RESEARCH ARTICLE

Clinical Application of Serum Tumor Abnormal Protein from Patients with Gastric Cancer

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Abstract

Background: To verify whether serum tumor abnormal protein (TAP) would correlate with the responsiveness of palliative chemotherapy in patients with advanced gastric cancer, and the variation of conventional serum tumor markers e.g., carcinoembryonic antigen (CEA), antigen 125 (CA125),carbohydrate antigen19-9 (CA19-9) of adjuvant chemotherapy in patients with early gastric cancer. Materials and Methods: Patients with histologically confirmed gastric cancer and treated with chemotherapy were enrolled into this study. TAP values of these patients were determined by detecting abnormal sugar chain glycoprotein in serum, combined with the area of agglomerated particles. For patients with advanced gastric cancer, responsiveness of palliative chemotherapy was compared with variation of TAP and the relation between variation of TAP and tumor markers in patients with early gastric cancer was analyzed. Results: Totally 82 gastric cancer patients were enrolled into this study. The value of TAP is more closely related to responsiveness of palliative chemotherapy for patients with advanced gastric cancer. The correlation between TAP and responsiveness to palliative chemotherapy is stronger than the correlation between several conventional serum tumor markers (CEA, CA125 and CA199) .The variation of TAP was also positively correlated with the trend of CA125 in adjuvant chemotherapy. Conclusions: TAP is sensitive in monitoring the responsiveness to palliative chemotherapy in patients with advanced gastric cancer. But this result should be confirmed by randomized clinical trials for patients with gastric cancer.

Keywords: Tumor abnormal protein (TAP) - tumor marker - gastric cancer- chemotherapy efficacy

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Introduction

Gastric cancer is one of the most common malignant tumors in China , and the morbidity and mortalit rate are still high in recent years (Jemal et al., 2006). For gastric cancer, chemotherapy is an effective method for patients with advanced disease (Bruckner et al., 2000). Some patients with early gastric cancer could accept operation and adjuvant chemotherapy. But some patients diagnosed with advanced gastric cancer have to be treated with palliative chemotherapy. For many patients, progress of the disease is unavoidable, therefore , it is crucial to predict the responsiveness of chemotherapy in treating patients with gastric cancer (Liu et al., 2014; Wu et al., 2014).

Tumor markers are substances expressed in different biological tissues which could indicate the cancer (Daniele Marrelli et al., 1999). The technology of detecting sugar chains is widely used in blood test for cancer patients, e.g., the related tumor markers in gastric cancer. The carcinoembryonic antigen (CEA) is one of the tumor markers related with alimentary tract. And high level of carbohydrate antigen 19-9 (CA199) in serum had also been observed in neoplasms of the alimentary tract and pancreas (Kornek et al., 1991). But at present, other tumor markers with higher specificity are required for patients with gastric cancer .

Structure of sugar chain is involved in regulating the cell-cycle of tumor cells (Meany et al., 2011). A variety of abnormal sugar chain glycoprotein produced during the multistep development of human tumors (Dube et al., 2005). These abnormal sugar chain combined with calcium - histone proteins, known as Tumor abnormal protein (TAP). TAP is a common feature in the process of abnormal cell proliferation (Hakomori, 2009; Meany et al., 2011).

The aim of this study was to compare the predictive value of TAP with several common tumor markers, eg., CEA, CA125 and CA199 in gastric cancer. And explore whether TAP could effectively predict the responsiveness of chemotherapy.

Materials and Methods

Eligibility criteria were as follows: 1. All inpatients were required to be pathologically diagnosed with gastric adenocarcinoma, and they had measureable lesion by CT scan. Blood samples were collected before and after chemotherapy from all patients in Jiangsu Cancer Hospital & Research Institute from September 2014 to February 2015; 2. to have a score of Karnofsky Performance Status

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(KPS) ≥ 70; 3. to be 25 to 75 years of age; 4.to sign an informed consent before treatment;. 5.to have blood examination and meet the following requirements: white blood cell count >3.0×10^9 and platelet count >135×10^9, bilirubin and transaminases <1.5 times the upper normal limit and creatinine leval <1.5 times the upper normal limit. Patients were excluded from this study if they1. failed to complete two cycles of chemotherapy; 2.had any serious medical or psychiatric condition; 3 suffered from other malignancies at the same time; 4.were pregnant or lactating women.

All patients were divided into two groups, group A with early gastric cancer treated with adjuvant chemotherapy, and group B with advanced gastric cancer treated with palliative chemotherapy. TAP tests were detected 1 day before chemotherapy and 2 weeks after chemotherapy. And the valus of serum tumor biomarkers, eg., CEA, CA125, CA199 were recorded concomitantly, which were measured by immunofluorescence. It is defined as negative as CEA <3.5ng/mL or defined as negative as CA125<35U/mL or defined as negative as CA19-9 <39U/mL. TAP detection, firstly, 2 mL venous blood was collected from patients and prepared for blood smear; secondly, agglutination-agent dropped on the surface of blood smear and air dried; thirdly, the agglomerated particles observed by TAP image analyzer, and the area of condensed particles were measured; finally, the values of TAP recorded.

