gene family. Recently, we were able to develop an immunofluorometric procedure for the quantitative measurement of hK6 in biologic fluids, including serum.

Methods: We have used an hK6-specific immunofluorometric assay to quantify hK6 protein in a large number of serum samples from normal individuals, as well as from patients with various malignancies.

Results: We report for the first time, significant increase of serum hK6 concentration in a large proportion of patients with ovarian carcinoma. The elevations of hK6 appear to be relatively specific for ovarian cancer because other malignancies did not cause any increase in the concentration of this biomarker in serum. Serial hK6 measurements appear to correlate with CA125 levels in patients monitored postsurgery.

Conclusions: This is the first report describing significant elevations of hK6 concentration in serum of ovarian cancer patients. These data suggest that hK6 may represent a potential new biomarker for diagnosis and monitoring of ovarian carcinoma. Copyright © 2000 The Canadian Society of Clinical Chemists

KEY WORDS: Human kallikrein 6; zyme; protease M; neurosin; tumor markers; ovarian cancer biomarkers.

Introduction

Until recently, the human kallikrein gene family was thought to consist of only genes: pancreatic/renal kallikrein (KLK1, encoding for hK1 protein), human glandular kallikrein 2 (KLK2, encoding for hK2 protein) and human kallikrein 3 (KLK3, encoding for hK3 protein or prostate-specific antigen [PSA]). The latter two kallikreins, PSA and hK2, are

relatively prostatic-specific and they have already found important applications as biomarkers for the diagnosis and monitoring of prostate cancer (1–6).

New members of the human kallikrein gene family have recently been discovered (1). This gene family now contains at least 14 genes that are all encoding for serine proteases, show significant homology at both the DNA and amino acid level and they are all localized at the chromosomal locus 19q13.3-q13.4, in tandem, without any intervention from other nonkallikrein genes. This area of investigation has recently been reviewed (1).

The KLK6 gene (encoding for human kallikrein 6, hK6) has been cloned independently by three groups of investigators and was previously given the names zyme (7), protease M (8), and neurosin (9). Recently, uniform nomenclature for all newly discovered and the traditional kallikrein genes has been established (10). The KLK6 gene encodes for a trypsin-like serine protease of 244 amino acids in length, of which 16 amino acids constitute the signal peptide and 5 amino acids, the activation peptide. The mature enzyme consists of 223 amino acids. It has been previously predicted that hK6 is a secreted protein (7–9,11). This was recently verified by finding hK6 protein in various biologic fluids, including cerebrospinal fluid, nipple aspirate fluid, breast cyst fluid, male and female serum, seminal plasma, amniotic fluid, and breast cancer cytosols (12). Little *et al.* (7) have demonstrated that this enzyme has amyloidogenic potential in the brain and may play a role in the development and progression of Alzheimer's disease. Others have cloned the same gene by the method of differential display, and found that it is down-regulated in aggressive forms of breast cancer (8). The same gene was cloned by Yamashiro *et al.* from the human colon adenocarcinoma cell line COLO 201 (9). The availability of a highly sensitive hK6 immunoassay made possible the measurement of hK6 in various biologic fluids (12). We here

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TABLE 1
Concentration of Human Kallikrein 6 (hK6) in Serum of Normal Individuals and Patients with Various Malignancies

Patient Group	Number of Samples	hK6, $\mu\text{g/L}$				95 th Percentile	No. of Patients with hK6 > 15 $\mu\text{g/L}$ (%)
		Min	Max	Median			
Normal males	41	3.2	11.4	7.5	11.1	0 (0)	
Normal females	40	3.5	13.7	7.0	10.8	0 (0)	
Breast cancer ^a	24	1.1	11.9	4.3	9.7	0 (0)	
Medullary thyroid carcinoma ^b	29	0	13.9	5.0	11.8	0 (0)	
Testicular cancer ^c	78	2.0	32.2	9.3	14.3	1 (2)	
Gastrointestinal cancer ^d	28	2.6	10.6	5.7	9.6	0 (0)	
Prostate cancer ^e	40	1.0	16.1	4.1	9.5	1 (2)	
Lung cancer	18	2.6	7.4	5.2	6.7	0 (0)	
Ovarian cancer ^f	80	1.0	206	23.0	148	53 (66)	

^aWith serum CA 15.3 levels > 414 KU/L (upper ref. range 5 KU/L).

^bWith calcitonin levels \geq 1,135 ng/L (upper ref. range 100 ng/L).

^cWith hCG levels \geq 69 IU/L (upper ref. range 10 IU/L) or AFP levels \geq 110 $\mu\text{g/L}$ (upper ref. range 10 $\mu\text{g/L}$).

