RESEARCH ARTICLE

The association of ABO and Rhesus blood type with the risks of developing SARS-CoV-2 infection: A meta-analysis

Soo, K-M.¹, Chung, K.M.², Mohd Azlan, M.A.A.², Lam, J.Y.¹, Ren, J.W.X.², Arvind, J.J.², Wong Y.P.¹, Chee, H.Y.¹, Amin-Nordin, S.¹

¹Department of Medical Microbiology, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia (UPM), Serdang, Selangor, Malaysia ²School of Science, Monash University, Subang Jaya, Selangor, Malaysia

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ABSTRACT

Coronavirus Disease 2019 (COVID-19) has been spreading like a wildfire everywhere in the globe. It has been challenging the global health care system ever since the end of 2019, with its virulence and pathogenicity. Recent studies have shown the association between ABO blood group, Rhesus blood type and susceptibility to COVID-19 infection. Various studies and few meta-analyses have been done and some might be inconsistent; therefore, this meta-analysis was done to assess the relationship between different ABO and Rhesus blood types on the susceptibility to COVID-19 infections. This meta-analysis assessed the odds ratio of COVID-19 infection of different ABO and Rhesus blood types. Subgroup analyses according to (1) age and gender matched; (2) different blood group antigens; (3) Rhesus positive and negative of each blood group were carried out. Publication bias and Quality Assessment of Diagnostic Accuracy Studies-2 (QUADAS-2) were also done to assess the risk of bias in these publications. It was found that blood group A showed significant difference in odds ratio of COVID-19 infection (OR, 1.16; 95% CI, 1.08-1.24). Blood group AB showed significant difference in odds ratio when studies with lower QUADAS-2 score were removed. This means that populations with blood group A and AB are more likely to be infected with COVID-19. As there is a higher tendency that blood group A and AB to be infected with COVID-19, precautious care should be taken by these populations.

Keywords: COVID-19; ABO blood group; Rhesus factor; susceptibility; relationship.

INTRODUCTION

The emergence of the novel coronavirus in December 2019 (COVID-19) is caused by a severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) (Zhou *et al.*, 2020). This has led to a worldwide pandemic and has been taxing the world's health care system, as many patients require ventilatory assistance for treatment (Heneka *et al.*, 2020).

Blood group has been shown to be associated with susceptibility to infections caused by viruses and bacteria such as norovirus, dengue virus, malaria, rotavirus, *N. gonorrhoeae*, and *H. pylori* (Fan *et al.*, 2020; Khalil *et al.*, 2020). This is because ABO antibodies are part of the innate immune system against some parasites, bacteria, and enveloped viruses, and blood group antigens are the receptor for immune and inflammatory responses (Fan *et al.*, 2020), as well as receptors for infection (Wu *et al.*, 2020). SARS-CoV-2 is a completely new virus. Recent studies and a few metanalyses have shown that there is an association between ABO blood group and susceptibility to COVID-19 (Franchini *et al.*, 2021; Kabrah *et al.*, 2021). However, the results are

inconsistent, as some studies have shown a lack of association of susceptibility with blood group (Dzik et al., 2020; Anderson et al., 2021) and studies are also confounded by factors such as gender, advanced age, and different ethnicities (Kabrah et al., 2021; Rahim et al., 2021). No subgroup analysis has been done on these factors. Therefore, this meta-analysis was done to assess the association between blood group and susceptibility to COVID-19, as well as subgrouping the data with gender and age matched, and into different blood group antigens as well as Rhesus positive and negative of each blood group. Subsequently a better insight can be seen on which specific blood type would have a higher risk of getting COVID-19. This can act as a biological marker that can predict the prevalence of this infection.

MATERIALS AND METHODS

Literature search

Preferred Reporting Item for Systemic Review and Metaanalysis Protocol (PRISMA) recommendations were used to

^{*}Corresponding author: syafinaz@upm.edu.my

guide this review (Refer to Supplementary file, Prisma 2020 Checklist). However, the review was not registered.

Data sources

Relevant studies were identified through systematic search of electronic databases such as PubMed, Scopus, and Medline (from 2019 until present, via Ovid). Only articles published in English were evaluated.

Search strategy

The search of relevant studies was carried out from 2-3 August 2021 by using subject headings and free text terms. The search was carried out with the keywords "(ABO blood group AND Rhesus factor OR Rhesus blood group OR blood type OR Rhesus positive OR Rhesus negative OR Rh- OR Rh+ OR Antigen A OR Antigen B) AND (association OR relation OR correlational) AND (SARS-CoV-2* OR COVID-19*) AND (risk OR susceptibility)".

Inclusion criteria

The inclusion criteria of this meta-analysis include: (1) ABO and Rh blood type that are clear and extractable from the articles; (2) research that includes COVID-19 patients as well as individuals without COVID-19; (3) study that provides original data; (4) study that includes research types such as case control studies, cross-sectional studies, cohort studies which are retrospective or prospective.

Exclusion criteria

The exclusion criteria of this meta-analysis include: 1) duplicate reports; 2) review papers that do not contain the original data; 3) case studies that contain small sample size; 4) the study that was not relevant to the subject; 5) articles with unavailability of full texts; 6) results that could not be pooled through calculation.

Data abstraction

Study selection

The titles and abstracts of potentially relevant studies from the literature search were screened by one reviewer in accordance with the eligibility criteria and further confirmed by a second reviewer. Duplicated records, studies without abstract, apparently irrelevant studies, review papers, and case studies were excluded. The full text articles of the remaining studies were screened by two reviewers. Any disagreements about study inclusion and exclusion were resolved between the reviewers by consensus.

