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Research Article

Association of Serum Vascular Endothelial Growth Factor (VEGF) with Colorectal Cancer: A Systemic Review and Meta-Analysis

Donglie Zhu[1#], Zelong Yang[1#], Xiangxia Miao[2#], Wanli Yang[3#], Mingzuo Jiang[3], Yong Chen[1*]

[#]These authors contributed equally to this work

[1]Department of Hepatobiliary Surgery, Fourth Military Medical University, Shaanxi, China
[2]Department of Respiratory Medicine, The First Hospital of Yulin, Shaanxi, China
[3]State key Laboratory of Cancer Biology, Fourth Military Medical University, Shaanxi, China

[*]Corresponding Author: Yong Chen, Department of Hepatobiliary Surgery, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi, 710032, China, E-mail: gdwkcy@fmmu.edu.cn

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Abstract

Vascular endothelial growth factor (VEGF) is one of the most important regulators in angiogenesis, affecting endothelial cell survival and function. Some studies have shown that serum VEGF is higher in CRC patients than in healthy control groups while other studies have given the opposite conclusion. Therefore, this meta-analysis is purposed to systemically review and evaluate the correlation between serum VEGF and CRC. Finally, 23 studies were included in this study. The meta-analysis demonstrated that serum VEGF in the cancer group was significantly higher than that in the control group (SMD: 1.5, 95% CI: 1.05-1.95, P<0.001). However, obvious heterogeneity existed among the studies (P<0.001, I2=96%) and subgroup analyses were performed to investigate the source of this heterogeneity. The results indicated that with respect to VEGF, the correlation was significant regarding tumor location, study region, age, and study size. The results of this meta-analysis showed that serum level of VEGF might be used as a candidate biomarker for CRC patients.

Keywords: VEGF; CRC; Meta-analysis; Heterogeneity

1. Introduction

Colorectal cancer (CRC) is one of the most frequently diagnosed cancers [1, 2]. It has been reported that about 1 million people develop CRC each year worldwide (3). Moreover, the disease-related mortality of CRC patients corresponds to about 33% [3]. Several factors have been found to be associated with CRC, including inflammatory bowel disease, CRC history in first-degree relatives, obesity, little physical activity, smoking and high intake of red meat [4]. And screening is an effective way to reduce the related mortality of CRC through colonoscopy and fecal immunochemical tests (FIT) [5]. Angiogenesis plays a critical role in tumor development and angiogenic factors are important targets of tumor therapy. As we know, a plenty of cytokines are involved in tumor angiogenesis, such as VEGF, Ang1, PDGF-A, PDGF-B, and IL-8 [6, 7]. VEGF has a close relationship with multiple kinds of tumors and it has been reported that VEGF has been described as overexpressed in lung cancer [8]. In addition, anti-VEGF treatment is proven to relieve nerve edema and deliver oxygen more efficiently into nerves to improve the nerve function of patients who have had / (or have) tumors of the nervous system [9]. In the digestive system, a high level of VEGF is associated with the development of CRC [10]. VEGF is a multi-functional cytokine and mainly acts on the vascular endothelium. According to the studies, VEGF can induce the mitotic activity in endothelial cells and capillary sprouting transferred by two high-affinity receptors (Flt-1 and Flk-1/KDR) [11, 12]. It has been reported that serum VEGF has a strong relationship with the CRC. However, whether CRC patients have a high level of serum VEGF or not remains to be determined. For example, Bünger S, et al. found that serum VEGF was obviously lower in CRC patients compared with healthy control groups [13]. Nevertheless, Landriscina, et al. demonstrated that serum VEGF is not significantly correlated with CRC [14]. Therefore, we conducted this analysis so as to assess the correlations between the serum VEGF and CRC. We also aim to clarify the role of serum VEGF in CRC development, and finally provide evidence for further studies and novel therapeutic methods for CRC patients through targeting VEGF.

2. Methods

2.1 Literature search

A comprehensive literature search was carried out using online databases including PubMed, EMBASE, and Web of Science. Key words for the literature search were as follows: “serum”, “serum level”, “vascular endothelial growth factor”, “VEGF”, “colorectal”, “CRC”, “colon”, “cancer” and “tumor”. The above search terms were only for human subjects. We searched for full-text articles and abstracts published in English and all relevant articles that were identified online were from March 1998 to June 2018. All potentially applicable studies were considered for review, regardless of the primary outcome. The full-text articles were screened independently by 2 members of the research team, and another member of the research team resolve any disagreements; the third member also reviewed all the excluded articles. We also performed a manual search in reference lists in order to find the additional relevant papers.

2.2 Inclusion and exclusion criteria

Three authors independently screened the suitable study records. Studies that met the following criteria could be included: (1) the cases of patients diagnosed with CRC where the controls were healthy people (2) reported serum VEGF levels in CRC patients and healthy controls; (3) studies that provided the means (M) and standard deviation (SD) of the serum VEGF levels in CRC patients and controls; (4) studies published in English. The exclusion criteria were based on the following: (1) the articles were reviewed, case report, abstract, or unpublished papers; (2)

patients' sample were not serum source; (3) studies without complete data.

2.3 Data extraction

Three researchers independently elicited the data of the included articles: the study title, name of first authors, year of publication, country, type of study, age and sex of participants, sample size, mean ± SD of VEGF levels, and clinical characteristics of participants.

2.4 Quality assessment

We adopted the Newcastle Ottawa Scale (NOS) to conduct the quality assessment independently by two researchers. The NOS tool contains nine items and each study was evaluated by an NOS score ranging from 0 to 9. We defined studies as poor quality if the NOS score ≤ 3, 4 to 6 corresponds to moderate quality and studies were high quality when their score was from 7 to 9.

