Pathol. Oncol. Res. (2009) 15:37–40
DOI 10.1007/s12253-008-9115-z

ORIGINAL PAPER

Investigation of Soluble HER2 and Transforming Growth Factor Beta-1 Serum Levels in Gestational Trophoblastic Disease

Alamtaj Samsami Dehaghani · Neda Rahimi Rad · Mohammad Javad Fattahi · Baharak Khadang · Mohammad Amin Kashef · Zahra Sarraf · Abbas Ghaderi

Received: 30 August 2008 / Accepted: 15 October 2008 / Published online: 31 October 2008

Abstract HER2/neu and TGF-β1 are over-expressed in various types of malignancies. It appears that they play an important role in the biologic behavior of tumors and have prognostic value. Gestational tropoblastic diseases (GTDs) comprise of a heterogeneous group characterized by abnormally proliferating trophoblastic tissues, ranging from benign to malignant. The objective of this study was to measure and compare the serum levels of s-HER2 and TGF-β between patients with GTDs and pregnant and non-pregnant controls. Serum levels of s-HER2 and TGF-β1 were determined by ELISA method in 95 GTD patients (55 complete moles, 32 persistent moles, and 8 choriocarcinoma), 30 normal pregnant controls, and 22 normal non-pregnant controls. Mean serum level of s-HER2 did not differ significantly between patients and controls. TGF-β1 serum level was significantly higher in GTD patients (20.29±10.68 pg/ml with 95% confidence interval (CI) of 18.10–22.48 pg/ml) compared with pregnant controls (10.26±11.84 pg/ml with 95% CI of 5.75–14.76 pg/ml) and non-pregnant controls (7.27±9.61 pg/ml with 95% CI of 3.01–11.53 pg/ml) ($P<0.001$). Our findings suggest that TGF-β1 serum levels in GTD patients may represent a potential prognostic marker. Further investigations with larger sample size and more frequent sampling are required to elucidate this issue.

Keywords HER2 · TGF-β1 · Gestational trophoblastic disease

A. S. Dehaghani (✉) · N. R. Rad · Z. Sarraf
Department of Obstetric and Gynecology, Hafez hospital,
Shiraz University of Medical Sciences,
71345-1798, Shiraz, Iran
e-mail: samsamia@sums.ac.ir

M. J. Fattahi · B. Khadang · M. A. Kashef · A. Ghaderi
Shiraz Institute for Cancer Research,
Shiraz University of Medical Sciences,
Shiraz, Iran

A. Ghaderi
Department of Immunology,
Shiraz University of Medical Sciences,
Shiraz, Iran

Introduction

Gestational trophoblastic diseases (GTDs) consist of a spectrum of disorders characterized by an abnormal proliferation of trophoblastic tissue. GTDs include hydatidiform mole (complete and partial), invasive mole, choriocarcinoma, and two rare variants called placental site trophoblastic tumor (PSTT) and epithelioid trophoblastic tumor (ETT). It is considered today the most curable gynecologic cancer [1, 2].

HER2/neu oncogene (also known as c-erbB-2) is a member of the epidermal growth factor receptor (EGFR) family. It is over-expressed in various types of cancers including breast, ovarian, lung, gastric, and oral cancers; it is one of the biomarkers used as a prognostic and predictive factor in some solid malignancies [3]. Clinical and molecular studies suggest that increased soluble HER2 (s-HER2) expression may contribute to disease progression by mediating angiogenesis, metabolic adaptation, and other aspects of metastasis that define the cancer phenotype [4].

Increased expression of HER2/neu in breast cancer cells results in transactivation of EGFR family [5]. Several investigations have shown that over-expression of HER2

is present in metastatic breast cancers and is associated with resistance to chemotherapy, resulting in decreased survival of patients [6, 7]. Over-expression of HER2/neu was also found to be a poor prognostic factor for survival from advanced-stage ovarian cancer and endometrial cancer [8].

