

RESEARCH ARTICLE

Clinical Significance of Serum Vascular Endothelial Growth Factor and Complement 3a Levels in Patients with Colorectal Cancer in Southern Iran

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Abstract

Background: Colon cancer (CRC) is perhaps the second most common cause of cancer mortality. This study determined the clinical significance of serum vascular endothelial growth factor (VEGF) and serum complement 3a (C3a) levels in patients with CRC in Fars province, southern Iran. **Materials and Methods:** Between June 2010 and June 2012, 110 patients with CRC of both genders and different age groups were divided into 3 groups. Group A included patients who had just undergone surgery; Group B had undergone chemotherapy after surgery; and Group C had undergone chemotherapy and radiotherapy after surgery. Twenty one healthy subjects with normal colonoscopy were considered as a control group. ELISA was undertaken to determine VEGF and C3a levels before and after treatment measures. **Results:** The mean age of patients was 53.9 ± 14.1 years. Considering VEGF level, a significant decrease was visible after treatment measures in groups A and B, but not Group C. For VEGF level, the difference was not statistically significant between two genders and various age groups before and after treatment. No significant difference was found for VEGF level between patients and normal group before any treatment. Regarding C3a levels in 101 subjects, they significantly decreased after treatment measures. Before and after treatment, the difference was statistically significant between two genders, but was not statistically significant among various age groups. **Conclusions:** As VEGF and C3a levels were significantly lower in patients after treatment, these may be beneficial markers in assessment of CRC therapy especially in early stages.

Keywords: Colorectal cancer - VEGF - C3a - Iran

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Introduction

Colorectal cancer (CRC) is considered as the third most common human cancer and the third leading cause of cancer mortality (Kargi et al., 2012). In developed countries, the mortality rate was shown to be about 33% with similar incidence rate among both genders occurring mostly in persons older than 50 years (Cunningham et al., 2010). CRC mortality rate has shown a decreasing trend in United States; however, disparities by socioeconomic status and race/ethnicity still persist (Enewold et al., 2014).

In developing countries, the cancer incidence rates were demonstrated to have an increasing trend, so it is important for policy makers to be familiar with cancer epidemiology in each locality for preventive measures (Talaiezhadeh et al., 2013).

In Fars province, southern Iran, during 1990-2005; CRC has been the 4th and 3rd prevalent cancer among males and females respectively. The average annual crude incidence rate and age-specific incidence rate of

the disease were 1.92 and 3.26 respectively and these figures among females were 1.51 and 2.41 (Mehrabani et al., 2008). In Fars Province, the incidence rate has shown a remarkable increase due to changes in the life style, decreased physical activity, heavy smoking habits, dietary changes and increased prevalence of obesity (Saberi-Firoozi et al., 2007). The 1, 3 and 5-year survival rates were reported 93.9, 50.3 and 27.2 percent respectively which is lower than developed countries (Mehrabani et al., 2012a; Mehrabani et al., 2012b). In Fars Province, a correlation between PD-1 gene polymorphism and CRC susceptibility was shown in patients (Yousefi et al., 2013).

The important risk factors for CRC include age, chronic inflammatory bowel diseases, colorectal polyps, a family or personal history of CRC, and inherited genetic alterations, such as familial adenomatous polyposis or hereditary nonpolyposis CRC (Lowery et al., 2014; Wang et al., 2014; Safarpour et al., 2013).

The diagnosis often occurs at the late stage of disease with a poor prognosis while an early detection may

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improve the prognosis (Ward et al., 2006). Therefore, screening programs can improve prognosis by the detection of cancer at its early stages (Zubero et al., 2014).

There is still a hope that the presence of malignant disease could be detected by specific changes in the composition of serum proteins. However, the use of serum markers, such as carcinoembryonic antigen (CEA), has so far failed to deliver diagnostic tests of high sensitivity and specificity for colon cancer. Complement C3a-desArg was shown to be present at significantly higher levels in serum from patients with colorectal adenomas and carcinomas than in healthy subjects suggesting that quantification of C3a-desArg level could ameliorate existing screening tests for colorectal cancer (Habermann et al., 2006).