2.5 Statistical analysis

Subgroup analysis and meta regression have been used for heterogeneity analysis. The heterogeneity of the studies was evaluated by the SMD and 95% CI. Significant heterogeneity was found in the studies by a p value < 0.1 for Q test or I2 > 50%. Heterogeneity among the studies was tested by using a random effect model when indicated. Publication bias was investigated both visually by using a funnel plot and statistically via Begg funnel plots and the Eggers bias test, which measures the degree of funnel plot asymmetry. RevMan 5.3 and State SE11.0 were performed for all analyses.

3. Results

3.1 Literature search

According to the search criteria, in total, 593 studies were collected by the literature search. 268 relevant articles after duplicates removed. 169 articles were excluded, 99 articles assessed for eligibility. Among them, 76 articles were excluded according to the exclusion criteria (25 papers were reviewed, 27 papers had no extractable date, 16 papers were without control groups, and 8 papers had sample overlap). Finally, 23 studies with 3400 subjects (2510 CRC patients and 890 controls) were included in this meta-analysis. Figure 1 presents the process of student selection.

3.2 Characteristics of the included studies

As shown in Table 1, we summarized the main characteristics of the included studies. The studies were published between March 1998 to June 2018 and contain 2510 CRC cases and 890 healthy people. All studies used serum samples. The enzyme-linked immune Sorbent assay (ELISA) was used to detect the VEGF levels. Thirteen studies were performed in Europe, nine studies were performed in Asia, and one was in Egypt. The 23 studies contain twenty-one case-control studies and two prospective studies. In accordance to NOS: five studies scored 5 [15-19], eleven studies scored 6 [13, 14, 20-28] and seven studies scored 7 [29-35] (Table 1).

3.3 Meta-analysis

As shown in Figure 2, The overall effect indicated that VEGF levels in CRC patients were strongly higher than that in healthy cases (SMD: 1.5, 95% CI: 1.05-1.95, P<0.001) according to the results, the heterogeneity across studies was significant (P<0.001, I2=96%), therefore we performed the random effect model.

3.4 Subgroup analysis

Subgroup analysis was carried out to investigate the heterogeneity among studies and evaluate the robustness of our findings. Tumor location, region and sample size of the studies were used to evaluate potential sources of heterogeneity (Table 2). Following tumor locations, all studies were divided into either a colon group or others, eight studies were reported in the colon group and seventeen studies were reported in others. As shown in

Figure 3, higher VEGF levels were detected in case compared with the control both in colon (SMD: 2.93, 95% CI: 1.65–4.20, P<0.0001) and others subgroup (SMD: 1.17, 95% CI:0.71–1.63, P<0.0001). Subgroup analysis which was based on the region of studies revealed that serum VEGF in CRC patients was higher than healthy control groups Asia: SMD: 1.36, 95% CI: 0.62-2.09, P=0.0003, Europe: SMD: 1.61, 95% CI: 1.02-2.20, P<0.0001) (Figure 4). Further subgroup analysis which was based on the sample size revealed that serum VEGF in CRC patients was higher than healthy controls in both the two subgroups (sample size < 50: SMD: 3.23, 95% CI: 1.81-4.64, P<0.0001; sample size ≥ 50: SMD: 0.91, 95% CI: 0.50-1.33, P<0.0001) (Figure 5). Moreover, studies which were based on age showed that serum VEGF in CRC patients was higher than the healthy controls (age < 50: SMD: 2.21, 95% CI: 1.07-3.34, P=0.0001, age ≥ 50: SMD: 0.96, 95% CI: 0.41-1.52, P=0.0007) (Figure 6). Still, all subgroup analysis showed large heterogeneity, and these variables did not contribute to finding the source of heterogeneity.

