Study of the Vascular Endothelial Growth Factor (VEGF) Expression and Microvascular Density (MVD) in Primary Colorectal Cancer Specimens

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Objective: *Determine the relationship between vascular endothelial growth factor (VEGF) expression and microvascular density (MVD) in primary colorectal cancer specimens including the prognostic value by evaluating the correlation between various common reported prognostic histopathologic indictors and these two angiogenic parameters. The Inter-observer reliability on VEGF and MVD measurement was also determined.*
Material and Method: *Anti-VEGF and anti-factor CD34 monoclonal antibodies immunohistochemical staining was performed in 40 randomly selected formalin-fixed paraffin-embedded colorectal cancer specimens of non-stage-IV patients who underwent curative resection using. Immunoreactive in 25% or more carcinoma cells was categorized as positive. The intensity of VEGF expression was graded in a semiquantitative fashion, ranging from 0 to 2 Tumor MVD was determined by counting any endothelial cells stained with CD34 per two randomly selected fields at x200 magnification in each slide. The correlation between VEGF expression and MVD was evaluated. Inter-observer agreement was assessed by comparing the results of VEGF and MVD measurements made by two pathologists.*
Results: *A moderate correlation was found between the percentage of positive immunoreactive cells and the intensity of VEGF immunoreactive staining (correlation value of 0.436, $p < 0.05$). MVD was found having no correlation with both the percentage of positive immunoreactive cells and intensity of VEGF immunoreactive staining (the correlation value of -0.056, $p = 0.732$ and 0.108, $p = 0.506$, respectively). Neither MVD nor VEGF expression in primary colorectal cancer tissue was found having a significant correlation with any common reported prognostic histopathologic indictors. In counting CD34-stained endothelial cells, this study revealed a high intra-observer correlation coefficient of 0.886 (95% CI: 0.715-0.955) for the first pathologist and 0.913 (95% CI: 0.782-0.965) for the second. High inter-observer reliability was found in both MVD and VEGF measurement with a substantial agreement (agreement: 95%, kappa = 0.643) between the two pathologists.*
Conclusion: *In primary colorectal cancer tissues, there was no significant relationship between MVD and VEGF expression. This study revealed a high intra and inter-observer reliability on VEGF and MVD measurement. Neither MVD nor VEGF expression provided predictive value of advanced or aggressiveness of disease. Further studies on larger sample size would help validate these results.*

Keywords: *Colorectal cancer, VEGF, Microvascular density, Reliability, Intra-observer, Inter-observer*

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The formation of new vessels (angiogenesis) is an essential process for primary tumor growth and distant metastasis[1]. Production of vascular endothelial growth factor (VEGF), a key angiogenic factor, from tumor cell promotes new vessels formation and tumor progression[1,3]. Several previous studies indicated that high-serum VEGF levels predict aggressiveness of disease and relate to outcomes following curative surgery for colorectal cancer[4,7]. Pertaining to primary tumor tissues, VEGF expression and microvascular density (MVD) in tumor tissues may be reliable markers of tumor angiogenesis and relate to the aggressiveness of the disease in an individual patient similar to serum VEGF. In order to assess the relationship of tumor angiogenesis with clinical outcomes, immunohistochemistry has been used to evaluate reactivity for VEGF, and to measure levels of MVD in primary colorectal cancer specimens. Jiang CQ et al[8] found that for colorectal tumor

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tissues, the positive expression rate of VEGF protein in the colorectal adenocarcinoma group was significantly higher than that in the colorectal adenoma group. Regarding the MVD, it was found to be significantly increased in tumors with higher stage[9]. From previous studies[8,9], these two angiogenic markers probably do have significant prognostic value in colorectal cancer patients. However, no consensus has been reached on the relationship between these two markers for angiogenesis. Since studies in various kinds of tumors, the studies in colorectal cancer tissues have neither succeeded nor failed to demonstrate a correlation between the expression of VEGF and MVD[10-13]. For this reason, the present study aimed to determine the relationship between VEGF expressions and MVD in colorectal cancer specimens. The authors also aimed to determine their prognostic value in the presented patients by evaluating their correlation to various common reported prognostic histopathologic indictors that would possibly be essential information for treatment planning after surgery or early indicators of responses to therapy. The Inter-observer reliability on VEGF and MVD measurement was also determined.

