Research Article

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Serum Homocysteine, VEGF and TGF- β 1 dynamic change in colorectal cancer patients prior and post-operation

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Abstract: Background: The aim of the study was to evaluate the serum homocysteine (Hcy), vascular endothelial growth factor (VEGF) and transforming growth factor β 1 (TGF- β 1) dynamic change in colorectal cancer patients pre- and post-operation.

Material and methods: One hundred and eighteen CRC patients treated with surgery (CRC group) and 56 healthy controls (Control group) were included in this work. The serum Hcy, VEGF TGF- β 1 were examined by enzymatic cycle and enzyme-linked immunosorbent assay (ELISA) of the two groups. We followed patients for 12 months and out of the 118 CRC patients, 14 patients had recurrent disease. Serum Hcy, VEGF and TGF- β 1 were measured before and after surgery and repeated every 2 months.

Results Serum Hcy, VEGF and TGF- β 1 were $16.20 \pm 4.79 \mu\text{mol/L}$, $492.36 \pm 97.32 \text{ pg/ml}$, $29.23 \pm 7.47 \text{ pg/ml}$ for the CRC group and $8.98 \pm 3.02 \mu\text{mol/L}$, $315.21 \pm 56.28 \text{ pg/ml}$, $7.69 \pm 2.31 \text{ pg/ml}$ for the control groups. Serum Hcy, VEGF and TGF- β 1 was significantly ($p < 0.05$) lower after surgery in both recurrent and nonrecurrent CRC patients ($p < 0.05$). Interestingly, serum Hcy, VEGF and TGF- β 1 gradually increased with time.

Conclusion Serum Hcy, VEGF and TGF- β 1 levels are elevated in CRC patients and may correlated with the post-operative disease recurrence.

Keywords: colorectal cancer; Hcy; VEGF; TGF- β 1 prognosis.

Introduction

Colorectal cancer (CRC) is the 3rd most commonly diagnosed cancer in the United States and one of the leading cause of cancer related death world-wide [1, 2]. Post operation tumor recurrence is the main cause of death and an independent factor for prognosis. Studies related to the risk of recurrence in CRC patients after surgery showed in advanced stages of the disease, lymph node metastasis and tumor differentiation were the independent factors for tumor recurrence.

Hcy, a sulfur-containing amino acid which is produced as an intermediate product as a result of demethylation during methionine metabolism [3, 4], is a risk factor for cardiovascular disease [5, 6]. In addition, some studies have also shown that Hcy is also associated with the development of malignant tumors [7, 8]. Vascular growth factor (VEGF), a vasculogenic factor that promotes endothelial cell proliferation and metastasis, improves vascular permeability and promotes tumor angiogenesis and recurrence [8-10]. Transforming growth factor β 1 (TGF- β 1) is closely related to tumor angiogenesis invasion and metastasis. TGF- β 1 can promote invasion metastasis and recurrence of tumors through paracrine and autocrine pathways [11, 12]. Intriguingly, the correlation between serum Hcy, VEGF, and TGF- β 1 with CRC recurrence has rarely been discussed. In this work, we evaluated serum Hcy, VEGF and TGF- β 1 dynamic change in CRC patients pre- and post-operation and discussed the correlation with disease recurrence.

Material and methods

Patients

One hundred and eighteen CRC patients treated with cancer surgery (CRC group) were included in this work. In all 118 CRC cases, histological analysis was used to

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confirm that patients had colorectal carcinoma. The control group included 56 healthy individuals with no malignant tumors. The study protocol was approved by the Institutional Ethical Committee of Tianjin Union Medical Center (Tianjin People's Hospital) of China. All subjects included in the study provided informed consent. The inclusion criteria for the CRC group were: (i) patients were histologically confirmed to have colorectal cancer; (ii) patients had received radical resection of colorectal cancer; (iii) no pre-operative chemoradiotherapy was performed; (iv) signed informed consent was obtained from all the subjects. The general characteristics of the 118 CRC patients were showed in **Table 1**.

Ethical approval: The research related to human use has been complied with all the relevant national regulations, institutional policies and in accordance the tenets of the Helsinki Declaration, and has been approved by the Institutional Ethical Committee of Tianjin Union Medical Center (Tianjin People's Hospital) of China.

Informed consent: Informed consent has been obtained from all individuals included in this study

Serum Hcy, VEGF and TGF- β 1 examination

We collected 5ml venous blood from CRC patients and controls, and the serum was separated by centrifugation and stored at -20°C until the next experiments. Serum Hcy levels were determined by circulating enzyme method and serum levels VEGF and TGF- β 1 were examined by enzyme-linked immunosorbent assay (ELISA).