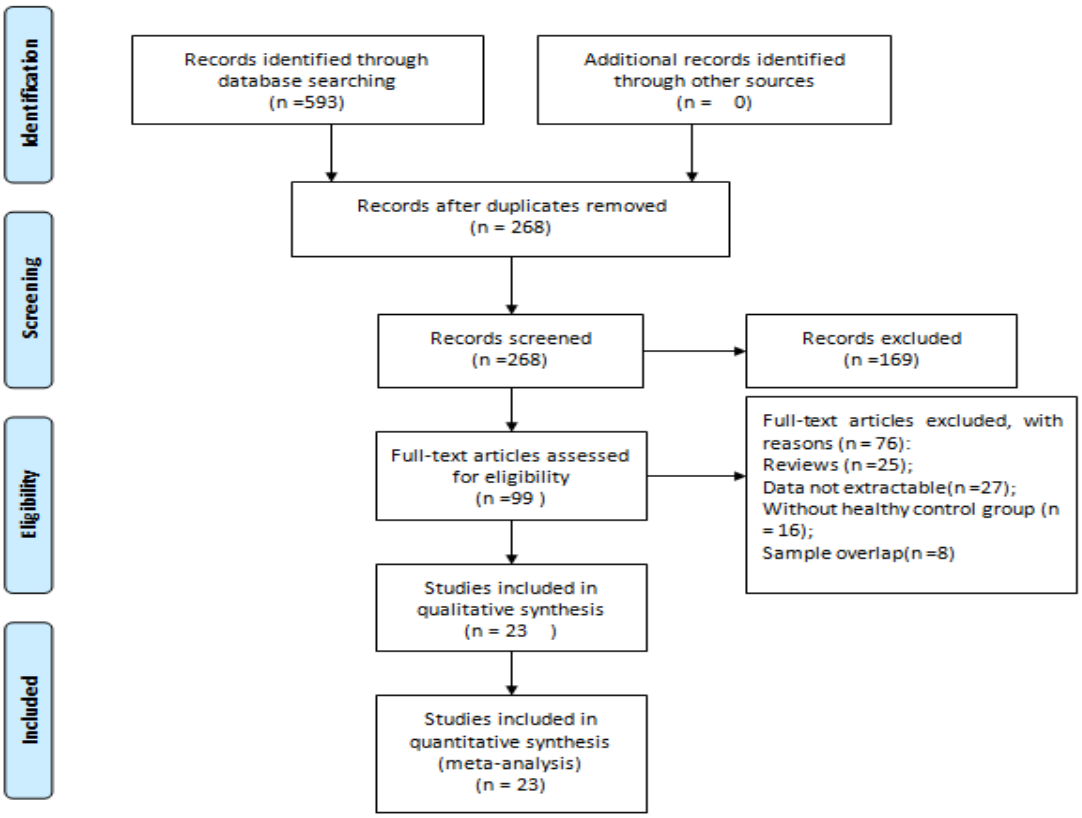

Figure 1: Flow diagram of literature search and selection.

Study	year	Country	Design	Sample Size	Sample	Method	Age		VEGF concentration		NOS
							Case	Controls	Case	Controls	
Abdel-Gawad et al.	2008	Egy pt	case-control	68	serum	ELISA	NA	NA	768.5 ± 45.5	70.5 ± 5.4	7
Broll et al.	2001	Germany	case-control	164	serum	ELISA	NA	NA	438 ± 396	203 ± 124	5
Bünger et al.	2011	Germany	case-control	100	serum	ELISA	71.2 ± 8.2	65.5 ± 11.3	190.9 ± 187.5	141.3 ± 103.5	6
Bünger et al.	2012	Germany	case-control	283	serum	ELISA	69.6 ± 9.8	62.4 ± 11.9	78 ± 57.6	59.9 ± 70	6
Coşkun et al.	2017	Turkey	case-control	60	serum	ELISA	60 ± 10	66 ± 11	859.1 ± 88	892 ± 137.8	7
Cressey et al.	2005	Thailand	case-control	85	serum	ELISA	NA	NA	1081 ± 652	543 ± 344	5
Dbouk et al.	2007	Lebanon	case-control	44	serum	ELISA	67 ± 16	40 ± 9	21.2 ± 14	4.5 ± 2.1	7
De Vita et al.	2003	Italy	case-control	131	serum	ELISA	NA	NA	504.1 ± 223	78.1 ± 22	7
Gonzalez et al.	2007	Spain	prospective study	87	serum	ELISA	67 ± 9.3	45 ± 8	251 ± 49.3	162 ± 27	6
Johdi et al.	2017	Malaysia	case-control	40	serum	ELISA	72±11	62 ± 12	97.7 ± 34.9	38.9±14.2	6
Karayiannakis et al.	2002	Greece	prospective study	128	serum	ELISA	NA	NA	492 ± 337	186 ± 128	7
Kemik et al.	2010	Turkey	case-control	162	serum	ELISA	43.5±10.7	40.4±11.3	629.3 ± 205.6	309.4 ± 135.8	6
Kemik et al.	2011	Turkey	case-control	74	serum	ELISA	49.5±12.3	40.4±11.3	789.7±200.8	309.4±135.8	6
Kushlinskii et al.	2014	Russia	case-control	143	serum	ELISA	52.5±10.8	56.5±9.2	513±47.3	349±84	7
Landriscina et al.	1998	Italy	case-control	30	serum	ELISA	NA	NA	11.2±4	11.8±5	6
Mehrabani et al.	2014	Iran	case-control	96	serum	ELISA	NA	NA	416.6±481.8	310.3±396.3	6
Nakamura et al.	2013	Japan	case-control	47	serum	ELISA	NA	NA	601.8±128.3	217.8±46.3	5
Spacek et al.	2018	Czech Republic	case-control	48	serum	ELISA	NA	NA	68±11.6	17.5±2.2	7
Sulkowski et al.	2009	Poland	case-control	141	serum	ELISA	NA	NA	128.4±146	5.9±1.2	6
Wei et al.	2009	Taiwan	case-control	116	serum	ELISA	NA	NA	79.7±193.2	60.9±19.7	5

Werther et al.	2000	Denmark	case-control	705	serum	ELISA	69±9.7	39±7.2	270±415.2	120±163.3	6
Werther et al.	2002	Denmark	case-control	74	serum	ELISA	NA	NA	388±249	391±175	5
Werther et al.	2002	Denmark	case-control	574	serum	ELISA	69±9.5	59±1.7	268±415.2	220±156.2	6

Abbreviations: NOS=Newcastle-Ottawa quality assessment scale; ELISA=enzyme-linked immune Sorbent assay; VEGF= Vascular endothelial growth factor.

Table 1: Characteristics of the included studies.

Subgroups	**SMD (95% CI)**	**Z**	***P***	**Test of Heterogeneity**	
				I^2	*P*
Tumor location					
Colon	2.93 (1.65,4.20)		*P*<0.0001	98%	*P*<0.0001
Others	1.17 (0.71,1.63)		*P*<0.0001	94%	*P*<0.0001
Region					
Asia	1.36 (0.62,2.09)		*P*=0.0003	94%	*P*<0.0001
Europe	1.61 (1.02,2.20)		*P*<0.0001	96%	*P*<0.0001
Sample size					
<50	3.23 (1.81,4.64)		*P*<0.0001	97%	*P*<0.0001
≥ 50	0.91 (0.50,1.33)		*P*<0.0001	94%	*P*<0.0001
Age					
<50	2.21 (1.07,3.34)		*P*=0.0001	89%	*P*=0.003
≥ 50	0.96 (0.41,1.52)		*P*=0.0007	95%	*P*<0.0001

Abbreviations: SMD=Standard Mean Difference; CI= Confidence Intervals.

Table 2: Subgroup analysis of VEGF level in CRC.

