Serum Endoglin and Hepatocyte Growth Factor; Correlation with the clinical presentation of breast cancer patients

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Key words:Breast Cancer, Clinical Presentation, CD 105, HGF.

Submitted: 23 October 2010 - Accepted: 30 December 2010

ISSN: 2070-254X

Abstract

Objective: To estimate the relationship between serum levels of endoglin (CD 105) and hepatocyte growth factor (HGF) and the clinical presentation of breast cancer patients which would correlate with tumor prognosis.
Methods: The current prospective study included 37 newly diagnosed female patients with breast cancer and 23 healthy women as a control group. All patients were carefully assessed by history taking, complete physical examination, and staging work up. Blood samples were collected from all candidates for measurements of serum levels of endoglin and HGF.
Results: The median age of the patients was 43 years (range 31–75 years) and about 65% of them (24 patients) were postmenopausal. Fourteen patients had negative estrogen and progesterone receptors. Staging work up revealed that 20 patients (54%) had T1, T2 disease, regional lymph nodes were involved in 34 patients (91.9%) and 22 patients (59.5%) had distant metastasis. Serum endoglin level was higher in breast cancer patients than that of controls (5.33 ± 2.13 Vs 3.99 ± 1.22 ng/ml) and the difference was statistically significant (p=0.045). However, the endoglin level did not differ significantly according to the menopausal status, primary tumor size, regional lymph nodes involvement or the presence of distant metastases. The significant difference was only noticed according to hormone receptor status. Serum HGF in breast cancer patients (2449 ± 1129 pg/ml) was higher than controls (2270 ± 760 pg/ml), but the difference was not statistically significant (p=0.57). The HGF level differed significantly according to the primary tumor size (T1,T2 Vs T3,T4) and the presence of metastatic spread (M0 Vs M1).
Conclusion: Serum endoglin correlated with breast cancer more than HGF and both reflected the severity of progression as well as the metastatic potential. Moreover, endoglin has the potential to be an ideal target for antiangiogenic therapy and a useful marker of early detection of breast cancer or tumor relapse.

Introduction

Angiogenesis refers to the formation of new blood vessels from preexisting vessels or circulating endothelial progenitor cells. It is a multistep process that promotes metastasis involving the spread from the primary tumor through blood and lymphatic vessels and subsequent growth of the secondary tumor at the anchored distant organs **(1,2).** Blocking tumor angiogenesis has been proven to be an important strategy for cancer therapy, and has been approved for clinical use **(3,4).**

Endoglin (CD105) has proven to be a useful marker of angiogenesis. CD105 serves as a receptor for transforming growth factor (TGF)-β 1 and TGF-β3, and modulates TGF-β signaling through its interaction with TGF-β receptor I and TGF-β receptor II (TGF- RII) **(5).** The TGF-β /ALK signaling pathway is activated by TGF-β 1, TGF-β RII, and CD105 leading to promotion of the proliferation of the endothelium **(6).** CD105 is highly expressed on proliferating endothelial cells of both the peritumoral and intratumoral blood vessels that participate in tumor angiogenesis, and is either weakly or negatively expressed in the vascular endothelium of normal tissues **(7,8).** CD105 expression on breast cancer cells enhances its invasiveness and in breast cancer patients, higher expression of tissue endoglin is associated with poorer treatment outcome **(9,11).**

Patients with metastatic disease had significantly elevated CD105 levels in comparison with metastasis-negative individuals and healthy controls. Chemotherapy significantly reduced the levels of serum CD105. Therefore, the circulating levels of CD105 can detect patients with more advanced disease, predict those at risk of metastasis, and alter in response to chemotherapy. CD105 levels might also help in measuring the response of an individual to treatment, especially to antiangiogenic modalities and in follow-up to monitor for disease recurrence **(12,13)**.