All data was analyzed by the STATA 8.0 software (Stata Corporation, 4905 Lakeway Drive College Station, Texas 77845 USA). Continuous variables were summarized by descriptive statistics. Counting data by logistic regression analysis; measurement data with the mean±standard deviation (x±s) and t test. Associations between the variation of TAP and patients' age, gender, organ metastasis and grade of malignancy were investigated with logistic regression analysis. Before and after chemotherapy, the relation between the responsiveness of Adjuvant therapy and tumor markers was analyzed with the regression analysis; the relation between the responsiveness of Palliative therapy and variation of TAP was analyzed with the correlation analysis. $P<0.05$ was considered statistically significance. The correlation coefficient is "r". When $|r|\geq 0.8$, it is considered highly correlated; $0.5\leq|r|<0.8$, it is considered moderately correlated; $0.3\leq|r|<0.5$, it is considered lowly correlated; if $|r|<0.3$, the correlation is negligibly.

Results

From September 2014 to February 2015, totally 82 gastric cancer patients, 57man and 25 women were enrolled into this study, with the mean age of 57.6, ranging from 30 to 75. All patients had been histologically confirmed with gastric cancer, 45 patients in group A, and 37 in group B (Table 1).

Table 1. Characteristics of All the Patients with Gastric Cancer

Character	Number (%)	*P*-value	OR (95%CI)
Gender			
Man (%)	57 (69.5%)	0.96	1.03 (0.40-2.63)
Women (%)	25 (30.5%)		
Age (year)			
Mean (Range)	57.6 (30-75)		
≥60	40	0.99	0.99 (0.42- 2.37)
<60	42		
Organ metastasis			
0-1	66	0.73	0.83 (0.28-2.48)
≥2	16		
Pathological grade			
High or medium	32	0.72	0.852 (0.350-2.071)
Low	50		

Table 2. The Correlation between TAP and the Responsiveness of Palliative Chemotherapy in Group B (n=37)

	CR+PR		SD		PD		r	*P*
	Increase	Decrease	Increase	Decrease	Increase	Decrease		
TAP*	2(5.4%)	16(43.3%)	2(5.4%)	4(10.8%)	13(35.1%)	0(0)	0.80	<0.001
CEA**	3(8.1%)	15(40.5%)	3(8.1%)	3(8.1%)	11(29.8%)	2(5.4%)	0.62	<0.001
CA125***	2(5.4%)	16(43.3%)	2(5.4%)	4(10.8%)	12(32.4%)	1(2.7%)	0.73	<0.001
CA19-9****	4(10.8%)	14(37.9%)	3(8.1%)	3(8.1%)	12(32.4%)	1(2.7%)	0.63	<0.001

CEA, carcinoembryonic antigen; CA, carbohydrate antigen; TAP, Tumor abnormal protein (TAP); CR, complete response; PR, partial response; SD, stable disease (SD); PD, progressive disease (PD). r, correlation coefficient; P, significance level. *r=0.80,*P<0.001, TAP compared with responsiveness of chemotherapy; **r=0.62,**P<0.001,CEA compared with the responsiveness of chemotherapy; ***r=0.73,***P<0.001,CA125 compared with the responsiveness of chemotherapy; ****r=0.63,****P<0.001,CA199 compared with the responsiveness of chemotherapy

Table 3. Comparison between the Variation of TAP and Tumor Markers CEA,CA125 and CA199 in Group A (n=45)

Tap	Coef.	Std. Err.	t	*P*>|t|	95%CI	
CEA	0.19	0.13	1.47	0.15	-0.07	0.45
CA125	0.49	0.13	3.74	0.001	0.22	0.75
CA199	0.21	0.12	1.66	0.10	-0.05	0.47
_cons	0.11	0.10	1.07	0.29	-0.10	0.32

Coef. ,correlation coefficient; Std. Err.,standard error; 95% Conf. Interval, 95% confidence intervals; R-squared, determination coefficient; Adj R-squared, adjusted determination coefficient; n=45; Prob > F=0.0002; R-squared =0.37; Adj R-squared=0.33; Root MSE= 0.41.