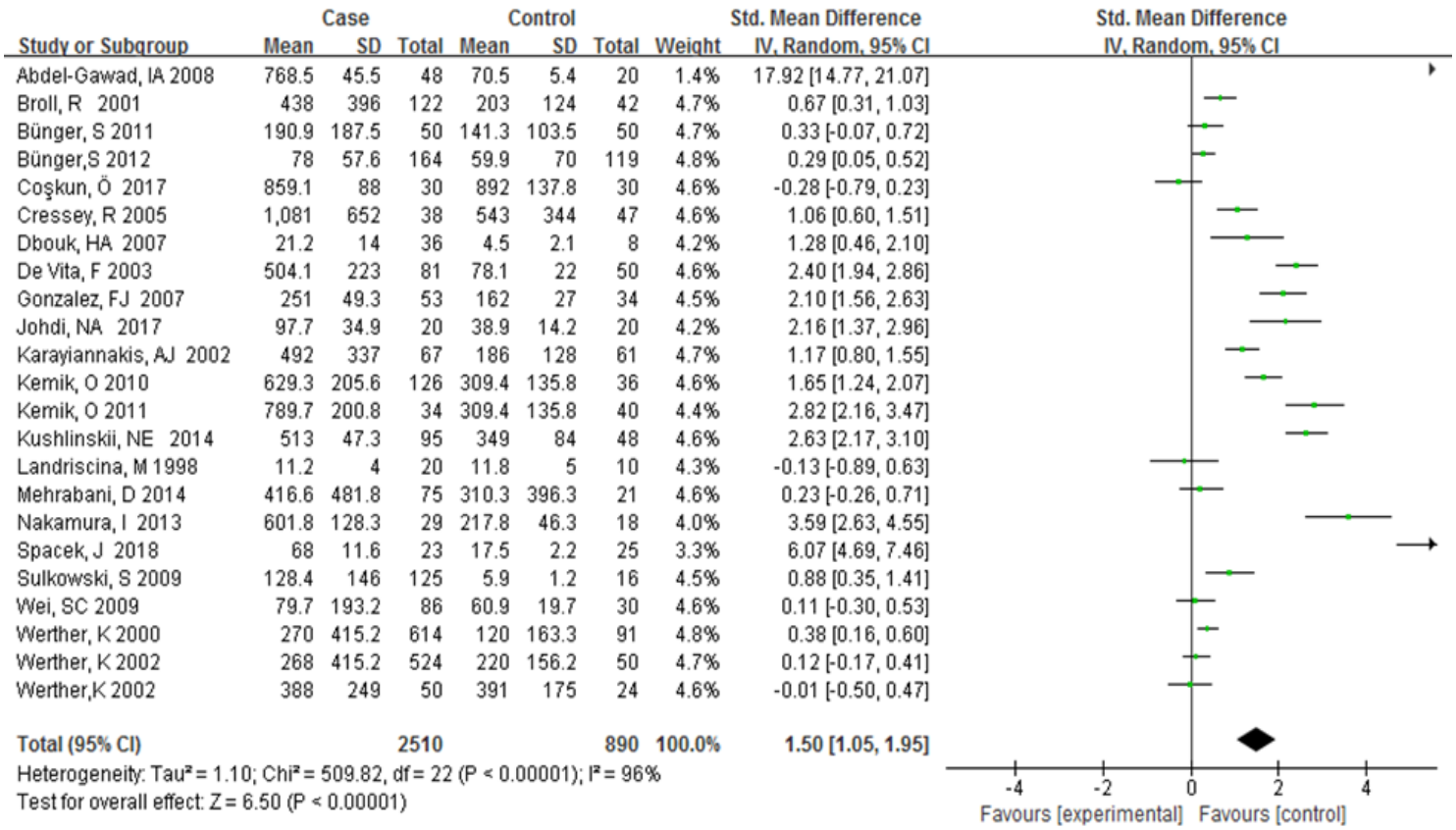

Figure 2: SMD analysis of serum VEGF level in CRC patients and the controls.

