Review Article

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Serum homocysteine level was elevated in ulcerative colitis and can be applied as diagnostic biomarker

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Abstract

Objective – To investigate the correlation between serum level of homocysteine (Hcy) and ulcerative colitis (UC) and evaluate its diagnostic performance by pooling the open published data.

Methods – The case–control or cohort studies relevant to serum level of Hcy and UC, published in Pubmed, Medline, EMBASE, China Wanfang and CNKI databases, were systematically screened by using the text word of "homocysteine," "hcy," "UC," "inflammatory bowel disease." The standard mean difference (SMD) was pooled through random effect model. The diagnostic sensitivity, specificity and area under the receiver operating characteristic (AUC) curve of serum Hcy for UC were also calculated.

Results – Eighteen relevant case–control studies were identified by electronic searching the related databases. The pooled results indicated that the serum levels of Hcy were statical different between UC and healthy controls with SMD = 0.95 (95% CI: 0.87–1.04). The serum levels of Hcy were 14.30 ± 3.08 (range: 10.10–21.73) and 10.09 ± 1.57 (range: 6.80–12.47) μmol/L for UC and healthy controls, respectively, of the included 18 studies. Using serum Hcy as biomarker for UC identification, the diagnostic sensitivity, specificity and AUC were 94.44% (95% CI: 72.71–99.86%), 72.22% (46.52–90.31%) and 0.88 (95% CI: 0.77–0.99, $P < 0.05$), respectively. Significant publication bias was identified in the present work.

Conclusion – Based on the present publications, serum Hcy was elevated in UC cases and can be applied as serological marker for UC diagnosis. However, due to significant publication bias, the diagnostic performance should be further validated by well-designed prospective diagnostic studies.

Keywords: homocysteine, ulcerative colitis, diagnosis, meta-analysis

1 Introduction

Ulcerative colitis (UC) is a kind of chronic non-specific inflammatory bowel disease (IBD). UC is mainly characterized by chronic diarrhea, abdominal pain and mucus purulent bloody stool [1]. UC can be accompanied by systemic symptoms and extraintestinal manifestations. At present, the exact pathogenesis of UC is not clear yet [2]. According to the current research, the genetic susceptibility of UC is stimulated by the intestinal flora under the influence of environmental factors, which starts the abnormal intestinal immune response, and then causes the inflammatory reaction, leading to the erosion and bleeding of the intestinal mucosa and the formation of ulcers [3,4]. Therefore, the abnormal inflammatory response caused by intestinal mucosal immune response plays an important role in the pathogenesis of UC. Homocysteine (Hcy) is an intermediate substance in the metabolic process of methionine. High level of Hcy can maintain the inflammatory state of intestinal mucosa by inducing oxidative stress, promoting inflammatory response and triggering endoplasmic reticulum stress [5]. Studies have explored the correlation between plasma Hcy level and UC, but the results are not completely consistent [6,7]. Therefore, the literatures on the relationship between Hcy and UC were systematically searched and data combinations were made to further explore the relationship between Hcy and UC and evaluate the value of serum Hcy as a biomarker in diagnosing UC.

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2 Materials and methods

2.1 Publication searching

The publication electronic systematic searching was performed by two reviewers (Zhang XP and Wang SS). The case–control or cohort studies relevant to serum levels of Hcy and UC, published in Pubmed, Medline, EMBASE, China Wanfang and CNKI databases, were systematically screened using the text word of "homocysteine," "hcy," "ulcerative colitis," "inflammatory bowel disease." The references of the included studies were also screened in order to find the potential suitable publication.

2.2 Publication inclusion and exclusion criteria

For the initial identified studies, the publications were further screened for inclusion or exclusion by two reviewers (Wang HM and Wu JX) independently. The publication inclusion criteria were (1) studies relevant to human beings, (2) case–control or cohort studies relevant to serum levels of Hcy and UC, (3) UC was diagnosed and confirmed by colonoscopy, (4) serum Hcy level can be extracted from the original study and (5) studies were published in English or Chinese. Publication exclusion criteria were (1) literature review or case report, (2) studies relevant to animals not human beings, (3) not enough data such as serum Hcy level can be extracted from the original publications and (4) studies published in other language neither English nor Chinese.