Table 4. Comparison between the Variation of TAP and Tumor Markers CEA,CA125 and CA199 (n=45) in Group B

Tap	Coef.	Std. Err.	t	*P*>|t|	95%CI	
CEA	0.18	0.16	1.08	0.29	-0.16	0.51
CA125	0.46	0.26	1.82	0.08	-0.055	0.98
CA199	0.08	0.27	0.30	0.77	-0.46	0.62
_cons	0.14	0.09	1.38	0.17	-0.06	0.34

Coef. ,correlation coefficient; Std. Err.,standard error; 95% Conf. Interval, 95% confidence intervals; R-squared, determination coefficient; Adj R-squared, adjusted determination coefficient; n=45; Prob > F=0.0002; R-squared =0.37; Adj R-squared=0.33; Root MSE= 0.41.

Variation in the value of TAP in patients with gastric cancer were compared with age, gender, organ metastasis and grade of malignancy, and $P>0.05$, suggesting the baseline characters e.g., age, gender, organ metastasis and grade of malignancy would not influence the Variation of TAP (Table 1).

The TAP, CEA, CA125 and CA199 were independently associated with the responsiveness of palliative chemotherapy. Before and after chemotherapy, the relation between TAP and the responsiveness of palliative chemotherapy in patients with gastric cancer was analyzed and $P<0.05$, r=0.80 (Table 3). The responsiveness of palliative chemotherapy is considered highly correlated with the variation of TAP, the level of TAP decreased when CT scan showed disease controlled and increased in disease progression. The relation between CEA and the responsiveness of palliative chemotherapy was analyzed and $P<0.05$, r=0.62; the correlation between CA125 and the responsiveness of palliative chemotherapy was analyzed and $P<0.05$, r=0.73; the correlation between CA199 and the responsiveness of palliative chemotherapy was analyzed and $P<0.05$, r=0.63. The correlation between responsiveness of palliative chemotherapy and TAP is stronger than the correlation between several conventional serum tumor markers (CEA, CA125 and CA199). The variation of TAP is closely related with the responsiveness of palliative chemotherapy. TAP is sensitive in monitoring responsiveness of palliative chemotherapy (Table 2).

Before and after chemotherapy, the relationship between the variation of TAP and tumor markers in group A was analyzed with the regression analysis in group A. The variation of TAP was compared with CEA, $P<0.05$ and 0 is not contained in the 95% confidence intervals (Table 3). The correlation of CA125 is 0.49, suggested a positive impact on TAP. The value of TAP increased with the value of CA125; conversely, the value of TAP decreased with the value of CA125. The variation of TAP was compared with CEA and CA199, $P>0.05$ and 0 is not contained in the 95% confidence intervals. It suggested the trend of the value of TAP is not associated with the trend of CEA and CA199. The variation of TAP was positively correlated with the trend of CA125, but not correlated with CEA and CA199 in adjuvant chemotherapy.

The variation of TAP in group B was compared with CEA, CA125 and CA199, $P>0.05$ and 0 is contained in the 95% confidence intervals. It suggested the trend of the value of TAP is not associated with the trend of CEA, CA125 and CA199 in patients with advanced gastric cancer in this study.

Discussion

Recently, many studies indicate that some aberrant glycosylation is a result of initial oncogenic transformation, as well as a key event in induction of invasion and metastasis. The tissue expression of these antigens has been found in a variety of epithelial malignant tumors (breast, colon, ovarian, endometrial, stomach and lung). Tumor markers are substances expressed in different biological fluids or tissues which could indicate the presence of a neoplasm. So, when the level of TAP reached a high degree, it could be detected in peripheral blood (Blomme et al., 2009). Today, tumor markers are primarily used in preoperative staging of neoplasms, postoperative monitoring of the treatment's effectiveness, and early diagnosis of recurrence. In the procession of invasion and metastasis in tumor, the serum sialic acid content increased several weeks before the clinical diagnosis. It was important for early detection and promptly treatment (Li et al., 2012). Many tumor markers were impossible to be detected in current clinical detection, but could be detected in the same reaction system with the TAP detection. The TAP detection kit contains agglomerant , which aided and promoted a variety of abnormal sugar chain to form crystal aggregates by gathering with each other. In the TAP detection system, difference could be observed with image analyzer or biological microscope comparing with images of coagulated blood without TAP.

Most gastric cancer patients were treated with palliative chemotherapy or adjuvant chemotherapy. The responsiveness of palliative chemotherapy and prognosis of gastric cancer were detected by tumor markers and CT scan. (Liu et al., 2015). More and more evidences show, the invasion or metastasis of tumor cells is directly related with the prognosis of cancer patients (Meyer T et al., 1998). And many recent researches indicated a close relation between the abnormal surface glycosylation and the invasion and metastasis of tumor cell. (Dube et al., 2005; Jiang et al., 2010). So, the invasion and metastasis of tumor could be predicted by detecting the level of special sugar based structures. In this study, TAP is considered sensitive in monitoring the responsiveness of palliative chemotherapy in patients with advanced gastric cancer. The variation of TAP was not correlated with CEA, CA125 and CA199 in palliative chemotherapy, but positively correlated with the trend of CA125 in adjuvant chemotherapy.