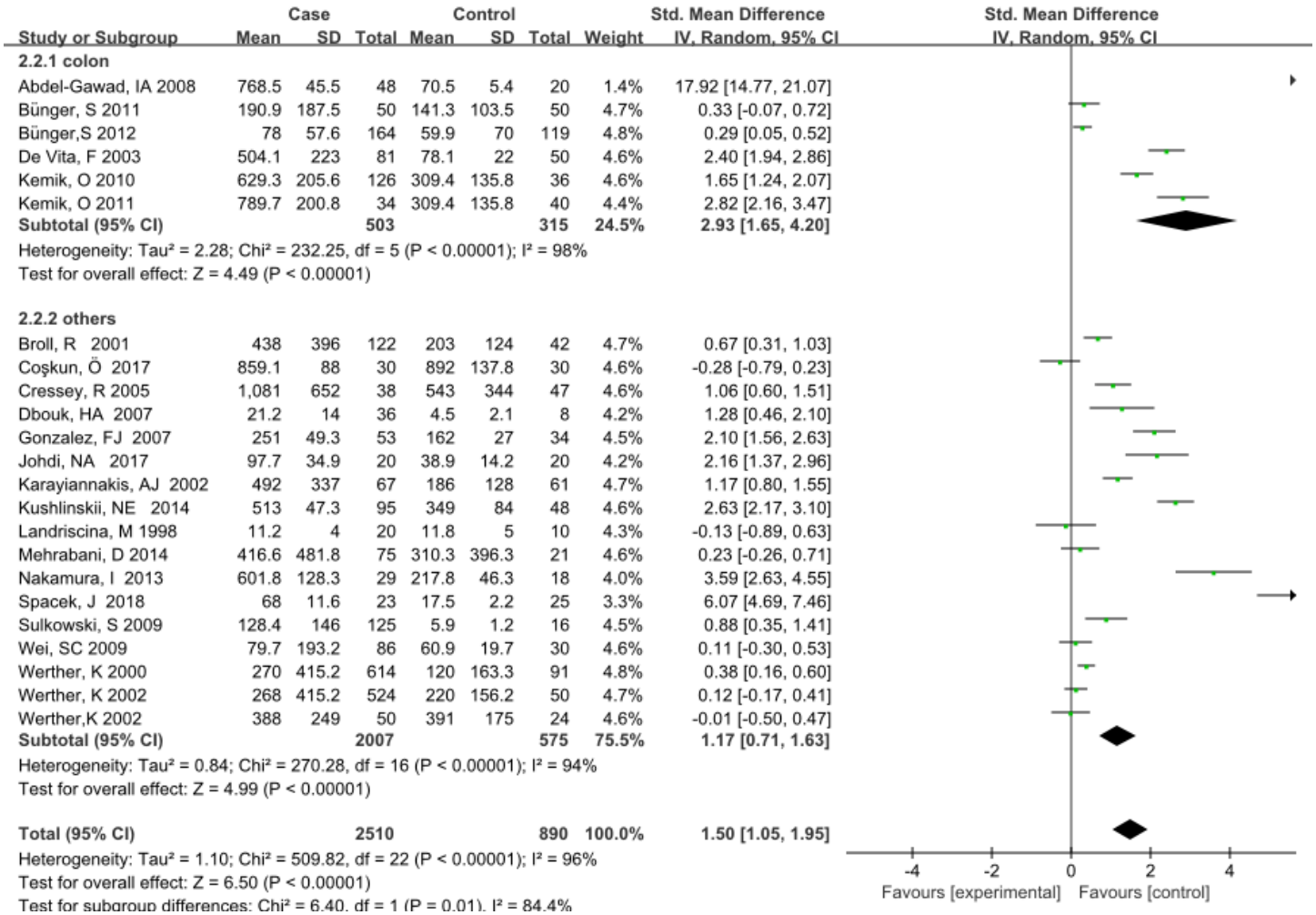

Figure 3: Subgroup analyses for relationship between serum VEGF and CRC according to tumor location.

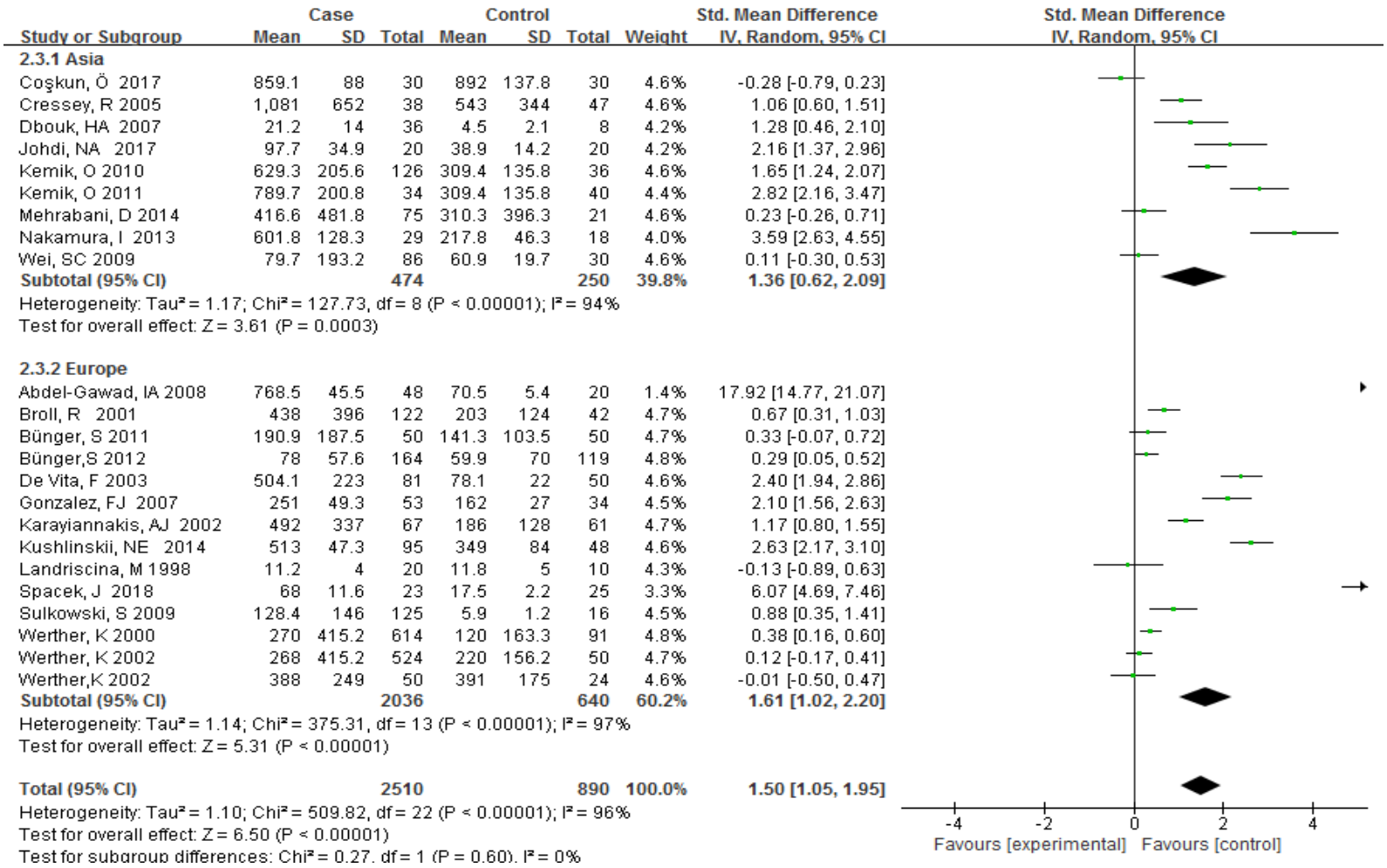

Figure 4: Subgroup analyses for relationship between serum VEGF and CRC according to region.