Transforming growth factor-β (TGF-β) is a cytokine that almost every cell in the body produces and has receptors for it. It acts as a modulatory cytokine released by T regulatory cells during the course of cell-mediated immune response. TGF-β regulates cellular proliferation by inhibition, thus in normal cells it works as a tumor suppressor. Mutations in the TGF-β pathway in cancer cells render these cells resistant to the growth inhibition of TGF-β, causing uncontrolled proliferation of the cells [9, 10].

There are three isoforms of TGF-β, each encoded by a distinct gene. TGF-β1 messenger RNA is expressed in endothelial, hematopoietic, and connective-tissue cells. The levels of TGF-β1 in serum can be measured and used as diagnostic or prognostic marker for human diseases [9]. Increased serum level of TGF-β1 has been detected in some solid malignancies such as gastric and breast cancer [11, 12]. High serum levels of TGF-β1 protein are also found in patients with invasive prostate cancer [13] and colorectal cancer [14].

TGF-β signaling pathway plays an important role in the pathogenesis and progression of GTDs [15, 16]. Increased expression of TGF-β1 mRNA has been found in tissues from molar pregnancy [17].

The aim of the present study was to determine the serum level of s-HER2 and TGF-β1 in GTD patients and to compare the values with those of pregnant and non-pregnant healthy women. No studies investigating serum level of s-HER2 and TGF-β1 in GTD patients have been published up to date.

Materials and Methods

Subjects A total of 95 patients with GTD (mean age of 27.56 years) referred from Obstetrics and Gynecology outpatient clinics or hospital wards of Shiraz University of Medical Sciences were studied, including 55 patients with complete hydatidiform mole, 32 patients with persistent hydatidiform mole, and 8 patients with choriocarcinoma. Diagnosis was made based on histopathologic examination of the tissue obtained from endometrial curettage. Six months after evacuation a follow-up sample was obtained from six patients with complete mole and five patients with persistent mole (selected from the total of 95 patients). Thirty healthy pregnant women regional volunteers with normal pregnancy and gestational ages of 10 to 22 weeks with single alive fetus and 22 healthy non-pregnant women in reproductive age were also recruited as controls in this case-control study. Controls were those attending Obstetrics and Gynecology outpatient clinics for routine prenatal check-ups or for routine visits. No significant difference was found between case and control groups regarding age, gravidity, parity, and gestational age.

This study was approved by our university ethics committee. Informed consent was obtained from all participants.

s-HER2 and TGF-β1 Serum Levels Determination Sera from patients (prior to molar evacuation) and controls were collected in sterile clean dry tubes, separated, and kept frozen at −70°C until used. s-HER2 and TGF-β1serum levels were determined using commercial ELISA kits (BenderMed System, Austria for sHER2 and Biosource, USA for TGF-β1), according to manufacturer recommendations.

Statistical Analysis The data were analyzed using SPSS software (version 11.5.0; SPSS Inc., Chicago, IL, USA). T-test, Mann–Whitney test, and one way ANOVA were used to compare groups, when appropriate. Serum levels are expressed as mean ± standard deviation (SD). A *P*-value of less than 0.05 was considered significant.

Results

Serum levels of s-HER2 and TGF-β1 were measured in 95 GTD patients, 30 pregnant controls, and 22 non-pregnant healthy women. The mean serum level of s-HER2 was 3.05± 1.30 ng/ml in GTD patients, 3.19±1.62 ng/ml in healthy pregnant controls, and 2.77±1.67 ng/ml in non-pregnant controls, as depicted in Table 1, the difference was not statistically significant ($P>0.05$).

