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

BRIAN MAWALLA (bsiza@yahoo.com)
Catholic University of Health and Allied Sciences

Xiaoxiao Luo
Huazhong University of Science and Technology Tongji Medical College

Yuan Xianglin
Huazhong University of Science and Technology Tongji Medical College

Research note

Keywords: gastric cancer, VEGF-C, clinicopathological features

Posted Date: May 11th, 2020

DOI: https://doi.org/10.21203/rs.2.20208/v4

Abstract

Objectives:

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

Results: 161 samples were analyzed from Chinese patients who had undergone either palliative or curative surgical procedure between October 2015 and September 2017 at Tongji medical hospital. Immunohistochemistry was used to analyses presence of VEGF-C in tissues. Among the 161 samples from gastric cancer patients, 101 (62.7%) samples showed strong VEGF-C expression and 60 (37.3%) samples showed weak VEGF-C expression. Multivariate logistic regression analysis was used to show statistical significance with strong expression of VEGF-C for tumor grade, invasion of lymph nodes and TNM staging. Strong expression of VEGF-C was significantly more likely in adenocarcinoma of gastric cancer.

VEGF-C may be used as a biological marker for assessing the biological characteristics of gastric cancer.

Introduction

Gastric cancer (GC) exhibits epidemiological variability by 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 most 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 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 are 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 demonstrated correlation with various clinicopathological parameters. Another study 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, invasion

of lymph tissues and lymph nodes [13-15]. In addition, expression of vascular endothelial growth factor-C seen in an earlier stage of gastric cancer was significantly correlated with lymphatic invasion, thus it can be clinically helpful to predict the outcomes of minimal or more extensive surgical manipulations and nodal clearance in GC patients [16].

This study was 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 an analytical cross-sectional study among patients undergoing surgery at TMH during the period of October 2015 to September 2017. This study was conducted in the oncology and pathology department at Tongji medical hospital in Wuhan, Hubei, China, a department with the capacity of 4,000-6,000 beds.

Sampling:

Convenient sampling was method deployed in this study. Tissue samples of patient who attended at Tongji Medical Hospital in oncology and pathology department were collected after filling precoded questionnaire. A precoded questionnaire was used to obtain information of the participants .The questionnaire was divided into demographic characteristics, clinical characteristics and staining analysis. All tissue samples of patients above 19 years of age of both sexes who underwent curative or palliative surgery were included into the study. Patients whose biopsy sample for histopathological analysis could not be obtained and did not consent for the study were excluded from the study. Their samples were taken for further analysis of VEGF-C by immunohistochemistry. To ensure internal validity of the study precoded questionnaire was tested. XY and BM were trained how to fill the questionnaire.

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 included: 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 a 4-µm thick paraffin-embedded section and then 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].

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. For categorical variables frequency and proportion was used. Chi-square test was used for comparison between the categorical variables groups. The odds ratio was used to measure strength of the association between predictor and outcome variables. A p-value of less than 0.05 was reported 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. Adjusted odds ratio presented at 95% Confidence Interval and P- value less than 0.05 were considered statistically significant.

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 the 161 gastric cancer patients, 101 (62.7%) gastric samples were regarded as strong VEGF-C expression group and 60 (37.3%) were regarded as weak VEGF-C expression group (Figure 1). 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%).

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).

There was a statistically significant association of VEGF -C expression and Bormann classification. Tumors with strong VEGF-C expression were 4.86 more likely to be ulcerative type of gastric cancer than those presented with weak VEGF-C expression (OR: 4.86,CI; 1.10-7.11, P=0.013). There was a statistically significant association between strong VEGF-C expression and tumor grade (P=0.005). Strong VEGF-C expression was 4.88 more likely to be found in poorly differentiated gastric tumors than tumors with weak VEGF-C expression (OR=4.44,95% CI; 2.55-9.89). VEGF-C expression was also significantly associated with lymphatic involvement (P= 0.001). Malignancies with strong VEGF-C expression were 3.34 more likely to involve lymphatic system than those with weak VEGF- C expression (OR=3.34, 95% CI; 1.65 -7.20).

VEGF- C expression was negatively associated with TNM stage (P=0.005). Gastric tumors with strong VEGF-C expression were less likely to develop advanced (TNM stage III- IV) gastric tumors than those with weak VEGF-C expression (OR=0.60,95% CI; 0.27- 0.94).

However, no statistically significant association was found between tumor location, tumor size, tumor stage, vascular involvement, histological classification and VEGF-C expression (Table 2).

Multivariate logistic regression

Multivariate logistic regression analysis revealed that VEGF-C expression

as a significant predictor 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).

Discussion

VEGF is an important angiogenesis facilitating factor and has been elevated in most malignant tumor tissues. VEGF can also regulate characteristics of metastatic tumor, which makes it an important focus in determining gastric tumors prognosis and treatment.