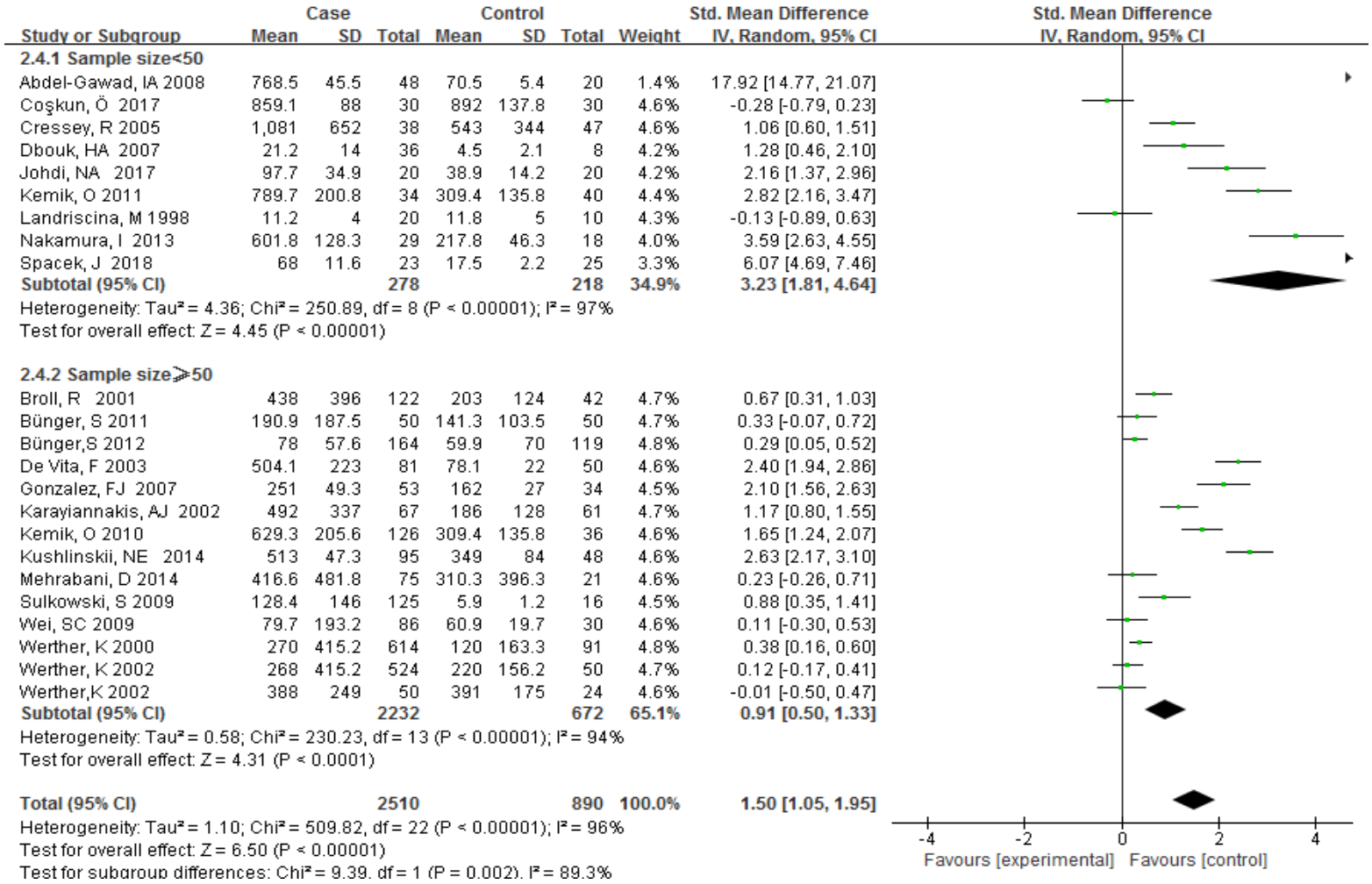

Figure 5: Subgroup analyses for relationship between serum VEGF and CRC according to sample size.

