

DIAGNOSTIC AND PROGNOSTIC PERFORMANCE OF HUMAN EPIDIDYMIS SECRETORY PROTEIN- 4 (HE4) IN CANCER TREATMENT: A REVIEW

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ABSTRACT

HE4 is one of several WAP proteins that are localized on human chromosome. HE4 is expressed in a number of tissues like glandular epithelium of the breast, female genital tract, epididymis, vas deferens, distal renal tubules, respiratory epithelium, colonic mucosa and salivary glands less often in gastric tissue and pancreas. HE4 has emerged as a valuable biomarker for distinguishing malignant from benign masses, prognostication, monitoring and screening of various cancers. It serves as a useful prognostic biomarker for ovarian and endometrial cancer. HE4 demonstrated comparable diagnostic performances to CA125 as a tumor marker for detecting ovarian cancer and proved as more sensitive and specific in detecting early stages of ovarian cancer. HE4 improves the utility of CA125 as a tumor marker in ovarian cancer, and using both markers simultaneously increases the tumor marker sensitivity. The use of this combination has enabled the detection of ovarian cancer as compared with use of either marker

alone for the discrimination of benign from malignant ovarian lesions.

HE4 has also emerged as a serum biomarker for lung cancer, pulmonary adenocarcinoma, chronic kidney disease, renal failure, and kidney fibrosis.

KEYWORDS: Human Epididymis- 4 protein, ovarian cancer, endometrial cancer, CA-125, ROMA.

INTRODUCTION

Human Epididymis- 4 protein (HE4) was discovered by Kirchhoff et al in 1991.^[1] HE4 first identified in males in the distal epithelium of the epididymis. HE4 is one of several WAP proteins (Whey acidic protein) that are localized on human chromosome 20q12–13.1. It functions as a protease inhibitor essential for sperm maturation.^[2] HE4 messenger RNA (mRNA) was localized to the distal regions of the epithelial cells of the epididymal duct, indicating a possible role for HE4 in sperm maturation^[1]. It has been found in other healthy epithelial tissues such as the respiratory tract and female reproductive organs, including the ovaries and uterus, but its function is not fully understood. It is normally secreted only in very low concentrations by healthy ovaries.^[3] HE4 was initially identified in epithelial cells of human epididymis WFDC2 gene and referred to as an epididymis-specific, fertility-related protein. HE4 is an 11 kDa protein belongs to a “four-disulfide core” family. It is made up of two whey acidic protein domains and a 4 disulfide core containing, eight cysteine.^[4] However, HE-4 is a 124 amino acid long polypeptide with two WFDC domains. HE-4 is secreted in the human seminal fluid as a disulfide-bonded homo-trimer and is a cross-class protease inhibitor inhibits some of the serine, aspartyl and cysteine proteases tested using hemoglobin as a substrate.^[5] HE4 is N-glycosylated and highly stable on a wide range of pH and temperature. HE4 gene generates five mRNA variants as a result of alternative splicing and utilization of alternative promoters. Amino acid sequence and domain analysis of the deduced peptides predicts that the HE4 *N*-terminal and *C*-terminal WAP domains are encoded by exon 2 and exon 5, respectively. The structural divergence, differential locations, and markedly different expression patterns suggest that they may be implicated in tissue-specific functions.^[6]

Tissue expression

HE4 is expressed in a number of normal and malignant tissues. Normal glandular epithelium of the breast, female genital tract, epididymis, vas deferens, distal renal tubules, respiratory epithelium, colonic mucosa and salivary glands, less often in gastric tissue and pancreas.^[7]

Among normal tissues, the highest levels of HE4 were found in the trachea and salivary gland. Lower expression was found in lung, prostate, pituitary gland, thyroid and kidney.