The hepatocyte growth factor (HGF) and its receptor (cMET) play an important role in the growth and spread of cancer making them a potential targets for the development of cancer-modifying therapy. The high serum HGF and its clinical application had been described in many cancers e.g. multiple myeloma, non-Hodgkin's lymphoma, liver and gastric cancers **(14,15).** The prognostic significance of HGF in breast cancer patients was questioned in some studies **(16,17).**

The aim of the current study was to estimate the relationship between serum levels of endoglin (CD 105) and HGF and the clinical presentation of breast cancer patients which would correlates with tumor prognosis.

Patients and Methods

The current prospective study included 37 newly diagnosed female patients with breast cancer referred to Kasr El-Aini Center of Radiation Oncology and Nuclear Medicine (NEMROCK) and 23 healthy women as a control group during the period between March 2009 and July 2010. Ethical Committee approval and informed oral consent was obtained from all included subjects.

All patients were assessed by **1-** Careful history taking. **2-** Complete physical examination including local examination. **3-** Laboratory investigations; complete blood picture, kidney function tests, liver function tests, CA15-3, and measurement of serum levels of endoglin and HGF. 4- Radiological assessment; bilateral mammography & breast ultrasonography, baseline chest radiograph, and abdominal ultrasound. 5- Baseline isotopic bone scanning.

Sample collection:

Five milliliter blood samples were collected from all candidates. Samples were collected on plain tubes and then centrifuged at 1000 rpm for 10 minutes. Serum was divided into aliquots and frozen at -70 °C till assay time. Routine investigations were done, in addition to serum HGF and endoglin that were quantified with an enzyme- linked immunosorbant assay kits (R&D Systems, Inc., 614 McKinley Place NE, Minneapolis, MN 55413, United States of America). The assay of CA 15-3 was done on Immulite 2000 by kit purchased from Siemens (Siemens Healthcare Diagnostic Products Ltd. LIanberis, Gwynedd LL55 4EL, United Kingdom).

Statistical analysis:

Quantitative data are presented as mean and standard deviation and comparison between two groups was done using t-test. For non-parametric data, median and 25^{th}-75^{th} percentile was used. Qualitative data were expressed as frequency and percentage and comparison was done by Chi square test. Quantitative data were compared using pearson correlation coefficient (r). The optimal cutoff for endoglin and HGF in serum and the area under the curve were calculated by constructing a receiver operating characteristic (ROC) curve. P value less than 0.05 was considered statistically significant. Calculations of the data were done on SPSS (SPSS incorporation, Chicago, Illinois).

Results

The median age of the patients was 43 years (range 31–75 years) and about 65% of them (24 patients) were postmenopausal. The majority of patients (n=35) had tumors having histopathology of infiltrating duct carcinoma (IDC) with moderate and well differentiation in 31 patients (83.8%). Fourteen patients (37.8%) had negative estrogen and progesterone receptors. Staging work up revealed that 20 patients (54%) had T1, T2 disease, regional lymph nodes were involved in 34 patients (91.9%) and 22 patients (59.5%) had distant metastasis. **L**ungs were the commonest site affected by the metastatic spread (10 patients) followed by bones (9 patients) and liver (5 patients). The median level of CA 15-3 in breast cancer patients was 51.5 IU/ml (25^{th}-75^{th} percentile: 16.5-287) **(Table 1).**

Table 1: Patient and tumor characteristics

Parameter	Result
Age at diagnosis	
Median	43 years
Range	31-75 years
Family history	
Positive	6 (16.2)
Negative	31 (83.8)
Menopause	
Yes	24 (64.9)
No	13 (35.1)
Grade	
Low	2 (5.4)
Intermediate	29 (78.4)
High	6 (16.2)
ER and PR status	
ER +ve	23 (62.2)
PR +ve	20 (54)
Both negative	14 (37.8)
T (primary tumor)	
T1	3 (8.1)
T2	17 (45.9)
T3	9 (24.4)
T4	8 (21.6)
N (regional lymph nodes)	
Not involved	3 (8.1)
Involved	34 (91.9)
M (distant metastasis)	
0	15 (40.5)
1	22 (59.5)
CA 15-3: IU/ml	
Median (25th-75th percentile)	51.5 (16.5-287)