2.3 Data extraction

The data of each included publication were extracted by two reviewers (Zhang XP and Tan QH) independently and cross-checked. In case of disagreement, the corresponding author was consulted and final decision was

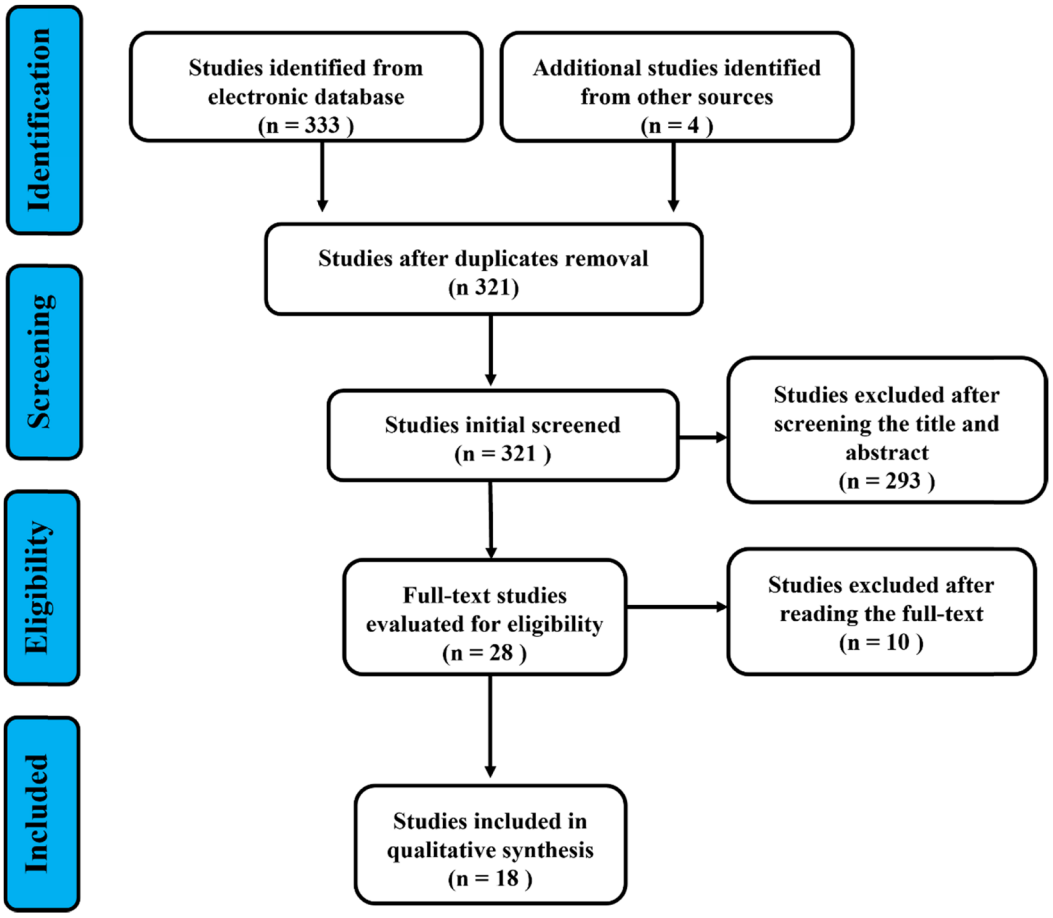

Figure 1: Publication searching flow-chart for serum Hcy level and UC.

