Impact of haematologic deficiencies on recurrent aphthous ulceration: a meta-analysis

H. Chen,[1] Q. Sui,[1] Y. Chen,[1] L. Ge*[2] and M. Lin*[3]

IN BRIEF

- Studies the aetiology of recurrent aphthous ulceration (RAU).
- Recognises haematologic deficiencies could be a significant risk factor for RAU.
- Stresses the importance of screening and treating any haematologic deficiencies in preventing the occurrence of RAU.

RESEARCH

Background Recurrent aphthous ulceration (RAU) is one of the most common oral mucosal diseases, some of which may be secondary to haematologic deficiencies. This meta-analysis aims to evaluate the association between haematologic deficiencies and RAU. **Materials and methods** Case control studies were identified using a predefined search strategy that compared the difference in haematologic deficiencies between a RAU group and a control group. A meta-analysis was performed to estimate the combined odds ratios (ORs) and 95% confidence intervals (95% CIs) in a fixed-effects model and a random-effects model, as appropriate. **Results** In this meta-analysis, nine case control studies, including total 710 cases in RAU groups and 602 cases in control groups, were considered eligible for inclusion. Overall, the combined results based on all studies showed that the rate of haematinic deficiencies was significantly high in the RAU group (vitamin B12: OR = 3.75, 95% CI: 2.38–5.94; folic acid: OR = 7.55, 95% CI: 3.91–14.60; ferritin: OR = 2.62, 95% CI: 1.69–4.06; and haemoglobin: OR = 1.77, 95% CI: 1.12–2.80). **Conclusion** This meta-analysis suggests that haematologic deficiencies could be a significant risk factor for RAU. Thus, screening and treating any haematologic deficiencies may play an important role in preventing the occurrence of RAU.

INTRODUCTION

Recurrent aphthous ulceration (RAU) is one of the most common oral mucosal diseases, with an average incidence between 5% and 60% over a lifetime in the general population.[1–2] This chronic, incurable condition is characterised by recurrent, round or ovoid ulcers with clean borders, erythematous haloes, and yellow or grey floors.[1–2] The aetiology of RAU is still unknown, although many conditions such as trauma, stress, microorganisms, family history, nutritional deficiencies, medications, immune disorders, and genetic predisposition are proposed as potential causative factors.[3–4]

Several studies revealed that haematologic deficiencies, such as those related to vitamin B12, folic acid, ferritin, or haemoglobin, occur in 20% of patients with RAU.[5–6] Several case control studies found the levels of these factors were significantly low in the RAU group,[7–8] and some studies reported haematinic replacement therapy was significantly useful to reduce or eliminate recurrences of RAU lesion.[9]

Although there is growing agreement that haematologic deficiencies (vitamin B12, folic acid, ferritin and haemoglobin) are associated with increased risk for RAU, their potential effect on RAU has not been established.[10–11] Researchers have found no statistically significant difference between RAU patients and control groups,[10–11] and no specific pathogens have been identified to suggest a correlation between haematologic deficiencies and RAU. Accordingly, more evidence is needed to evaluate the strength of a correlation between haematologic deficiencies and RAU susceptibility. In this study, we reviewed the existing literature and performed a meta-analysis of several published case control studies to evaluate the association between haematologic deficiencies and RAU.

MATERIALS AND METHODS

Search strategy

We conducted a computerised literature search of Medline, PubMed, and CNKI (China national knowledge infrastructure whole article database) before May 2014, using the following search terms: 'haematologic deficiency' or ' haematinic deficiency' or 'haematologic parameter' or 'haematologic*' or ' haematinic*' or 'vitamin B12' or 'cobalamin' or 'folic acid' or 'folate' or ' folacin' or 'vitamin M' or ' folvite' or 'ferritin' or 'ferroprotein' or 'iron' or 'Fe' or 'haemoglobin' or 'haemoglobin' or 'haematocrystallin' or 'oxyphorase' or 'HB' or 'HGB' or 'HbA') and ('recurrent aphthous ulceration' or 'recurrent aphthous stomatitis' or 'canker sores' or 'oral ulcer' or 'dental ulcer' and 'case control study'. The search was restricted to English and Chinese and to human studies. Additional articles were acquired from references cited by the identified original studies and relevant reviews. Furthermore, we contacted some experts in the area to identify unpublished or ongoing trials nearing completion.