Material and Method

The Royal Thai Army Institutional Review Board approved the present study before enrolling any subjects. Tumor specimens of 565 colorectal cancer patients who underwent resection in Phramongkutklao Hospital between January 1, 2005 and December 31, 2010 were archived and reviewed. Tissues of patients who received preoperative chemotherapy, preoperative radiation, or prior anti-angiogenic therapy were excluded. Anti-VEGF and anti-CD34 monoclonal antibodies (Labeled Dextran Polymer Antibody Complex Technique) have been used to demonstrate reactivity for vascular endothelial growth factor (VEGF), and to measure levels of MVD. Forty randomly selected formalin-fixed paraffin-embedded colorectal cancer specimens of non-stage-IV patients who underwent curative resection were immunohisto-chemically stained. Every slide of the archived colorectal cancer tissues was reviewed and categorized according to the American Joint Committee on Cancer (AJCC) staging system. Information regarding the originally reported histopathological features was reviewed for confirmation of the preexisting results, such as grade of the cancer (well differentiation, moderate differentiation, and poor differentiation), depth of penetration (pT staging), number of positive lymph nodes (pN staging), and presence of lymphatic or vascular invasion.

Immunohistochemistry

Immunohistochemical staining was performed using the Labeled Dextran Polymer Antibody Complex Technique. Staining for VEGF and vascular endothelial cells was performed using an anti-VEGF monoclonal antibody (Diagnostic Biosystem, USA) and anti-CD34 monoclonal antibody (Dako, Denmark), respectively. The formalin-fixed, paraffin-embedded, 5-µm tissue sections were deparaffinized with xylene, dehydrated in ethanol and incubated with 3% hydrogen peroxidase for 5 minutes, and washed with phosphate-buffered saline (PBS). After washing, the incubation of the tissue sections in 10% normal horse serum was done, followed by overnight incubation with anti-VEGF (1:200) antibody or anti-CD34 antibody (1:500). After the slides were dropped with the superenhancer TM, they were incubated, and washed with working PBS wash buffer. A drop of labeled dextran polymer conjugated polyhorseradish peroxidase (HRP) was added onto each slide and incubated for 30 minutes. The sections were then washed in two changes of cold PBS for 10 minutes each and wiped carefully to remove excess PBS. Finally, a drop of freshly prepared DAB (32-Diamino benzidine Tetra Hydrochloride—a substrate Chromogen) was added onto the sections. Slides were then washed in running distilled water to remove excess DAB and counter-stained with Hematoxylin.

MVD assessment

For the assessment of MVD, each tissue section was examined at five times magnification and two areas of highest MVD were identified. After that, individual vessel counts were performed at x200 magnification. Any single brown-stained cell that indicated an endothelial cell stained with CD34 was counted as a single vessel. Branching structures were counted as a single vessel, unless there was a break in the continuity of the structure. Tumor MVD was determined by an average number of counted CD34 endothelial cells from two selected fields at x200 magnification in each slide.

VEGF expression

For the evaluation of VEGF expression, the percentage of VEGF immunoreactive carcinoma cells was recorded. Level of VEGF expression was assessed based on the percentage of immunoreactive tumor cells

and the intensity of VEGF expression. Corresponding to the percentage of immunoreactive cells, the samples were categorized as positive (immunoreactive in 25% or more carcinoma cells), and negative (immunoreactive in less than 25% carcinoma cells). The intensity of VEGF expression was graded in a semiquantitative fashion, ranging from 0-2 (0: negative, 1: weakly positive, and 2: strongly positive).

Inter-observer reliability

VEGF expression and MVD were measured independently by each of two pathologists, one working in Department of Pathology, Phramongkutklao College of Medicine, and the other working in the Department of Pathology, Army Institute of Pathology. Inter-observer variability of VEGF and MVD measurements were determined. Inter-observer agreement was assessed by comparing the results of VEGF and MVD measurements made by the two pathologists.

Statistical analysis

The correlation between VEGF expression and MVD was determined by Spearman's correlation method. The correlation between individual histopathologic features and angiogenetic markers (VEGF expression and MVD) was determined by either Pearson correlation or Spearman rank correlation method according to types of data. The Pearson correlation method reflects the degree of linear relationship between two variables ranging from +1 to -1. A correlation of "+1" means that there is a perfect positive linear relationship between variables. A correlation of "-1" means that there is a perfect negative linear relationship between variables. A correlation of "0" means that there is no linear relationship between the two variables. A probability value of less than 0.05 was considered to be significant.

