Expression of VEGF-C and its association with clinicopathological features among patients with gastric cancer undergoing gastric surgery at Tongji medical hospital, China.

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Abstract

Objectives: Gastric cancer shows epidemiological variability according to geographic locations, these differences have been demonstrated both regionally and within countries. There is no baseline protocol regarding using VEGF-C as tumor marker in local setting therefore it was necessary to conduct this study, the current study conducted to analyze the correlation exists between VEGF-C and clinicopathological parameters in patients with gastric cancer undergoing a surgical procedure at Tongji medical hospital.

Results: 161 samples analyzed were from Chinese patients who had undergone either palliative or curative surgical procedure between October 2015 and September 2017 at Tongji medical hospital. Immunohistochemistry was method was used to analyses presence of VEGF-C in tissues. Among 161 gastric cancer patients, 101 (62.7%) gastric samples showed strong VEGF-C expression and 60 (37.3%) gastric samples showed weak VEGF-C expression. Using multivariate logistic regression analysis, tumor grade, invasion of lymph nodes and TNM staging were statistically significantly found to associate with strong expression of VEGF-C. VEGF-C strong expression was significantly seen more in adenocarcinoma of gastric cancer, and Therefore, VEGF-C may be used as a biological marker for assessing the biological characteristics of gastric.

Introduction

Gastric cancer (GC) exhibits epidemiological variability per geographic location, and these differences have been demonstrated both regionally and within countries [1]. Gastric cancer is positioned as the third most frequent cause of death in patients with cancer and is ranked fifth in the common types of cancer globally [2]. Despite the dramatic decrease in gastric cancer’s incidence in other countries, China has seen an increase in both younger and older groups, though there is a decreased incidence in women [3]. A study was done, and it showed that the age of onset of developing gastric cancer among the Chinese population is earlier than that of Westerners. Also, the mortality and incidence of gastric cancer in China varies from province to province. (It is more common in the north than the south.) [4,5]. The disease is curable when it presents itself at an early stage, at which point surgical manipulations are still possible, and radiotherapy or adjuvant chemotherapy is part of curative
management [6]. However, patients with late-stage gastric cancer are left with a poor prognosis despite the sophisticated and modern technologies used in the management of gastric cancer [7]. In gastric cancer patients, VEGF subfamilies’ status has been implicated in correlating with various clinicopathological parameters. Also, another study was conducted and showed that strong expression of VEGF-C was significantly associated with lymphatic involvement, TNM staging and vascular involvement in gastric cancer (p<0.01). Furthermore, the same study showed that VEGF-C was not seen to be associated with gender, age at time of surgery, tumor size, or tumor location. [12]. Similar studies demonstrated that VEGF-C strong expression on gastric tissue was positively associated with lymphatic system invasion (lymph tissues and lymph nodes invasion) [13-15]. Also, expression of vascular endothelial growth factor-C seen in an earlier stage of gastric cancer was significantly correlated with lymphatic invasion, and so can be clinically helpful to predict the outcomes of minimal or more extensive surgical manipulations and nodal clearance in GC patients [16]. This study conducted to find out expression of VEGF-C and its association with clinicopathological features among patients with gastric cancer undergoing gastric surgery at Tongji Medical Hospital, (TMH). Findings from this study can be used to establish management protocols of VEGF-C, as a molecular tumor marker in patients with gastric cancer at Tongji hospital

Methods

Study design.

This was analytical cross-section study among patients undergoing surgery at TMH in a period October 2015 to September 2017. This was conducted in oncology and pathology department at Tongji medical hospital in Wuhan,Hubei,CHINA; with capacity of 4000-6000 beds.

Specimen Collection

Tissue samples analyzed in this study were collected from Chinese patients (n=161) who had undergone either a palliative surgical procedure or a therapeutic surgical procedure between October 2015 and September 2017 at Tongji medical hospital (Hubei, Wuhan, China). Patient data retrieved were age, sex, date of birth, history of smoking and alcohol consumption, TNM stage, anatomical location of tumor, histopathological pattern of the cancer, tumor grade, Bormann’s classification,
vascular and lymphatic involvement by tumor, tumor grading (well differentiated, moderate differentiated, and poorly differentiated), and positive VEGF status. TMN staging criteria were used according to the American Joint Committee on Cancer (AJCC) [17]. Tissues were then taken for further analysis of expression of VEGF-C.

