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Correlation of serum HE4 with tumor size and myometrial invasion in endometrial cancer☆

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ABSTRACT

Objective. To evaluate the utility of serum (HE4) as a marker for high risk disease in patients with endometrial cancer (EC).

Methods. Preoperative serum HE4 levels were measured from a cohort of 75 patients surgically treated for EC. Cases were compared to matched controls without a history of cancer. HE4 levels were analyzed as a function of primary tumor diameter, grade, stage and histological subtype. Wilcoxon rank-sum test, ROC curve, Spearman rank correlation coefficient and contingency tables were used for statistical analyses.

Results. Stage distribution was as follows: 49 stage I, 2 stage II, 20 stage III, 4 stage IV. Type I EC was present in 54 patients, type II in 21. Median HE4 was significantly elevated in both types I and II EC compared to controls ($P<0.001$ and $P=0.019$, respectively). There was significant correlation between type I EC, median HE4, deep myometrial invasion (MI) (>50%, $P<0.001$) and primary tumor diameter (PTD) (>2 cm, $P=0.002$). Low risk patients (type I, MI ≤ 50% and PTD ≤ 2 cm) had significantly lower median HE4 compared to all other type I EC patients ($P<0.01$). In comparison to prior investigations, HE4 (cutoff of 8 mfi) was more sensitive than CA125 in detecting advanced stage disease.

Conclusion. Our data suggest that HE4 is elevated in a high proportion of EC patients, is correlated with PTD and MI, and is more sensitive than CA125 in EC. These observations suggest potential utility of HE4 in the preoperative prediction of high risk disease and the necessity for definitive surgical staging.

Introduction

Endometrial carcinoma (EC) is the most common gynecologic malignancy, accounting for more than half of all gynecologic cancers and 6% of all cancers in women in the United States. In 2010, there were an estimated 43,470 new cases and 7,950 cancer deaths; the latter represents twice the number of estimated EC deaths observed two decades ago, placing it among the ten leading causes of death from malignancy in women in the United States [1,2]. It is expected to become an even greater public health concern as the prevalence of obesity, one of the most common risk factors for EC, increases worldwide [3]. Fortunately, most cases are diagnosed at an early stage by virtue of early presentation of symptoms and surgery alone is often adequate for cure.

At present no serum marker is universally utilized for patients with endometrial cancer. A sensitive serum marker could help monitor response to treatment, facilitate surveillance and may serve as a predictor of extrauterine disease to aid in surgical planning and prognostication in patients with a new diagnosis. Post-treatment surveillance consists of monitoring clinical symptoms and the use of imaging modalities, which often will not detect disease until larger tumor burdens are present [4]. Although CA125 is routinely used in some practices, it has poor sensitivity and specificity [5–8]. Only 10% to 20% of patients with early-stage EC and approximately 25% of patients with asymptomatic recurrent disease will have an elevated CA125 level [9,10]. On the contrary, for patients with ovarian cancer serum CA125 correlates closely with regression or progression of disease [11]. A rising postoperative CA125 level is predictive of tumor relapse with a sensitivity of 84–94% [12,13]. These data emphasize the critical importance of identifying a more reliable biomarker for patients with EC.

Abbreviations: HE4, Human epididymis protein 4; EC, endometrial cancer; FHCRC, Fred Hutchinson Cancer Research Center; mfi, median fluorescence intensity units; AUC, area under the curve; PTD, primary tumor diameter; MI, myometrial invasion.

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HE4 (Human Epididymis Protein 4), also known as WFDC2, was first cloned as one of four cDNAs highly expressed in the human epididymis [14]. It is one of 14 homologous genes on chromosome 20q12-13.1 which encode proteins with a whey-acidic-protein (WAP)-type four disulphide core (WFDC) domain. HE4 cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors [15]. Although its physiological role is yet to be determined, genes at the WFDC locus are variably conserved across species and presumably share a role in natural immunity with both antimicrobial and anti-inflammatory activity [16,17]. Cumulative data indicate that WAP domain family members are implicated in cancer pathogenesis. Expression of elafin and SLPI (Secreted Leukocyte Protease Inhibitor, or anti-leukoproteinase 1), which are the two best studied WAP proteins, have been identified in various carcinomas, suggesting a potential role in cancer development and/or progression [18–22]. Relative to elafin and SLPI, HE4 has been poorly studied and little is known regarding its potential role in carcinogenesis. Galgano et al. found significant HE4 gene expression in some pulmonary, endometrial, breast and ovarian adenocarcinomas, and less often, in gastrointestinal and urological carcinomas [23]; these results are in concordance with other investigations [17,24,25].

Multiple studies have reported upregulation of HE4 gene expression in epithelial ovarian carcinomas [24–32] and, hence, several research groups have explored its potential role as an ovarian cancer biomarker. Drapkin et al. confirmed elevated HE4 protein levels in 100% of endometrioid and 93% of serous ovarian carcinomas [24]. These investigators also demonstrated that HE4 is a secreted glycoprotein that is present in the circulation and other body fluids. Recent publications suggested HE4 to be superior to CA125 as an ovarian cancer biomarker. Moore et al. [33] found that of all the tumor markers in their study, CA125 included, HE4 had the highest sensitivity as a single marker. The combined use of CA125 and HE4 improved sensitivity when compared to either marker alone. These findings have been corroborated by other investigators [27].

