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Original Article

Vimentin gene promoter hypermethylation in stool as a biomarker for colorectal neoplasm diagnosis: a meta-analysis

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Abstract: Objective: The purpose of this meta-analysis was to assess the clinical value of vimentin gene promoter hypermethylation in stool as a biomarker for colorectal neoplasm diagnosis. Methods: Prospective clinical diagnostic studies about vimentin gene promoter hypermethylation in stool as a biomarker for colorectal neoplasm diagnosis were electronic screened in the databases of Medline, Cochrane Library, EMBASE, ISI Web of Knowledge, CNKI and Wanfang. The number of true positive (tp), false positive (fp), false negative (fn), true negative (tn) were extracted from each individual publications. The combined diagnostic sensitivity, specificity, positive likelyhood ratio (+lr), negative likelyhood ratio (-lr), diagnostic odds ratio (DOR) and area under the symmetric ROC curve were calculated by metadisc-1.4 software. Results: Six prospective diagnostic studies were finally included in our present meta-analysis. The pooled sensitivity was 0.64 (95% CI: 0.58-0.69) and 0.92 (95% CI: 0.89-0.95) by random effect model and fixed effect model for colorectal cancer and colorectal adenoma respectively. The pooled specificity were 0.89 (0.86-0.92) and 0.92 (0.87-0.95) for colorectal cancer and colorectal adenoma respectively by fixed effect model; The pooled +lr were 5.68 (4.16-7.75) and 4.15 (2.28-7.56) for colorectal cancer and colorectal adenoma respectively by fixed effect model without statistical heterogeneity; The pooled -lr were 0.40 (0.26-0.61) and 0.54 (0.29-1.04) for colorectal cancer and colorectal adenoma respectively by random effect model because of statistical heterogeneity across the included studies. The pooled DOR were 7.16 (10.78-27.32) and 7.88 (2.61-23) for colorectal cancer and colorectal adenoma by fixed and random effect model respectively. The pooled areas under the ROC curve were 0.90 and 0.91 of vimentin gene promoter hypermethylation in stool for colorectal cancer and colorectal adenoma diagnoses respectively. Conclusion: Vimentin gene promoter hypermethylation rate in stool of colorectal cancer or colorectal adenomas were much higher than that of healthy controls, which could be a promising biomarker for colorectal neoplasm diagnosis.

Keywords: Colorectal neoplasm, hypermethylation, vimentin gene, stool, diagnosis, meta-analysis

Introduction

Colorectal carcinoma is one of the most diagnosed malignant tumors world-wide [1]. Its morbidity is still on the rise for recent years [2]. Colorectal neoplasm included colorectal cancer and colorectal adenoma. Colorectal adenoma is one of the most precancerous lesions of colorectal cancer. It has been reported that the development and progression of colorectal adenoma to colorectal cancer will take 5 to 10 years. This made the colorectal cancer early screen possible [3, 4].

DNA hymethylation, one of the commonly detected changes in cancers, is deemed as an important epigenetic mechanism for tumor suppressor genes silence. Several studies have evaluated the possibility of tumor suppressor genes promoter hypermethylation as biomarkers for colorectal cancer and colorectal adenoma diagnoses [5-7]. Chen et al [8] evaluated vimentin gene promoter hypermethyliation as biomarker in stool for colorectal cancer or colorectal adenoma diagnosis. The author found hypermethyliation rate of vimentin gene was significant higher in stool of colorectal cancer