Table 1: General characteristics of the included 18 studies

Author	Year	Country	UC			Control			Hcy detection	NOS score
			Sample size	Age	Hcy (μmol/L)	Sample size	Age	Hcy (μmol/L)		
Koutroubakis et al. [8]	2000	Greece	55	NA	15.90 ± 10.30	74	NA	9.60 ± 3.40	IMx assay	6
Danese et al. [9]	2005	Italy	83	NA	11.1 ± 4.9	70	NA	7.1 ± 1.7	HPLC	7
Drzewoski et al. [10]	2006	Greece	30	50.3 ± 11.7	10.10 ± 3.10	21	53.1 ± 12.8	6.8 ± 2.5	HPLC	7
Jiang et al. [11]	2010	China	88	NA	21.73 ± 6.59	100	NA	12.47 ± 5.01	Enzymatic cycling assay	6
Akbulut et al. [7]	2010	Turkey	55	NA	13.30 ± 1.93	45	NA	11.20 ± 3.80	HPLC	6
Chen et al. [12]	2012	China	112	39.4 ± 11.7	11.27 ± 7.26	110	40.3 ± 10.8	8.19 ± 4.81	HPLC	7
Sun and Zhu [13]	2012	China	60	NA	14.69 ± 6.77	20	NA	11.16 ± 2.67	Enzymatic cycling assay	6
Ning [14]	2013	China	52	47.40 ± 13.80	13.70 ± 1.92	50	46.40 ± 13.89	11.10 ± 3.58	NA	6
Wang [15]	2014	China	68	45.80 ± 12.70	14.90 ± 5.39	60	NA	11.38 ± 4.39	Enzymatic cycling assay	5
Liu et al. [16]	2014	China	50	46.70 ± 13.2	13.73 ± 1.91	50	46.30 ± 13.90	11.24 ± 3.58	NA	6
Ju and Bai [17]	2014	China	60	58.12 ± 7.49	14.74 ± 1.69	60	68.29 ± 7.33	10.26 ± 1.48	Enzymatic cycling assay	6
Owczarek et al. [18]	2014	Poland	47	37.94 ± 13.44	10.34 ± 4.31	65	34.38 ± 12.12	9.71 ± 2.81	IMx assay	7
Zhou and Guan [19]	2015	China	60	47.0 ± 12.8	18.60 ± 2.90	55	49.0 ± 10.8	10.50 ± 4.30	Enzymatic cycling assay	5
Zhuo et al. [20]	2016	China	84	42.15 ± 9.84	14.28 ± 1.21	30	40.83 ± 8.53	11.16 ± 1.64	Immunoturbidimetry	5
Xue et al. [21]	2017	China	112	41.13 ± 9.14	16.93 ± 9.49	100	41.79 ± 8.95	10.33 ± 7.48	Enzymatic cycling assay	5
Wang et al. [22]	2018	China	60	46.26 ± 4.16	17.56 ± 4.56	60	46.45 ± 4.09	8.25 ± 2.31	Enzymatic cycling assay	5
Liu et al. [23]	2018	China	63	47.1 ± 6.0	13.74 ± 1.90	63	46.6 ± 6.1	11.23 ± 3.57	NA	5
Zheng et al. [6]	2017	China	128	41.5 ± 9.6	10.78 ± 3.33	138	40.3 ± 8.7	9.91 ± 2.88	Enzymatic cycling assay	7

made. The extracted data and information were as follows: (1) the first author's name, (2) publication time, (3) source of literature, (4) number of cases and controls in the original study, (5) source of patients (region), (6) serum level of Hcy in UC and control groups, (7) serum Hcy detection methods and (8) NOS score of each included studies.

2.4 Statistical analysis

STATA16.0 statistical software was applied for data analysis. Before pooling the results, the data were examined for statistical heterogeneity by I^2 test. If statistical heterogeneity existed ($I^2 > 50\%$, $P < 0.05$), then data were combined through random effect model; otherwise, the data were pooled by fixed effect mode. Two tails $P < 0.05$ were considered statistical difference.

3 Results

3.1 General quality of the included 18 case–control studies

After systematic electronic searching of the databases, 18 case–control studies were finally identified. The study's identifying, inclusion and exclusion processes are demonstrated in Figure 1. Of the included 18 studies, ten were