Positive HE4 immunoreactivity was prominent and consistent in ovarian cancer, while some positivity was observed in other cancers, including mesothelioma, lung, endometrial, breast, gastrointestinal, renal and transitional cell carcinomas. Among the different tumor sites, the highest expression levels were found in ovarian cancer; moderate levels in lung adenocarcinoma and lower levels in breast, transitional cell, gastric and pancreatic carcinomas. Among malignant conditions, the highest levels of HE4 have been noted in ovarian cancer for women and in lung cancer for men.^[7]

Schummer *et al.* found that using the cDNA microarray technique *HE4* is over expressed in ovarian cancer.^[8] Bingle *et al.* used Northern blot hybridization in order to characterize its tissue-specific expression and found that HE4 mRNA is abundant in lung, kidney and the salivary gland.^[6] Le Bleu *et al.*,^[9] identified HE4 as a significantly and the most frequently—upregulated gene in fibrosis associated myofibroblasts in patients with kidney fibrosis. From the cancer genome atlas (TCGA) database, compared the expression of HE4 across various cancer types utilizing the RNA-seq data. It is clearly found that the overexpression of HE4 in ovarian and uterine cancer. Lower expression is also noted for cervical cancer, lung adenocarcinoma and kidney chromophobe renal cell cancer. Sporadic extreme elevation was noted in head and neck cancers and little or no expression was noted for all other cancers.

Physiological variation of HE4

HE4 levels seem not to be affected by time of sampling, fasting, exercise, or seasonal factors urban *et al.*,^[10] identified two main determinants of HE4 levels in healthy individuals includes, smoking and particularly in younger subjects, active smoking is related to a clear increase in HE4 levels. The mechanism behind elevated HE4 levels in smokers is somewhat unclear. As HE4 is also expressed in airway epithelium, inflammation might contribute to the elevated serum levels of HE4 in active smokers, but this remains speculative until further studies pinpoint the association between smoking and HE4. The second determinant is associated with age found to be increased in the elderly subjects. HE4 increases with increasing age. Richard G. Moore, *et al.*,^[11] showed that significant difference in serum HE4 concentrations in by age, with a significant rise that starts at age 60 years. When premenopausal women compared with postmenopausal women, there was a significantly higher serum HE4 level in the postmenopausal patient population. HE4 levels are also

affected by pregnancy: pregnant women have significantly lowered levels of HE4 in comparison with age-matched non pregnant premenopausal women. Menstrual cycle, endometriosis and estrogen and progestin contraceptive usage do not alter serum levels of HE4.^[12]

HE4 Biomarker Utility

HE4 has emerged as a valuable biomarker for both ovarian and endometrial in 2003 cancer.^[15] HE4 has been evaluated for distinguishing malignant from benign masses, prognostication, monitoring and screening.

HE4 as a tumor marker

Ovarian cancer is heterogeneous disease, fifth most common causes of cancer-related deaths of among women worldwide.^[13] Epithelial ovarian cancer set by the World Health Organization (WHO) recognizes eight histological tumor subtypes: serous, mucinous, endometrioid, clear cell, transitional cell, squamous cell, mixed epithelial and undifferentiated.^[14] Within each subtype, tumors are further described as benign, malignant, or borderline, and depending upon tumor subtype; classified as low or high-grade. Borderline tumors are considered to have low malignant potential and/or indolent behavior. Early stage ovarian cancer has an excellent prognosis if treated, but 70% of patients are diagnosed in advanced stage which is associated with a poor survival rate of only 10–30%. There is a close correlation between stage at presentation and survival; the success of treatment for early stage disease, limitations of treatment for advanced ovarian cancer a screening test is intuitively appealing. However, the low prevalence of ovarian cancer limits the achievable sensitivity and specificity of any single screening test. Therefore the search of new biomarker in combination with existing or which can replace the old is highly recommended. Serum CA125 which is more commonly used tumor marker found to be elevated in many primary tumors, such as ovarian, endometrial, colorectal, breast and lung cancers. It is also elevated in a number of other conditions, including pregnancy, inflammation, endometriosis, fibroids, benign ovarian cysts, cirrhosis and abdominal surgery. It can be detected in the blood, pleural effusion, ascites and amniotic fluid.^[13] So as a tumor marker, it has low sensitivity and specificity for early diagnosis of endometrial carcinoma. In addition, there is a group of women with epithelial ovarian cancers, mostly those with mucinous tumors, in whom CA125 levels are never increased. This explains the tremendous amount of effort that has been expended over the past few decades to find new ovarian cancer biomarkers which are

sensitive and specific and can improve diagnosis that could be used together with, or instead of, CA125. There is a pressing need for novel markers which are sensitive and specific and can improve diagnosis when used in combination with CA125 or can replace it. HE4 is a promising biomarker either independent or in combination with CA125 substantially improves the accuracy of screening and/or disease monitoring.

