Pepsin in Saliva as a Diagnostic Marker for Gastroesophageal Reflux Disease: A Meta-Analysis

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Gastroesophageal reflux disease (GERD) is very common. Salivary pepsin detection has previously been considered as a method for GERD diagnosis. We performed a meta-analysis to investigate the utility of salivary pepsin assay as a diagnostic tool of GERD.

Material/Methods: PubMed, Web of Science, the Cochran Library, and EMBASE (from January 1980 to 23 October 2018) were searched for pepsin in saliva for GERD diagnosis. We summarized the retrieved specificity, sensitivity, negative likelihood ratio (NLR), positive likelihood ratio (PLR), diagnostic odds ratio (DOR), and receiver operating characteristic (ROC) curves data in the meta-analysis.

Results: In final analysis, a total of 5 studies were included. The summary sensitivity, specificity, NLR, and PLR were 0.60 (95% CI 0.41–0.76), 0.71 (95% CI 0.51–0.86), 0.56 (95% CI 0.34–0.93), and 2.1 (95% CI 1.1–4.1), respectively. The pooled DOR was 4 (95% CI 1.0–11.0) and area under the ROC was 0.70 (95% CI 0.66–0.74).

Conclusions: The meta-analysis showed that pepsin in saliva has moderate diagnostic value for GERD, and is not as helpful as previously thought.

MeSH Keywords: Gastroesophageal Reflux • Laryngopharyngeal Reflux • Meta-Analysis as Topic • Pepsin A • Sensitivity and Specificity

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Background

Gastroesophageal reflux disease (GERD) is one of the most common esophageal diseases, and its prevalence is increasing worldwide [1,2]. GERD is the retrograde movement of gastric contents, resulting in mucosal damage and pathologic changes [3,4].

Diagnostic methods of GERD include empirical PPIs treatment, questionnaires, endoscopy, and ambulatory reflux monitoring. Ambulatory reflux monitoring can provide confirmatory evidence of GERD, such as 24-h pH and multichannel intraluminal impedancometry (24-h pH-MII). However, it is invasive, expensive, and uncomfortable for the patient [5]. Empirical PPIs treatment, also called "PPI test", lack sensitivity and specificity for GERD diagnosis, as shown by a systematic review [6]. When compared with objective evidence of GERD as defined by pH-metry or endoscopy, questionnaires such as the reflux disease questionnaire (RDQ) and gastroesophageal reflux disease questionnaire (GERDQ) lack sensitivity and specificity [7]. Endoscopy is performed with patients with alarm signs or in refractory GERD patients. However, non-erosive reflux disease (NERD) and most extra-esophageal reflux (EER) have no endoscopic evidence of mucosa damage [8,9].

Pepsin is proteolytic enzyme of pepsinogen, which is released solely by gastric chief cells. There is no production of pepsin in the airway or esophagus, thus its presence in the esophagus or more proximally (pharynx or airways) suggests gastro-oesophageal reflux (GOR) [10,11]. Salivary pepsin detection has previously been considered as a method for GERD diagnosis. Many studies have assessed the value of salivary pepsin assay for GERD diagnosis, but the diagnostic values differed [12–26].

In the present study, a meta-analysis was performed to investigate the diagnostic value of pepsin in saliva for diagnosis of GERD.

Material and Methods

Search strategy and study inclusion

The present research was performed according to the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) guideline [27]. Two investigators (Guo and Wu) independently searched the published articles in multiple databases: PubMed, Web of Science, the Cochran Library, and EMBASE (from January 1980 to 23 October 2018). The search strategies in PubMed, Web of Science, the Cochran Library, and EMBASE were "(saliva[Title/Abstract]) OR salivary[Title/Abstract]) OR spit[Title/Abstract])) OR "Saliva"[Mesh])) AND (pepsin[Title/Abstract])", "TS=(saliva AND pepsin)", "saliva in

Title Abstract Keyword AND pepsin in Title Abstract Keyword" and "'saliva': ab,ti AND 'pepsin': ab,ti", respectively. References of review articles or previously published meta-analyses were also searched manually.