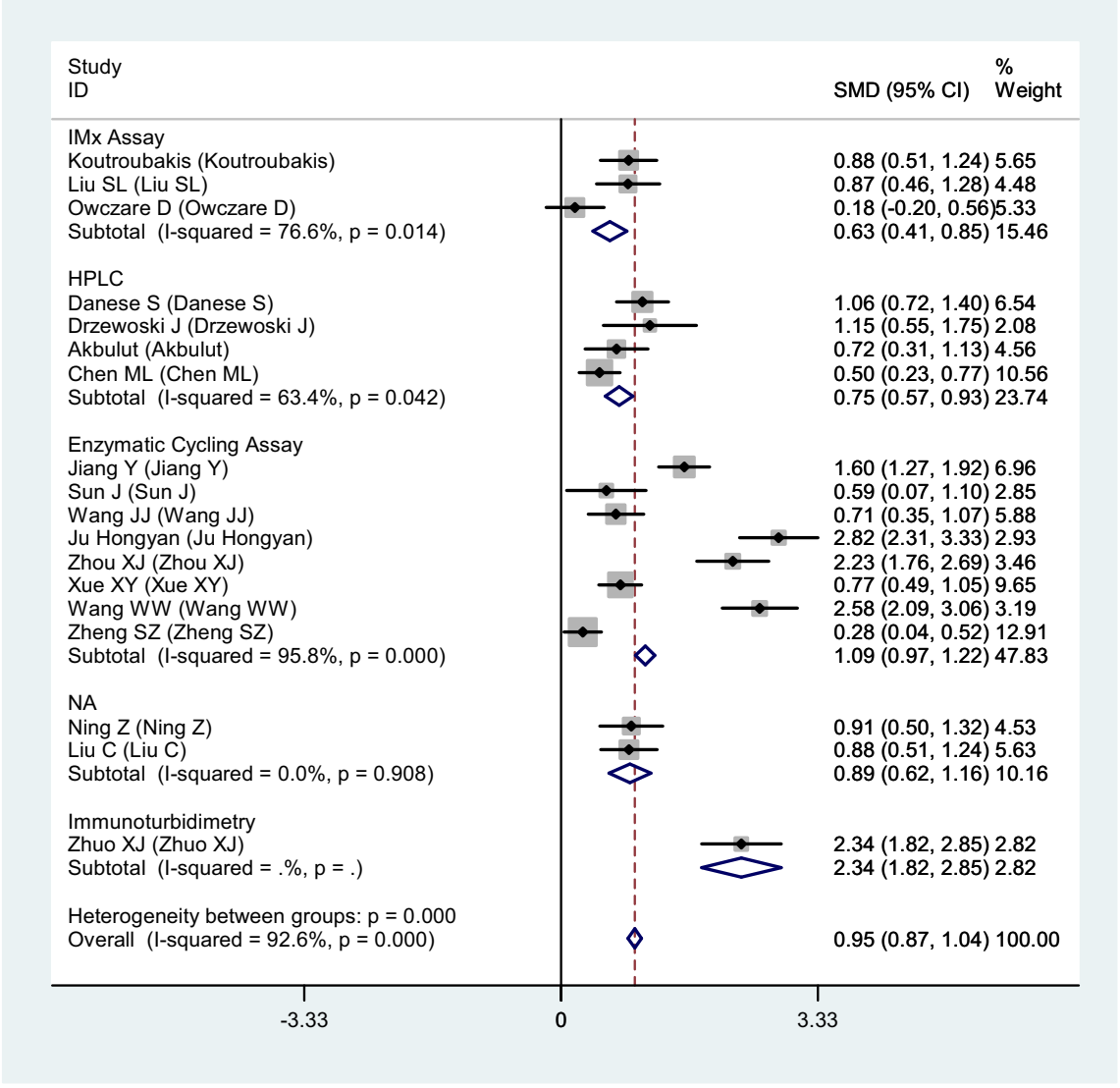

Figure 2: Forest plot of serum Hcy level and UC.

Table 2: Sub-group analysis for serum Hcy between UC and healthy controls

Detection methods	SMD	95% CI	No of studies	I^2
IMx assay	0.63	0.41–0.85	3	76.6%
HPLC	0.75	0.57–0.93	4	63.4%
Enzymatic cycling assay	1.09	0.97–1.22	8	95.8%
Immunoturbidimetry	2.34	1.82–2.85	1	NA
NA	0.89	0.62–1.16	2	0.0%

published in Chinese, two in Greek, one in Italian and one in Turks. The general characteristics of the included 18 publications are shown in Table 1.

3.2 Pooled standard mean difference (SMD)

Statistical heterogeneity across the included 18 studies was statistically significant. Therefore, the SMD was pooled by random effect model. The pooled data indicated that serum level of Hcy was statical different between UC and healthy controls with the SMD = 0.95 (95% CI: 0.87–1.04; $P < 0.01$), Figure 2. According to the serum Hcy detection methods, the SMD between UC and controls was also evaluated (Table 2).

3.3 Diagnostic performance of serum Hcy for UC

The serum levels of Hcy were 14.30 ± 3.08 (range: 10.10–21.73) and 10.09 ± 1.57 (range: 6.80–12.47) μmol/L for UC and healthy controls, respectively, of the included 18 studies (Figure 3a). Serum Hcy level in UC was significantly higher than that of corresponding controls ($P < 0.05$), Figure 3b. Using serum Hcy as biomarker for UC identification, the diagnostic sensitivity, specificity and area under the receiver operating characteristic (ROC) (AUC) curve were 94.44% (95% CI: 72.71–99.86%), 72.22% (46.52–90.31%) and 0.88 (95% CI: 0.77–0.99, $P < 0.05$), respectively, Figure 3c.