This study demonstrates that vascular endothelial growth factor C does correlate with some clinicopathological parameters; and it 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]. Yong Dai et al in his study also reported correlation of VEGF-C and lymph node metastasis in gastric cancer and recommended the use of VEGF-C in evaluating prognosis in gastric cancer patients [22]. Lin Wang et al also reported a significant correlation between VEGF-C strong-expression, TNM staging, tumor grading, and lymphatic node invasion [23]. This is also supported by another study [24], VEGF-C has been implicated to be a lymphangiogenic factor, but exact mechanisms are not precise [25]. 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 (26-33). Yonemura et al. [34] and Kabashima et al. [35] showed that VEGF-C expression was correlated with lymphatic nodal invasion.

Also, this study results showed there was no association found between the size of gastric tumor, gender, age, and VEGF-C strong-expression. This finding is in agreement with Lin Wang et al. [23]. Yong Dai et al. [22] also reported no significant correlation of VEGF-C and age, sex. Unlike the present study which reported statistically significant association of VEGF-C expression and tumor grade also Yong Dai et al. [22] found no significant difference of VEGF-C and tumor grade. This study found no association between VEGF-C expression and tumor location. This finding is consistent with Bin Wang et al. [36], in his study he demonstrated no significant association of proximal, middle and distal gastric cancer and VEGF-C expression.

In our study, tumor grading, lymph node invasion and TNM stage in multivariate logistic regression analysis were found to be statistically significantly associated with strong VEGF-C expression.

Conclusion

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
2. Lauren classification was not used in this

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

Funding

There was no funding available for this study

Acknowledgments

The authors acknowledge the tireless technical support given by colleagues in the departments of oncology and pathology and the surgical unit.

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

Prior informed verbal consent was obtained from the participants and written approval to conduct the study was obtained from Tongji ethical committee.

Consent for publication

Not applicable

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.

References

1. Ferlay J, Shin HR, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010; 127:2893.
2. Torre LA, Bray F, Siegel RL, Ferlay J, Lortet-Tieulent J. Global cancer statistics, 2012.CA Cancer J clin.2005 Mar, 65(2): 87-108.
3. Jemal A, Siegel R, Ward E, et al. Cancer statistics, 2006. CA Cancer J Clin 2006; 56:106.
4. Wong BC, Ching CK, Lam SK, et al. Differential north to south gastric cancer-duodenal ulcer gradient in China. China Ulcer Study Group. J GastroenterolHepatol 1998; 13:1050.
5. Chen JS, Campbell TC, Li JY, et al. Life-style and Mortality in China. A Study of the Characteristics of 65 Chinese Counties, Oxford University Press, Oxford 1990.
6. Cunningham D, Allum WH, Stenning SP, et al.: Perioperative chemotherapy versus surgery alone for resectable gastroesophageal cancer. N Engl J Med. 2006, 355:11–20.
7. Lordick F, Allum W, Carneiro F, Mitry E, Tabernero J, Tan P, Van Cutsem E, van de Velde C, Cervantes A: Unmet needs and challenges in gastric cancer: the way forward. Cancer Treat Rev. 2014, 40:692–700.
8. Wei B, Tai Y, Tong H, Wen S, Tang S, et al. Correlations between VEGF-A expression and prognosis in patients with gastric adenocarcinomas. Int J clinExp Pathol.2017; 10(8): 8461-8469.
9. Ding S, Li C, Lin S, Han Y, Yang Y, et al. Distinct roles of VEGF-A, Distinct roles of VEGF-A and VEGF-C in tumor metastasis of gastric carcinoma. Oncol Rep.2007 Feb;(2): 369-375.
10. Tanigawa N, Amaya H, Matsumura M, Shimomatsuya T. Correlation between expression of vascular endothelial growth factor and tumor vascularity, and patient outcome in human gastric carcinoma. JClin Oncol.1997 Feb;15:826-832
11. N. Chen, F. J. Hsieh, Y. M. Cheng et al., "The significance of placenta growth factor in angiogenesis and clinical outcome of human gastric cancer," *Cancer Letters*, vol. 213, no. 1, pp. 73–82, 2004.
12. Takahashi A, Kono K, Itakura J, Amemiya H, Feng Tang R, et al. Correlation of vascular endothelial growth factor-C expression with tumor-infiltrating dendritic cells in gastric cancer. Oncology. 2002; 62(2): 121-127.
13. Shi H, Xu JM, Hu NZ, Xie HJ. Prognostic significance of expression of cyclooxygenase-2 and vascular endothelial growth factor in human gastric carcinoma. World J Gastroenterol 2003; 9: 1421-1426.
14. Lauren, "The two histological main types of gastric carcinoma: diffuse and so-called intestinal type carcinoma," ActaPathologicaetMicrobiologicaScandinavica, vol. 64, pp. 31–49, 1965.
15. Kinoshita J, Kitamura K, Kabashima A, Saeki H, Tanaka S, et al. Clinical significance of vascular endothelial growth factor-C (VEGF-C) in breast cancer. Breast Cancer Res Treat. 2001 Mar; 66(2): 159–164.
16. Ann Driessen, MD, PhD, * Willy Landuyt, PhD, † Sylvia Pastorekova, PhD,‡ Johnny Moons, RN, MScN,Laurence Goethals, MD,§ Karin Haustermans, MD, PhD,§ Philippe Nafteux,