As presented in Table 2, mean serum level of TGF-β1 was significantly higher in GTD patients (20.29±10.68 pg/ml with 95% confidence interval (CI) of 18.10–22.48 pg/ml) compared with pregnant controls (10.26±11.84 pg/ml with

Table 1 Mean serum level of s-HER2 in GTD patients, healthy pregnant controls, healthy non-pregnant controls, and follow-up group*

	Number	Mean s-HER2 serum level (ng/ml)
Total GTD patients	95	3.05±1.30
Normal pregnant controls	30	3.19±1.62
Normal non-pregnant controls	22	2.77±1.67
Follow-up group	11	3.12±0.68

*No statistically significant difference was found between patients and controls

Table 2 Mean serum level of TGF-β1 in GTD patients, healthy pregnant controls, healthy non-pregnant controls, and follow-up group

	Mean TGF-β1 serum level (pg/ml)	95% CI	*P*-value*
Total GTD patients	20.29±10.68	18.10–22.48	N/A
Normal pregnant controls	10.26±11.84	5.75–14.76	<0.001
Normal non-pregnant controls	7.27±9.61	3.01–11.53	<0.001
Follow-up group	11.12±10.46	5.08–17.16	<0.001

**P*-value comparing patients with other groups
CI: Confidence interval; Mean ± SD

95% CI of 5.75–14.76 pg/ml) and non-pregnant controls (7.27±9.61 pg/ml with 95% CI of 3.01–11.53 pg/ml) ($P<0.001$). Statistically significant differences existed ($P<0.001$) when comparing patients with the follow-up group. In addition, the difference between TGF-β1 serum level of patients 6 months following evacuation and serum level of control groups (pregnant or non-pregnant) did not prove to be statistically significant ($P>0.05$).

Comparing serum levels of s-HER2 and TGF-β1 between subgroups of patients (55 complete hydatidiform mole, 32 persistent hydatidiform mole, and eight choriocarcinoma patients) revealed no statistically significant difference (data not given).

Discussion

GTDs comprise a spectrum of diseases with different clinical presentations that continue to be a significant reproductive health problem worldwide. The rate of metastasis in this neoplastic disease is high due to increased vascularization [2].

Tumor markers and certain immune regulatory mediators have previously been investigated in molar pregnancy and choriocarcinoma [18, 19]. The potential role of s-HER2 and TGF-β1 as tumor markers in patients with GTDs was investigated in the present study.

HER2/neu is a member of EGFR family that has been investigated in various neoplasms; release of soluble form of HER2/neu and its alterations in the serum during malignancy course remains as a potential biomarker in certain tumors particularly in the case of breast cancer [6, 7].

In the current study, the serum level of s-HER2 was determined in GTD patients and was compared with normal healthy pregnant and non-pregnant controls. Mean concentration of s-HER2 in GTD patients and controls was not statistically different.

The role of Her2/neu in GTDs has been studied previously [15, 16]; however, no data on the serum level of HER2 in GTDs was available in the literature. In a study conducted by Tuncer et al. [15], expression of c-erbB-2 gene product was investigated immunohistochemically (IHC) on sections obtained from GTD patients; they reported the importance of EGFR-related family of oncogenes in the pathogenesis of GTDs and proposed that the increased expression of EGFR in complete mole may influence the development of persistent gestational trophoblastic tumor. Utilizing IHC, Yang et al. [16], found the overexpression of c-erbB-2 protein as a strong predictor for the malignant transformation of complete mole.

Although previous studies [15, 16] found an increased serum level of s-HER2 in GTD patients, we failed to find an association between s-HER2 determination by ELISA in serum and GTDs. This could be explained based on the fact that characteristics of antibodies used in ELISA and IHC and also sensitivity of these tests are different. As IHC is done on formalin fixed tissue compared with sera in our assay, evacuation of tissue and handling of sera should also be considered. On the other hand, differences in the proportion of GTD subtypes in our study and others [15, 16], status of treatment, and even age and ethnicity of the studied populations should be considered. Performing reverse transcription polymerase chain reaction (RT-PCR) to analyse mRNA on tissue to determine the level of HER2 gene expression could be suggested to elaborate more on this issue.