ER: estrogen receptor, PR: progesterone receptor

Serum endoglin level was higher in breast cancer patients than that of controls (5.33 ± 2.13 Vs 3.99 ± 1.22 ng/ml) and the difference was statistically significant (p=0.045). However, the endoglin level did not differ significantly according to the menopausal status, primary tumor size, regional lymph nodes involvement or presence of distant metastases. The significant difference was only noticed according to hormone receptor status. Serum HGF in breast cancer patients (2449 ± 1129 pg/ml) was higher than controls (2270 ± 760 pg/ml), but the difference was not statistically significant (p=0.57). The HGF level differed significantly according to the primary tumor size (T1,T2 Vs T3,T4) and the presence of metastatic spread (M0 Vs M1) **(Table 2)**.

Table 2: Mean Level of Serum Endoglin and HGF According to Patient and Tumor Characteristics

Characteristics	No. of cases	Endoglin, ng/ml		HGF, pg/ml	
		Mean (SD)	P value	Mean (SD)	P value
Age at diagnosis					
≤ 35 years	4	5.5 (2.2)	0.9	2620 (1732.4)	0.83
> 35 years	33	5.3 (2.2)		2438.4 (1121.6)	
Family history					
Negative	30	5.2 (2)	0.41	2443.6 (1202.4)	0.93
Positive	7	6.3 (2.7)		2473.7 (751.9)	
Menopausal status					
Premenopausal	14	5.3 (1.4)	0.8	2719.8 (1129.2)	0.46
Postmenopausal	23	5.4 (2.4)		2413.5 (1287.4)	
CA 15.3 level					
Normal	6	4.5 (1.6)	0.27	1833.1 (929.3)	0.13
Elevated	31	5.5 (2.1)		2645.5 (1236.8)	
ER and PR status					
Both negative	14	4.4 (1.7)	0.037	2397.3 (858.4)	0.83
At least one positive	23	5.9 (2.2)		2475.6 (1164)	
T (primary tumor)					
T1,2	20	5.5 (2.2)	0.7	2113.0 (1009.7)	0.04
T3,4	17	5.2 (2.1)		2896.4 (1155.2)	
N (regional L.N)					
Negative	4	5.8 (1.5)	0.72	1860.5 (1111.5)	0.35
Positive	33	5.3 (2.2)		2503.9 (1110)	
M(distant metastasis)					
Negative	15	4.8 (1.4)	0.27	1940 (789.2)	0.007
Positive	22	5.7 (2.5)		2955.3 (1312.7)	

On constructing ROC curve for diagnosis, serum endoglin showed the highest area under the curve (57%) with P=0.045, followed by HGF (40%) and CA 15-3 (27%) (**Figure1**). The cutoff point of endoglin was 5.31 ng/ml which yielded a specificity of 100% and a sensitivity of 56% in diagnosing breast cancer. However, the HGF showed the greatest area under the curve on constructing ROC curve for prognosis (77%) with P=0.02 and cutoff of 1838 pg/ml to discriminate between localized and metastatic tumor (sensitivity 86% and specificity (53%), but serum endoglin showed the smallest area (56%) with CA 15-3 in between (73%) (**Figure2**). Serum endoglin correlated positively with HGF and this correlation was statistically significant (r=0.44, P=0.002) (**Figure3**). Also, there was a significant positive correlation between HGF and CA 15-3 (r=0.351, P=0.023).

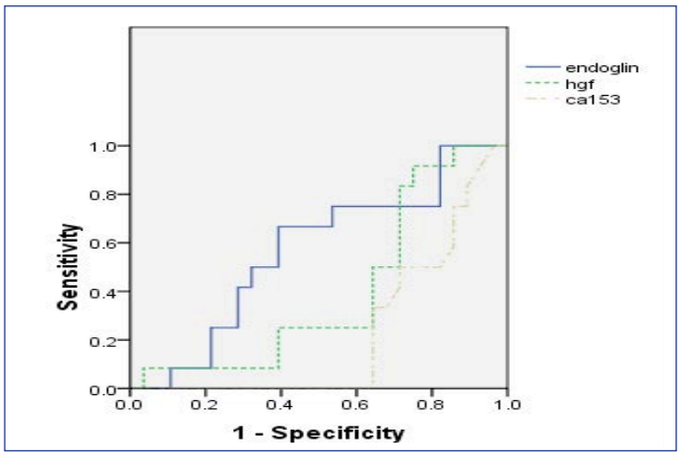

Fig. 1: Multiple ROC curves of the three serum markers to discriminate between patients and controls.