Role in ovarian cancer and endometrial cancer

HE4 promotes migration and adhesion of ovarian cancer cells. Human epididymis protein 4 might inhibit cell proliferation by regulating the mitogen-activated protein kinase and phosphoinositide 3-kinase/AKT signal transduction pathways *in vitro*.^[16]

HE4 is found in high levels in the serum of women with serous epithelial ovarian cancer. Serum levels are less affected by menstruation, ovulation and other benign ovarian conditions (e.g. endometriosis) compared with CA125. In premenopausal women, HE4 is the more sensitive and specific marker of ovarian malignancy, including early stage ovarian cancer. In postmenopausal women, the very non-specificity of CA125 can be helpful in determining whether an ovarian mass is malignant or not, as the incidence of secondary malignancy to the ovary in this group of women is more common and the occurrence of minor rises due to benign ovarian conditions is less likely. CA125 however, can be quite elevated in conditions such as pulmonary embolus; cirrhosis; peritoneal dialysis; and pleural, pericardial and peritoneal effusions.

In a study by Huhtinen, K. *et al.*, serum CA 125 and HE4 concentrations of healthy patients were compared with patients with endometriosis (Endo) and epithelial ovarian cancer (EOC), HE4 level is highly elevated in epithelial ovarian cancer, HE4 shows high differentiation between ovarian cancer vs, endometriosis and healthy controls.^[17]

In a study by Moore, R.G. *et al.*,^[18] and Montagnana *et al.*,^[19] levels of HE4 were measured in benign diseases prior to surgery for pelvic mass and found HE4 not elevated in benign diseases, including ovarian cysts and endometriosis, higher the value poorer outcomes suggesting HE4 plays an important role in ovarian cancer.

In a study evaluating multiple biomarkers for ovarian cancer, the combination of CA125 and HE4 was superior compared with any other marker alone or 2 markers in combination. Abdel-Azeez *et al.*,^[20] measured CA125, HE4 and mesothelin in serum from patients with

pelvic mass, including with ovarian cancer and with benign disease. As a single marker, HE4 had the highest sensitivity for detecting ovarian carcinoma (82.9%) including early disease (76.9%) and a combination of CA125 with HE4 was a better predictor of ovarian malignancy than either alone.

CA 125 and HE4 can be combined in a mathematical algorithm to better assess the risk of epithelial ovarian cancer in women with pelvic mass.

Risk assessment with the ROMA (Risk of Ovarian Malignancy Algorithm) score

A risk of malignancy algorithm (ROMA) that combines the diagnostic power of the CA 125–HE4 marker panel with menopausal status has been approved by the FDA for distinguishing malignant from benign pelvic masses.^[21] In a multicenter prospective study that included a total of 531 patients, preoperative levels of CA 125 and HE4 were measured and separate logistic regression algorithms were utilized for premenopausal and postmenopausal women. The ROMA algorithm combines CA 125 and HE4 values along with the menopausal status into a predictive index, which in turn is used to calculate the predicted probability of ovarian cancer (from 0 to 100%). The regression formulae are as follows, where LN is the natural logarithm.

ROMA (Risk of Ovarian Malignancy Algorithm).^[22]

- A quantitative serum test that combines HE4, CA 125 and menopausal status into a numerical score.
- To assess whether a woman who presents with an ovarian adnexal mass is at high or low likelihood of finding malignancy on surgery.

- **Calculation**

□ A cut off of ≥ 1.31 and ≥ 2.77 were used for pre- and postmenopausal women with an ovarian adnexal mass, respectively, to provide a specificity level of 75%.

ROMA classifies patients as being at a low or at a high risk for malignant disease using the following algorithms.

Premenopausal: predictive index (PI) = $-12.0 + (2.38 \times \text{LN}(\text{HE4})) + (0.0626 \times \text{LN}(\text{CA125}))$.

Postmenopausal: PI = $-8.09 + (1.04 \times \text{LN}(\text{HE4})) + [0.732 \times \text{LN}(\text{CA125})]$.

Predicted probability: (PP) = $100 \times \exp(\text{PI}) / (1 + \exp(\text{PI}))$.

According to the manufacturer's insert, the following thresholds were selected for ROMA.