The intra-observer reliability assessed the reproducibility of each observer for each MVD measurement. Inter-observer agreement on MVD and VEGF measurement was expressed as Interclass Correlation Coefficients (InterCC). Cohen's kappa was used for assessing inter-rater reliability; the kappa coefficient of more than 0.8 was defined as excellent correlation, between 0.6 and 0.8 as good correlation (exceeding chance), between 0.4 and 0.6 as moderate correlation, and less than 0.4 as poor correlation.

Results

VEGF immunoreactivity was observed mainly in the cytoplasm of tumor cells and in stromal cells. In all, 35 of 40 cases (87.5%) revealed positive VEGF expression, in which more than 25% of carcinoma cells were immunoreactive for VEGF. The group determined as VEGF-positive revealed that the median (min-max) percentage of VEGF immunoreactive cells was 80% (30% to 90%). A moderate correlation was found between the

Table 1. Patients' demographics (n = 40)

	Number of patients, n (%)
Location of tumors	
Right side colon	9 (22.5)
Left side colon	2 (5.0)
Sigmoid colon	14 (35.0)
Rectosigmoid	4 (10.0)
Rectum	11 (27.5)
Differentiation	
Well	1 (2.5)
Moderately	34 (85.0)
Poorly	5 (12.5)
Nodal status	
N0	14 (35.0)
N1	15 (37.5)
N2	11 (27.5)
Depth of invasion	
T2	1 (2.5)
T3	32 (80.0)
T4	7 (17.5)
AJCC staging	
1	1 (2.5)
2A	8 (20.0)
2B	7 (17.5)
3B	12 (30.0)
3	12 (30.0)
Lymphovascular invasion	
Yes	24 (60.0)
No	16 (40.0)

Table 2. Mean MVD comparison between the VEGF positive and VEGF negative group (n = 40)

VEGF immunoreactivity	Number (%)	MVD (mean (SD))	p-value
Negative	5 (12.5)	97.00 (41.7)	0.751
Positive	35 (87.5)	104.62 (50.8)	

Table 3. Correlation between various common histopathologic features and angiogenic markers (n = 40)

Histopathologic features	Correlation coefficients		
	VEGF intensity	VEGF percentage	Microvascular density (MVD)
Number of LN involvement	0.188	0.116	-0.097
AJCC stage	0.216	0.091	-0.119
Differentiation	-0.015	0.060	0.028
Depth of invasion	-0.115	-0.166	-0.054
Lymphovascular invasion	-0.109	0.050	-0.259
Microvascular density (MVD)	0.161	0.014	1.000

percentage of positive immunoreactive cells and the intensity of VEGF immunoreactive staining (correlation value of 0.436, $p < 0.05$). The mean (SD) microvascular density was 103.67 (49.37). The result from Spearman's correlation test also indicated that MVD was not correlated with both the percentage of positive immunoreactive cells and intensity of VEGF immunoreactive staining (the correlation value of -0.056, $p = 0.732$ and 0.108, $p = 0.506$, respectively). No significant differences were found in MVD between the VEGF-positive and VEGF-negative groups (104.62 versus 97.0; $p = 0.751$). From the result of this small number of samples, neither MVD nor VEGF expression in primary colorectal cancer tissue was found having a significant correlation with any common reported prognostic histopathologic indictors including the tumor differentiation, depth of tumor penetration (pT staging), number of positive lymph nodes (pN staging), and presence of lymphatic or vascular invasion, and the American Joint Committee on Cancer stage as shown in Table 3. In counting CD34-stained endothelial cells, the present study revealed a high intra-observer correlation coefficient of 0.886 (95% CI: 0.715-0.955) for the first pathologist and 0.913 (95% CI: 0.782-0.965) for the second pathologist (Table 4). High inter-observer reliability was observed in both MVD and VEGF measurements with a substantial agreement (agreement 95%, kappa = 0.643)between the two pathologists (Table 5).