Inclusion and exclusion criteria

Studies were included if they: (1) provided sufficient information to construct a 2×2 contingency table, (2) assessed the accuracy of salivary pepsin assay for diagnosis of GERD, and (3) only involved adult patients. We only included original articles written in English. Reviews, animal experiments, case reports, conference abstract, correspondences, and editorials were excluded.

Procedures

The data was extracted and the methodological quality was assessed independently by 2 investigators (GZH and WH). The extracted data included year, methodology characteristics, country of origin, study population characteristics (adults or pediatric), details of the pepsin assays, and cut-off value. The Quality Assessment of Diagnostic Accuracy Studies-2(QUADAS-2) was used to assess the methodological quality of the studies [28]. If there were discrepancies between the 2 investigators, a consensus meeting was held by referral to a third investigator.

Statistical analysis

All the extracted data were inputted in STATA ver. 12.0 (Stata Corp, College Station, TX, USA) and Review Manager (version 5.2 of Windows; Cochrane Collaboration, Oxford, UK). The specificity, sensitivity, pooled negative likelihood ratio (NLR), pooled positive likelihood ratio (PLR), corresponding 95% confidence intervals (CIs), and diagnostic odds ratio (DOR) were calculated from false positive, true positive, true negative and false negative, which was extracted from each study before data pooling. The overall performance of salivary pepsin assay in GERD patients were evaluated by the summary receiver operator characteristic (SROC) curve.

The I^2 statistic were used to assess the statistical heterogeneity among the studies. $P \leq 0.10$ indicated significant heterogeneity, while $P > 0.10$ indicated no significant heterogeneity for the Q statistic [29]. A random-effect models was used when the heterogeneity was high ($P < 0.05$, $I^2 > 50\%$). Meta-regression analyses were conducted on the basis of sample size, cut-off value, and region [30]. Potential publication bias was evaluated by Deeks' asymmetry test [31].
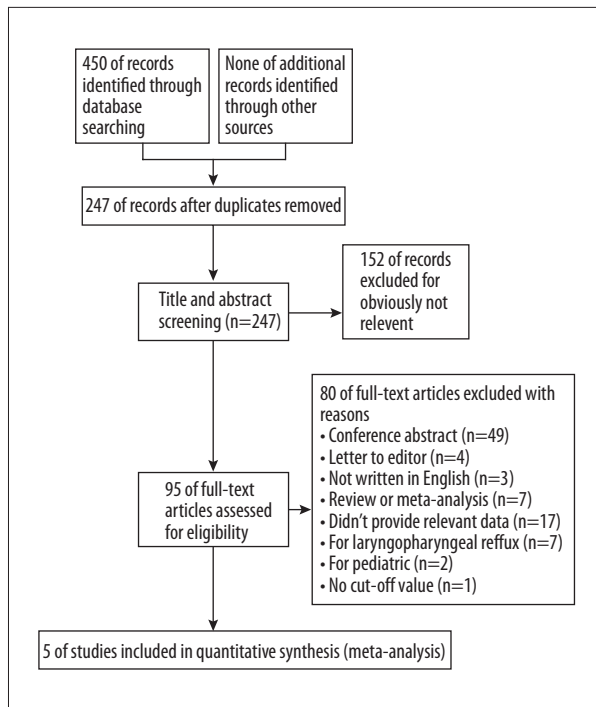
Figure 1. Retrieval flowchart to obtain study data for meta-analysis.

Results

Study characteristics

Detailed search steps are described in Figure 1. A total of 420 potentially relevant articles were identified for retrieval. After screening the titles and abstracts, 152 were excluded

because they were irrelevant. No more relevant articles were identified by searching of the reference lists of previous systematic reviews [32,33]. After detailed full-article inspection, 2 articles for pediatrics were excluded [16,17], and 1 article using Western blotting analysis without cut-off value of pepsin for GERD was excluded [24]. Finally, 5 articles met our predefined inclusion criteria and were included in the final analysis [12,14,15,19,23]. Because diagnostic accuracy in 1 study was reported separately for different diagnostic criteria [12], the study was divided into 2 parts; as the result, 6 datasets were analyzed. Details of these studies are shown in Table 1. The risk of bias for the enrolled studies is presented in Figure 2.

