The Use of Breast Magnetic Resonance Imaging Parameters to Identify Possible Signaling Pathways of a Serum Biomarker, HE4

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Objectives: This study aimed to investigate the relationship between breast magnetic resonance imaging (MRI) parameters; clinical features such as age, tumor diameter, N, T, and TNM stages; and serum human epididymis protein 4 (HE4) levels in patients with breast carcinoma and use this as a means of estimating possible signaling pathways of the biomarker, HE4.

Methods: Thirty-seven patients with breast cancer were evaluated by breast MRI and serum HE4 levels before therapy. Correlations between parameters including age, tumor diameter T and N, dynamic curve type, enhancement ratio (ER), slope washin (S-WI), time to peak (TTP), slope washout (S-WO), and the serum level of HE4 were investigated statistically. Human epididymis protein 4 levels of early and advanced stage of disease were also compared statistically.

Results: Breast MRI parameters showed correlation to serum HE4 levels and correlations were statistically significant. Of these MRI parameters, S-WI had higher correlation coefficient than the others. Human epididymis protein 4 levels were not statistically different in early and advanced stage of disease.

Conclusions: High correlation with MRI parameters related to neoangiogenesis may indicate signaling pathway of HE4.

Key Words: breast cancer, HE4, magnetic resonance imaging

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The kinetics of contrast materials on breast magnetic resonance imaging (MRI) are reported to mirror the vascular landscapes of tumors. Malignant lesions display fast and intense enhancement with a clear washout pattern. The initial phase of contrast enhancement is termed the *washin* phase. The maximum enhancement rate and slope during the washin phase have been linked with the microvascular density of the lesions.[1,2] Pathogenesis is thought to depend on increased vascularity and capillary permeability. In contrast to malignant lesions, those that are benign demonstrate a slow increase in enhancement.[3–6] The washout pattern of malignant lesions, on the other hand, is correlated with the interstitial structure of the tumor.[7] All these important kinetic features can be ascertained from time–signal intensity curves of the lesions.

Kuhl et al[4] divided the time–signal intensity curves of breast lesions into the following 3 types: persistently increasing, plateau, and washout enhancement. The last 2 types are considered to be suspicious. A positive predictive value for the washout pattern has been reported to be 87%.[4] In addition, certain quantitative measures have been introduced such as enhancement rate, time to peak (TTP), and slope washin (S-WI).[3,5,8,9] Szabó et al,[3] in their article on the correlation between dynamic MR features and prognostic factors in invasive breast cancer, found the enhancement ratio (ER) and the initial slope (in other words, S-WI) to be positively correlated with tumor size. Buadu et al[10] reported that the steepest slope of contrast medium uptake correlated with microvascular counts. High histologic grade, positive c-erbB-2 status and negative ER status were also associated with short TTP, and washout ratios showed a positive correlation with histologic grade and tumor size. The washout curve pattern was positively correlated with Ki-67 status.[3]

There are several well-known prognostic factors for breast cancer, including lymph node status, tumor size, histologic type and grade, the expression of oestrogen and progesterone receptors, oncoprotein c-erbB-2, and p53 tumor suppressor gene product, Ki-67.[11–15] Some authors consider microvascular density to be a prognostic factor. Angiogenesis is a crucial element in carcinogenesis which influences tumor growth and invasion.[16] Vascular endothelial growth factor (VEGF) is the most important proangiogenic molecule, acting on tumor growth and the development of metastases.[17–19] For breast tumors, MR contrast enhancement is said to be primarily based on VEGF expression.[20]

One of the most promising new biomarkers is human epididymis protein 4 (HE4). Human epididymis protein 4 gene expression is highest in normal human trachea and salivary glands, but also active in the lungs, prostate, pituitary gland, thyroid, and kidneys. Up-regulation of HE4 gene expression has been reported in ovarian cancer, allowing this biomarker to be used in diagnosis.[21–33] In addition, pulmonary, endometrial, breast, gastrointestinal, and urological cancers may lead to gene up-regulation and immunoreactivity.[33]

There is an increasing trend for estimation of prognostic biomarkers by radiological imaging. To our knowledge, there have been no previous studies on the relationship between breast MRI findings and serum HE4 levels; moreover, no studies have been conducted to estimate the possible pathways of a biomarker by means of the interpretation of breast MRI findings. The aim of our study was to identify the relation between certain breast MRI parameters and serum HE levels in newly diagnosed breast carcinoma patients, and through this, ascertain a possible signaling pathway for this poorly understood biomarker. The secondary aim was to correlate clinical features (N, T stages) with serum HE4 levels in patients with breast cancer and investigate whether HE4 levels differ for early and advanced stages of the disease.