Table 4. Intra-observer variability for average value of counted CD34 endothelial cells stained

Intra-observer correlation in counting CD34 endothelial cells stained			
First pathologist		Second pathologist	
ICC	95% CI	ICC	95% CI
0.886	0.715-0.955	0.913	0.782-0.965

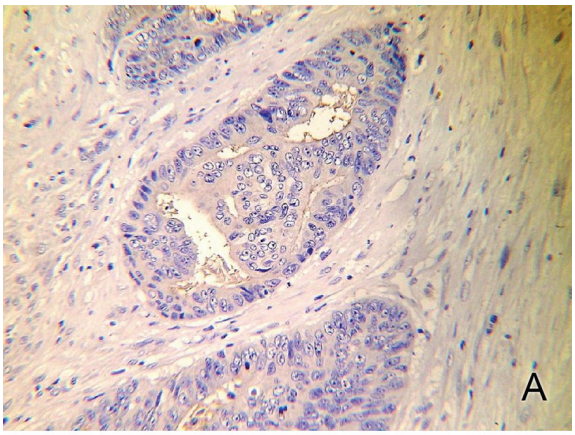
Discussion

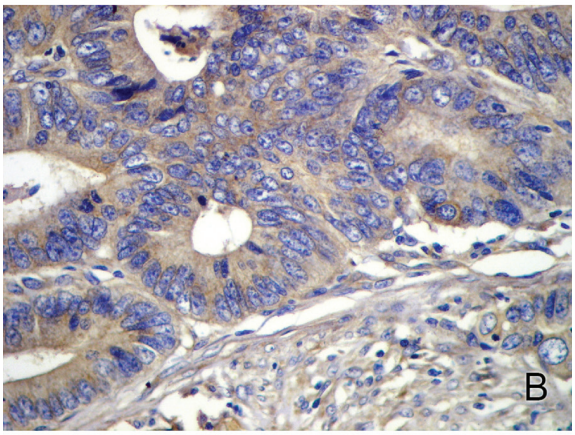
Prognostic value of angiogenic markers (VEGF expression and MVD in tumor tissues) has gained a substantial interest in recent years. Des Guetz et al[14] performed a meta-analysis of all published studies relating to MVD and VEGF expression. The present study revealed that VEGF expression significantly predicted poor recurrence-free survival (RR = 2.84; 95% CI: 1.95-4.16) and overall survival (RR = 1.65; 95% CI: 1.27-2.14). The study of Zheng et al[15] examined 97 cases of colorectal carcinomas that were immuno histochemically stained using anti-

Table 5. VEGF expression and MVD: Inter-observer reliability classification between two observers for 40 tumor specimens

Inter-observer reliability: VEGF and MVD measurement	Correlation value	p-value
Percentage of VEGF immunoreactive cell	0.671	<0.001*
Intensity of immunoreactive staining	0.927	0.001*
Counted CD34 endothelial cells stained (MVD)	0.977	<0.001**
	Agreement	Kappa
Determination of positive VEGF immunoreactive staining (> 25%)	95.0%	0.6429

* Spearman's correlation
** Pearson's correlation





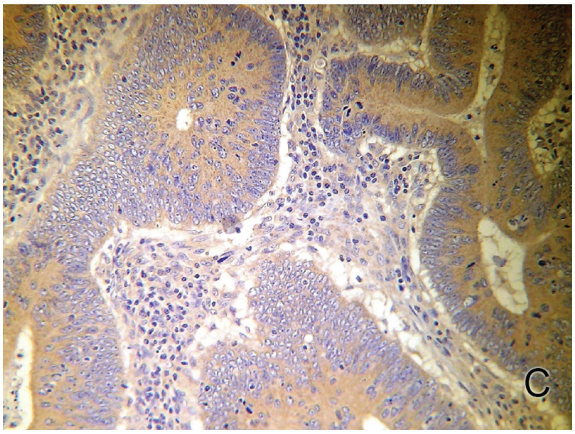


Fig. 1 Immunohistochemical analysis of VEGF expression in colorectal tumor specimen. VEGF expression was scored as A: negative, B: weakly positive and C: strongly positive

VEGF and anti-CD34 monoclonal antibodies and revealed that VEGF expression was more intense in poorly-differentiated adenocarcinoma, but the author concluded that only MVD had prognostic value