Inclusion and exclusion criteria

The following criteria were used to include published studies: (i) case control studies were conducted to assess the association between at least one of these haematologic parameters (vitamin B12, folic acid, ferritin and haemoglobin) and RAU; (ii) the article clearly described the diagnosis protocol for haematologic deficiencies; (iii) sufficient data were presented to calculate the odds ratios (ORs) as well as 95% confidence intervals (CIs). If samples of two studies

[1]Graduate Student in State Key Laboratory of Oral Diseases, Sichuan University; [2]Associate Professor in Department of Oral medicine, West China School of Stomatology, Sichuan University; [3]Professor in Department of Oral medicine, West China School of Stomatology, Sichuan University
*Correspondence to: Dr Lin Ge and Dr Mei Lin
Email: v1i2c3@163.com, linmei2k@163.com

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Table 1 Baseline characteristics of studies included in the meta-analysis

Authors	Year	Country	Design	Source of control	B12			Folate			Ferrin			Hemoglobin		
					Specimen	No. of RAU	No. of control	Specimen	No. of RAU	No. of control	Specimen	No. of RAU	No. of control	Specimen	No. of RAU	No. of control
Burgan	2006	Jordan	Case control trial	HCC	serum	143	143	serum	143	143	serum	143	143	RBC	143	143
Challacombe	1983	England	Case control trial	PCC							serum	105	78			
Compilato	2010	The Mediterranean area (western Sicily)	Case control trial	PCC	serum	32	29	serum	32	29	serum	32	29	RBC	23	29
Khan	2013	Pakistan	Case control trial	HCC	serum	60	60	RBC	60	60	serum	60	60	RBC	60	60
Lopez-Jornet	2014	Spain	Case control trial	HCC	serum	92	94	serum	92	94	serum	92	94			
Olson	1982	America	Case control trial	HCC	serum	90	23	RBC or serum	90	23	serum	90	23	RBC	90	23
Piskin	2002	Turkey	Case control trial	HCC	serum	35	26	serum	35	26	serum	35	26			
Thongprasom	2002	Thailand	Case control trial	PCC	serum	23	19	Serum and RBC	23	19				RBC	23	19
Wray	1975	England	Case control trial	HCC	serum	130	130	RBC or serum	130	130	serum	130	130			

HCC, hospital-based case-control; PCC, population-based case-control; RBC: red blood cell

overlapped, the study which held the larger sample that was identified. Major reasons for exclusion of studies were (i) study type was review, editorial, letter, comment, or case report; (ii) duplicated studies; (iii) the data were not complete or available; (iv) studies subjects had Behcet's disease, haematological deficiencies, celiac disease and other gastrointestinal symptoms or diseases, acute ulcerative gingivitis, herpes simplex and zoster, or other ulcerative or erosive oral lesions (pemphigus, pemphigoid, erosive lichen planus, erythema multiforme).

Data extraction

Data were extracted by two authors (Hong Chen and Qiuli Sui) from all eligible publications according to the inclusion criteria listed above. The following data were extracted: first author, year of publication, country of origin of participants, study design, number of RAU and control subjects, number of subjects with haematinic deficiencies in RAU and control groups, and data on any of the above haematologic parameters. Importantly, cut-off values for haematinic deficiencies varied among regions, and the WHO also did not have consistent standards. Therefore, in our study, deficiencies were defined according to the original articles, as done previously by Kirke.[12]

Table 2 Pooled data for hematologic deficiencies (vitamin B12, folic acid, ferritin, hemoglobin) and RAU in meta-analyses

Hematologic parameters	No. of study	OR (95% CI)	P-publication bias		Heterogeneity test	
			Egger**	Begg††	P value	I^2†
Vitamin B12	8	3.76 (2.38,5.94)	0.03	0.13	0.60	0%
Folic acid	8	7.55 (3.91,14.60)	0.40	0.55	0.68	0%
Ferritin	8	2.62 (1.69, 4.06)	0.12	0.13	0.24	26.1%
Hemoglobin	5	1.77 (1.12,2.80)	0.72	1.00	0.23	30.4%

Fixed effects models were used, weighted by the inverse variance. All statistical tests are two sided; P < 0.1 is considered statistically significant for Q statistics; I^2 is interpreted as the proportion of total variation contributed by between-study variation; **Egger's test to evaluate publication bias, p <0.05 is considered statistically significant; ††Begg's test to evaluate publication bias, p <0.05 is considered statistically significant.