- Pre-menopausal women

* PP >12.5%=high risk of finding EOC.

* PP <12.5%=low risk of finding EOC.

➤ Post-menopausal women

* PP >14.4%=high risk of finding EOC.

* PP <14.4%=low risk of finding EOC.

The ROMA algorithm performed better in the premenopausal population than in the postmenopausal cohort. In the premenopausal group, the algorithm had a sensitivity of 92.3% and a specificity of 75.0%; in the postmenopausal group, the sensitivity and specificity were 76.5% and 74.8%, respectively. Predicted probability (PP) values greater than 13.1% indicated high risk in premenopausal population, and PP values higher than 27.7% indicated high risk in the postmenopausal population. Using this algorithm, 93.8% of epithelial ovarian cancers were correctly classified as high risk, prompting referral for treatment by a gynecologic oncologist.^[23]

Benefits of ROMA

1. Compared to testing CA 125 alone, ROMA has higher specificity for ovarian cancer detection. There was a significant difference between benign and malignant disease with respect to serum CA125, HE4 and ROMA levels. This means. Among 10 women with benign gynecologic diseases, ROMA dismisses 1 more patient which CA125 alone would include as having ovarian cancer. With regard to benign tumours, it was interesting to see that the fibromas/thecomas group and the endometriomas had the highest levels of CA125, whereas for HE4, the endometriomas had the lowest level. As already mentioned by Huhtinen et al.^[17] measuring both CA125 and HE4 together could be of particular interest in differentiating endometriosis from ovarian cancer, as ovarian cancer will cause a raised CA125 and HE4, whereas endometriosis will only cause a raised CA125.
2. ROMA accurately identifies 94% (45/48) of the patients with pelvic mass that have ovarian cancer. Use of HE4 and CA 125 with ROMA has a high sensitivity for the prediction of epithelial ovarian cancer in women with pelvic mass.
3. Compared to testing CA 125 alone, ROMA has higher sensitivity for ovarian cancer detection Among 10 women with ovarian cancer, this means-ROMA identifies 1 more patient than CA 125 alone.
4. Compared to testing CA 125 alone, ROMA increases sensitivity by 10% in the detection of stages I/II ovarian cancer.

5. The study concluded that ROMA increases differentiation of ovarian cancer from other pelvic masses than CA 125 alone, even in early stage ovarian cancer. ROMA might be valuable as a first line biomarker for selecting high risk patients for referral to a tertiary center and further diagnosis.^[24]

HE4 for Screening, Prognostication, Recurrence and Monitoring

HE4 may serve as a useful prognostic biomarker for ovarian and endometrial cancer. Elevated levels of HE4 are associated with increases in International Federation of Gynecology and Obstetrics (FIGO) stage, grade, preoperative CA 125 levels and residual tumor.^[25] Elevated HE4 levels also correlate with the aggressiveness of ovarian cancers, poor prognosis, and overall survival.^[26,27] Recent results from the multicenter European project “OVCAD” showed that higher HE4 levels corresponded with poor surgical outcome with respect to residual tumor mass and platinum resistance. Advanced age and stage, lymph node involvement, presence of ascites, and suboptimal cytoreduction correlated with elevated levels of ROMA, HE4 and CA 125.^[28] Elevated HE4 and higher ROMA score were independent predictors of poor prognosis, defined in terms of shorter overall survival, progression-free survival and disease-free survival. In comparing CA 125 and HE4, HE4 was better at identifying patients with optimal cytoreduction.^[29] Higher HE4 levels also correlated with primary tumor diameter and increased myometrial invasion, supporting the biomarker’s utility in predicting a higher risk of metastatic disease preoperatively.^[30]

HE4 levels may aid in monitoring response to therapy. Serum levels of HE4 obtained at the time of diagnosis of ovarian cancer differed significantly from levels following complete clinical remission (324.1 pM vs 23.3 pM,) indicating a possible role in monitoring response to therapy.^[31] It is found that HE4 is a good indicator for the remission from the disease was reported by a follow up study by Allard et al. in which it was shown that the values of HE4 correlated with the clinical response to treatment or remission from the disease, as documented by CT imaging.^[32] In a recent prospective controlled study, it is found that HE4 was able to detect recurrent ovarian cancer with 74% sensitivity and 100% specificity at a cutoff of 70 pmol/L. Using a combination of HE4 and CA 125 increased overall sensitivity to 76%. A combination of CA 125 and HE4 may offer better lead times and sensitivity for the detection of recurrent ovarian cancer.^[33]