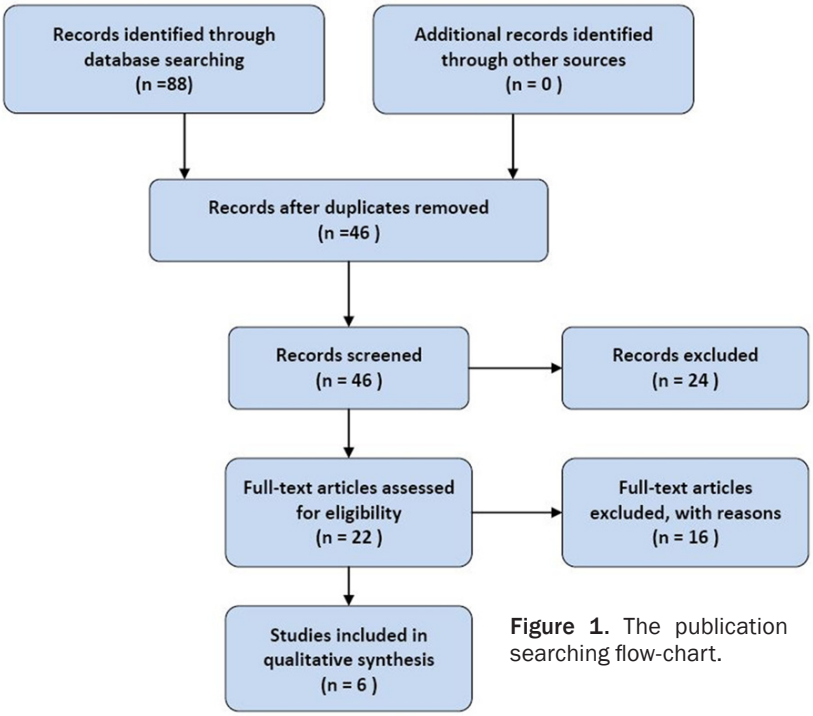

Figure 1. The publication searching flow-chart.

Table 1. The general information of included studies

Study	Year	Sample size	CRC		CRA		Control		Country	Methods
			+	-	+	-	+	-		
Li	2009	80	9	13	9	11	2	36	U.S.	Methl-BEAMing
Itzkowitz	2007	162	31	9	-	-	19	103	U.S.	MSP
Chen	2005	263	43	51	6	44	8	111	U.S.	MSP
Zhu	2011	107	52	8	13	4	4	26	China	MSP
Fu	2010	22	5	9	-	-	0	8	China	MSP
Baek	2009	149	45	15	31	21	5	32	Korea	MSP

CRC = Colorectal cancer; CRA = Colorectal Adenomas; MSP = Methylation Specific PCR.

or colorectal adenoma patients than that of healthy controls, which could be a potential biomarker for colorectal cancer diagnosis. However, the sample size of this study was small and the clinical vale was limited. Therefore, we performed this meta-analysis to further evaluate the diagnostic value of vimentin gene promoter hypermethylation in stool for colorectal cancer and colorectal adenoma patients.

Material and methods

Study electronic searching

Prospective clinical diagnostic studies about vimentin gene promoter hypermethylation in stool as a biomarker for colorectal neoplasm diagnosis were electronic screened in the databases of Medline, Cochrane Library, EMBASE, ISI Web of Knowledge, CNKI and Wanfang. The searching items were: "colorectal cancer, colon cancer, rectal cancer; colorectal carcinoma; colorectal neoplasm, stool, methylation, hypermethylation and vimentin". All potential relevant studies were assessed in details and additional all citations of the included articles were further evaluated in order to identify additional suitable studies. The publication searching was done by two reviewers (Ma Peng, Dai Shaojun) independently. The publication searching procedure was demonstrated in **Figure 1**.

Data collection

The data and main information for each included study was extracted by two reviewers (Dai Shaojun and Mingdong Wu) independently and checked by another reviewer (Wangyue Wang). The year of publication, first and corresponding author and area the study performed were extracted from the 6 included studies. The patients of true positive (tp), false positive (fp), false negative (fn) and true negative (tn) detected by vimentin gene promoter hypermethylation in stool of each included publication were also extracted and cross checked by two reviewers independently.

Study quality assessment

The methodological qualities of each included study were evaluated by two reviewers (Lixian Liu and Wangyue Wang) independently by a questionnaire included 11 items according to the Cochrane Reviews Handbook [9].