Meta-analysis results on diagnostic value of salivary pepsin assay for GERD

The 5 studies (6 datasets) were pooled for assessment of diagnostic accuracy. As shown in Figure 3, pooled specificity was 0.71 (95% CI 0.51–0.86) and pooled sensitivity was 0.60 (95% CI 0.41–0.76). The pooled negative likelihood ratio and pooled positive likelihood ratio were 0.56 (95% CI 0.34–0.93) and 2.1(95% CI 1.1–4.1), respectively. The diagnostic odds ratio (DOR) was 4 (95% CI 1.0–11.0). The area under the receiver operating characteristic curve was 0.70 (95% CI 0.66–0.74; Figure 4). The likelihood ratio plot showed the saliva pepsin assay for GERD neither confirmed nor excluded its utility (Figure 5).

Heterogeneity, subgroup analysis, and publication bias

The proportion of heterogeneity probably was small (sensitivity: I^2=91.02%, p=0.00; specificity: I^2=89.64%, p=0.00). We did a meta-regression analyses to identify the source of

Table 1. Characteristics of studies included in the meta-analysis.

No.	Author	Year	Country	Sample size	Age	Diagnostic criteria	Pepsin assay method	Sample time/total sample number	Cut-off (ng/ml)
1	Dolina(a) [12]	2018	Czech	32GERD/11 HC	Adult	24h pH-MII (acid reflux)	PepTest	Waking up/1	36
	Dolina(b)					Weakly acid reflux			36
2	Du [15]	2017	China	122GERD/128SC/35HC	Adult	24h pH-MII	PepTest	Waking, 1–2 h after lunch and dinner/3	76
3	Hashizume [14]	2017	Japan	15GERD/11SC	Adult	24h pH-MII	PepTest	Fasting	16
4	Hayat [19]	2015	UK	111 GERD symptoms/100HC	Adult	24h pH-MII	PepTest	Waking, 1 h after lunch and dinner/3	16
5	Yuksel [23]	2012	UK	22GERD/25SC	Adult	Wireless 48 h pH-monitoring	PepTest	Not mention	16