Angiogenesis is a definition for the growth of new capillary blood vessels in the body and is critical in healing of tissue injuries and cancer growth. Formation of tumor vessel network, including blood and lymph vessels, is an important stage in carcinogenesis process. The discovery of vascular growth factors has resulted into a better understanding of tumor biology, so opening new possibilities in treatment of cancer to target angiogenesis within tumor-associated stroma, including therapy for colon cancer patients is of great importance (Szajewski et al., 2014).

Tumors such as colon cancer can release angiogenic growth factors stimulating the growth of blood vessels into tumors thus providing oxygen and nutrients enabling an exponential growth. Vascular endothelial growth factor (VEGF) is the most potent angiogenic growth factor. Many studies have revealed the VEGF role in colon cancer, especially in angiogenesis stimulation (Ahluwalia et al., 2013). It was shown that VEGF generated by colon cancer cells could stimulate their growth directly through an autocrine mechanism which was independent of its primary function in induction of angiogenesis (Ahluwalia et al., 2014).

Several preclinical reports denote to development of resistance to anti-angiogenic therapy and the limited clinical effectiveness of anti-VEGF antibodies (Miyazaki et al., 2013) even in animal studies, it was shown that DNA vaccine against VEGF had an anti-angiogenic effect, leading to prolonged survival in mouse cancer model (Kyutoku et al., 2013).

C3a was shown to be a multifunctional proinflammatory mediator that can increase vascular permeability, be spasmogenic and chemotactic, and induce the release of pharmacologically active mediators from several cell types. The in vivo production of C3a may also initiate, contribute to or exacerbate the inflammatory reactions (Mandel et al., 2000). This complement may also be activated whenever the exposure to tumor antigens is happened (Winawer et al., 1993) and the translational application of proteomics technology can identify the pretreatment serum levels of C3a and C4a as predictive biomarkers of response too (Maher et al., 2011). The role of 8130 m/z C3a fragment as a potential marker for the early detection of HCV-related HCC was previously demonstrated (Kanmura et al., 2010).

As late diagnosis of colorectal carcinomas results in

a significant reduction of average survival times and yet, despite screening programs about 70% of tumors are detected at advanced stages (UICC III/IV) (Habermann et al., 2006), this study was undertaken to determine whether detection of colorectal cancer would be possible through identification of tumor specific protein biomarkers of VEGF and C3a in serum samples of patients in Fars province, southern Iran.

Materials and Methods

Study population

A total of 110 (66 men and 44 women) patients with CRC were enrolled. All patients underwent resection of primary CRC between June 2010 and June 2012 in Nemazee and Faghihi hospitals affiliated to Shiraz University of Medical Sciences of Shiraz, Southern Iran. None of patients had any history of receiving blood transfusion, radiotherapy or chemotherapy before surgery. A control group was recruited from normal healthy patients with a normal colonoscopy and without any history of cancer and any recent trauma or surgery. The control group included 21 persons (11 men and 10 women). This study was approved by the Ethics Committee of Shiraz University of Medical Sciences, and informed consent was received from each participant. Blood sample (5 ml) was provided from the peripheral vein of patients in two steps of 24 hours before and 24 hours after treatment measures in the three groups. Group A included patients who had just undergone surgery; Group B had undergone chemotherapy after surgery; and Group C had undergone chemotherapy and radiotherapy after surgery.

Samples were immediately centrifuged and their serum was stored at -70°C till experimental analysis. ELISA was undertaken using commercially available VEGF (R&D system kits, USA) and C3a (BD Bioscience, USA) kits according to the manufacturer's instructions. Each sample was evaluated in triplicate, and the mean value was used for analysis. If R2 of standard solution was less than 0.98, data from the plate were excluded.

Statistical analyses

Data of the case and control groups were compared using independent two samples T and Fisher Exact tests. Pearson and Kendalls correlation coefficient was determined. The relationship between age and VEGF level was calculated by Kendalls correlation coefficient. Values of $p < 0.05$ were considered statistically significant.