Here serum level of TGF-β1 was also determined in the studied population. In normal cells TGF-β inhibits cellular proliferation. After tumor cells become resistant to growth inhibition by TGF-β, the tumor cells often increase their production of TGF-β. In response to increased production of TGF-β, the tumor cells, whose growth can no longer be inhibited by TGF-β, become more invasive and metastasize to distant organs due to TGF-β mediated stimulation of angiogenesis and cell motility, TGF-β also enhances the production and influences the adhesive characteristics of the extracellular matrix and suppresses the activities of infiltrating immune cells, helping the tumor escape host immunosurveillance [20–22].

Changes in serum level of TGF-β and variation in its genes have been studied in most reproductive related disorders; in various cancers elevated levels of TGF-β have been reported including gastric carcinoma [23], esophageal cancer [24], and skin malignancy [25]. Measurement of TGF- β protein level in breast cancer patients might help to identify the high-risk population early in tumor progression [11].

In the current study, TGF-β1 serum levels were significantly higher in GTD patients compared with normal pregnant and non-pregnant controls. In addition to the

notion that TGF-β1 is released by replicating tumor cells and facilitates tumor growth and invasion by promoting angiogenesis, affecting extracellular matrix and immunosuppression, it could also be postulated that production of self-antigens during cellular proliferation in GTD will trigger T regulatory cells and consequently more TGF-β1 will be synthesized. TGF-β1 released by T regulatory cells functionally suppresses the immune effector mechanism [10], giving more chance to neoplastic cells to grow and to migrate to other organs and tissues.

Our findings raise the possibility that TGF-β1 serum level determination in GTD patients may have prognostic significance. Failing to detect a statistically significant difference in TGF-β1 serum level between complete mole, persistent mole, or choriocarcinoma in the current study restricts its value in prediction of tumor behavior; however, the limited number of patients in the present study should be considered while interpreting the results.

Further investigations with larger sample sizes and more frequent sampling are required to elucidate the role of TGF-β1 serum level as a predictive and prognostic factor in the management of GTDs, to nominate it as a serum marker that can be measured to have a clue of persistence or remission of GTD following diagnosis, or to follow the response to treatment.

Acknowledgement This work was financially supported by a grant from Shiraz University of Medical Sciences (grant No.82-1791) and in part by Shiraz Institute for Cancer Research.