GERD – gastroesophageal reflux disease; HC – health control; SC – symptomatic control.
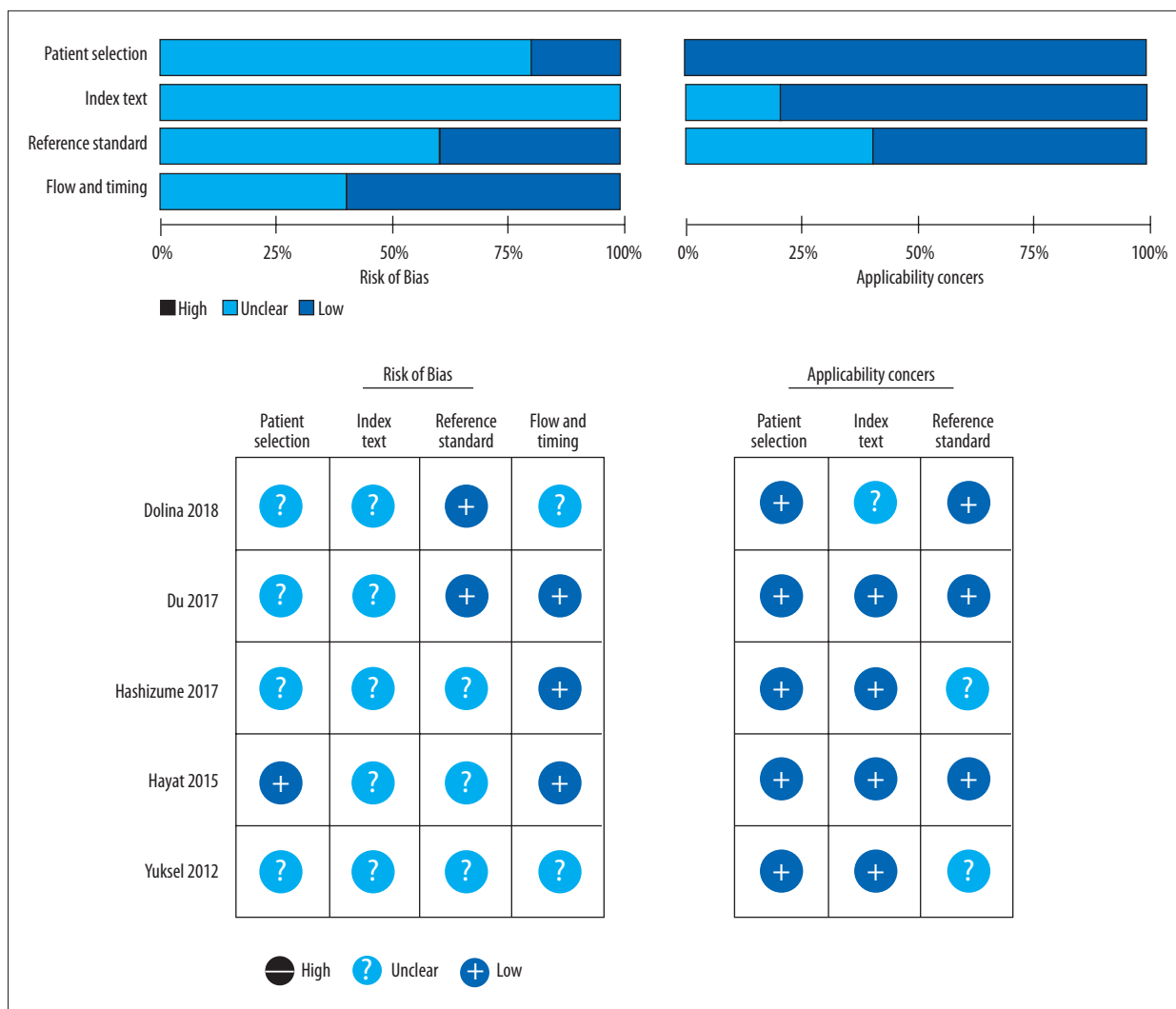
Figure 2. Quality of the studies as assessed by the Quality Assessment of Diagnostic Accuracy Studies questionnaire. Deep blue indicates absence of bias, black indicates the presence of bias, and light blue indicates unclear.

heterogeneity. There was lower heterogeneity when subgroup analysis (Table 2) was conducted according to sample size (≥50 versus <50) ($I2$=2.15%, p=0.34), research region (Asia or Western countries) (I^2=2.58%, p=0.28), and cut-off value (≥25 ng/ml or <25 ng/ml) (I^2=2.15%, p=0.34), which indicated that they were not the heterogeneity source. No evidence of publication bias was shown by the Deeks' asymmetry test (p=0.44; Figure 6).

Discussion

Pepsin is only produced in the stomach, so it is a specific biomarker for gastric reflux and can be detected in saliva, sputum, secretary otitis media, and even in tears [34,35]. Detecting pepsin in saliva is a noninvasive and convenient diagnostic tool compared with endoscopy and 24-h pH-MII monitoring.