^dWith CA 19.9 levels \geq 629 KU/L (upper ref. range 37 KU/L) and CEA levels \geq 1,000 $\mu\text{g/L}$ (upper ref. range 5 $\mu\text{g/L}$).

^eWith PSA \geq 324 $\mu\text{g/L}$ (upper ref. range 4 $\mu\text{g/L}$).

^fWith CA 125 \geq 372 KU/L (upper ref. range 35 KU/L).

hypothesize that hK6 concentration in serum may be altered during various disease processes, and especially, in cancer. In this paper, we report measurement of hK6 protein in a large number of serum samples obtained from patients with diverse malignancies. Although, in most cancer cases, hK6 concentration was not elevated, we found that hK6 concentration in serum is significantly increased in a large proportion of patients with ovarian cancer. These data suggest that hK6 may constitute a new biomarker for diagnosis and monitoring of ovarian carcinoma.

Materials and methods

IMMUNOFLUOROMETRIC ASSAY FOR hK6

The details of this immunofluorometric assay have been recently described (12). The assay utilizes two hK6-specific polyclonal antibodies, one raised in mouse and the other raised in rabbit. This is a noncompetitive immunofluorometric procedure that incorporates the principles of time-resolved fluorometry for detection. The assay measures hK6 in the range of 0.5 to 200 $\mu\text{g/L}$ with precision < 10%. Serum samples were analyzed without sample pretreatment.

CLINICAL SAMPLES

For this investigation, we used leftover serum samples obtained from patients with various malignancies (Table 1). We have deliberately included patients with relatively high tumor burden (as indicated by tumor marker levels of at least 10-fold higher than the upper limit of normal) to increase the chance of detecting possible hK6 elevations in serum. All serum samples were stored at -20°C

until analysis for a maximum time of 1 yr. Our procedures are in accordance with the Ethical Standards of the Helsinki Declaration of 1975, as revised in 1983.

ANALYSIS OF TUMOR MARKERS

The tumor markers CA125, PSA, CEA, and AFP were analyzed on the Elecsys immunoassay analyzer (Roche Diagnostics, Indianapolis, IN, USA). CA15.3, CA19.9, and hCG were analyzed on the Immuno 1 immunoassay analyzer (Bayer Diagnostics, Tarrytown, NY, USA) and calcitonin was measured with a radioimmunoassay kit from Diasorin, Italy. The upper limit of normal values for the tumor markers were 35 KU/L (CA125), 4 $\mu\text{g/L}$ (PSA), 10 $\mu\text{g/L}$ (AFP), 5 $\mu\text{g/L}$ (CEA), 35 KU/L (CA15.3), 37 KU/L (CA19.9), 10 IU/L (hCG), and 100 ng/L (calcitonin).

Results

A total of 378 serum samples were analyzed with the previously described immunofluorometric assay for hK6 (13). These samples were from either normal individuals (male and female) or from patients with various malignancies. The obtained data are shown in Table 1. While in none of the normal controls and in only two samples from patients with nonovarian malignancies the hK6 concentration was above 15 $\mu\text{g/L}$ (an arbitrary cutoff), the majority of patients with ovarian carcinoma (~66%) had highly elevated hK6 concentrations in their serum (>15 $\mu\text{g/L}$). The distribution of hK6 values in serum of ovarian cancer patients is shown in Figure 1. As shown in Figure 2, the correlation between hK6 concentra-

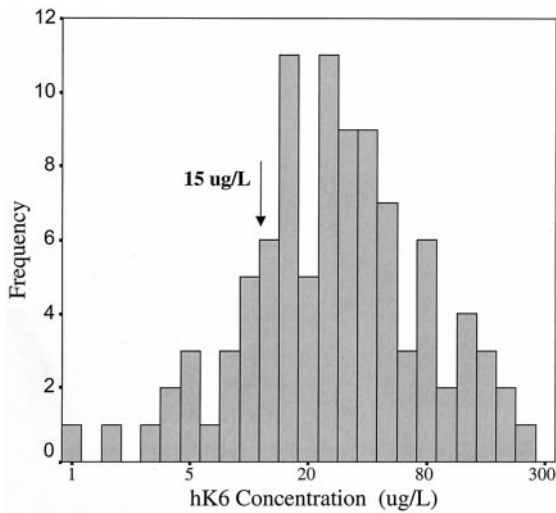


Figure 1 — Frequency distribution of hK6 concentrations in the serum of 80 patients with ovarian carcinoma. The level of 15 $\mu\text{g/L}$ that was used as a cutoff in Table 1 is indicated by an arrow. Approximately 66% of ovarian cancer patients have serum hK6 concentration higher than this cutoff value. From another 298 serum samples with nonovarian cancer, only 2 sera had values slightly higher than 15 $\mu\text{g/L}$ (see Table 1).

tions and CA125 levels is poor and not statistically significant.