References

1. Genest DR, Berkowitz RS, Fisher RA, Newlands ES, Fehr M (2003) Gestational trophoblastic disease. In: Tavassoli FA, Devilee P (eds) World Health Organization Classification of Tumors. Pathology and Genetics. Tumors of the Breast and Female Genital Organs. IARC, Lyon, pp 250–254
2. Sivanesaratnam V (2003) Management of gestational trophoblastic disease in developing countries. Best Pract Res Clin Obstet Gynaecol 17:925–42
3. Hung MC, Lau YK (1999) Basic science of HER2/neu: a review. Semin Oncol 26:51–59
4. Hung MC, Schechter AL, Chevray PY, Stern DF, Weinberg RA (1986) Molecular cloning of the neu gene: absence of gross structural alteration in oncogenic alleles. Proc Natl Acad Sci U S A 83:261–264
5. Worthylake R, Opresko LK, Wiley HS (1999) ErbB-2 amplification inhibits down-regulation and induces constitutive activation of both ErbB-2 and epidermal growth factor receptors. J Biol Chem 274:8865–8874
6. Nunes RA, Harris LN (2002) The HER2 extracellular domain as a prognostic and predictive factor in breast cancer. Clin Breast Cancer 3:125–135
7. Tsuda H, Hirohashi S, Shimosato Y, Tanaka Y, Hirota T, Tsugane S, Shiraishi M, Toyoshima K, Yamamoto T, Terada M (1990) Immunohistochemical study on overexpression of c-erbB-2 protein in human breast cancer: its correlation with gene amplification and long-term survival of patients. Jpn J Cancer Res 81:327–332
8. Cirisano FD, Karlan BY (1996) The role of the HER2/neu oncogene in gynecologic cancers. J Soc Gynecol Investig 3:99–105
9. Blobe GC, Schiemann WP, Lodish HF (2000) Role of transforming growth factor beta in human disease. N Engl J Med 342:1350–1358
10. Romagnani S (2006) Regulation of the T cell response. Clin Exp Allergy 36:1357–1366
11. Desruisseau S, Palmari J, Giusti C, Romain S, Martin PM, Berthois Y (2006) Determination of TGFbeta1 protein level in human primary breast cancers and its relationship with survival. Br J Cancer 94:239–246
12. Lin Y, Kikuchi S, Obata Y, Yagyu K, Tokyo Research Group on Prevention of Gastric Cancer (2006) Serum levels of transforming growth factor beta1 are significantly correlated with venous invasion in patients with gastric cancer. J Gastroenterol Hepatol 21:432–437
13. Ivanovic V, Melman A, Davis-Joseph B, Valcic M, Geliebter J (1995) Elevated plasma levels of TGF-b 1 in patients with invasive prostate cancer. Nat Med 1:282–284
14. Shim KS, Kim KH, Han WS, Park EB (1999) Elevated serum levels of transforming growth factor-b1 in patients with colorectal carcinoma: its association with tumor progression and its significant decrease after curative surgical resection. Cancer 85:554–561
15. Tuncer ZS, Vegh GL, Fulop V, Genest DR, Mok SC, Berkowitz RS (2000) Expression of epidermal growth factor receptor-related family products in gestational trophoblastic diseases and normal placenta and its relationship with development of postmolar tumor. Gynecol Oncol 77:389–393
16. Yang X, Zhang Z, Jia C, Li J, Yin L, Jiang S (2002) The relationship between expression of c-ras, c-erbB-2, nm23, and p53 gene products and development of trophoblastic tumor and their predictive significance for the malignant transformation of complete hydatidiform mole. Gynecol Oncol 85:438–444
17. Pang ZJ, Xing FQ (2003) Expression of transforming growth factor-beta and insulin-like growth factor in molar and placental tissues. Arch Gynecol Obstet 269:1–4
18. Hegab HM, Schindler AE, Rizk M, Ramadan M (2003) Evaluation of tumour markers in molar pregnancy. Arch Gynecol Obstet 268:151–154
19. Kohorn EI (2000) Measurement of CA-125 in trophoblastic disease. Gynecol Oncol 78:39–42
20. Hojo M, Morimoto T, Maluccio M, Asano T, Morimoto K, Lagman M, Shimbo T, Suthanthiran M (1999) Cyclosporine induces cancer progression by a cell-autonomous mechanism. Nature 397:530–534
21. Massague J (1990) The transforming growth factor-b family. Annu Rev Cell Biol 6:597–641
22. Norgaard P, Hougaard S, Poulsen HS, Spang-Thomsen M (1995) Transforming growth factor b and cancer. Cancer Treat Rev 21:367–403
23. Saito H, Tsuj itani S, Oka S, Kondo A, Ikeguchi M, Maeta M, Kaibara N (2000) An elevated serum level of TGF-β1 significantly correlated with lymph node metastasis and poor prognosis in patients with gastric carcinoma. Anticancer Res 20:4489–4493
24. Fukuchi M, Miyazaki T, Fukai Y, Nakajima M, Sohda M, Masuda N, Manda R, Tsukada K, Kato H, Kuwano H (2004) Plasma level of transforming growth factor beta1 measured from the azygos vein predicts prognosis in patients with esophageal cancer. Clin Cancer Res 10:2738–2741
25. Glick AB, Kulkarni AB, Tennenbaum T, Hennings H, Flanders KC, O'Reilly M (1993) Loss of expression of transforming growth factor beta in skin and skin tumors is associated with hyperproliferation and a high risk for malignant conversion. Proc Natl Acad Sci U S A 90:6076–6080