MD,FreddyPenninckx, MD, PhD,¶ KarelGeboes, MD, PhD,** Toni Lerut, MD, 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

17. Edge SB, Byrd DR, Compton CC, Fritz AG, Greene FL, Trotti A, editors. AJCC cancer staging manual (7th ed). New York, NY: Springer; 2010.
18. Igarashi H, Sugimura H, Maruyama K, et al. Alteration of immunoreactivity by hydrated autoclaving, microwave treatment, and simple heating of paraffin-embedded tissue sections. APMIS. 1994; 102:295–307.
19. Baillie R, Carlile J, Pendleton N, et al. Prognostic value of vascularity and vascular endothelial growth factor in non-small cell lung cancer. J ClinPathol. 2001; 54:116–120.
20. Shimizu M, Saitoh Y, Itoh H. Immunochemistry staining of Ha-ras oncogene in normal benign and malignant pancreatic tissue. Hum pathology 1990,21(6): 607-612
21. Cristina Angelescu, Florin Burada, Mihailoana, RaduAngelescu, Emil Moraru, et al. VEGF-A and VEGF-B mRNA expression in gastro-oesophageal cancers. Clinical and Translational Oncology. 2013 Apr; 15(4): 13–320.
22. Yong Dai, Jinbo Jiang, Yanlei Wang, Zutao Jin, Sanyuan Hu, The correlation and clinical implication of VEGF-C expression in microvascular density and lymph node metastasis of gastric carcinoma
23. Lin Wang 1, Hai-Gang Li 1 , Jian-Ming Wen 3 , Ting-Sheng Peng 3 , Hong Zeng 1 , and Ling-Yun Wang 2Expression of CD44v3, erythropoietin and VEGF-C in gastric adenocarcinomas: correlations with clinicopathological features
24. Takahashi A, Kono K, Itakura J, Amemiya H, Feng Tang R, et al. Correlation of vascular endothelial growth factor-C expression with tumor-infiltrating dendritic cells in gastric cancer. Oncology. 2002; 62(2): 121-127.
25. Jia L, Du Y, Li T, Lv Y, Wang Y, Zhang Y, Zhou X and Liu W. Differential expression of vascular endothelial growth factor-A, -C and -D for the diagnosis and prognosis of cancer patients with malignant effusions. OncolLett 2015; 10: 667-674.
26. Maeda K, Chung YS, Ogawa Y, Takatsuka S, Kang SM,et al. Prognostic value of vascular endothelial growth factor expression in gastric carcinoma. Cancer. 1996 Mar; 77(5): 858-863.
27. Saito H, Tsujitani S, Kondo A, Ikeguchi M, Maeta M, et al. Expression of vascular endothelial growth factor correlates with hematogenous recurrence in gastric carcinoma. Surgery. 1999 Feb; 125(2): 195-201.
28. Kakeji Y, Koga T, Sumiyoshi Y, Shibahara K, Oda S, et al. Clinical significance of vascular endothelial growth factor expression in gastric cancer. J ExpClin Cancer Res. 2002 Mar; 21(1): 125-129.
29. Karpanen T, Egeblad M, Karkkainen MJ, Kubo H, Ylä-Herttuala S, et al. Vascular endothelial growth factor C promotes tumor lymphangiogenesis and intralymphatic tumor growth. Cancer Res. 2001 Mar; 61(5): 1786-1790.