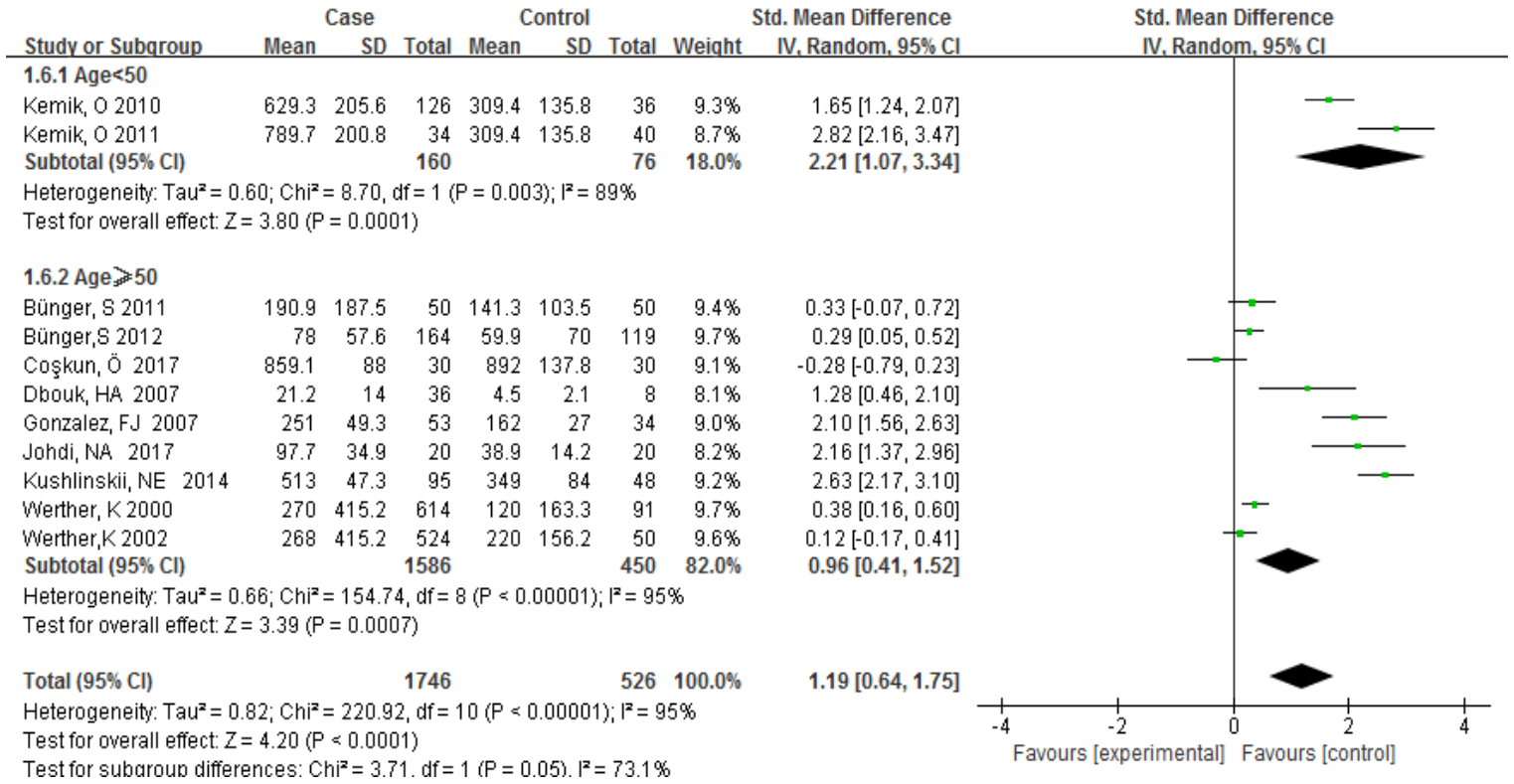

Figure 6: Subgroup analyses for relationship between serum VEGF and CRC according to age.

3.5 Sensitivity

Sensitivity analysis was performed to examine the resulting stability, in which one study was removed at a time. The results suggested that the omission of any study had no obvious effect on the findings, which also reflected the robustness of the conclusions (Table 3).

Study	SMD (95% CI)	*P* heterogeneity	I^2
Abdel-Gawad et al.	1.24 (0.84-1.64)	*P*<0.0001	95%
Broll et al.	1.56(1.08-2.04)	*P*<0.0001	96%
Bünger et al.	1.57(1.09-2.04)	*P*<0.0001	96%
Bünger et al.	1.59(1.10-2.07)	*P*<0.0001	96%
Coşkun et al.	1.59(1.12-2.05)	*P*<0.0001	96%
Cressey et al.	1.53(1.06-2.00)	*P*<0.0001	96%
Dbouk et al.	1.51(1.05-1.98)	*P*<0.0001	96%
De Vita et al.	1.44(0.99-1.89)	*P*<0.0001	95%
Gonzalez et al.	1.47(1.01-1.93)	*P*<0.0001	96%
Johdi et al.	1.47(1.01-1.93)	*P*<0.0001	96%
Karayiannakis et al.	1.53(1.05-2.00)	*P*<0.0001	96%
Kemik et al.	1.50(1.03-1.96)	*P*<0.0001	96%
Kemik et al.	1.43(0.98-1.88)	*P*<0.0001	96%
Kushlinskii et al.	1.43(0.98-1.87)	*P*<0.0001	95%
Landriscina et al.	1.57(1.11-2.04)	*P*<0.0001	96%
Mehrabani et al.	1.57(1.10-2.04)	*P*<0.0001	96%
Nakamura et al.	1.40(0.95-1.85)	*P*<0.0001	96%
Spacek et al.	1.32(0.89-1.76)	*P*<0.0001	95%
Sulkowski et al.	1.54(1.07-2.01)	*P*<0.0001	96%
Wei et al.	1.57(1.10-2.04)	*P*<0.0001	96%
Werther et al.	1.59(1.09-2.08)	*P*<0.0001	96%
Werther et al.	1.58(1.11-2.04)	*P*<0.0001	96%
Werther et al.	1.58(1.11-2.06)	*P*<0.0001	96%

Abbreviations: SMD= Standard Mean Difference; CI= Confidence Intervals.

Table 3: Sensitivity analysis.

3.6 Meta regression

To determine the source of heterogeneity, meta regression analyses were performed. The results of random-effects model meta-regression based on age, published year, region, design, NOS, and the sample size showed that they cannot explain the heterogeneity of the included studies. Table 4 presented the results of univariate analysis. Only region was considered as a key factor that might be weakly responsible for the heterogeneity among the included studies. After introducing all the covariates, the heterogeneity changes from 96% to 96.19% and the covariates did not contribute to heterogeneity in any of the preplanned comparisons (Table 4).