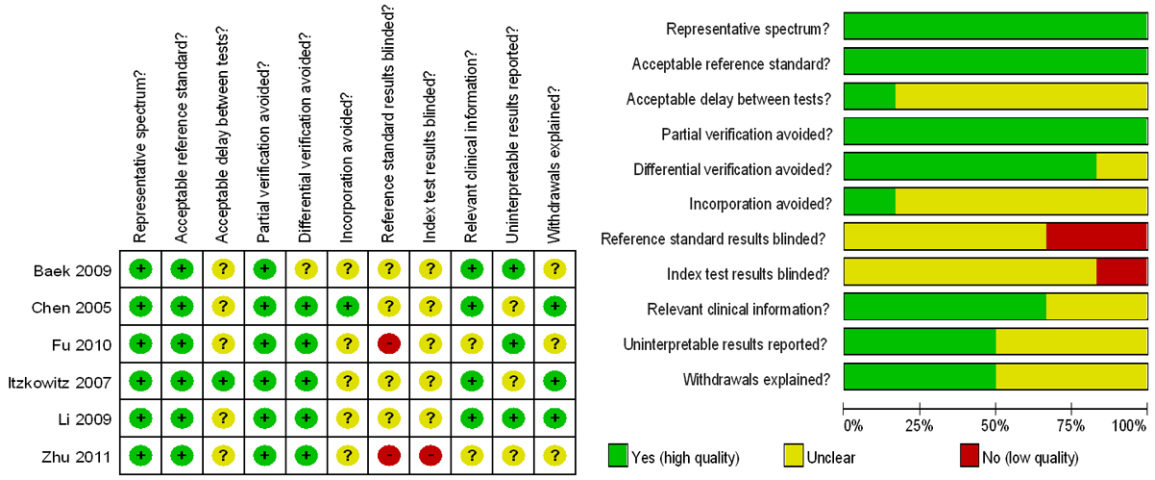

Figure 2. Quality assessment for included 6 studies. (The authors' judgments for each risk of bias item. + is "low risk"; - is "high risk''; is "moderate risk").

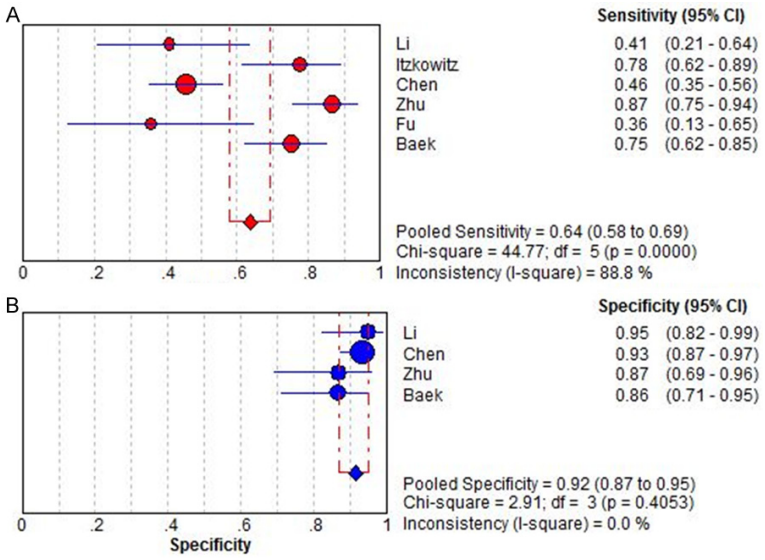

Figure 3. The sensitivity of S vimentin gene promoter hypermethylation in stool for colorectal neoplasm diagnosis (A: Colorectal cancer; B: Colorectal adenoma).

Statistical analysis

The statistical analysis was done by Meta-DiSc1.4 (http://www.biomedsearch.com/nih/Meta-DiSc-software-meta-analysis/16836745.html) and Stata11.0 (http://www.stata.com; Stata Corporation, College Station, TX) software. Chi-square and I^2 test were used to calculate the statistical heterogeneity across the studies. Fixed or random effect model was use to pooled the sensitivity, specificity, positive likelyhood ratio (+lr), negative likelyhood ratio (-lr), DOR according to the statistical heterogeneity evaluation results. The publication bias was evaluated by funnel plot and Egger's line regression test.