In Figure 3, we present data on temporal changes of serial serum hK6 and CA125 concentration in four patients with ovarian cancer. The hK6 concentration changes during the monitoring period, similarly to CA125, suggesting that this new biomarker may have value for patient management.

Discussion

Ovarian cancer is a serious disease that causes more deaths than any other cancer of the female reproductive system (13). Since survival could be dramatically improved if the disease is diagnosed early (14), there is great interest in the identification of biomarkers that could aid in the early detection and facilitate grading and/or staging (15). Unfortunately, the current serologic markers for ovarian carcinoma, including CA125 (16–19), inhibin (20–23), OVX1 (24), as well as many other markers (reviewed in 25) have shown some promise but have not gained wide clinical acceptance. Another potential ovarian cancer marker, lysophosphatidic acid, appears to also have some value for this purpose (26).

Among the classical human kallikreins, PSA has proven to be the most valuable biomarker for prostate cancer and is currently used for diagnosis and monitoring of this disease (2–4). Another potential prostatic biomarker, hK2, has also been recently introduced (5,6). Among the newly discovered kallikreins (1), none of them has been examined as a serologic marker for any malignancy because no methods currently exist to measure the secreted proteins with high sensitivity and specificity. The availability of a highly sensitive and specific assay for hK6 (developed by our group) (12) enabled us to perform this study and examine if hK6 analysis in serum has value for cancer diagnosis and monitoring.

The data of Table 1 summarize our findings and demonstrate that among all cancer types tested (normal males and females vs. breast, thyroid, tes-

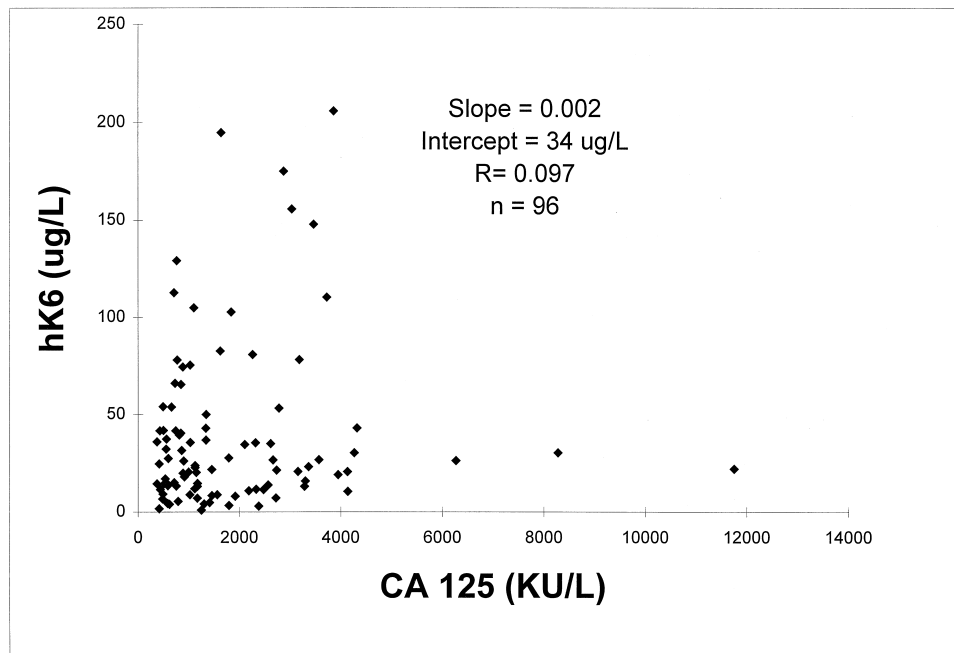


Figure 2 — Correlation between hK6 and CA125 concentration in 96 serum samples from ovarian cancer patients. There is no significant correlation between these two ovarian cancer biomarkers.