Results

The mean age of enrolled 110 patients (66 men and 44 women) with CRC was 53.9 ± 14.1 years and in the 21 control group was 47.5 ± 15.1 years (Range=23-73 years old). After treatment measures, the serum VEGF level was determined in just 75 patients due to death of other patients. Based on types of treatment for serum VEGF level as described in Table 1, a significant decrease was visible after treatment measures in groups A and B. The decrease was not statistically significant in Group C. The VEGF level in the control group was 310.3 ± 396.3 pg/

ml. No significant difference was found for VEGF level between patients and normal group before any treatment measure.

The VEGF value in males (43 patients) was 307.7±326.9 pg/ml before; and 290.6±330.4 pg/ml after treatment. In females (32 patients), the value was 466.6±523.3 pg/ml before; and 381.1±529.7 pg/ml after treatment. The difference was not statistically significant between two genders before and after treatment (p=0.1). In the control group, no significant difference was also noticed between two genders for VEGF level (p=0.2).

In patients with age less than 30 years, the serum VEGF level was 499.6±593.3 pg/ml, between 31 and 49 years was 350.5±360.8 and in patients older than 50 years was 369±435.1 pg/ml. The relationship between age and VEGF level which was calculated by Kendall's correlation coefficient which was +0.25 and was not statistically significant between various age groups (Pearson correlation coefficient=0.024) (p=0.4).

Regarding C3a level in 101 patients with CRC (59 males and 42 females), after treatment measure, the serum C3a level was determined in 76 patients due to death of other patients. Based on types of treatment for serum C3a level as described in Table 2, a significant decrease was visible after treatment measures in all groups (p≤0.0001). In male group, the C3a level was 2.5±0.5 ng/ml before and 1.5±0.5 ng/ml after treatment and the decrease was statistically significant (p=0.002). In female group, the C3a level was 2.4±0.4 ng/ml before and 1.4±0.5 ng/ml after treatment and the decrease was statistically significant (p=0.01). In control group, among males; C3a level was 2.5±0.4 ng/ml and in the females was 2.5±0.4 ng/ml. No significant difference was visible between the two genders (p=0.4).

In 7 patients with age <30 years, C3a level was 2.5±0.4 ng/ml, and in 30 patients between 31 and 49 years, it was 2.5±0.5 ng/ml and in 64 patients with age >50 years, was 2.4±0.4 ng/ml. There was no statistically significant

Table 1. Comparison of VEGF Level (pg/ml) among Colorectal Cancer Patients Regarding type of Treatment

Group	No.	VEGF		P value
		Before treatment	After treatment	
A	75	416.6±481.8	329.3±426.1	0.003
B	43	379.8±464.9	351.4±476.6	0.003
C	19	313.9±383.5	527.3±665.8	0.4

*Group A: The patients who had just undergone surgery; Group B: The patients who had undergone surgery + chemotherapy; Group C: The patients who had undergone surgery + chemotherapy + radiotherapy

Table 2. Comparison of Serum C3a Levels (ng/ml) between 3 Study Groups

Group	N	C3a		P value
		Before treatment	After treatment	
A	76	2.6±0.4	1.5±0.5	0.001
B	43	2.6±0.4	1.5±0.5	0.001
C	19	2.7±0.4	1.6±0.5	0.001

*Group A: The patients who had just undergone surgery; Group B: The patients who had surgery + chemotherapy; and Group C: The patients who had surgery + chemotherapy + radiotherapy

difference between these 3 age groups (p=0.4).

Discussion

CRC accounts for approximately 1,234,000 cancer cases each year and causes approximately 608,000 deaths each year responsible for the fourth most common cause of cancer mortality (Ferlay et al., 2011). So, early detection of this cancer is of great importance using approached such as fecal occult blood test (FOBT) (Pignone et al., 2002).

Vascular endothelial growth factor (VEGF) is the most potent angiogenic growth factor. Many studies have revealed the VEGF role in colon cancer, especially in angiogenesis stimulation. (Ahluwalia et al., 2014). As VEGF is expressed in a variety of human tumors, the determination of VEGF serum concentration could be a diagnostic method in cancer patients (Dube et al., 2007). In a multivariate analysis of molecular indicators for postoperative liver metastasis in colorectal cancer cases, it was demonstrated that the combination detection of CEA, VEGF, and EGFR might be an effective way to predict CRC liver metastasis (Qian et al., 2012) and also for negative expression of VEGF as an important prognostic factor in metastatic colorectal carcinoma (Kara et al., 2012).