PATIENTS AND METHODS

Patients

The study protocol was approved by the institutional ethics committee. Patients with newly diagnosed breast carcinoma were

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enrolled. Breast MR images and serum samples were obtained pretreatment. Patients underwent surgery on average 42 days after imaging (14–58 days). Serum samples were obtained when the patient was hospitalized just before surgery or chemotherapy. All the patients were diagnosed by a core biopsy before the surgery and histopathologic examination of mastectomy or lumpectomy specimens confirmed the diagnosis of invasive breast carcinoma. All patients gave informed consent to participate in the study. There were no patients with renal failure. Histopathological and multimodal evaluation (sonography, positron emission tomography) findings staging the disease were recorded.

Breast MRIs

Breast MRIs were performed using a 3-T (Magnetom, Skyra; Siemens, Germany) scanner with dedicated breast coils in prone position. A standard protocol was used: precontrast sagittal fat saturated turbo spin echo (TSE) T2-weighted imaging (T2-WI), coronal short τ inversion recovery (STIR), transverse TSE T1-WI, transverse diffusion-WI using single-shot echo-planar imaging, transverse dynamic precontrast and postcontrast fat-saturated fast low-angle shot (FLASH) 3-dimensional were obtained. For all the patients, gadolinium chelate was injected intravenously at a dose of 0.1 mmol/kg followed by a 20-mL saline flush. The injection rate was 2 mL/s and a power injector was used. Dynamic imaging was started after a fixed delay of 30 seconds following contrast material injection. Each sequence lasted approximately 65 seconds. A total of 5 series were acquired.

Interpretations

An experienced radiologist, with more than 5 (I.D.S.) years of experience reading breast MRI, evaluated the data set on standard image interpretation workstation (Syngo.via, Siemens Healthcare, Forchheim, Germany) using breast MRI application software. She was blinded to the serum HE4 levels. After loading patient data, primary breast carcinoma lesion enhancement was characterized by assessing the enhancement kinetic curves. Signal intensities were obtained precontrast and for each postcontrast series using operator-defined regions of interest (ROIs) by using a color-coded spectrum. During evaluation, the mostly color-coded areas in washin map were chosen. Measurements were performed in at least 3 areas of the tumor that showed high contrast uptake. Of these measurements, the maximally enhancing ROI and those displaying a more suspicious curve pattern were selected for analysis. The smallest possible pixel size was used for the ROIs excluding necrotic areas of the lesion.

Three enhancement patterns could be identified on the basis of the time–signal intensity curve. Progressive enhancement was defined as a continuous increase in signal intensity. A plateau pattern was delineated with an initial increase in signal intensity and followed by a flattening of the enhancement curve. The washout enhancement pattern involved an initial increase and subsequent decrease in signal intensity.[4] The enhancement kinetic curves of the primary carcinoma were assessed using 5% as a cutoff value (signal intensity percentage change >5% was considered persistent, a change between −5% and 5% was considered plateau, and a change less than −5% was considered washout). In addition to the dynamic curve pattern of the lesions, parameters of ER, TTP, and S-WI were calculated.

The ER was calculated as follows: (the peak contrast-enhanced signal intensity − unenhanced signal intensity)/unenhanced signal intensity × 100. Calculations were carried out automatically by the breast MRI application software for each drawn ROI. Time to peak was determined by the time to reach maximum signal intensity after contrast administration. It was also calculated by the breast MRI application software automatically. Slope washin was the rate of ER to TTP.