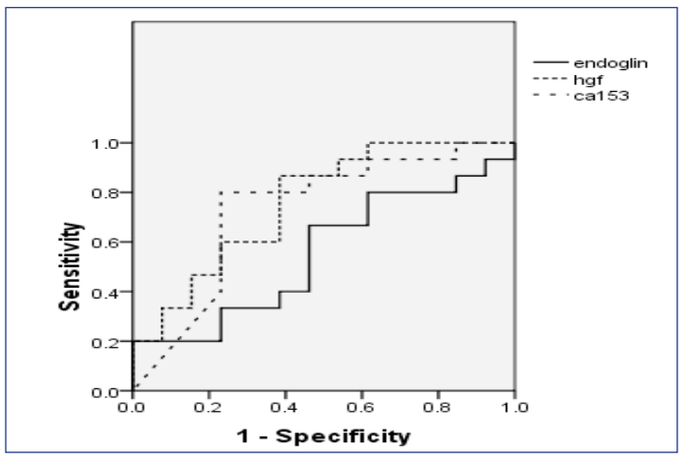

Fig. 2: Multiple ROC curve to discriminate between localized versus metastatic cancer.

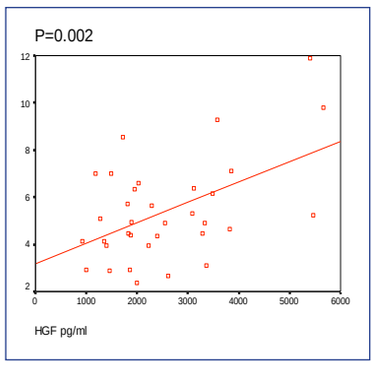

Fig. 3: Scatter diagram showing the correlation between serum endoglin and HGF.

Discussion

The current study was designed to investigate the significance of endoglin and HGF in the clinical presentation of breast cancer by examining blood samples from both breast cancer patients and healthy individuals. Endoglin is found in the circulation, but no clear information exists regarding the prognostic value of plasma levels of endoglin in cancer patients. Previous investigations using histochemical staining showed that endoglin is over expressed in blood vessels at sites of neovascularisation **(18,19)**. These findings provide circumstantial evidence that endoglin may be involved in tumor angiogenesis and hence tumor progression. Raised levels of endoglin were found in plasma samples from breast cancer patients, but its source remains unknown. Based on available information that CD105 is strongly expressed in blood vessels and in cancer tissues, it is reasonable to propose that increase of CD105 in the circulation of patients with metastatic disease resulted from angiogenesis both within and in the immediate vicinity of tumor mass **(7,20)**.

The mechanism responsible for the augmented expression of CD105 in endothelial cells of tumor tissues has not been elucidated. It is possible that CD105 expression is regulated by a number of cytokines and growth factors as implied by the observation that CD105 expression is increased in human umbilical vein endothelial cells (HUVECs) by irradiation and was thought to be a secondary event, the primary event being the production and release of up-regulators **(21)**.

It is known that tumor growth and metastasis are angiogenesis dependent and that the microvessel density in tumor tissues is inversely correlated with prognosis. Thus, circulating molecules that mark the degree of angiogenesis can be useful prognostic indicators **(20).** The current study demonstrated that serum endoglin level could be correlated with the clinical presentation of breast cancer. It was higher in breast cancer patients than that of controls (5.33 ± 2.13 Vs 3.99 ± 1.22 ng/ml) and the difference was statistically significant (p=0.045) with a cutoff point at 5.31 ng/ml which yielded a specificity of 100% and a sensitivity of 56% in diagnosing breast cancer. However, the endoglin level did not differ significantly according to the menopausal status, primary tumor size, regional lymph nodes involvement or presence of distant metastases. The significant difference was only noticed according to hormone receptor status.