In 2003, Potluri et al. used the fibrinogen digestion method to assay saliva/sputum pepsin for detection of gastric reflux, which was the first study on use of the salivary pepsin assay for GERD [26]. Western blot and enzyme-linked immunosorbent assay (ELISA) have also been used for pepsin assay [22–25]. In 2012, a rapid lateral flow test (Peptest) to detect pepsin in saliva/sputum was developed by Saritas Yuksel et al. [23], which has shortened time needed for the salivary pepsin assay to several minutes and offers a strong predictive value for GERD diagnosis. In our study, the summary sensitivity, specificity, NLR, and PLR were 0.60 (95% CI 0.41–0.76), 0.71 (95% CI 0.51–0.86), 0.56 (95% CI 0.34–0.93), and 2.1 (95% CI 1.1–4.1), respectively. Furthermore, the area under the ROC was 0.70 (95% CI 0.66–0.74). The likelihood ratio plot showed the saliva pepsin assay for GERD neither confirmed nor excluded its utility in diagnosis. Thus, these results suggest that the salivary pepsin assay does not have high utility in diagnosis of GERD.
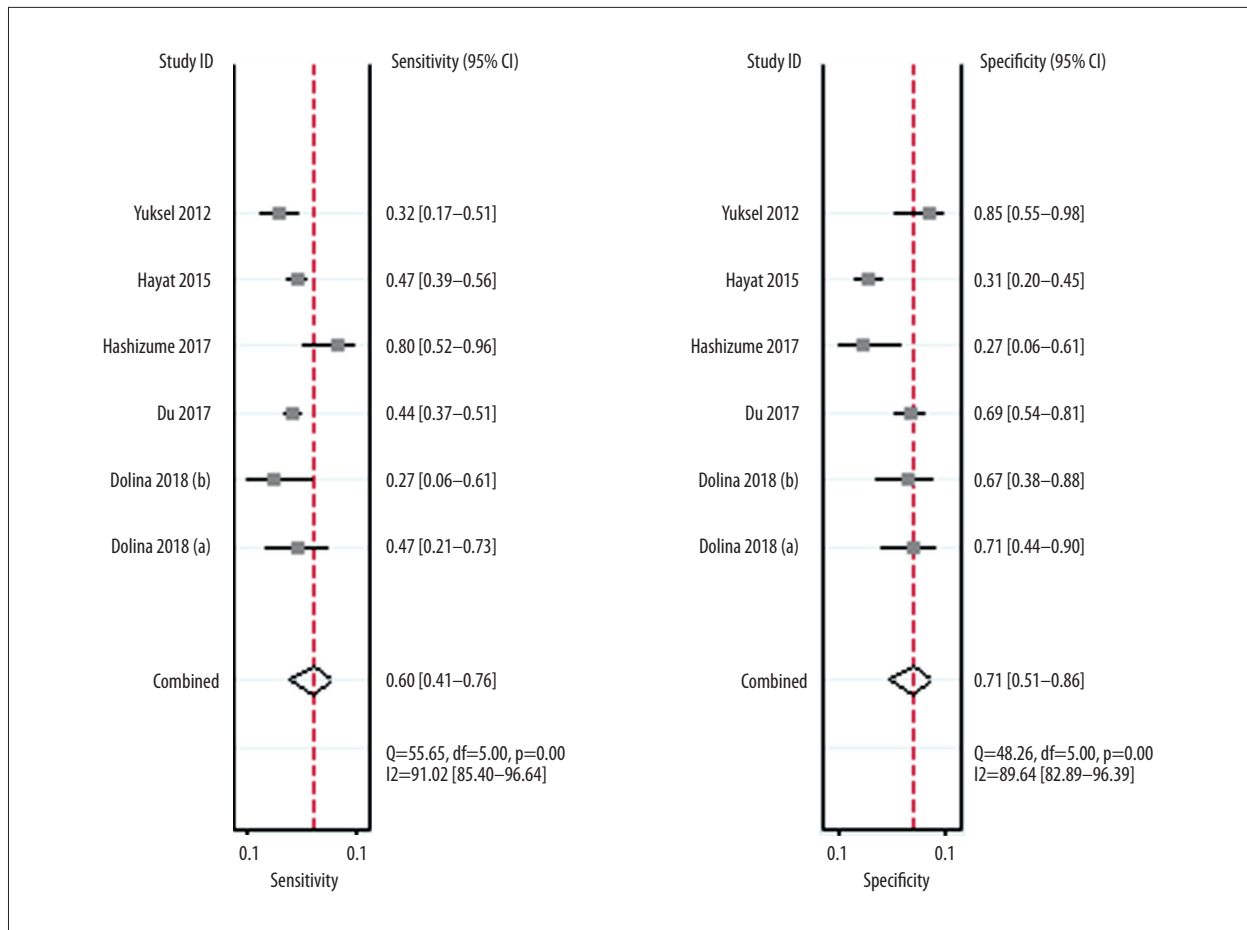
Figure 3. Sensitivity and specificity of saliva pepsin assay for diagnosis of GERD.
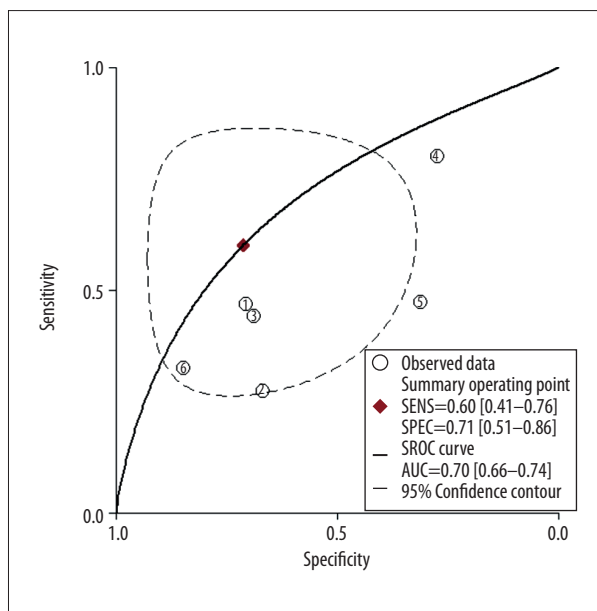