Results

Characteristics of included studies

Six prospective diagnostic studies [8, 10-14] were found fulfilled the inclusion criteria and include in this meta-analysis. Three studies were performed in US, 2 in China and 1 in Korea. Hypermethylation of vimentin gene promoter region was tested by methylation specific PCR in 5 publications and Methl-BEAMing assay was used in 1 study. Six studies and 4 publications assessed the diagnostic value of vimentin gene promoter hypermethylation in stool as a biomarker for colorectal cancer and colorectal adenoma respectively. The summarized characteristics of included 6 publications are demonstrated in **Table 1**.

Quality assessment

The methodological quality assessment was made by a questionnaire included 11 items: 1) Representative spectrum? 2) Acceptable refer-

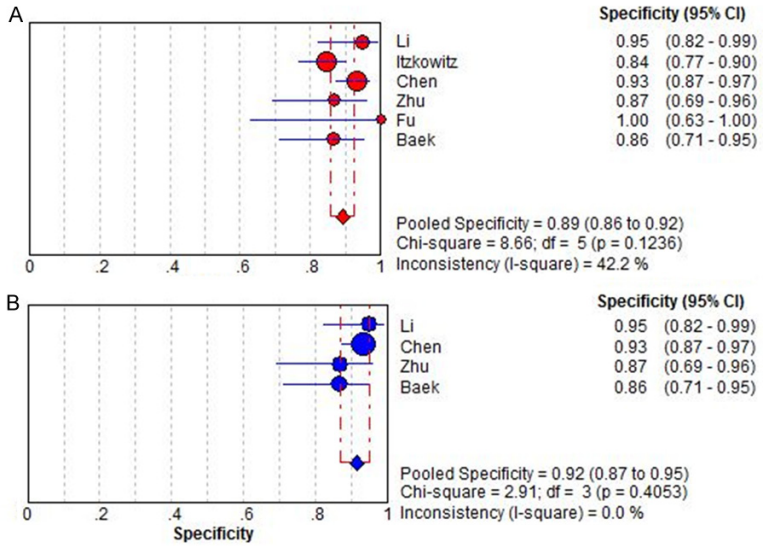

Figure 4. The specificity of vimentin gene promoter hypermethylation in stool for colorectal neoplasm diagnosis (A: Colorectal cancer; B: Colorectal adenoma).

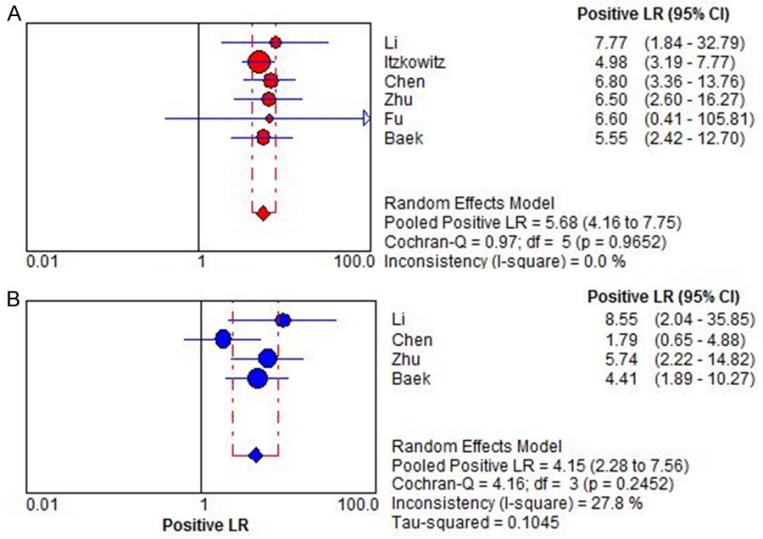

Figure 5. The +lr of vimentin gene promoter hypermethylation in stool for colorectal neoplasm diagnosis (A: Colorectal cancer; B: Colorectal adenoma).

ence standard? 3) Acceptable delay between tests? 4) Partial verification avoided? 5) Differential verification avoided? 6) Incorporation avoided? 7) Reference standard results blinded? 8) Index test results blinded? 9) Relevant clinical information? 10) Uninterpretable results reported? 11) Withdrawals explained? The general methodological quality was well and showed in **Figure 2**.