Serum Samples and Enzyme-Linked Immunosorbent Assay

Blood samples were taken just before the surgery or chemotherapy. Peripheral blood was collected in the morning before a meal. Samples were then aliquoted and stored at 80°C until the analysis date. Serum concentrations of HE4 were analyzed by ELISA according to the manufacturer's instructions (Fujirebio Diagnostics, Göteburg, Sweden). The limit of detection for HE4 using the measurement kit was 2.5 pmol/L.

Statistical Analysis

Correlations between age, dynamic curve type, ER, S-WI, TTP, T and N stage (obtained from MRI and histopathological examination), and the serum level of HE4 were investigated by Pearson correlation tests. On the basis of TNM stage (obtained from multimodal evaluation and/or histopathological examination), patients were divided into 2 groups: Early stage (stage IIB and earlier) and advanced stage (stage IIIA and later). Human epididymis protein 4 levels for these 2 groups were compared by independent samples *t* test. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Thirty-seven female patients were prospectively enrolled in our study (Table 1). The clinical indications for breast MRI were to assess extent of disease, and contralateral breast examination. Primary lesion diameter, histopathological results T, N, and TNM stages of the patients can be seen in Table 1. There were 25 patients with early and 12 patients with advanced stage of disease.

TABLE 1. Age, Primary Lesion Diameter, Histology, T, N, and TNM Stage Features of the Patients

	Mean/Number	SD/%
Age (32–81 y)	50.8	10.9
Primary lesion diameter (15–100 mm)	32.14	16.07
Histology		
Invasive ductal	33	89.2
Mixed invasive ductal-mucinous	1	2.7
Mixed invasive ductal-lobular	3	8.1
T stage		
T1	5	13.5
T2	23	62.2
T3	4	10.8
T4	5	13.5
N stage		
N0	7	18.9
N1	19	51.4
N2	6	16.2
N3	5	13.5
TNM stage		
2A	12	32.4
2B	13	35.1
3A	5	13.5
3B	1	2.7
3C	3	8.1
4	3	8.1

On dynamic MRI, there were 2 (5.4%) lesions with the progressive pattern, 16 (43.2%) with the plateau curve, and 19 (51.4%) tumors had washout curves. Mean ER was 252 ± 81 SD (75–442). The mean TTP was 176 seconds (±52 SD, 97–263 seconds). Mean S-WI ± SD was 1.54 ± 0.6 (0.35–3.02). Mean serum HE4 level ± SD was 69.7 ± 28.3 pmol/L (19–125).

The Pearson correlation test showed weak correlations of HE4 with age, tumor diameter N, and T (r values were, respectively, 0.151, 0.059, 0.271, and 0.047). Time–intensity curve showed a moderate correlation ($r = 0.484$). However, this correlation was statistically significant ($P = 0.002$). ER ($r = 0.597$, $P = 0.000$), S-WI ($r = 0.832$, $P = 0.000$), and TTP ($r = -0.485$, $P = 0.002$) showed also statistically significant correlations (Figs. 1–3).

Differences of HE4 levels for early and advanced stages of disease were not statistically significant ($P = 0.656$). The mean HE4 levels and standard deviations were 68.2 ± 27.4 pmol/L for early stages and 72.8 ± 31.1 pmol/L for advanced stages.

DISCUSSION

Our study showed that serum HE4 levels were significantly correlated with breast MRI parameters. Correlation with clinical parameters such as age, tumor diameter, and N and T stages were not statistically significant. Mean HE4 level for advanced stage of the disease was higher but not statistically significant.

Human epididymis protein 4 was first identified in the epithelium of the distal epididymis.[34] It is now considered to be a biomarker for the early screening, differential diagnosis, monitoring, and progression of ovarian carcinoma,[23,25,35–39] as epithelial ovarian cancer is thought to overexpress HE4 and benign processes do not cause elevated serum levels.[40] However, the influence of HE4 on ovarian cancer has not been characterized in detail.[41]

In fact, HE4 is not tumor specific and its immunoreactivity can be seen in other carcinomas.[42–45] Human epididymis protein 4 may have a role in the NFκB signaling pathway as its promoter includes this binding motif.[45–47] This pathway includes down-regulation of apoptosis, up-regulation of cell proliferation, and angiogenesis.[48–50] In our study, we tried to investigate the relationship of serum HE4 levels with MRI parameters that are thought to be associated with tumor angiogenesis. Our results demonstrated a strong correlation of the biomarker with MR parameters. These results may indicate that HE4 may have roles acting related to angiogenesis.