Data synthesis

All data from eligible studies were extracted. We assessed statistical heterogeneity by calculating the Cochran's Q statistic[13] and the I^2 statistic,[14] where p <0.1 was considered significant heterogeneity, and I^2 >50% represented a large heterogeneity. If heterogeneity existed, we synthesised data using a random-effects model (the DerSimonian and Laird method);[15] otherwise, a fixed-effects model (the Mantel-Haenszel method) was adopted.[16] The reliability of results was evaluated by performing sensitivity analysis. For meta-analysis, results were presented as ORs for dichotomous data and the corresponding 95% CIs. A p-value less than 0.05 were considered to be statistically significant. Several methods were used to assess potential publication and other biases. Not only visual inspection of funnel plot asymmetry but also Begg's rank correlation method[17] and the Egger's weighted regression method[18] were adopted to statistically assess publication bias (p <0.05 was considered statistically significant). All analyses were performed using STATA software, version 12.0 (StataCorp., College Station, TX, USA) and RevMan software,version 5.0.24 (The Nordic Cochrane Centre, Rigshospitalet, Denmark). All p values were two-sided.

RESULTS

Screening process

Fifteen studies were identified by screening the title and reading the abstract.[5,7–8,10,11,19–28] Of all identified studies, one was a review,[24] and three lacked sufficient data to obtain ORs and 95% CIs for the RAU and control groups.[25,27–28] Moreover, two articles[5,26] did not describe the cut-off value for haematologic deficiencies. Finally, nine articles were excluded after full text assessment.[7–8,10,11,19–23] The flow chart on selection of studies and reasons for exclusion is presented in Figure 1.

Nine studies citing the total 710 cases in the RAU and 602 cases in control groups were considered eligible for inclusion.[7–8,10,11,19–23] The sample size of these studies ranged from 42–286. All studies were case control studies. The subjects of these studies were from different countries, including Jordan, the UK and the USA. Cases were diagnosed by professionals and received a clinical diagnosis of RAU.[1–2] Controls had no clinical evidence of RAU and were matched with cases by age and/or sex. Controls were either hospital based or population based.

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

Evaluation of haematologic deficiencies in RAU

In this meta-analysis, statistically significant heterogeneity was not observed between studies based on the Chi-squared test and I^2 statistics (vitamin B12: p = 0.60, I^2 = 0%, folic acid: p = 0.68, I^2 = 0%, ferritin: p = 0.24, I^2 = 26.1%, and haemoglobin: p = 0.23, I^2 = 30.4%). Therefore, a fixed-effect model was adopted.

Eight of the eligible studies reported haematinic deficiencies, and five involved deficiency of haemoglobin in the RAU group and control group.[7–8,10,11,19–23] The combined results based on all studies showed that the rate of haematinic deficiencies was significantly high in the RAU group (vitamin B12: OR = 3.75, 95% CI: 2.38–5.94; folic acid: OR = 7.55, 95% CI: 3.91–14.60; ferritin: OR = 2.62, 95% CI: 1.69–4.06; and haemoglobin: OR = 1.77, 95% CI: 1.12–2.80) (Fig. 2). When stratifying by source of controls, a significantly increased RAU risk was observed except for vitamin B12 in population-based controls (PCC) and for haemoglobin in hospital-based controls (HCC) (vitamin B12: HCC: OR = 3.59, 95% CI: 2.25–5.72; PCC: OR = 11.80, 95% CI: 0.62–223.50; folate: HCC: OR = 6.53, 95% CI: 3.25–13.13; PCC: OR = 16.80, 95% CI: 2.15–131.26; ferritin: HCC: OR = 6.53, 95% CI: 3.25–13.13; PCC: OR = 16.80, 95% CI: 2.15–131.26; haemoglobin: HCC: OR = 1.50, 95% CI: 0.92–2.44; PCC: OR = 7.07, 95% CI: 1.41–35.41).