Pooled sensitivity

Six studies reported the vimentin gene promoter hypermethylation in stool as a biomarker for colorectal cancer diagnosis and 4 for colorectal adenoma diagnosis. Significant heterogeneity was found in colorectal cancer (I^2=88.8%). The pooled sensitivity was 0.64 (95% CI: 0.58-0.69) by random effect model. For colorectal adenoma, the pooled sensitivity was 0.92 (95% CI: 0.89-0.95) by fixed effect model without significant heterogeneity (I^2=0.0%) (**Figure 3**).

Pooled specificity

The pooled specificity were 0.89 (0.86-0.92) and 0.92 (0.87-0.95) for colorectal cancer and colorectal adenoma respectively by fixed effect model with no statistical heterogeneity across the included studies (**Figure 4**).

Pooled +lr

The pooled +lr were 5.68 (4.16-7.75) and 4.15 (2.28-7.56) for colorectal cancer and colorectal adenoma respectively by fixed effect model with no statistical heterogeneity across the included studies (**Figure 5**).

Pooled -lr

The pooled -lr were 0.40 (0.26-0.61) and 0.54 0.29-1.04) for colorectal cancer and colorectal adenoma respectively by random effect model because of statistical heterogeneity across the included studies (**Figure 6**).

Pooled DOR

The pooled DOR were 17.16 (10.78-27.32) and 7.88 (2.61-23) for colorectal cancer and colorectal adenoma by fixed and random effect model

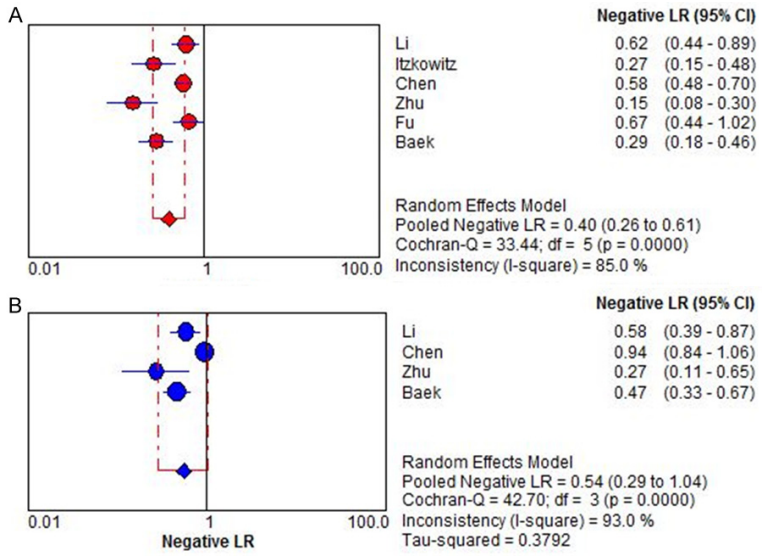

Figure 6. The -lr of vimentin gene promoter hypermethylation in stool for colorectal neoplasm diagnosis (A: Colorectal cancer; B: Colorectal adenoma).

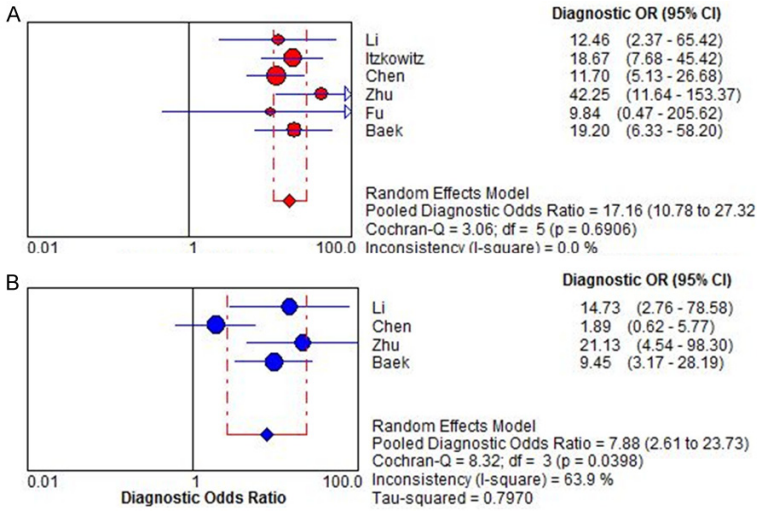

Figure 7. The DOR of vimentin gene promoter hypermethylation in stool for colorectal neoplasm diagnosis (A: Colorectal cancer; B: Colorectal adenoma).

respectively for without and with statistical heterogeneity across the included studies (**Figure 7**).