Immunohistochemical analysis of VEGF-C

Immunohistochemical staining was done using 4-μm thick paraffin-embedded sections and treated with 0.3% hydrogen peroxide at room temperature for ten minutes. These sections were heated in a solution (pH 6.0) of 1% mmol/L of trisodium citrate in a microwave for the extraction of antigen. Incubation of sections was done at a humidity condition of 4°C of primary antibodies (mouse monoclonal VEGF-C antibody) [1:100, DAKO]. Chosen slides were then washed three times in 0.1 mmol/L PBS for about 2 minutes and then incubated at standard room temperature with horseradish peroxidase (Envision, DAKO) conjugated mouse secondary antibody for 30 minutes. Negative control for VEGF-C detection was done using normal rabbit antibody IgG after development was done with 3,3′-Diaminobenzidine.

VEGF-C scoring according to immunohistochemistry

Two pathologists who were unaware of the clinical outcome of the patients did a pathological analysis of immunohistochemistry. The analysis of the staining was exclusively restricted to tumor cell reactions. VEGF-C stained results were classified, according to the intensity of color and percentage of epithelial cells that showed specific immunoreactivity, into 0, 1, 2, and 3 designations. If the summation of intensity and percentage was in the 0-2 ranges, it was considered a weak expression of VEGF-C, while a range of 3-6 was regarded as a strong expression of VEGF-C [18, 19, 20].

Data collection

All demographic data and data of tumor characteristics plus results of VEGF-C status after immunochemistry analysis were recorded into pre-coded questionnaire accordingly. All this were done by BM and XY, then data were entered into Stat software version 13.v

Statistical data analysis

Data were entered into Excel, imported for analysis into Stat software version 13.v, and analyzed
according to the objectives. Continuous variables were analyzed using mean and standard deviation. The significance of the associations between the predictor and outcome variables was calculated using a Chi-square test on the categorical variables. The odds ratio was used to test the strength of the association between predictor and outcome variables. A p-value of less than 0.05 was seen as significant. Predictor variables, which were found to be significant on univariate analysis, were subjected to multivariate logistic regression analysis to test the significance of the association between these variables and the outcome.

Results

Populations studied and demographic characteristics

During the study, 1260 patients underwent gastric surgeries at TMH. Out of these, 268 were found to be positive for VEGF. Of these, 107 were excluded from the study because of failure to meet inclusion criteria. Thus, 161 patients were studied. Among 161 gastric cancer patients, 101 (62.7%) gastric samples were regarded as strong VEGF-C expression group and 60 (37.3%) as weak VEGF-C expression group. (Figure1). Patients' ages ranged from 28 years to 81 years. The mean was 58 years, and the standard deviation was 10.44 years. The distribution between females and males was 29 females (18%) and 132 males (82%).

Figure 1. Flow chart diagram.

Table 1 below shows clinical characteristics associated with expression of VEGF-C according to univariate analysis. There was no statistically significant association of VEGF-C expression in relation to age and sex. There was also no significant association between VEGF-C, smoking and alcohol intake (Table 1).

Table 1 Demographic characteristics correlated with expression of VEGF- C according to univariate analysis

Table 2 below shows Tumor characteristics associated with VEGF -C

Expression on univariate analysis. There was a statistically significant association between strong VEGF-C expression and tumor grade. Strong VEGF-C expression were 4.88 more likely to be found in poorly differentiated gastric tumors than tumors with weak VEGF-C expression.
VEGF-C expression was also significantly associated with lymphatic involvement. Tumors with strong VEGF-C expression were 3.34 more likely to involve lymphatic system than those with weak VEGF-C expression. However, no statistical significant association was found between tumor location, tumor size, tumor stage, vascular involvement, histological classification and VEGF-C expression (Table 2).