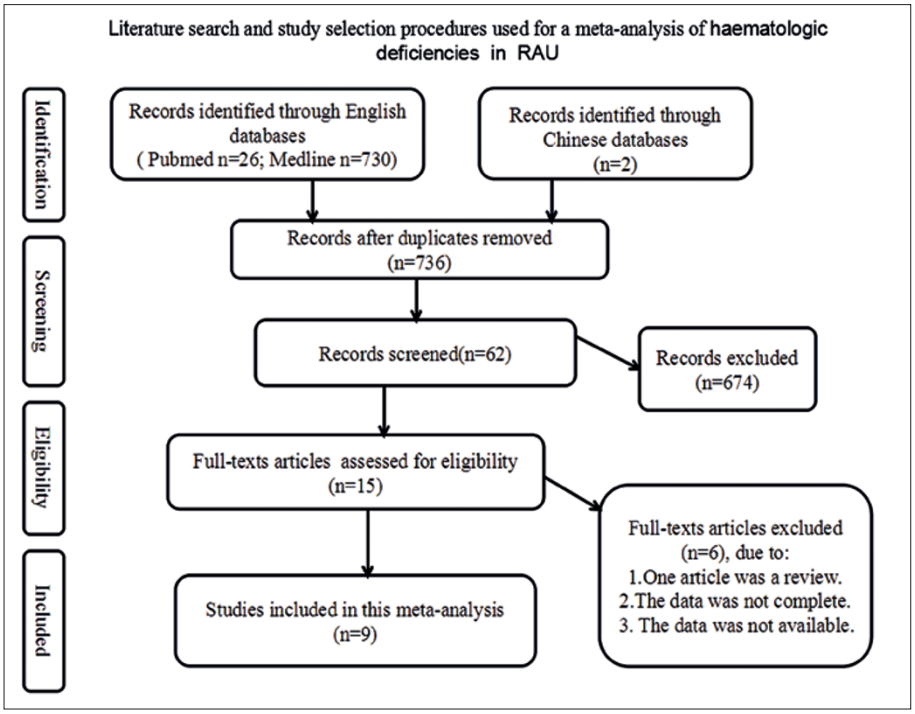

Fig. 1 Literature search and study selection procedures used for meta-analysis of haematologic deficiences in RAU

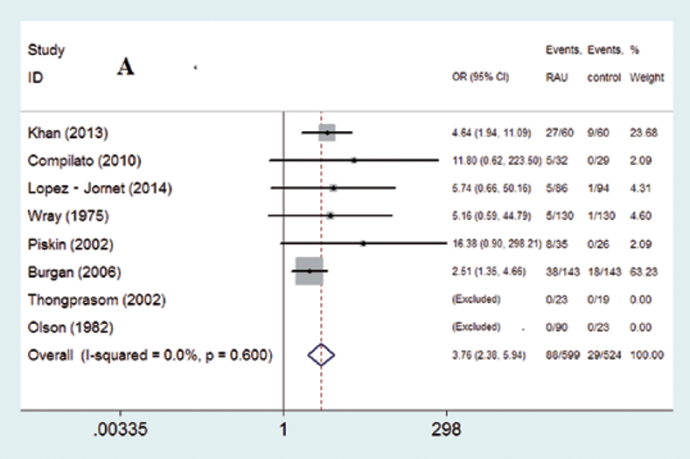

Fig. 2a Meta-analysis of vitamin B12 levels comparing in RAU group with control group