Pooled ROC

The pooled area under the ROC curve was 0.90 and 0.91 of vimentin gene promoter hypermethylation in stool for colorectal l cancer and colorectal adenoma diagnoses respectively (**Figure 8**).

Publication bias

The publication bias was evaluated by Deeks' funnel plot (**Figure 9**) and Egger's line regression test. The Deeks' funnel plot and Egger's line regression test indicated no publication bias for colorectal cancer (t=0.32, P=0.76) and colorectal adenoma (t=0.97, P=0.39).

Discussion

Colorectal cancer is a main cause of cancer related death globally [15]. The prognosis for early stage patients is relative well. However, the 5-year survival was rather low for advanced stage diseases. So, colorectal cancer screening for high risk population or early stage diagnosis is one of the important way to improve the prognosis [16]. The National Comprehensive Cancer Network (NCCN) colorectal cancer guideline described several colorectal cancer screening or early diagnosis procedures such as colonoscopy, flexible sigmoidoscopy, double-contrast barium enema, computed tomographic colonography and fecal occult blood test. The above screening or early diagnosis methods have been widely discussed in the previously studies. For each of the above mentioned screening or early diagnosis method, it has its own advantages and limitations. The main limitations of the present screening or early stage diagnosis for colorectal cancer were low sensitivity, low specificity, invasive or complex operation procedure [17, 18]. So, the ideal colorectal cancer screening or early diagnosis method should be high sensitivity, high specificity, mini-invasive and easy operation [19]. Detection tumor suppressor genes promoter hypermethylation in peripheral blood or stool may suitable for the colorectal screening or early diagnosis [20]. Promoter

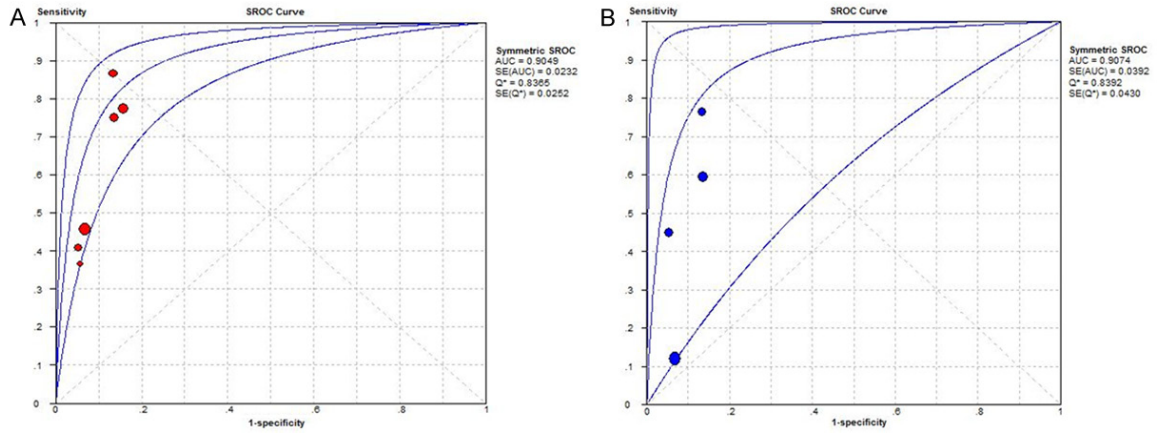

Figure 8. ROC cure of vimentin gene promoter hypermethylation in stool for colorectal neoplasm diagnosis (A: Colorectal cancer; B: Colorectal adenoma).