Figure 4. Summary receiver operating characteristic curve with 95% confidence contour and 95% prediction contour.
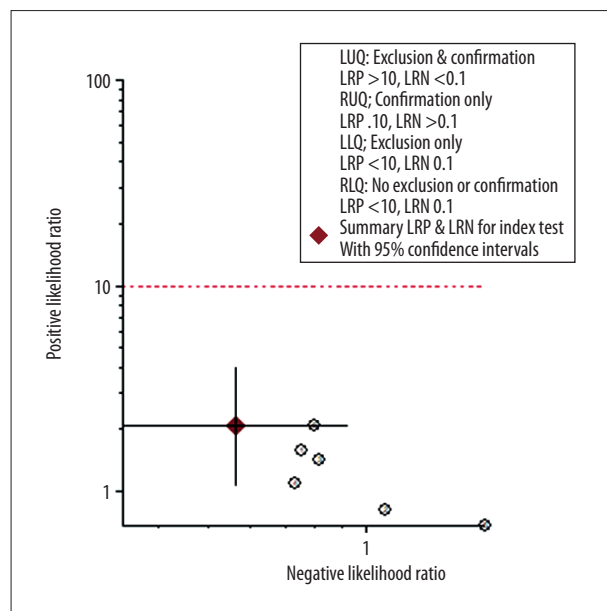

Figure 5. Likelihood ratio plot for saliva pepsin assay for diagnosis of GERD.

Table 2. Subgroup analysis.

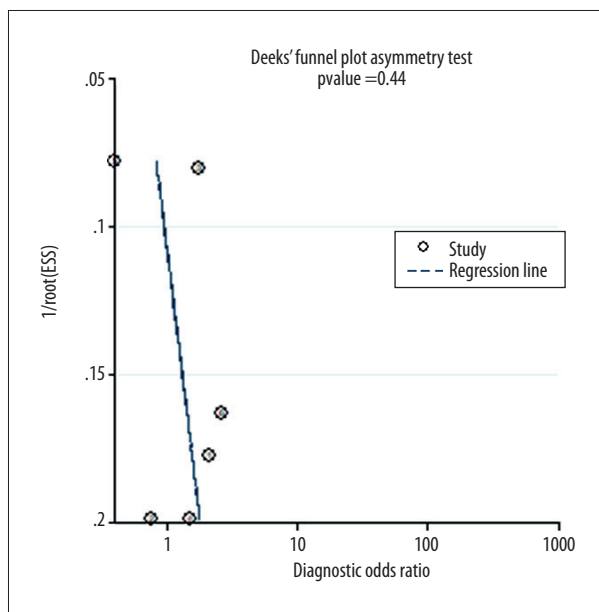| Subgroup (number of studies)	Sensitivity	p	Specificity	p
Region		0.25		0.74
Western country (n=4)	0.51 [0.31–0.71]		0.73 [0.52–0.94]	
Asia (n=2)	0.77 [0.57–0.97]		0.68 [0.35–1.00]	
Sample size		0.16		0.78
≥50 (n=3)	0.48 [0.24–0.72]		0.75 [0.53–0.97]	
<50 (n=3)	0.71 [0.52–0.90]		0.67 [0.41–0.93]	
Cut-off value		0.16		0.78
≥25 ng/ml (n=3)	0.48 [0.24–0.72]		0.75 [0.53–0.97]	
<25 ng/ml (n=3)	0.71 [0.52–0.90]		0.67 [0.41–0.93]	