Preliminary data has demonstrated overexpression of HE4 in endometrial carcinomas generating interest in HE4 as an EC biomarker [23,34–36]. Congruent with these data, a proteomics study recently performed in our laboratory found HE4 to be significantly upregulated in primary EC tissues [unpublished data]. Nevertheless, only a few research groups have begun investigating HE4's adequacy as a serum marker for EC. Moore et al. concluded that HE4 is elevated in all stages of EC and is more sensitive in early-stage EC compared to CA125 [36,37].

The purpose of this study was to assess the utility of serum HE4 as a marker for preoperative risk stratification in patients with a known diagnosis of EC. HE4 serum concentrations were measured in patients with type I and type II EC and compared to matched controls. HE4 levels were also analyzed among the type I EC cases as a function of primary tumor diameter, grade, surgical stage and histological subtype.

Methods

We conducted a pilot study of 75 patients treated surgically for primary endometrial cancer (cases) between January 1, 2007 and January 13, 2009 in Mayo Clinic. Cases were chosen to include a variety of stages, grades and histologies for HE4 evaluation. Special emphasis was given so that this selection reflected specifically a wide range of stage I patients with varying primary tumor diameter and myometrial invasion. Malignant mixed müllerian tumor (MMMT) is widely recognized as a biologically distinct entity and was therefore excluded from this study. Control blood samples were obtained from women with no known cancer diagnosis enrolled in the Center of Excellence (COE) mammography cohort at Fred Hutchinson Cancer Research Center (FHCRC) and with at least 10 ml serum available. For each case, one control was randomly selected from the COE cohort using an optimal matching algorithm on the Mahalanobis distance after transforming the matching factors (age and serum sample collection date) to have a mean of 0 and a standard deviation of 1. The medical records of the EC cases were abstracted including demographic, histologic and therapeutic parameters.

The tissue, serum and plasma specimens of the EC cases were collected after written informed consent was obtained. This investigation was approved by the Institutional Review Board of Mayo Foundation. In accordance with the Minnesota Statute for Use of Medical Information in Research, only those patients who consented to the use of their medical records were included.

Patients were instructed to fast overnight prior to the venipuncture. Both serum and plasma samples from the cases were collected and processed simultaneously in the pre-operative period, but the timing and duration of processing from collection to freezing was not ascertainable. The patients subsequently underwent surgery consisting of a hysterectomy and bilateral salpingo-oophorectomy at a minimum. Surgical staging during the collection time frame was uniform as demonstrated by intermittent quality assessment reviews and has been described in detail separately [38]. Histologic grade and subtype were confirmed on central pathology review.

Serum samples of both cases and controls were analyzed for HE4 analysis at FHCRC. Serum levels of HE4 were determined using a novel bead-based assay (HE4 BioPlex Assay) developed by Scholler et al. at the FHCRC [39]. This new assay was highly correlated with the originally developed double-determinant ("sandwich") ELISA (Pearson's correlation coefficient, $r = 0.89$), which has been successfully used for the serum detection of HE4 as a diagnostic tumor marker for ovarian cancer, had better reproducibility and used a smaller sample volume [39]. The monoclonal antibodies used in the originally developed ELISA were sold to the Fujirebio Diagnostics Inc. and subsequently commercialized as an FDA approved ELISA test (HE4 EIA, Fujirebio Diagnostics Inc.). HE4 serum levels were measured using the HE4 BioPlex Assay in median fluorescence intensity units (mfi).

Since the distribution of HE4 serum levels was positively skewed, the median was reported as the measure of central tendency. HE4 and CA125 serum levels were each compared between two independent groups using the two-sided Wilcoxon rank-sum test (Mann–Whitney test). Receiver operator characteristic (ROC) curves were constructed and the area under the curve (AUC) was used as an estimate of the ability of HE4 to discriminate between the EC cases and controls. The correlation between HE4 levels and primary tumor diameter (PTD), percent myometrial invasion (MI) and age was assessed using a non-parametric correlation coefficient, the Spearman rank correlation coefficient, as the distribution of HE4 was highly skewed. Using multiple cutoff points, contingency tables were constructed and the specificity of HE4 in detecting $MI > 50\%$ (versus $MI \leq 50\%$), $PTD > 2$ cm (versus ≤ 2 cm) and non-stage I disease (versus stage I) was calculated. Multivariate linear regression analysis was used to investigate the relationship of age and stage with the logarithm of serum HE4. A level of $P < 0.05$ was accepted as statistically significant for all statistical comparisons. Statistical analyses were performed using the SAS software package (version 9.2; SAS Institute, Inc.; Cary, NC).

Results

A total of 75 EC patients were selected to reflect a spectrum of stages, grades and histologies for HE4 evaluation. After matching cases and controls 1:1, the selected cases were on average 5 months younger and with blood samples collected on average 5 days earlier than their matched controls. The characteristics of the EC cases are summarized in Table 1.

The median HE4 serum levels were significantly elevated among all EC cases relative to their controls (median (interquartile range), 6 (4, 27.5) vs. 4 (4, 5) mfi, respectively; $P < 0.001$). The area under