Statistical method

Serum levels of Hcy, VEGF and TGF- β 1 were expressed as mean $\pm$ SD. Student-t test (two tailed, independent) was used to analyse the difference between the groups. Significance was defined as a P value less than 0.05, and all statistical tests were 2-sided. Analyses were conducted using SPSS19.0 software (SPSS, Inc., Chicago, IL, USA).

Results

Comparison of serum Hcy VEGF and TGF- β 1 of CRC and controls

The serum Hcy, VEGF and TGF- β 1 were 16.20 ± 4.79 μ mol/L, 492.36 ± 97.32 pg/ml, 29.23 ± 7.47 pg/ml for the CRC group

Table 1: The main features of the include 118 CRC patients.

Characteristics	n=118(%)
Age (year)	
≤ 50	35(29.7)
> 50	83(70.3)
Gender	
Male	66(55.9)
Female	52(44.1)
Tumor location	
Colon	50(42.4)
Rectal	68(57.6)
Tumors Differentiation	
High/moderate	27(22.9)
Low	91(77.1)
Duke stage	
A/B	70(59.3)
C/D	48(40.7)

Table 2: The serum Hcy VEGF and TGF- β 1 of CRC and controls.

Groups	Hcy(μ mol/L)	VEGF(pg/ml)	TGF- β 1(pg/ml)
Control(n=56)	8.98 ± 3.02	315.21 ± 56.28	7.69 ± 2.31
CRC(n=118)	$16.20 \pm 4.79^*$	$492.36 \pm 97.32^*$	$29.23 \pm 7.47^*$

*p<0.05 compared to control group

and 8.98 ± 3.02 μ mol/L, 315.21 ± 56.28 pg/ml, 7.69 ± 2.31 pg/ml for the control groups respectively. Serum Hcy, VEGF and TGF- β 1 levels in the CRC group were significantly higher than those of the control group (p<0.05) **Table 2**.

Serum Hcy, VEGF and TGF- β of CRC changes prior and post-surgery

Serum Hcy, VEGF and TGF- β 1 dynamic change in colorectal cancer patients are shown in **Table 3**. The serum levels of Hcy, VEGF and TGF- β 1 were significantly reduced after surgery in both recurrent and non-recurrent CRC patients (p<0.05). However, serum Hcy (**Figure 1**), VEGF (**Figure 2**) and TGF- β 1 (**Figure 3**) levels gradually increased as the follow-up time increased.

Table 3: The serum Hcy, VEGF and TGF- β 1 dynamic change in colorectal cancer patients.

Time point	Recurrence(n=14)			Non-recurrence(104)		
	Hcy (μ mol/L)	VEGF (pg/ml)	TGF- β 1 (pg/ml)	Hcy (μ mol/L)	VEGF (pg/ml)	TGF- β 1 (pg/ml)
Prior surgery	16.63 $\pm$ 4.12	478.33 $\pm$ 88.64	28.89 $\pm$ 7.21	16.12 $\pm$ 4.87	496.32 $\pm$ 98.54	29.34 $\pm$ 7.30
Post-surgery						
2-month	14.33 $\pm$ 3.17	412.34 $\pm$ 78.26	18.21 $\pm$ 4.65	14.57 $\pm$ 2.96	403.21 $\pm$ 68.63	19.32 $\pm$ 5.21
4-month	12.65 $\pm$ 2.98	387.34 $\pm$ 87.54	16.22 $\pm$ 3.88	13.34 $\pm$ 3.33	369.47 $\pm$ 70.21	14.23 $\pm$ 4.74
6-month	12.23 $\pm$ 3.08	366.41 $\pm$ 69.31	14.32 $\pm$ 4.01*	12.08 $\pm$ 2.74	341.23 $\pm$ 67.24	12.36 $\pm$ 3.36
8-month	13.57 $\pm$ 2.74	395.14 $\pm$ 77.81*	14.67 $\pm$ 3.84*	12.14 $\pm$ 2.69	330.21 $\pm$ 74.11	11.68 $\pm$ 3.74
10-month	15.74 $\pm$ 3.47*	421.27 $\pm$ 68.30*	15.26 $\pm$ 4.11*	11.36 $\pm$ 3.02	338.54 $\pm$ 70.29	12.05 $\pm$ 3.59
12-month	16.65 $\pm$ 4.11*	432.14 $\pm$ 70.23*	16.24 $\pm$ 3.78*	12.08 $\pm$ 3.07	341.12 $\pm$ 68.47	11.97 $\pm$ 4.15

*p<0.05 compared to control recurrence group

Figure 1: Serum Hcy dynamic changes in recurrence and non-recurrence colorectal cancer patients prior and post-operation (0 month means prior surgery). **Figure 3:** Serum TGF- β 1 dynamic changes in recurrent and non-recurrent colorectal cancer patients prior and post-operation (0 month means prior surgery). **Figure 2:** Serum VEGF dynamic changes in recurrent and non-recurrent colorectal cancer patients prior and post-operation (0 month means prior surgery).