HE4 in Lung cancer

Lung cancer is the leading cause of cancer related death in men and women. Despite modern diagnostic, staging and therapeutic advances, the 5-year survival rate of all cases diagnosed with lung cancer does not exceed 15%.^[34] The main histological types of lung cancer are non-small-cell lung carcinoma (NSCLC) and small-cell carcinoma (SCLC). To improve the survival rate of NSCLC, it is critical to detect lung cancer at an early stage using sensitive diagnostic and prognostic biomarkers. Surgical resection is the most effective treatment in patients with lung cancer, 5-year survival rate following surgical resection has only been improved in patients with early stages of disease. Thus, research efforts have focused on early detection and intervention at an earlier stage to decrease the high mortality, which implies the significance of diagnostic methods in lung cancer. To date, circulating tumor markers for lung cancer have become a major focus. Lines of evidence demonstrated that serum carcinoembryonic antigen (CEA), neuron specific enolase (NSE), cytokeratin fragment (CYFRA21-1), tissue polypeptide specific antigen (TPS) and progastrin-releasing peptide (ProGRP) were believed as potential markers to diagnosis of lung cancer. However, due to low sensitivity and specificity, the clinical values of them are limited. Therefore, early and accurate diagnostic tool for lung cancer is especially important for lung cancer management. Several primary studies based on small numbers of patients have recently suggested that elevated serum HE4 levels are associated with poor prognosis of pulmonary adenocarcinoma.^[35]

Hertlein et al.,^[36] reported significantly higher HE4 values for lung cancer and also showed significantly higher values for HE4 in lung cancer than benign diseases both in men and women. Values were reported by Yamashita^[37] and Iwahor X.Y. et al.,^[38] also found same. Serum HE4 levels were elevated in (90.0%) non-small cell lung cancer patients, (88.9%) small cell lung cancer patients. High levels of serum HE4 (>15ng/ml) after chemotherapy were significantly correlated with worse overall survival after the treatment. Liu W et al.,^[39] found that Serum HE4 may be used as a potential marker to differentiate lung cancer from PTB and healthy controls. In addition, higher levels of HE4 predict poor prognosis in (non-small-cell lung cancer) NSCLC patients. In the lung cancer subgroups, patients with adenocarcinoma had the highest serum HE4 levels, followed by patients with SCLC and SCC.

HE4 may be a candidate as a “leading-marker” for the discrimination of lung cancer because of its high sensitivity. These findings suggest that serum HE4 is a potential diagnostic and prognostic marker for lung cancer patients.

However, the role and molecular mechanisms of HE4 in the progression of lung cancer remain largely unknown and require further investigation.

HE4 in Breast cancer

As recently reported, HE4 is also expressed in ductal carcinoma of the breast; however, the function of this protein in breast cancer remains unclear. Galgano et al.,^[7] reported the positive expression of HE4 in breast cancer with a clear immunohistochemical staining of HE4 protein in the cytoplasm of breast cancer. Furthermore, HE4 expression was not only found at the protein level but also at the mRNA level in breast cancer cells by RT-PCR. These results may lead to the speculation that a high expression of HE4 has a critical role in tumor progression; however, the prognostic significance of HE4 remains unclear. Fisher B, Bauer M, Wickerham DL, et al.,^[40] found that approximately 25-30% of breast cancer patients with negative lymph nodes has developed distant metastases within ten years of surgery. There is a need to find an improved marker to stratify breast cancer patients into different risk groups more accurately than can be achieved with current clinico pathological factors; therefore, low-risk favourable patients can be spared unnecessary treatment, avoiding side-effects and reducing the cost of treatment. HE4 expression can be associated with lymph node metastases and disease-free survival in breast cancer. Mirei Kamei, et al.,^[41] in their study, hypothesised that the alternative expression of HE4 is associated with breast carcinogenesis or tumour progression. These findings suggest that HE4 is a possible predictive marker of lymph node metastasis and has a critical role in its recurrence.