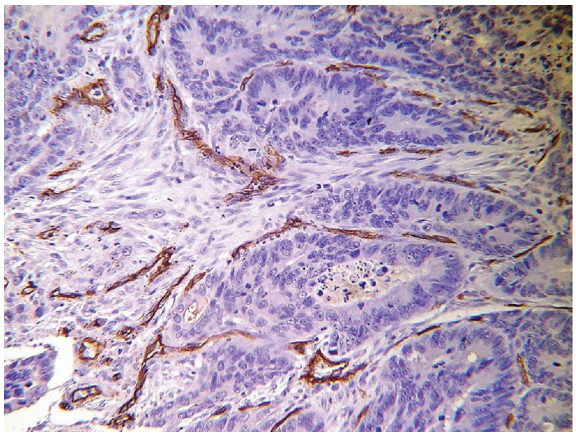


Fig. 2 Microvascular density in colorectal cancer specimen. The single brown-stained cell indicates an endothelial cell that was stained for the presence of CD34

in colon cancer. The same technique of immunohistochemical in the study of Zheng et al[16] has been performed in the present study for describing of reactivity for VEGF and measurement of MVD in colorectal cancer. Although there are many studies that indicated a correlation between MVD and VEGF expression in colorectal cancer[15,16] but the result of the present study found no significant correlation between these two angiogenic markers (MVD and VEGF expression) in the primary colorectal cancer tissues. This may be explained by the vessel formation that can occur by a number of different processes. The present study also revealed that neither MVD nor VEGF expression in the primary colorectal cancer tissues provide any value on prediction of advanced disease or outcomes of treatment. These special immohistochemical staining in primary colorectal tissues may not provide additional advantages for implementation to the routine practice. Further studies on new tissue samples and larger sample size would help validate these results.

To the authors' knowledge, the present study is the first to assess the intra-observer and inter-observer variability of VEGF and MVD measurements in a masked setting that was important to determine the accuracy of the measurement technique. It is therefore possible to have VEGF and MVD measured by different examiners and still make a valid conclusion on levels of VEGF expression and MVD. Some limitations were noted with respect to the retrospective nature of the present study and long duration of tissue archive that may have affected the intensity of

immunohistochemical staining and the accuracy of interpretation. Further studies on new tissue samples and larger sample size would help validate these results.

Conclusion

In primary colorectal cancer tissues, no significant relationship between MVD and VEGF expression but a moderate correlation was found between the percentage of positive immunoreactive cells and the intensity of VEGF immunoreactive staining. Neither MVD nor VEGF expression in primary colorectal cancer tissue was found providing any predictive value on advanced disease when comparison to various common reported prognostic indicators. The present study revealed a high intra and inter-observer reliability on VEGF and MVD measurement. Further studies on a larger sample size would help to determine the benefit of implementation of both special staining in routine practice.

Potential conflicts of interest

None.