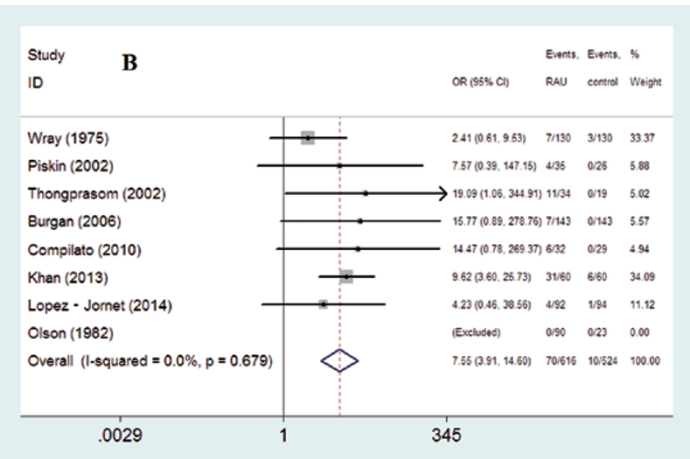

Fig. 2b Meta-analysis of folic acid levels comparing in RAU group with control group

Sensitivity analysis

Sensitivity analysis was conducted to estimate the influence of a single study on the overall meta-analysis, which was done by omitting one study at a time. In this meta-analysis, the omission of any study made no significant difference, except for haemoglobin levels, indicating that our results were statistically reliable (Fig. 3).

Cumulative meta-analyses

Cumulative meta-analyses of the impact of haematologic deficiencies on RAU were conducted by assortment of the studies by publication time. The results from the cumulative meta-analysis are displayed in Figure 4. Trends to no significant associations were evident as more data were gradually accumulated, except for haemoglobin.

Publication bias

Publication bias can artificially expand the apparent magnitude of an effect because no significant findings remain unpublished. Funnel plots of overall haematologic deficiencies comparing the RAU group with the control group showed basic symmetry, which suggested no publication bias.

However, visual inspection of the funnel plots could not obviate the potential publication bias for all analyses. In this meta-analysis, Begg's rank correlation method and Egger's weighted regression method were performed and revealed that publication bias was not evident except for vitamin B12 levels in RAU group with control group (vitamin B12: Begg's test p = 0.13, Egger's test p = 0.03; folic acid: Begg's test p = 0.55, Egger's test p = 0.40; ferritin: Begg's test p = 0.13, Egger's test p = 0.12; haemoglobin: Begg's test p = 1.00, Egger's test p = 0.72) (Table 2).

DISCUSSION

In this meta-analysis, we used nine case control studies[7–8,10,11,19–23] to evaluate the association between haematologic deficiencies and RAU. The results showed that haematologic deficiencies were significantly associated with decreased RAU risk. When subgroup analyses were performed by source of controls, results were a little different in HCC and PCC populations. Our results showed that significant association between vitamin B12 deficiency and RAU was observed among HCC, but not among PCC; however, we had opposite results with haemoglobin deficiency. As HCC studies may have some selection bias because such controls might belong to an ill population and not be representative of the general population, a proper population-based control subject may be better to our studies; therefore, the results of this meta-analysis should be interpreted with caution. For further study, we performed cumulative meta-analyses, in chronologic order, of the impact of haematologic deficiencies (vitamin B12, folic acid, ferritin, and haemoglobin) on RAU. Inclinations toward non-significant associations were evident with the gradual accumulation of more data over time, except for haemoglobin levels. Because there were only five studies concerning haemoglobin levels, it is important that well-designed

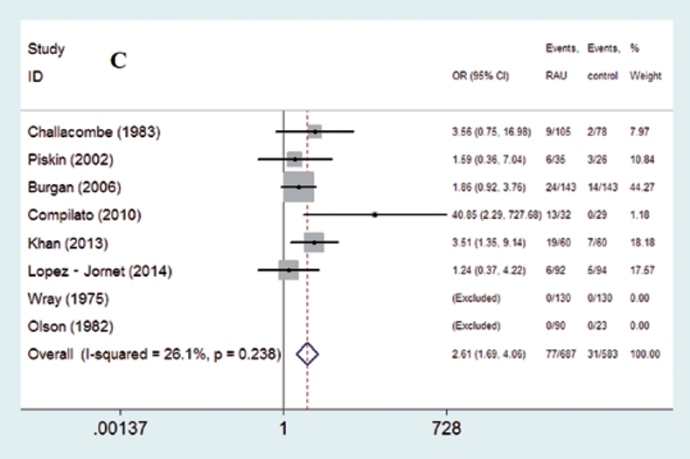

Fig. 2c Meta-analysis of ferritin levels comparing in RAU group with control group