Covariates	No. of studies	Coefficient	Standard error	*t*	*P*	95% Confidence interval	
Age	11	-1.251	0.824	-1.52	0.163	-3.116	0.613
Year	23	0.110	0. 128	0.86	0.397	-0.156	0. 378
Region	23	2.368	1.230	1.92	0.068	-0.191	4.927
Design	23	0.017	2.663	0.01	0.995	-5.520	5.554
NOS	23	1.614	0.980	1.65	0.115	-0.425	3.653
Sample size	23	-0.254	1.821	-0.14	0.890	-4.042	3.532

Table 4: Univariate meta-regression analysis for the potential variables between studies.

3.7 Publication bias

We conducted the funnel plot analysis and Egger's linear regression test to explore the potential publication bias. The Egger's (t=-1.80, P=0.087) suggested that there were no significant publication biases in the included studies (Figure 7).

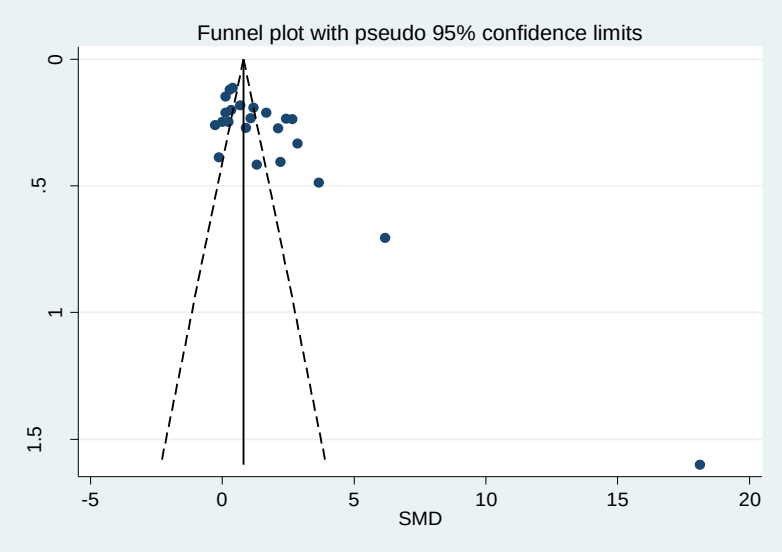

Figure 7: A funnel plot analysis of publication bias.

4. Discussion

CRC is one of the gastrointestinal cancers with the highest incidence [36]. Angiogenesis is an essential process for the growth and proliferation of cancer [37]. Numerous experimental and clinical studies have shown that high serum VEGF expression is related to CRC development. VEGF is a glycosylated dimeric polypeptide which is abundantly expressed and secreted by most human tumors. Some studies have found that VEGF can be a potential serum diagnostic marker for malignant disease since high VEGF mRNA levels can be detected in most human tumors by situ hybridization [38]. Furthermore, studies have also shown that VEGF and its receptors? are highly expressed in metastatic colon cancer cells and tumor associated endothelial cells [39]. These findings indicate that VEGF is an important angiogenic factor in CRC development [40].

Currently, many researchers are focusing on the association of serum VEGF and CRC. However, the results might be contradictory. For example, Spacek et al. have pointed out that serum VEGF and CRC are positively correlated [35]. While some studies gave different results. Coşkun et al. found that serum VEGF was lower in CRC patients compared with the healthy control groups [30].To the best of our knowledge, this is the first systematic analysis to explore the relationship of serum VEGF level and CRC. In this study, our results have shown that CRC patients had a higher serum VEGF level than the healthy controls. When interpreting these findings, we should take the following issues into consideration. First, VEGF plays an important role in CRC cells through the intracrine mechanism, that is, by regulating the activity of multiple receptor tyrosine kinases and downstream AKT signaling [10]. Second, inhibition of intracrine VEGF pathways can strongly suppress CRC invasion and migration via modulating the cell motility related molecules [41]. Third, Chronic inhibition of extracellular VEGF resulted in resistance to hypoxia-induced apoptosis and an increased sphere formation ability in CRC cell lines [42].

To explore the heterogeneity among studies, we performed subgroup analyses according to tumor location, region, sample size and age. It is known that VEGF polymorphism was found to be associated with malignancy susceptibility in CRC [43], and different types of gastrointestinal cancers that have disparate mechanisms of a carcinogenic effect. Therefore, we stratified subgroup analysis according to tumor location (Table 2). The results showed that the serum VEGF level was significantly higher in case groups than that in the control groups. However, the heterogeneity was obvious. In addition, Age is the main risk factor for CRC. It is reported that the risk of CRC increases significantly when people are over 50 years old [44]. Therefore, we performed subgroup analyses based on age, whether the age was <50 or ≥ 50. However, heterogeneity significantly still existed. Moreover, subgroup analysis, which was based on region and sample size revealed that serum VEGF in CRC patients was obviously higher than in the healthy controls and the heterogeneity still existed. According to the sensitivity analysis, the correlation was stable. In order to find out the source of heterogeneity, meta regression analysis was performed, the results showed that the region might be weakly responsible for the overall heterogeneity (P=0.068). Still, this study had several limitations. Firstly, significant heterogeneity existed among the studies. Although we have performed subgroup analysis, sensitivity analysis and regression analysis to explore the source of heterogeneity, the explanation was unsatisfactory. Secondly, our study was based on unadjusted estimates. And the confounding factors such as tumor stage, before and after intervention, and environmental factors should be considered. Thirdly, the sizes of subjects in several studies were small, and the backgrounds of patients varied, which might cause a lower statistical power and even the inconsistent results. Finally, we should be cautious in drawing conclusions with these limitations. Our study found that the serum level of VEGF in CRC patients was obviously high compared to healthy controls, suggesting that VEGF can be a potential serum diagnostic marker for CRC. In order to investigate whether high VEGF levels are affected by the region, larger sample sizes and adjustments to mixing factors need to be designed.

Acknowledgements

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Compliance with Ethics Guidelines

Not required.

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