References

1. Thielemann A, Kopczynski Z, Filas V, Breborowicz J, Grodecka-Gazdecka S, Baszczuk A. The determination of VEGF and MVD, among patients with primary breast cancer. Pathol Oncol Res 2008; 14: 137-44.
2. Giatromanolaki A, Koukourakis MI, Stathopoulos GP, Kapsoritakis A, Paspatis G, Kakolyris S, et al. Angiogenic interactions of vascular endothelial growth factor, of thymidine phosphorylase, and of p53 protein expression in locally advanced gastric cancer. Oncol Res 2000; 12: 33-41.
3. Burian M, Quint C, Neuchrist C. Angiogenic factors in laryngeal carcinomas: do they have prognostic relevance? Acta Otolaryngol 1999; 119: 289-92.
4. Abdel-Gawad IA, Hassanein HM, Bahgat NA, Abdel Sattar MA, El Sissy AH, Altaweel MA, et al. Study of endothelin-1 and vascular endothelial growth factor in patients with cancer colon. J Egypt Natl Canc Inst 2008; 20: 216-23.
5. Alabi AA, Suppiah A, Madden LA, Monson JR, Greenman J. Preoperative serum vascular endothelial growth factor-a is a marker for subsequent recurrence in colorectal cancer patients. Dis Colon Rectum 2009; 52: 993-9.
6. Alabi AA, Suppiah A, Madden LA, Monson JR, Greenman J. Preoperative serum levels of serum VEGF-C is associated with distant metastasis in colorectal cancer patients. Int J Colorectal Dis 2009; 24: 269-74.
7. Broll R, Erdmann H, Duchrow M, Oevermann E, Schwandner O, Markert U, et al. Vascular endothelial growth factor (VEGF)—a valuable serum tumour marker in patients with colorectal cancer? Eur J Surg Oncol 2001; 27: 37-42.
8. Jiang CQ, Liu ZS, Qian Q, He YM, Yuan YF, Ai ZL. Relationship of hypoxia-inducible factor 1 alpha (HIF-1alpha) gene expression with vascular endothelial growth factor (VEGF) and microvessel density (MVD) in human colorectal adenoma and adenocarcinoma. Ai Zheng 2003; 22: 1170-4.
9. Staton CA, Chetwood AS, Cameron IC, Cross SS, Brown NJ, Reed MW. The angiogenic switch occurs at the adenoma stage of the adenoma carcinoma sequence in colorectal cancer. Gut 2007; 56: 1426-32.
10. Wang Z, Ji W, Tang Q, Pan Z. Relationship among expression of the VEGF gene and MVD with cervical lymph nodes metastasis in laryngeal squamous cell carcinoma. Lin Chuang Er Bi Yan Hou Ke Za Zhi 2004; 18: 100-2.
11. Dong P, Yoshimi M, Tadashi N. The expression difference of VEGF, PDGF/dThdPase and MVD on primary hypopharyngeal carcinoma and metastasis lymph nodes. Lin Chuang Er Bi Yan Hou Ke Za Zhi 2002; 16: 451-3.
12. Cao Q, Zhang T, Jiang L, Wang L, Luo S. Expression and significance of STAT3 and VEGF with MVD in the nasal polyps. Lin Chung Er Bi Yan Hou Tou Jing Wai Ke Za Zhi 2010; 24: 641-4, 647.
13. Decaussin M, Sartelet H, Robert C, Moro D, Claraz C, Brambilla C, et al. Expression of vascular endothelial growth factor (VEGF) and its two receptors (VEGF-R1-Flt1 and VEGF-R2-Flk1/KDR) in non-small cell lung carcinomas (NSCLCs): correlation with angiogenesis and survival. J Pathol 1999; 188: 369-77.
14. Des Guetz G, Uzzan B, Nicolas P, Cucherat M, Morere JF, Benamouzig R, et al. Microvessel density and VEGF expression are prognostic factors in colorectal cancer. Meta-analysis of the literature. Br J Cancer 2006; 94: 1823-32.
15. Zheng S, Han MY, Xiao ZX, Peng JP, Dong Q. Clinical significance of vascular endothelial growth factor expression and neovascularization in colorectal carcinoma. World J Gastroenterol

2003; 9: 1227-30.
16. Takahashi Y, Tucker SL, Kitadai Y, Koura AN, Bucana CD, Cleary KR, et al. Vessel counts and expression of vascular endothelial growth factor as prognostic factors in node-negative colon cancer. Arch Surg 1997; 132: 541-6.

การศึกษาความสัมพันธ์ระหว่างการแสดงออกของวาสคิวล่าเอนโดธีเรียลโกรธแฟคเตอร์ (วีอีจีเอฟ) และความหนาแน่นของหลอดเลือดขนาดเล็กด้วยการย้อมอิมมิวโนฮีสโตเคมิคอลในก้อนเนื้อมะเร็งลำไส้ใหญ่และลำไส้ตรง

สหพล อนันต์นำเจริญ, ธีรยสถ์ นิมมานนท์

วัตถุประสงค์: *วาสคิวล่าเอนโดธีเรียลโกรธแฟคเตอร์ (วีอีจีเอฟ) เป็นสารที่สำคัญในการส่งเสริมให้เกิดการสร้างหลอดเลือดใหม่ และการแพร่กระจายของก้อนมะเร็ง จากการศึกษาในอดีตพบว่าระดับของสารวีอีจีเอฟในกระแสเลือดสามารถบ่งบอกถึงการพยากรณ์โรคได้ แต่ยังไม่มีข้อสรุปถึงบทบาทของการแสดงออกของวาสคิวล่าเอนโดธีเรียลโกรธแฟคเตอร์ (วีอีจีเอฟ) ในก้อนเนื้อมะเร็งลำไส้ใหญ่และลำไส้ตรงต่อผลการดำเนินโรคและการสร้างหลอดเลือดใหม่ การวิจัยนี้จึงมีวัตถุประสงค์เพื่อศึกษาถึงความสัมพันธ์ระหว่างการแสดงออกของวาสคิวล่าเอนโดธีเรียลโกรธแฟคเตอร์ (วีอีจีเอฟ) และการสร้างหลอดเลือดใหม่ด้วยการย้อมอิมมิวโนฮีสโตเคมิคอลในก้อนเนื้อมะเร็งลำไส้ใหญ่ และลำไส้ตรงโดยตรงตลอดจนวิเคราะห์ความสัมพันธ์กับผลตรวจทางพยาธิวิทยาที่บ่งชี้ถึงระยะ และความรุนแรงของการดำเนินโรคนอกจากนี้ยังศึกษาถึงความสอดคล้องของการวัดผลเปรียบเทียบระหว่างพยาธิแพทย์*