In breast cancer tissue, CD105 expression is inversely correlated with both overall and disease-free survival. **Kumar et al., 1999** assessed microvessel density in 106 patients using monoclonal antibodies to CD105 and the panendothelial marker CD34. CD105 expression correlated significantly with overall (P=0.0029) and disease-free (P=0.0362) survival, whereas CD34 counts showed no such relationship **(22). Dales et al., 2004** examined the prognostic significance of CD105 and CD31 immunocytochemical expression in 905 breast cancers. A greater number of CD105-positive microvessels correlated significantly with a poorer survival (P=0.001) both on uni- and multivariate analysis. This correlation was also seen in a subgroup of node-negative patients (P=0.035). CD31 expression correlated with poor survival, but not in the node-negative subgroup and not on multivariate analysis **(23)**. It should be noted, however, that there is cross reactivity of CD31 antibodies with fibroblasts and plasma cells leading to a degree of over-staining, which is not seen when using the more specific CD34**.** CD105 might turn out to be a superior prognostic indicator to established pan endothelial markers **(24)**.

Blocking tumor angiogenesis has been proven to be an important strategy for cancer therapy **(4).** Additional studies have also demonstrated that the systemic administration of naked anti-human CD105 monoclonal antibody suppresses established tumors in animal models, and that its efficacy is markedly enhanced when used in combination with a chemotherapeutic drug **(25)**. **Furstenberger et al., 2006** looked at changes in serum levels of soluble angiogenesis-related factors in breast cancer patients receiving anthracycline or taxane-based neoadjuvant chemotherapy. Although there were no differences in baseline levels of soluble CD105 between patients and controls, they found that patients demonstrated significant decrease in circulating levels of soluble CD105 after two cycles of chemotherapy (P<0.01) **(26)**.

HGF is a mitogen, morphogen, and survival factor for cells expressing Met, the HGF high affinity tyrosine kinase receptor. Both HGF and c-Met are expressed by neoplastic cells enabling autocrine and paracrine regulation of these processes **(27,28)**. HGF is secreted as an inactive pro-peptide which is cleaved by HGF activator into its active form. HGF activator is regulated by two inhibitors, HGF activator inhibitor type 1 (HAI-1) and type 2 (HAI-2); both transmembrane proteins that are secreted as proteolytically truncated forms **(29,30)**. Both HGF and c-Met are induced by hypoxia and have a critical role in cell migration under low oxygen tension **(31).** In human breast cancer, HGF plays a role in tumor metastasis and is an independent predictor of disease progression and survival **(32).**

The current study revealed that serum HGF in breast cancer patients (2449 ± 1129 pg/ml) was higher than controls (2270 ± 760 pg/ml), but the difference was not statistically significant (p=0.57). The HGF level differed significantly according to the primary tumor size (T1,T2 Vs T3,T4) and the presence of metastatic spread (M0 Vs M1).

Sheen-Chen et al., 2005 reported that there is a close correlation between the appearance of HGF in sera and tumor progression and monitoring of the serum HGF level might be useful as a tumor marker, particularly for the early detection of liver metastases **(33).** In the current study, the area under the curve of HGF was the highest (77 %, P=0.02) when ROC curve was constructed for the prognostic purpose with cutoff value of 1838 pg/ml of which yielded a sensitivity of 86% and a specificity of 53%. Thus, preoperative serum soluble HGF levels might reflect the severity of invasive breast cancer and deserve further evaluation.

Conclusion

Serum endoglin correlated with breast cancer more than HGF with and both reflected the severity of progression as well as the metastatic potential. Moreover, endoglin has the potential to be an ideal target for antiangiogenic therapy and a useful marker of early detection of breast cancer or tumor relapse.

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