A few authors have reported results on the relationship of HE4 in breast cancer.[33,41] Kamei et al[41] investigated the potential of HE4 to predict disease-free survival for patients with breast cancer. In their study, immunohistochemical analysis and reverse transcription polymerase chain reaction were used to determine the expression of HE4. These were compared with the clinicopathological factors or prognosis. They concluded that lymph node involvement was closely associated with HE4 expression. In addition, HE4 expression was reported to be a possible predictive factor of breast cancer recurrence. However in our study, the nodal status of the patients was weakly correlated with serum HE4 levels.

Breast MR with dynamic contrast enhancement provides many parameters that can allow more accurate and specific interpretation of tumors.[4,42] Angiogenesis of invasive breast tumors has been shown to be one of the main factors that affects MR contrast agent uptake, as it is dependent on microvascular density and endothelial permeability.[3] Microvascular density has been correlated with decreased disease-free period and survival in breast cancer.[43–46]

Angiogenesis is essential for tumor progression, invasion, and spread and is known to be essential in the development of breast carcinoma.[47] The mechanism of initiation of angiogenesis in tumor cells is not well characterized. However, proangiogenic and antiangiogenic factors are well described.[48–50] Among these, VEGF is the most important marker leading to endothelial cell

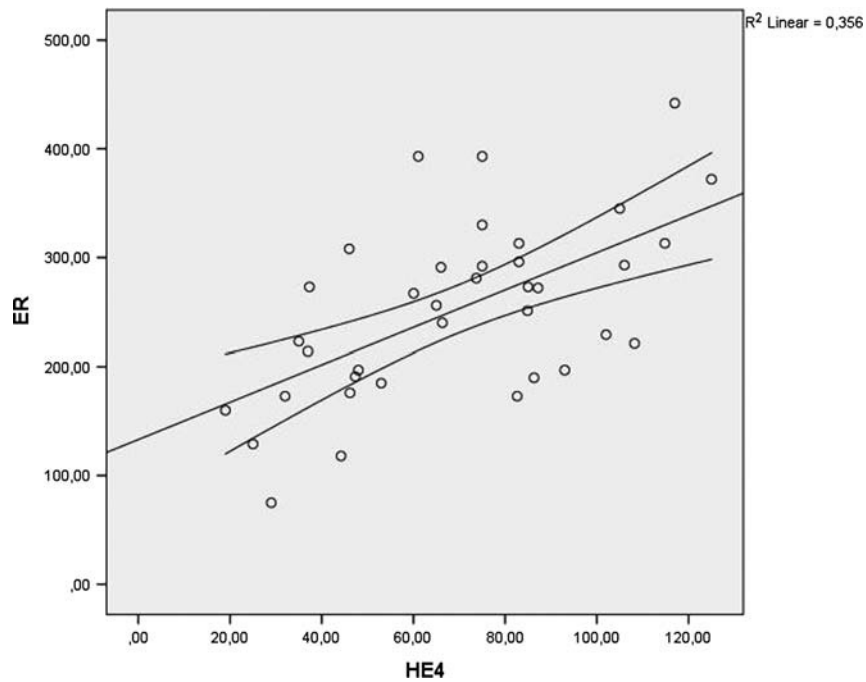

FIGURE 1. Correlation of ER values to serum level of HE4.