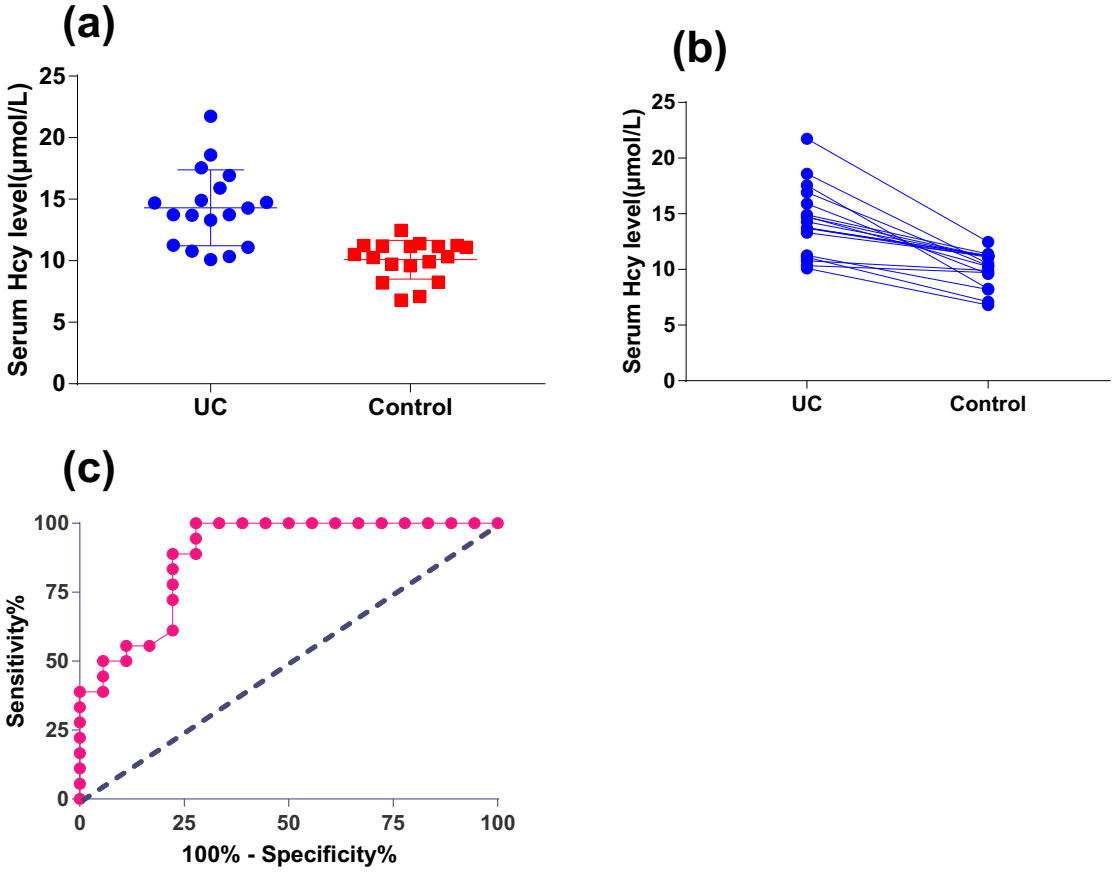

Figure 3: Serum Hcy level in UC and controls and its diagnostic performance. (a) Serum Hcy level in UC and controls. (b) Serum Hcy level in UC was significantly higher than that of corresponding controls. (c) ROC curve of serum Hcy as diagnostic biomarker for UC.

3.4 Publication bias

The Begg's funnel plot was bilateral asymmetry (Figure 4), which indicated that the publication bias was obvious. Furthermore, the Egger's line regression test also showed significant publication bias ($t = 3.29$, $P < 0.05$).