Figure 6. Deeks' plot for saliva pepsin assay for diagnosis of GERD.

The reported salivary pepsin for GERD diagnosis data, such as sensitivity and specificity, are highly variable among the research groups. The variation may be due to lack of a standard protocol in each study, which leaves unanswered questions such as: When should the saliva sample be collected? At the time of waking up, or after meals, or at the symptoms, or before bedtime, or before catheter placement? How many samples should be collected? Should samples be collected once or several times? Dolina et al. showed that the Peptest reaction was not finished at 15 min, as mentioned in previous articles, and the intensity continued to increase further until 40 min. The relative standard deviation of measurement was good when using the same device, but the reproducibility of 10 different individual devices was poor [12]. The subgroup analysis in our study showed that sample size (≥50 versus <50), research region (Asia or Western country), and the cut-off value (≥25 ng/ml or <25 ng/ml) had low heterogeneity. As a result, differences in study design and different enrollment criteria might be the source of heterogeneity. Some studies compared diagnosed GERD patients with health controls [12], while some compared GERD patients with symptomatic controls [14,15,19,23]. The study of Hayat et al. provided detailed diagnosis of non-GERD group (healthy controls, hypersensitive oesophagus, and functional heartburn) [19], but other studies just divided the GERD and non-GERD according to 24-h pH-MII or wireless 48-h pH monitoring, not giving the detailed diagnostic information of non-GERD patients [12,14,15,23].

The present study differs in several ways from previously meta-analyses and systematic reviews [3,32]. Firstly, we excluded studies on laryngopharyngeal reflux disease (LPRD) [13,18,20–22,25,26]. LPRD is a supra-esophageal form of GERD, which is defined as the back flow of gastric or gastroduodenal contents into the laryngopharynx. Although LPR and GERD are both caused by the reflux of stomach contents, it has been reported that LPR is distinct from GERD, with differing presenting symptoms and diagnostic criteria [36]. As a result, we excluded the studies on saliva pepsin assay for LPR patients. Secondly, the studies on pediatric patients were excluded because this study included infant GERD patients around 1 year old who cannot complain of symptoms. These entities are completely different from adult GERD. Thirdly, only the results of Peptest were included. There were several methods for pepsin assay, such as ELISA, Western blot, fibrinogen digestion, and Peptest, among which Peptest is now most widely used. To reduce heterogeneity, a study with 9 GERD patients and 31 symptomatic controls was excluded based on the Western blot method used [24]. Finally, ambulatory reflux monitoring was used as the criterion standards of GERD diagnosis for all the studies included, which reduces heterogeneity compared with the meta-analyses including studies using symptoms as diagnostic criteria.

The strengths of this study are that a comprehensive search strategy was used and a standard protocol was followed. In addition, the findings of a study with a large pooled sample are more robust than any individual study.

There are several limitations to our study. Firstly, the differences in study design and groups of each studies might be a source of heterogeneity. Secondly, as we only included published studies, publication bias is an inevitable problem. Finally, the summarized data in some articles limited our ability to conduct a more detailed analysis.

Conclusions

This meta-analysis shows that pepsin in saliva has only moderate diagnostic value for GERD, and is not as helpful as previously believed. More standard protocols for salivary pepsin detection are needed, and further large-scale studies with clear subgroup information should be done to verify the diagnostic value.

Conflict of interest

None.

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