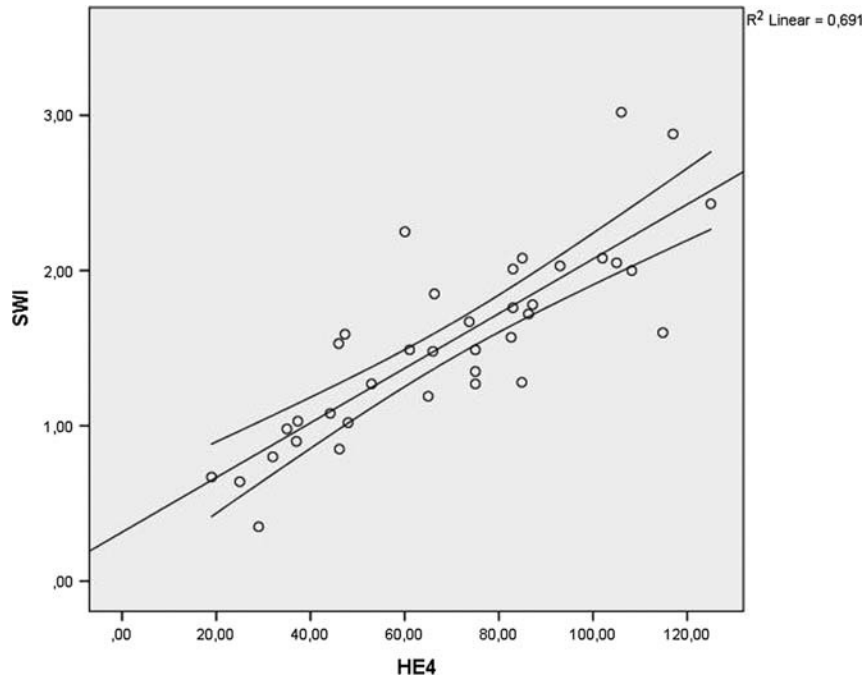

FIGURE 2. Correlation of S-WI values to serum level of HE4.

proliferation and vascular permeability. Higher expression of VEGF has also been proven to be associated with shorter disease-free intervals and reduced survival in patients with breast cancer.[47] In addition, Knopp et al[20] showed that breast MR contrast enhancement is based mainly on VEGF expression.

This study has some limitations. First of all, there is no control group in terms of serum HE4 level. Data from literature indicate normal serum level of HE4 as 50 to 70 pmol/L. Some of our patients had elevated serum levels. In advanced stages, although our results showed a higher mean serum level, some of which were in normal limits also. Therefore, wider studies are required. In addition, one radiologist had evaluated the images. This was another limitation of our study.

In conclusion, our results indicate that HE4 may act as an angiogenesis factor in breast carcinoma. Although high serum levels of HE4 could be observed, larger studies are required to investigate this molecule further especially for breast carcinoma.

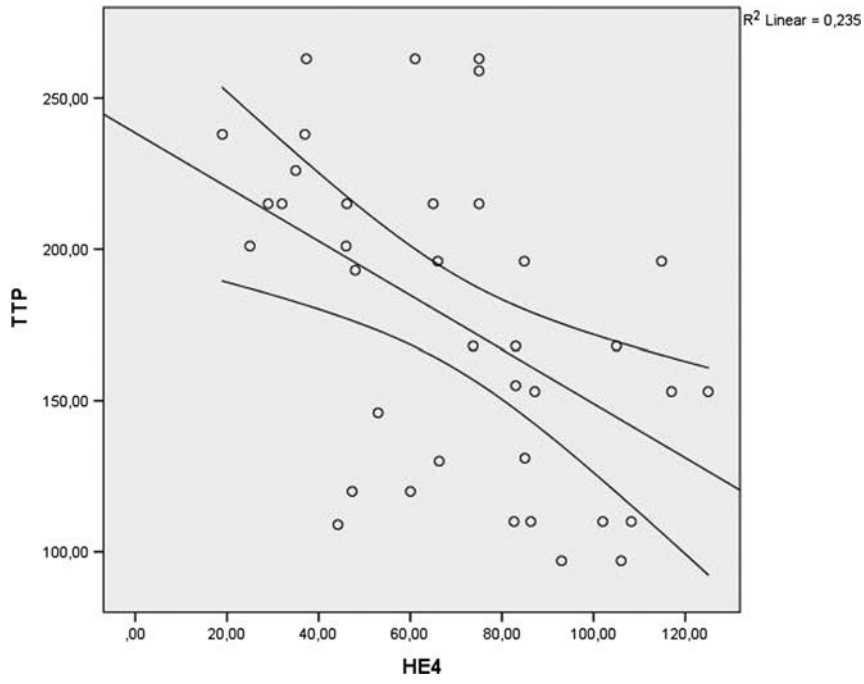

FIGURE 3. Correlation of TTP values to serum level of HE4.

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