วัสดุและวิธีการ: *ดำเนินการย้อมพิเศษด้วยสารแอนติวีอีจีเอฟและแอนติแฟคเตอร์ซีดี 34 ในชิ้นเนื้อมะเร็งลำไส้ใหญ่ และลำไส้ตรงของผู้ป่วยจำนวน 40 รายที่ได้รับการผ่าตัดโดยหวังผลให้หายขาดจากโรคการติดสีอิมมิวโนรีแอคทีฟมากกว่า หรือเท่ากับร้อยละ 25 ของเซลล์มะเร็งในชิ้นเนื้อจะถือว่าการแสดงออกให้ผลเป็นบวก ความเข้มของการแสดงออกของวีอีจีเอฟประเมินด้วยสายตาของพยาธิแพทย์ และแบ่งระดับตามความเข้มในการแสดงออกของการติดสีเป็น 0 ถึง 2 สำหรับการนับความหนาแน่นของหลอดเลือดขนาดเล็กตัดสินจากการนับจำนวนหลอดเลือดที่ติดสีซีดี 34 แบบสุ่มสองครั้งแล้วคำนวณหาค่าเฉลี่ยด้วยกำลังขยาย 200 เท่า ประเมินหาความสัมพันธ์ระหว่างการแสดงออกของวีอีจีเอฟ และความหนาแน่นของหลอดเลือดขนาดเล็กด้วยวิธีการทางสถิติ ผลต่อการพยากรณ์โรคประเมินโดยการหาความสัมพันธ์กับผลพยาธิวิทยาที่บ่งบอกระยะ และความรุนแรงของโรค สำหรับความสอดคล้องในการวัดผลทำการเปรียบเทียบระหว่างผลการประเมินพยาธิแพทย์สองคนที่ทำการประเมินเป็นอิสระต่อกัน*

ผลการศึกษา: *พบว่ามีความสัมพันธ์ในระดับปานกลางระหว่างจำนวนของเซลล์ที่ย้อมติดสี และความเข้มของการติดสี (correlation value of 0.436, $p < 0.05$) การศึกษานี้ไม่พบว่ามีความสัมพันธ์กันระหว่างความหนาแน่นของหลอดเลือดขนาดเล็กต่อทั้งจำนวนและความเข้มในการการแสดงออกของวาสคิวล่าเอนโดธีเรียลโกรธแฟคเตอร์ (วีอีจีเอฟ) สำหรับในการพยากรณ์โรคนั้นพบว่าทั้งความหนาแน่นของหลอดเลือดขนาดเล็ก และการแสดงออกของวาสคิวล่าเอนโดธีเรียลโกรธแฟคเตอร์ (วีอีจีเอฟ) ในก้อนเนื้อมะเร็งลำไส้ใหญ่และลำไส้ตรงไม่ได้สัมพันธ์กับระยะ และความรุนแรงของโรคจากผลการตรวจทางพยาธิวิทยา การศึกษานี้พบว่ามีความสอดคล้องกันของผลการประเมินระหว่างพยาธิแพทย์ทั้งสองคนอยู่ในระดับสูง (agreement: 95%, kappa = 0.643)*

สรุป: *จากผลการศึกษานี้การแสดงออกของวาสคิวล่าเอนโดธีเรียลโกรธแฟคเตอร์ (วีอีจีเอฟ) ในก้อนเนื้อมะเร็งลำไส้ใหญ่และลำไส้ตรงไม่ได้สัมพันธ์กับความหนาแน่นของหลอดเลือดขนาดเล็กทั้งความหนาแน่นของหลอดเลือดขนาดเล็ก และการแสดงออกของวาสคิวล่าเอนโดธีเรียลโกรธแฟคเตอร์ (วีอีจีเอฟ) ในก้อนเนื้อมะเร็งลำไส้ใหญ่และลำไส้ตรงไม่ได้สัมพันธ์กับระยะ และความรุนแรงของโรคจากผลการตรวจทางพยาธิวิทยา*