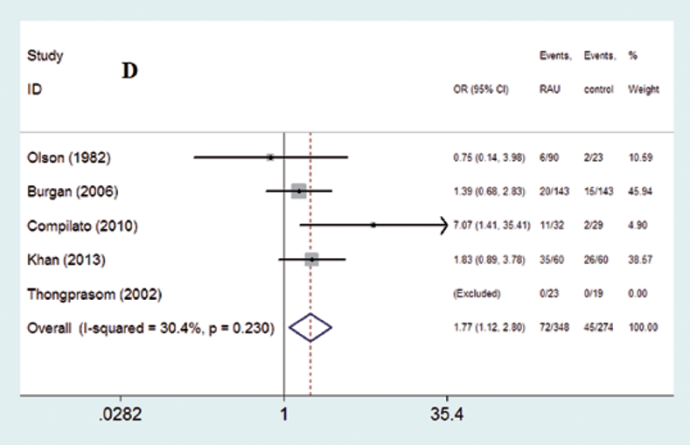

Fig. 2d Meta-analysis of hemoglobin levels comparing in RAU group with control group

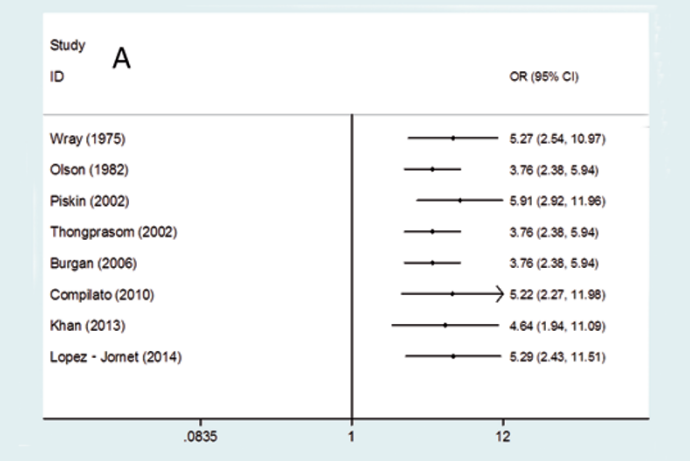

Fig. 3a Cumulative meta-analysis of vitamin B12 deficiency in RAU

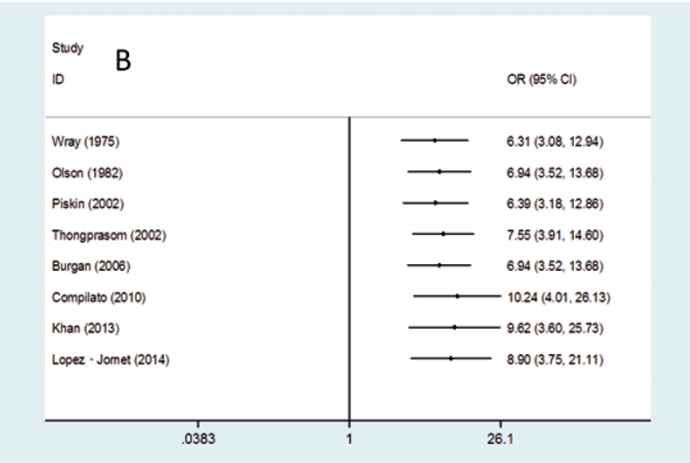

Fig. 3b Cumulative meta-analysis of folic acid deficiency in RAU

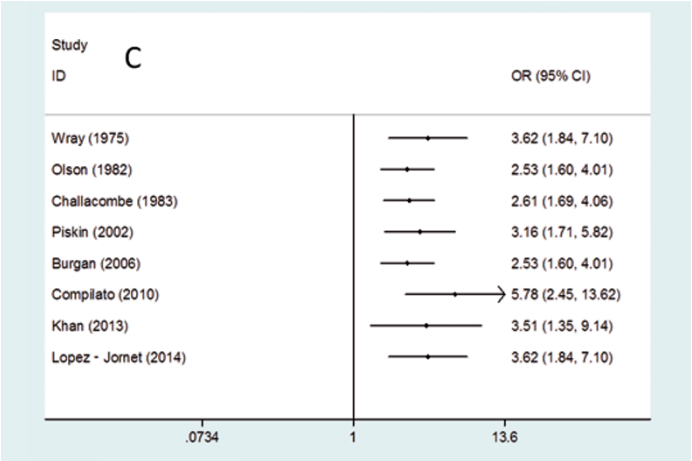

Fig. 3c Cumulative meta-analysis of ferritin deficiency in RAU

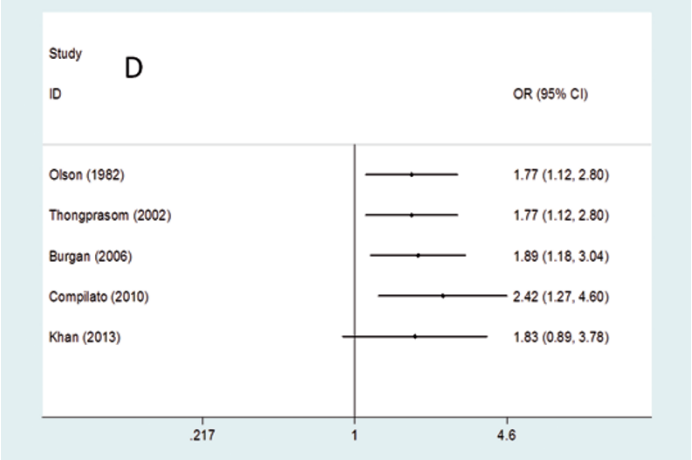

Fig. 3d Cumulative meta-analysis of hemoglobin deficiency in RAU

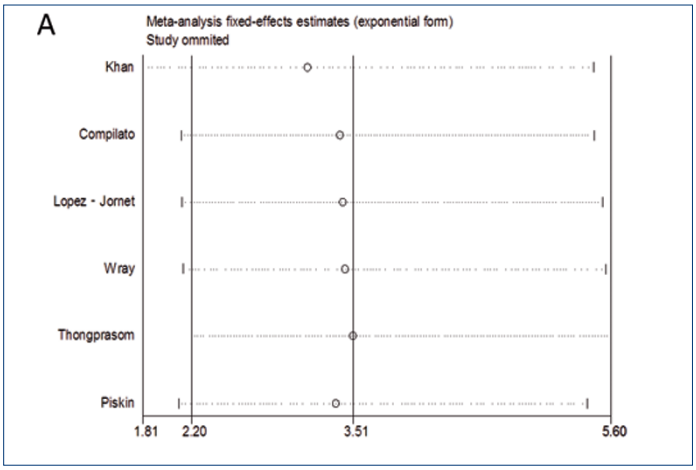

Fig. 4a Sensitivity analysis of vitamin B12 levels in RAU

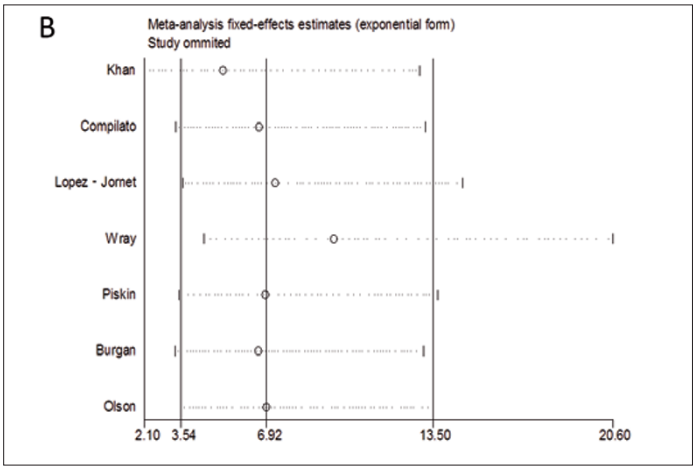

Fig. 4b Sensitivity analysis of folic acid levels in RAU

studies be performed to re-evaluate the association. Conversely, future studies regarding the impact of these haematologic parameters on RAU seem unnecessary. The degree of heterogeneity in a meta-analysis partly determines the difficulty in drawing overall conclusions.