30. Mattila MM, Ruohola JK, Karpanen T, Jackson DG, Alitalo K, et al. VEGF-C induced lymphangiogenesis is associated with lymph node metastasis in orthotopic MCF-7 tumors. Int J Cancer. 2002 Apr; 98(6): 946-951.
31. Stacker SA, Caesar C, Baldwin ME, Thornton GE, Williams RA, et al. VEGF- D promotes the metastatic spread of tumor cells via the lymphatics. Nat Med. 2001; 7(2): 151-152.
32. Skobe M, Hawighorst T, Jackson DG, Prevo R, Janes L, et al. Induction of tumor lymphangiogenesis by VEGF-C promotes breast cancer metastasis. Nat Med. 2001; 7(2): 192-198.
33. Cristina Angelescu, Florin Burada, Mihailoana, RaduAngelescu, Emil Moraru, et al. VEGF-A and VEGF-B mRNA expression in gastro-oesophageal cancers. Clinical and Translational Oncology. 2013 Apr; 15(4): 13–320.
34. Yonemura Y, Endo Y, Fujita H, Fushida S, Ninomiya I, Bandou E, et al. Role of vascular endothelial growth factor C expression in the development of lymph node metastasis in gastric cancer. Clin Cancer Res. 1999; 5:1823-9.
35. Kabashima A, Maehara Y, Kakeji Y, Sugimachi K. Overexpression of vascular endothelial growth factor C is related to lymphogenous metastasis in early gastric carcinoma. Oncology 2001; 60:146–50.
36. Bin wang,Peng dong Xi-zhen wangshi Zhuang zhang ,Xin cheng, Proximal compared with middle and distal gastric carcinoma: Expression of VEGF and VEGF-C

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	
	<60 years	46 (66.7%)	23 (33.3%)	0.980 (0.42-9.67)	0.577
Sex					
	Male	86 (65.2%)	46 (34.8%)	1	0.488
	Female	15 (51.7%)	14 (48.3%)	0.833 (0.34-1.88)	0.688
Smoking					
	Yes	14 (22.9%)	47 (77.1 %)	1	
	No	87 (87.0%)	13 (13.0 %)	0.654 (0.405-2.32)	0.614
Alcohol intake					
	Yes	31 (77.5%)	9 (22.5%)	1	
	No	70 (57.9)	51 (42.1%)	1.66 (0.499 -4.10)	0.752

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

Predictor variable	VEGF C Expression (%)		Un-adjusted	P-Value
	Strong	Weak	OR (95% CI)	
	101 (n)	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%)	8 (47.1%)	0.44 (0.26-6.64)	0.074
Ulcerating	90 (64.3%)	50 (35.7%)	4.86 (1.10-7.11)	0.013
Tumor grade				
Well differentiated	3 (27.3%)	8 (72.7%)	1	
Moderate differentiated	16 (40%)	24 (60%)	0.56 (0.11-8.14)	0.076
Poor differentiated	82 (74.5%)	28 (25.5%)	4.88 (2.55-9.89)	0.005
Histological classification				
Aden carcinoma	12 (21.1%)	45 (78.9%)	1	
Mixed type	89 (85.6%)	15 (14.4%)	8.16 (0.84 – 3.78)	0.714
Tumor size				
<3cm	3 (56.5%)	20 (43.5%)	1	
>3cm	98 (48.6%)	40 (51.4%)	3.16 (0.78-6.34)	0.078
Vascular involvement				
Yes	61 (85.9%)	10 (14.1%)	1	
No	40 (44.4%)	50 (55.6%)	4.33 (0.08-16.45)	0.062
Lymphatic involvement				
Yes	7 (58.3%)	5 (41.7%)	1	
No	94 (63.1%)	55(36.9%)	3.34 (1.65-7.20)	0.001
TNM stage				
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

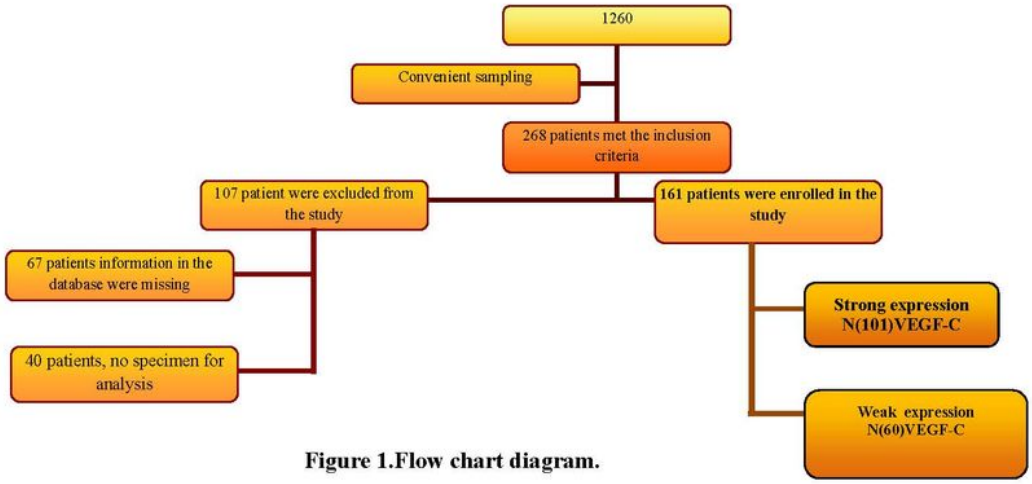

Figure 1.Flow chart diagram.

Figure 1

Flow chart diagram

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- TableS1.docx