In a study in Japan, it was shown that the survival rate in patients with low VEGF level was significantly more than those with high VEGF level and the response to chemotherapy was significantly more in patients with lower VEGF level in comparison with higher VEGF level (Mandel et al., 2000). In another study in Germany among 122 patients with CRC, VEGF level had a significant correlation with tumor size and patients with VEGF level greater than cut-off point had a poorer prognosis than those patients with VEGF level less than cut-off point (Dube et al., 2007). Another study in Italy among 81 patients with CRC revealed that there was a significant difference between serum VEGF level in patients with CRC and normal healthy group. For CEA level and age, there was no significant correlation between preoperative VEGF level and disease site, gender, tumor size and grade (Lieberman et al., 2000).

Other study in UK among 120 patients with CRC demonstrated that VEGF level in the control group was higher than the CRC patients which is different from the previous studies. There was no significant relationship between VEGF level and variables such as age, sex and lymph node involvement (Smith, 2001). In Lebanon among 36 patients with CRC, no significant difference was noticed between for VEGF level between normal and patients group and also between different age groups and both genders (Greene, 2002). Also in another study, it was shown that Serum LDH and neutrophil levels were the main prognostic factors in predicting survival, followed by progression free survival and VEGF (Cetin et al., 2012).

In another study in Japan among 127 CRC patients, the VEGF level was not significantly different between patients and healthy controls, however, elevated level of VEGF-C showed a correlation with increasing tumor stage while high levels of VEGF denoted to a poor outcome. No correlation was observed between VEGF level and

age or sex (Pignone et al., 2002). In another study in UK, a decrease in VEGF level was associated with a lower survival rate, but the difference was not statistically significant (Smith, 2001). In Korea among 143 patients with CRC, high VEGF levels demonstrated a significant correlation with a lower survival rate but, there was no relationship between VEGF level and age or sex (Greene, 2002).

In our study, no significant difference was found for VEGF level between patients and normal subjects before treatment similar to findings of another study in Lebanon (Greene, 2002) and in Japan (Mandel et al., 2000), but was different to results of other studies in Japan (Pignone et al., 2002) and in Germany (Dube et al., 2007).

We showed a statistically significant difference for VEGF level in all age groups in group B who underwent chemotherapy after surgery. Radiotherapy after surgery was not as effective as chemotherapy in lowering the VEGF level that may be due to a decline in immunity activity of the patients in response to radiotherapy.

C3a is a complement of acute phase which has association with cardiovascular disease, obesity, diabetes, and dyslipidemia and is produced mainly in the hepatocytes and adipocytes (Levin et al., 2003). This complement can also be activated whenever the exposure to tumor antigens is happened (Levin et al., 2003), so an increase of this complement may be beneficial in diagnosis of CRC in earlier stages while a decrease in its level may be a sign of response to a specific therapeutic measure (Levin et al., 2003).

The relationship between C3a level and clinic pathological factors was less described. In 2006, Habermann et al. determined the C3a-desArg level in 59 patients with colorectal carcinoma. They showed that C3a-desArg level was significantly higher in patients with colorectal adenomas and carcinomas in comparison to normal group (Levin et al., 2003).

Our study revealed that serum C3a level in CRC patients (2.5 ± 0.3 ng/ml) was obviously higher than normal subjects (< 2.20 U/ml). So, it was shown that serum C3a level may be used as a tumor marker in CRC. The same findings were previously reported (Levin et al., 2003). In our study, a significant difference was reported for the first time for C3a level between the 3 different treatment groups. Our results denoted to a significant difference between serum C3a level before and after treatment in all three treatment groups. Our study indicates that C3a is an important clinical parameter for early diagnosis of CRC as serum C3a level was significantly lower in patients who received radiotherapy and chemotherapy after surgery when compared to healthy controls. We can conclude that both VEGF and C3a serum levels can be beneficial in assessment of therapy of CRC patients especially in earlier stages.

Conflict of interest: No competing interest.

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