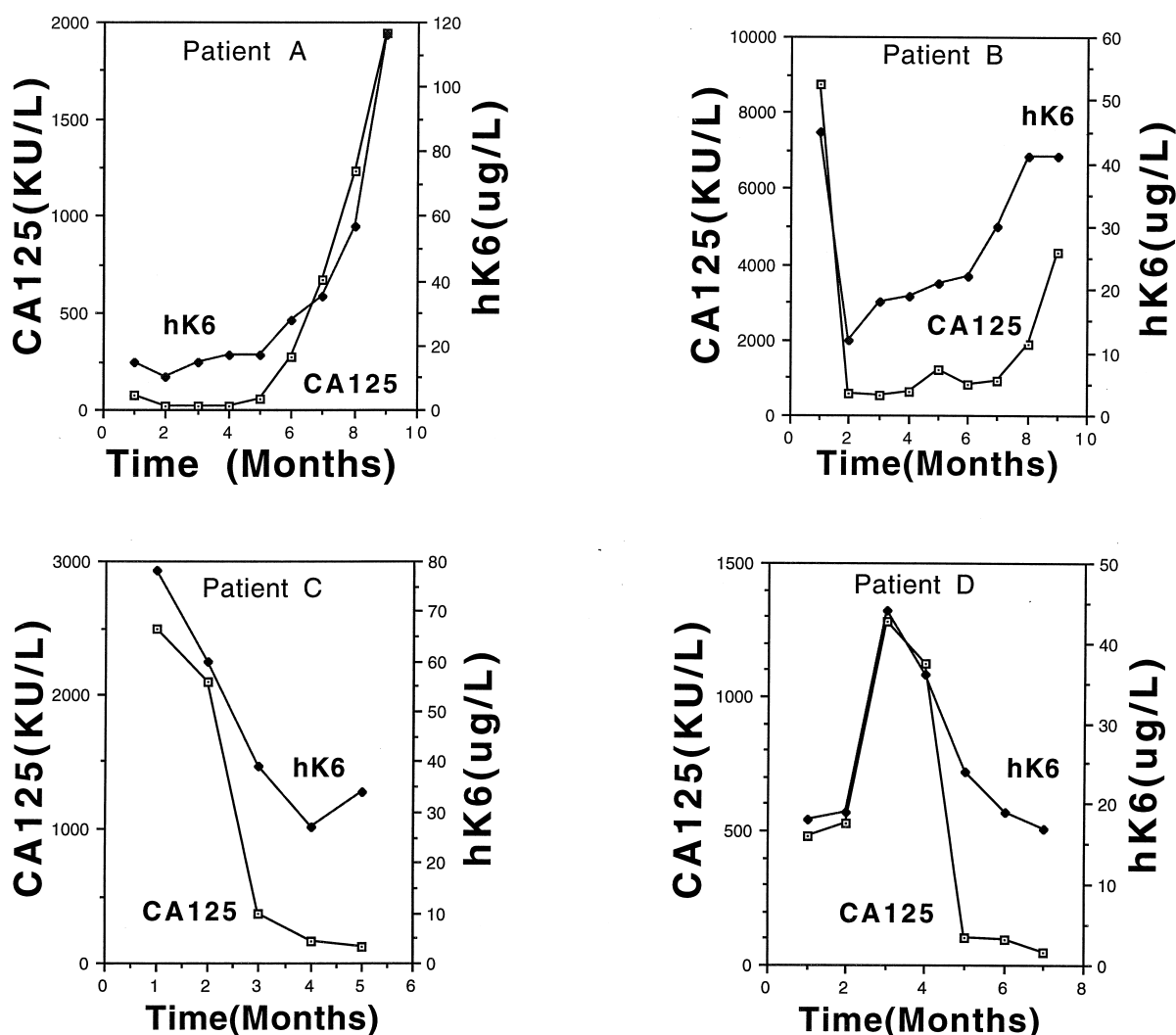


Figure 3 — Analysis of hK6 and CA125 in serial serum samples from patients with ovarian cancer. These data suggest that hK6 may have value for patient monitoring.

ticular, gastrointestinal, prostate, lung, and ovarian cancer), only ovarian cancer patients show significantly elevated levels of this biomarker in the circulation. Approximately 66% of patients had levels higher than 15 $\mu\text{g/L}$, a cutoff that affords 98% to 100% specificity for all other cancers tested. Although these data are highly promising, regarding value of hK6 as a circulating biomarker for ovarian carcinoma, it should be taken into consideration that all patients with ovarian cancer had relatively high levels of CA125 (≥ 372 KU/L, which is $\sim 10\times$ higher than the upper reference range). It will be worthwhile to analyze presurgical serum samples from ovarian cancer patients at various disease stages to examine if hK6 concentration in serum increases early during ovarian cancer development and progression. Furthermore, the analysis of sera from ovarian cancer patients, who do not have any serum CA125 increases, will indicate if this biomarker is complementary to CA125. Our data of Figure 3 indicate that serum levels of hK6 change with time during ovarian cancer monitoring, suggesting that

this biomarker may be useful for monitoring patients after primary treatment.

As is evident from Figure 2, there is no significant correlation between hK6 concentration and CA125, suggesting that these two biomarkers may be complementary for the diagnosis and management of ovarian carcinoma. This possibility merits further investigation.

In conclusion, we here provide the first evidence that serum hK6 concentration is significantly increased in about 66% of ovarian cancer patients. The test seems to be specific for ovarian cancer since no such increases were seen in various other malignancies. We thus propose that hK6 represents a novel serum biomarker for ovarian cancer, which may find applicability for disease diagnosis and monitoring.

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