Table 2. Tumor characteristics related to VEGF C expression on univariate analysis.

Multivariate logistic regression

Multivariate logistic regression analysis revealed that VEGF-C expression were significant predictors for tumor grade (Adjusted odds ratio(AOR) 7.78, 95% confidence interval (CI) ;2.78 to 9.29, P-value = 0.001) , lymph node invasion (AOR 18.11;95% CI 4.32 to 22.81, P =0.013) and TNM staging (AOR 4.12,95% CI;2.30 to 15.92;P = 0.005 ) in patient with gastric cancer (Table S1).

Table S1. Multivariate logistic regressions

Discussion

This study has shown that vascular endothelial growth factor C has correlations with some clinicopathological parameters, and also has a negative relationship with some of the clinicopathological settings. In the present study, VEGF-C expression was found to be significantly associated with lymphatic nodal invasion, and this result is supported by research done by Cristina et al. [21]. Also, the present study results showed there was no association found between the size of gastric tumor, gender, age, tumor location, and VEGF-C strong-expression. This finding is in agreement with Lin Wang et al. [22]; although there was a significant correlation between VEGF-C strong-expression, TNM staging, tumor grading, and lymphatic node invasion. This is in agreement with another study [23], and the reason for this is the fact that VEGF-C has been implicated to be a lymphangiogenic factor, but exact mechanisms are not precise [24]. Previous clinical studies have established that strong expression of VEGF-C is well established in primary tumors and correlates with increased migration of tumor cells to regional lymph nodes in different human carcinomas (25-32). Yonemura et al. [33] and Kabashima et al. [34] showed that VEGF-C expression was correlated with lymphatic nodal invasion. In our study, tumor grading, lymph node invasion and TNM stage in multivariate logistic regression analysis were found to be statistically significantly associated with
Conclusion

In the current study, VEGF-C expression was seen significantly more in adenocarcinoma histological type of gastric cancer. In multivariate analysis, VEGF-C was found to be statistically significantly associated with tumor grade, lymph node invasion and TNM stage. In conclusion, VEGF-C may be of use as a tumor marker for assessing the biological characteristics in gastric cancer patients presenting with adenocarcinoma types of gastric malignancies.

Limitations of the study:

1. The study population included only those of Asian descent.
2. Lauren classification was not used in this study.

Abbreviations

**AJCC:** American Joint Committee on Cancer  
**GC:** gastric cancer  
**PBS:** phosphate buffered saline  
**TMH:** Tongji Medical Hospital  
**TNM:** tumor, lymph node and metastasis  
**VEGF:** vascular endothelial growth factor  
**VEGF-C:** vascular endothelial growth factor C

Declarations

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Conflicts of interest

There were no conflicts of interest.

Availability of data and materials

The datasets analyzed during the current study are available from the corresponding author upon
reasonable request.

Ethics approval and consent to participate

Written approval to conduct the study was obtained from Tongji ethical committee.

Consent for publication

Written consent for publication was obtained from respective patients.

Author’s contribution

BM participated in specimen’s collection and XL participated in paper designing while XY participated in data analysis and manuscript writing. All authors have read and approved this manuscript.

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PhD, and Nadine Ectors, MD, PhD**Expression of Carbonic Anhydrase IX (CA IX), a Hypoxia-Related Protein, Rather Than Vascular-Endothelial Growth Factor (VEGF), a Pro-Angiogenic Factor, Correlates With an Extremely Poor Prognosis in Esophageal and Gastric Adenocarcinomas

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Tables

Table 1. Demographic characteristics correlated with expression of VEGF-C according to univariate analysis.