[29] In this meta-analysis, we used the Q test and I^2 statistics to quantify the impact of heterogeneity, where $p < 0.1$ was considered significant heterogeneity, and $I^2 > 50\%$ represented a large heterogeneity. Fortunately, statistically significant heterogeneity was not observed between studies for overall analysis. Moreover, we conducted a sensitivity analysis by omitting one study at a time. Consequently, the omission of any study in this meta-analysis made no significant difference except for haemoglobin levels, indicating that our results were statistically reliable.

However, there are still some limitations in this meta-analysis. First, unadjusted OR estimates were adopted because we could not obtain or calculate adjusted ORs with potential confounders, such as age and sex. Serious confounding bias may arise from the lack of this information. Second, the number of articles and the number of subjects in the meta-analysis of haemoglobin levels were small; thus, the reliability of our result may need validation by other studies. Third, we only included articles published in English and Chinese, which might induce selection bias. Finally, meta-analyses belong to retrospective research, the quality of which is subject to the original studies and methodological limitations. To minimise bias, some efforts were carried out in this study. First, we created a detailed protocol before initiating the study, and performed a meticulous, predefined search strategy. Second, the quality of the eligible studies included in this meta-analysis was satisfactory and met our inclusion criteria.

In conclusion, this meta-analysis assessed the combined effect of haematologic deficiencies in RAU and found that there could be a significant correlation. Thus, screening and treating any haematologic deficiencies may play an important role in preventing, to a great extent, the occurrence of RAU. However, to date, precise pathological mechanisms have not been identified to suggest a correlation between haematologic deficiencies and RAU. Further studies are still needed to clarify this relationship and reduce the occurrence of RAU.

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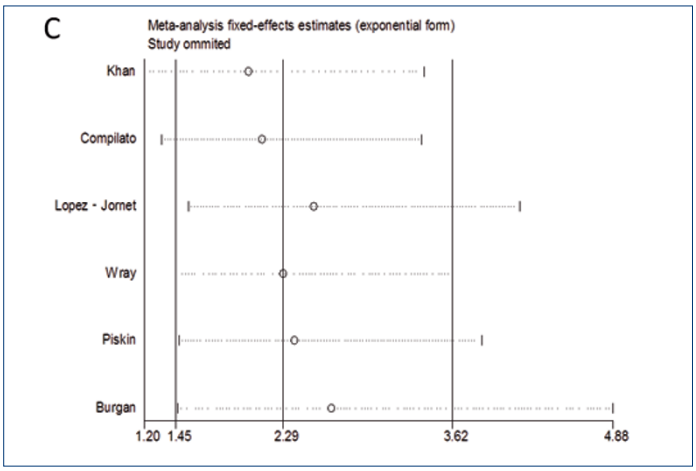

Fig. 4a Sensitivity analysis of ferritin levels in RAU

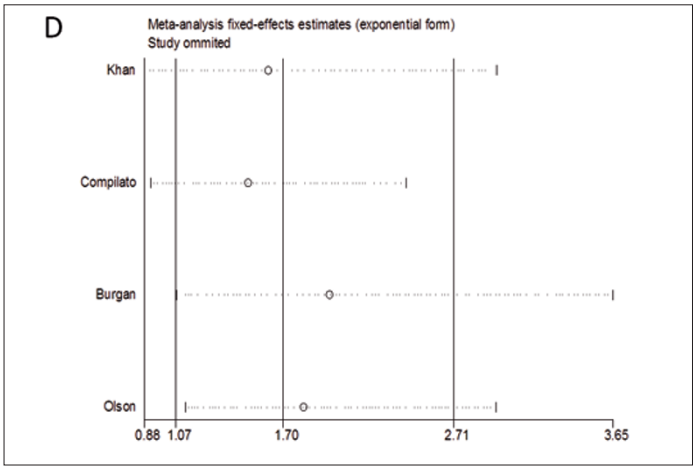

Fig. 4b Sensitivity analysis of hemoglobin levels in RAU