4 Discussion

In the present work, 18 case–control studies were included and the combined results demonstrated that the serum levels of Hcy were statical different between UC and healthy controls with SMD = 0.95 (95% CI: 0.87–1.04). The serum levels of Hcy were 14.30 ± 3.08 (range: 10.10–21.73) and 10.09 ± 1.57 (range: 6.80–12.47) μmol/L for UC and healthy controls, respectively, of the included 18 studies. The elevated serum Hcy indicated that Hcy may play an important role in UC development. Applying Hcy as serological biomarker for UC, the diagnostic sensitivity, specificity and AUC were 94.44% (95% CI: 72.71–99.86%), 72.22% (46.52–90.31%) and 0.88 (95% CI: 0.77–0.99, $P < 0.05$), respectively.

Hcy is an intermediate substance in the metabolic process of methionine. There are three main pathways for Hcy in the body [24,25]: (1) re-methylation to generate methionine, (2) in cystathionine – cystathionine was synthesized under the catalysis of synthase and (3) released into the blood or discharged with urine. The above metabolic pathway abnormalities can cause the serum Hcy level elevation. High level Hcy can maintain the inflammatory state of intestinal mucosa by inducing oxidative stress, promoting inflammatory response, triggering endoplasmic reticulum stress, etc., and participate in atherosclerosis and thrombosis by damaging vascular endothelial cells, changing blood coagulation state and platelet function, stimulating low-density lipoprotein oxidation and other mechanisms. Thromboembolic disease, as a serious complication of UC, is one of the causes of death in UC patients. Under normal physiological conditions, Hcy maintains a low level *in vivo* and is affected by folic acid (FA), Vit B12 levels and their related metabolic enzymes [26]. When the genes of key enzymes of Hcy metabolism (such as methionine synthetase and methylenetetrahydrofolate reductase) are defective or mutated, the normal metabolism of Hcy *in vivo* and the plasma Hcy is affected.

The mechanism of high Hcy aggravating UC injury may include the following points [27–29]: (1) Hcy is transported into the cell through the concentration gradient and transport device inside and outside the cell, and autoxidation occurs in the presence of metal ions to generate free oxygen clusters, which damages the intestinal mucosa; (2) induce ER stress, mediate cell adhesion molecules and cytochemical factors to maintain the inflammatory state of intestinal mucosa epithelium; (3) inhibiting the activity of inducible nitric oxide synthase reduces the production of nitric oxide, destroys the antibacterial system of phagocytes and increases the susceptibility of UC patients to intestinal pathogens; (4) promote the proliferation of vascular smooth muscle cells, damage vascular endothelial cells, change the coagulation state of blood, change platelet function, stimulate low-density lipoprotein oxidation and other mechanisms to participate in atherosclerosis and thrombosis. Therefore, hyper-Hcy may play a role in the occurrence and development of UC. It is necessary to measure and intervene the serum Hcy of UC patients early, and reduce the plasma Hcy level by supplementing FA and Vit B12 to slow down the inflammatory reaction caused by Hcy.

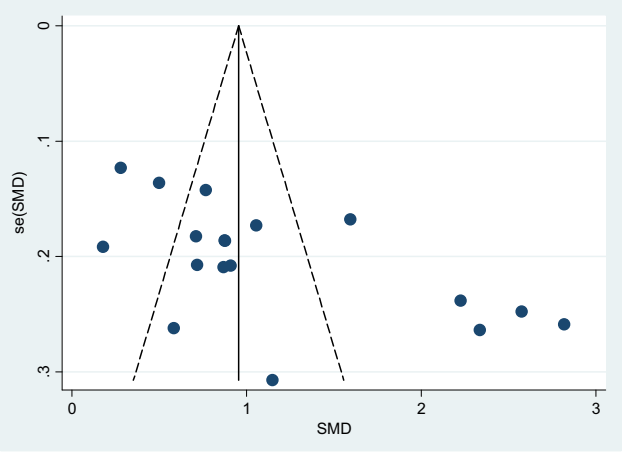

Figure 4: Begg's funnel plot for serum Hcy and UC.

5 Limitation

The present work also had significant limitations including significant publication bias, statistical heterogeneity and publication language restriction. Therefore, due to the above limitations, the diagnostic performance of serum Hcy should be further validated by well-designed prospective diagnostic studies.

6 Conclusion

Based on the present publications, serum Hcy of UC cases was significantly higher than that of healthy controls, which indicated that Hcy may play an important role in

UC development. Furthermore, the elevated serum Hcy can be applied as serological marker for UC diagnosis.

Conflict of interest: All authors declare no conflict of interest.

Data availability statement: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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