TAP is an independent predictor for the responsiveness of chemotherapy in this study. Though it followed similar rationale with the detection of tumor marker, TAP could not be replaced.The result of CT scan is considered the gold-standard for evaluating the efficacy of palliative chemotherapy for patients with advanced gastric cancer. The overall accuracy of CT was 78.64% in T stage and 74.09% in N stage of gastric cancer. The diagnostic sensitivity, specificity and accuracy of CT for determining distant metastases of gastric cancer were 65.63%, 99.47% and 94.55%, respectively (Chao et al., 2007). The variation of TAP is closely related with the responsiveness of palliative chemotherapy. The level of TAP increased when CT scan showed the procession of disease, and decreased when the disease is well controlled. So, the variation of TAP would provide important guidance for the individualized treatment of patients with gastric cancer. But this result should be confirmed by randomized clinical trials for patients with gastric cancer. However, further clinical trials should be conducted to evaluate.

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References

Blomme B, Van Steenkiste C, Callewaert N, et al (2009). Alteration of protein glycosylation in liver diseases. *J Hepatol*, **50**, 592-60.

Boucher D, Cournoyer D, Stanners CP, et al (1989). Studies on the control of gene expression of the carcinoembryonic antigen family in human tissue. *Cancer Res*, **49**, 847-52.

Bruckner H, Morris JC, Mansfield P, et al (2000). Neoplasms of the stomach cancer medicine.5 m ed. Hamilton: *BC Decker*, **35**, 1355-90.

Daniele M, Franco R, Alfonso D, et al (1999). Prognostic significance of CEA, CA 19-9 and CA 72-4 preoperative serum levels in gastric carcinoma. *Oncology*, **57**, 55-62.

Dube D H, Bertozzi C (2005). Glycans in cancer and inflammation-potential for therapeutics and diagnostic. *Nat Drug Discov*, **4**, 477-88

Duffy MJ (2006). Serum tumor markers in breast cancer: are they of clinical value? *Clin Chem*, **52**, 345-51.

Fujiyama S, Tanaka M, Maeda S, et al (2002). Tumor markers in early diagnosis, follow-up and management of patients with hepatocellular carcinoma. *Oncology*, **62**, 57-63.

Hakomori S (2002). Glycosylation defining cancer malignancy: New wine in an old bottle. *Proc Natl Acad Sci U S A*, **99**, 10231-3.

Jemal A, Siegel R, Ward E, et al (2006). Cancer statistics. *CA Cancer J Clin*, **56**, 106-30.

Jiang M, Pu R (2010). Study on serum tumor related material (BXTM) with early diagnosis in malignant tumor. *National Med Frontiers China*, **5**, 80-1.

Jin L, Huang XE (2014). Efficacy of bifidobacterium tetragenous viable bacteria tablets for cancer patients with functional constipation. *Asian Pac J Cancer Prev*, **15**, 10241-4.

Kornek G, Depisch D, Temsch EM, et al (1991). Comparative analysis of cancer-associated antigen CA-195, CA 19-9 and carcinoembryonic antigen in diagnosis, follow-up and monitoring to response to chemotherapy in patients with gastrointestinal cancer. *J Cancer Res Clin Oncol*, **117**, 493-6.

Liu J, Huang XE, Feng JF (2014). Further study on pemetrexed based chemotherapy in treating patients with advanced gastric cancer (AGC). *Asian Pac J Cancer Prev*, **15**, 6587-90.

Liu J, Huang XE (2014). Efficacy of Bifidobacterium tetragenous viable bacteria tablets for cancer patients with functional constipation. *Asian Pac J Cancer Prev*, **15**, 10241-4.

Meany DL, Chan DW (2011). Aberrant glycosylation associated with enzymes as cancer biomarkers. *Clin Proteomics*, **8**, 7-9.

Meyert T, Hart R (1998). Mechanisms of tumor metastasis. *Eur J Cancer*, **3**, 214-221.

Steinberg W (1990). The clinical utility of the CA 19-9 tumor-associated antigen. *Am J Gastroenterol*, **85**, 350-5.

Tatli A, Urakci Z, Kalender M, et al (2015). Alpha-fetoprotein (AFP) elevation gastric adenocarcinoma and importance of AFP change in tumor response evaluation. *Asian Pac J Cancer Prev*, **16**, 2003-7.

Varkia, Cummings R, Esko J, et al (1999). Essential of glycobiology. New York: cold spring harbor laboratory press, 483-491

Wu XY, Huang XE, You SX, et al (2013). Phase II study of pemetrexed as second or third line combined chemotherapy in patients with colorectal cancer. *Asian Pac J Cancer Prev*, **14**, 2019-22.