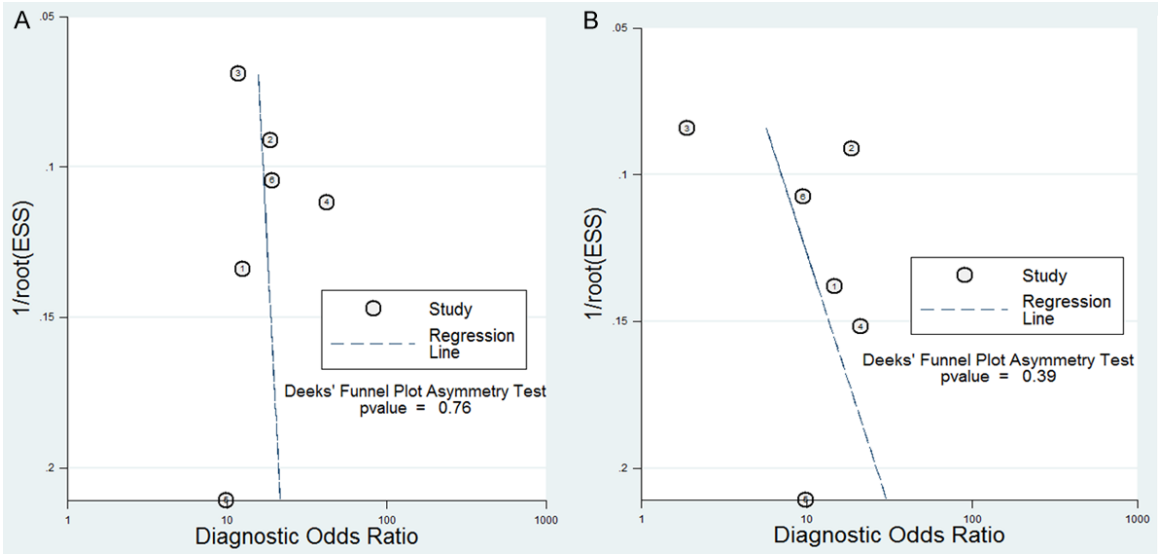

Figure 9. Publication bias evaluated by Deeks' funnel plot.

hypermethylation is a kind of epigenetic modifications, which affects the cytosines in the dinucleotide: cytosine-phosphate-guanine (CpG). The covalent addition of a methyl group on the C5 of the Cp transforms it into a methylated cytosine (Cm). Tumor suppressor gene transcriptional silencing by CpG island hypermethylation of its promoter region is one of the important component in the initiation and progression of various types of cancer. Various studies have demonstrated the high hypermethylation rate in tumor suppressor genes in cancer tissue or serum than those of healthy controls [21-23]. This indicated that tumor suppressor gene promoter hypermethylation detection can be a promising method for colorectal cancer early detection or screening. Hypermethylation of vimentin gene promoter region has been discussed in several previous published studies [11, 13]. However, because of small sample size for each individual study, the results were inconclusive and clinical value was limited. Therefore, we collected open published studies about vimentin gene promoter hypermethylation in stool as a biomarker for colorectal neoplasm diagnosis and pooled the combined sensitivity, specificity and ROC in order to further evaluate its clinical usefulness. In our present meta-analysis, we found that the pooled sensitivity was 0.64 (95% CI: 0.58-0.69) and 0.92

(95% CI: 0.89-0.95), pooled specificity were 0.89 (0.86-0.92) and 0.92 (0.87-0.95) for colorectal cancer and colorectal adenoma respectively. The sensitivity and specificity were relative high for colorectal cancer and colorectal adenoma. And the sensitivity, specificity also had good consistency for colorectal cancer and colorectal adenoma. This indicated detection of vimentin gene promoter hypermethylation was a promising method for colorectal cancer or colorectal adenoma diagnosis. However, hypermethylation in promoter region of tumor-suppressor genes may link and interact with each other, indicating analysis of a single gene promoter methylation status may be not enough for colorectal cancer screening or early diagnosis. Multiple genes simultaneous detection for promoter hymethylation may provide more accuracy for colorectal cancer diagnosis than a single gene.

Disclosure of conflict of interest

None.

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