HE4 and Renal Fibrosis

Renal fibrosis is considered the end-stage for chronic kidney diseases of various origins, resulting in kidney failure. Fibrosis is thought to result from wound healing processes that, either due to the inability to remove the antigen/irritation or resolve inflammation, fail to terminate. In the initial priming phase, injury to the tubular cells within the kidney results in the formation of local inflammation. Activation of the innate immune system via toll-like receptors (TLRs) results in the production of pro-inflammatory cytokines [interleukin-1 β (IL-1 β), IL-6, tumour necrosis factor alpha (TNF- α)], as well as growth factors (bone morphogenetic protein 7, connective tissue growth factor, epidermal growth factor, platelet-

derived growth factor B, vascular endothelial growth factor), which contribute to the recruitment of inflammatory cells: T cells, monocytes/macrophages, fibrocytes. These cells synthesize and release pro-inflammatory and fibrotic cytokines, such as TNF- α , transforming growth factor-beta, chemokine (C-C motif) ligand 2 (CCL2)/monocyte chemoattractant protein-1, chemokine (C-C motif) ligand 5 (CCL5)/regulated on activation, normal T cell expressed and secreted (RANTES) and plasminogen activator inhibitor-1 (PAI-1), angiotensin II (Ang II). This leads inflammation - tissue damage- fibrosis.^[42] Le Bleu *et al.*,^[9] identified a number of genes including matrix proteins (Bgn, biglycan; Des, desmin; Dcn, decorin), serine proteases (Prss 23 and Prss 35) and protease inhibitors (SerpinF1 and Serpina10), all of which are known to be involved in fibrosis. HE4 is most up regulated gene in fibrosis-associated myofibroblasts. The HE4 gene encodes for a putative serine protease inhibitor that is up regulated in human and mouse fibrotic kidneys and is elevated in the serum of patients with kidney fibrosis. HE4 suppresses the activity of multiple proteases, including serine proteases and matrix metalloproteinase and specifically inhibits their capacity to degrade type I collagen. Collectively these studies suggest that HE4 is a potential biomarker of renal fibrosis and a new therapeutic target.

HE4 as a biomarker and its future use

Monitor for recurrence and disease progression in patients with epithelial ovarian cancer. HE4 correlated better with the PET/CT results as compared to CA 125. HE4 increased 5-8 month before CA 125 in relapsed ovarian cancer. A change in HE4 concentration of ≥ 25 percent is considered significant. An increase of this magnitude suggests recurrence or disease progression, while a decrease of this magnitude suggests therapeutic response. Elevated levels were found in several tumour cell lines including ovarian, lung and colon and breast cancer and can be used as biomarker for such condition.

Reference Values

Females: $< \text{or } = 140 \text{ pmol/L}$.

Males: Not applicable.

Limitations

Certain histological types of ovarian cancer (e.g., mucinous or germ-cell tumors) rarely express HE4. Therefore, HE4 is not recommended for monitoring of patients with known mucinous or germ-cell ovarian cancer. Elevated concentrations of HE4 may be present in individuals with non-malignant disease. Therefore, concentrations of HE4 cannot be used as

absolute evidence for the presence or absence of malignant disease and the HE4 test should not be used in cancer screening or diagnosis. There are limited data on the diagnostic test performance of the human epididymis protein 4 (HE4) test used to diagnose ovarian cancer or to monitor disease progression and recurrence in women after initial treatment for epithelial ovarian cancer. There is no established cut-off for determining when an HE4 test is positive, when used for identifying disease progression or recurrence. Moreover, a survival advantage of early detection of ovarian cancer recurrence using HE4 levels or other biomarkers has not been established. No published studies were identified evaluating use of the HE4 test to screen asymptomatic women for ovarian cancer.

CONCLUSION

HE4 has emerged as one of the most promising markers for improving the sensitivity and specificity. The use of HE4 might enable to improve detection of ovarian cancer as compared with use of either marker alone for the discrimination of benign from malignant ovarian lesions. HE4 demonstrated comparable diagnostic performances to CA125 as a tumor marker for detecting ovarian cancer with more sensitive in detecting early stages of ovarian cancer and more specific. HE4 also has potential role as a serum biomarker for lung cancer, pulmonary adenocarcinoma, chronic kidney disease, renal failure and kidney fibrosis.

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