| Predictor variable | VEGF C Expression (%) | Un-adjusted OR (95% CI) | P-Value |
|--------------------|------------------------|--------------------------|---------|
|                    | Strong 101 (n) | Weak 60 (n) | 
| Age                | >60 years | 55 (59.8%) | 37 (40.2%) | 1 | 0.980 (0.42-9.67) | 0.577 |
|                    | <60 years | 46 (66.7%) | 23 (33.3%) | 1 | 0.833 (0.34-1.88) | 0.688 |
| Sex                | Male | 86 (65.2%) | 46 (34.8%) | 1 | 0.833 (0.34-1.88) | 0.688 |
|                    | Female | 15 (51.7%) | 14 (48.3%) | 4.46 (0.34-5.11) | 0.066 |
| Smoking            | Yes | 14 (22.9%) | 47 (77.1%) | 1 | 0.654 (0.405-2.32) | 0.614 |
|                    | No | 87 (87.0%) | 13 (13.0%) | 1.66 (0.499-4.10) | 0.752 |
| Alcohol intake     | Yes | 31 (77.5%) | 9 (22.5%) | 1 | 0.44 (0.26-6.64) | 0.074 |
|                    | No | 70 (57.9) | 51 (42.1%) | 1 | 0.44 (0.26-6.64) | 0.074 |

Table 2. Tumor characteristics related to VEGF C expression on univariate analysis.

| Predictor variable | VEGF C Expression (%) | Un-adjusted OR (95% CI) | P-Value |
|--------------------|------------------------|--------------------------|---------|
|                    | Strong 101 (n) | Weak 60 (n) | 
| Tumor location     | Proximal | 70 (74.5%) | 24 (25.5%) | 1 |
|                    | Distal | 31 (46.2%) | 36 (53.8%) | 4.46 (0.34-5.11) | 0.066 |
| Bormann classification | Fungating | 2 (50%) | 2 (50%) | 1 |
|                    | Infiltrative | 9 (52.9%) | 0.44 (0.26-6.64) | 0.074 |
| Tumor grade               | Ulcerating | Normal | HR (95% CI) | p-value |
|--------------------------|------------|--------|-------------|---------|
| Well differentiated      | 3 (27.3%)  | 8 (72.7%) | 1           | 0.076   |
| Moderate differentiated  | 16 (40%)   | 24 (60%) | 0.56 (0.11-8.14) | 0.714   |
| Poor differentiated      | 82 (74.5%) | 28 (25.5%) | 4.88 (2.55-9.89) | 0.005   |

| Histological classification | Aden carcinoma | Mixed type | HR (95% CI) | p-value |
|-----------------------------|----------------|------------|-------------|---------|
| Aden carcinoma              | 12 (21.1%)     | 45 (78.9%) | 1           | 0.714   |
| Mixed type                  | 89 (85.6%)     | 15 (14.4%) | 8.16 (0.84 - 3.78) | 0.078   |

| Tumor size | Ulcerating | Normal | HR (95% CI) | p-value |
|------------|------------|--------|-------------|---------|
| <3cm       | 3 (56.5%)  | 20 (43.5%) | 1           | 0.078   |
| >3cm       | 98 (48.6%) | 40 (51.4%) | 3.16 (0.78-6.34) | 0.062   |

| Vascular involvement | Ulcerating | Normal | HR (95% CI) | p-value |
|----------------------|------------|--------|-------------|---------|
| Yes                  | 61 (85.9%) | 10 (14.1%) | 1           | 0.062   |
| No                   | 40 (44.4%) | 50 (55.6%) | 4.33 (0.08-16.45) | 0.001   |

| Lymphatic involvement | Ulcerating | Normal | HR (95% CI) | p-value |
|-----------------------|------------|--------|-------------|---------|
| Yes                   | 7 (58.3%)  | 5 (41.7%) | 1           | 0.001   |
| No                    | 94 (63.1%) | 55 (36.9%) | 3.34 (1.65-7.20) | 0.001   |

| TNM stage |
|-----------|
|     | Cases 1 | Cases 2 | p     |
|-----|---------|---------|-------|
| I-II| 11 (33.3%) | 22 (66.7%) | 1     |
| III-IV | 90 (70.3%) | 38 (29.7%) | 0.60 (0.27-0.94) | 0.005 |

**Figures**
Figure 1. Flow chart diagram.
Supplementary Files

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Table S1.docx