Discussion

In our work, we found that serum Hcy, VEGF and TGF- β 1 expression were significantly higher than that of control groups. We also found that serum Hcy, VEGF and TGF- β 1 levels were significantly reduced post-surgery compared to pre-surgery in both recurrent and non-recurrent CRC patients. These results suggest that serum Hcy, VEGF and TGF- β 1 levels are elevated in CRC patients and may correlate with post-operative disease recurrence.

Cancer statistical analysis showed that CRC is the third most commonly diagnosed carcinoma among both men and women, second only to lung and breast cancer in the United States [2]. Due to improved treatment modality, target drugs, early diagnosis, and screening methods the prognosis for CRC patients has improved over the past

several decades. However, CRC is still a significant cause of cancer related death globally. Recurrent or metastatic disease is still the main cause of death for CRC patients who undergo surgical treatment. Many studies [13-16] have investigated the risk factors related to post-operative recurrence or metastasis for CRC patients. Most of the relevant studies suggest that at the clinical stage, local-regional lymph node status and tumor differentiation are involved in post-operative recurrence or metastasis. Iida [16] and his colleges evaluated the risk factors involved in postoperative recurrence in patients with pathologically classified T1 CRC by including 284 patients with pT1 CRC who had undergone radical surgery. They found that lymphatic invasion was an independent risk factor for recurrence in pT1 CRC patients. Tokodai [13] investigated the risk factors for recurrence in stage II/III CRC patients treated with curative surgery. The authors included 206 stage II and 180 stage III patients who underwent surgery and followed up with median times of 51 and 45 months. They found that perioperative serum tumor marker levels, lymphatic invasion, and infiltrative growth patterns were associated with disease recurrence.

Hcy is a sulfur-containing amino acid, which is one of the intermediate products produced by demethylation during methionine metabolism. Miller and his colleges [17] discussed the homocysteine, cysteine, and the risk of incident colorectal cancer in a cohort study. They found that high plasma homocysteine is associated with an increased risk of CRC. However, whether elevated serum Hcy is correlated with the post-operative recurrence of CRC was not clear and is seldom reported in the literature. In our present study, we evaluated serum Hcy, VEGF and TGF- β 1 dynamic change in CRC patients pre- and post-surgery and investigated their correlation with the disease recurrence. We found that serum Hcy, VEGF and TGF- β 1 level were elevated in CRC patients compared to healthy controls and may be linked with disease recurrence.

Folic acid is closely associated with tumorigenesis. Inadequate folic acid can lead to an accumulation of Hcy. As a sensitive marker, Hcy can reflect the levels of folic acid and, therefore, predict the risk of tumorigenesis. Folic acid and vitamin B12 deficiency is related to inadequate intake and the high metabolism of tumors. Due to the accelerated division and proliferation of cancer cells, more folic acid and vitamin B12 are needed as coenzymes to participate in the synthesis of nucleic acids. In cancer patients this results in folic acid and vitamin B12 deficiency leading to Hcy accumulation. Serum levels of Hcy can therefore be used to reflect cancer growth to a certain extent. Sun et al. [18] reported that increasing levels of Hcy is significantly associated with the proliferative activity of cancer cells,

and positively correlated with the number of living cells. Some studies have shown that serum Hcy levels have clinical value in the diagnosis, and prediction of recurrent of digestive tract cancer [19], cervical cancer [20, 21] and breast cancer [7, 22]. Liu et al. [23] found that the level of Hcy increased again 3 to 6 months before diagnosis of recurrence, and suggested that the re-elevated serum Hcy was an important sign for disease recurrence.

Conclusion

Serum Hcy, VEGF and TGF- β 1 level are elevated in CRC patients and may be involved in post-operative disease recurrence. Therefore, continuous monitoring of serum Hcy, VEGF and TGF- β 1 concentrations may be useful for early detection disease recurrence and improve the prognosis.

Conflict of interest: Authors state no conflict of interest

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