Data extraction and quality assessment

Two independent reviewers (Ka Mun and Amir) extracted data from the eligible studies and reached a consensus on all data. The extracted data includes the following information: (1) citations of the studies; (2) characteristics of participants (age, population); (3) characteristics of the study (sample size, types of study, time of study); (4) ABO blood group distribution. Disagreements were resolved by the consensus of the two reviewers. OpenMeta[Analyst] software (Brown School of Public Health, USA) was used to calculate the combined estimate of the studies. QUADAS-2 was performed to assess the quality of studies. It was done by referring to appendix 2 of the study conducted by Vengesai *et al.* (Vengesai *et al.*, 2021). Any discrepancies were either resolved or removed.

Data statistical analysis

Statistical analysis was carried out by using OpenMeta[Analyst] software (Brown School of Public Health, USA). The I-squared index was used to assess heterogeneity between studies. Random effect and fixed effect models were used to calculate the mean effect size of the studies with significant heterogeneity ($I^2 > 75\%$) and without significant heterogeneity ($I^2 < 75\%$), respectively.

Publication bias was carried out by using Meta-Essentials program (Erasmus Research Institute of Management, Netherlands). The publication bias was carried out using six methods: observing for an asymmetrical funnel plot, significant difference (p < 0.05) in Egger's analysis and Begg's analysis, high Rosenthal's fail-safe N and Orwin's fail safe N (n > 1000), and significant difference between the combined estimate and unbiased estimate (p < 0.05), which is generated by the Trim and fill method. General agreement of the results among these methods (at least four of six methods showed the presence of publication bias) was interpreted as sufficient evidence of publication bias (Ferguson, 2007).

Definition and outcomes

COVID-19 patients were confirmed by using real time polymerase chain reactions (PCR), reverse transcription PCR or virus neutralization tests. Negative control was defined as individuals that are negative for COVID-19 infections, including hospitalized controls, blood donors, and healthy controls. The outcome was defined as the effect size of odds ratio of a blood group with positive COVID-19 infection. Formula of odds ratio is shown below: Secondary outcome was the subgroup analyses according to (1) studies with age and gender matched; (2) different blood group antigens; (3) Rhesus positive and negative of each blood group.



a = number of COVID patients who are certain blood group

b = number of controls who are certain blood group

c = number of COVID patients who are not certain blood group

d = number of controls who are not certain blood group

RESULTS

Results of literature search

A comprehensive search yielded 735 potentially relevant studies from different databases, PubMed (n = 484), Ovid (n = 247) and Scopus (n = 4). 24 duplicate reports were excluded. The remaining 711 studies were filtered and screened based on the title and abstract, of which 699 studies were excluded due to topics that were unrelated to the study. The remaining 12 studies met the inclusion criteria of our meta-analysis, in which they contain original data, are not case study with low sample size, and contain the relevant data of odds ratio of susceptibility to COVID-19 infections. In addition, we manually screened through the reference lists of the published meta-analysis (Franchini *et al.*, 2021; Kabrah *et al.*, 2021) and other studies, and found 11 relevant studies. Therefore, there are a total of 23 studies included in our meta-analysis. There is no disagreement between the

reviewers (Ka Mun and Amir) during the selection process. The study selection is shown in Figure 1. Table 1 presents the time of study, sample size, median of age, and location of the studies. Among the studies, thirteen are case control studies, seven cohort studies and three cross sectional studies.

Associations between ABO blood group and Rhesus factor with COVID-19 susceptibility

In this meta-analysis, 53, 835 COVID patients and 1, 328, 484 negative controls were analyzed. In this meta-analysis, the percentages of A, B, AB and O blood groups in patients and controls were 40%, 14%, 5% and 42%, respectively, whereas the percentage of both Rhesus positive and Rhesus negative blood group were 88% and 12%, respectively.

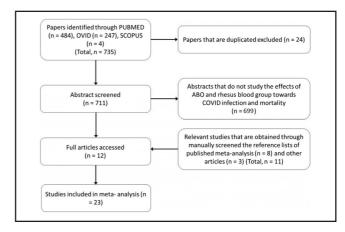


Figure 1. Study selection process for meta-analysis.

Table 1. Demographic, time and location of the included studies

	Study	Time of study	Sample	Mean or median of age (years)	Location
1	(Abdollahi et al., 2020)	March 2020	397	53.7 ± 5.1 ^b	Tehran, Iran
2	(Anderson et al., 2021)	March-November 2020	107 796	44.9 ± 0.2 ^b	Utah, Idaho and Nevada, USA
3	(Arac et al., 2020)	March-May 2020	392	NA	Diyarbakır Gazi Yaşargil Training and Research Hospital, Kayapınar/Diyarbakır, Turkey
4	(Barnkob et al., 2020)	February-July 2020	473 654	Control: 50 (36-64), Case: 52 (40-67)	Denmark
5	(Bhandari et al., 2020)	March-June 2020	825	56.5 ± 19.2	Elmhurst Hospital Center, New York, USA
6	(Boudin et al., 2020)	April 2020	1 688	28 (23-35)	Toulon, France
7	(Deleers et al., 2021)	March-June 2020	290	55.2 ± 12.5 ^b	Bruxelles, Belgium
8	(Dzik et al., 2020)	February-May 2020	6 797	NA	Boston, USA
9	(Fan et al., 2020)	January-March 2020	105	55.1 ± 1.1 ^b	Zhongnan Hospital, Wuhan, China
10	(Gallian <i>et al.</i> , 2020)	March-April 2020	998	Median 41.0	Bouches-du-Rhône, Haut-Rhin, Oise, Seine-Saint-Denis, France
11	(Göker <i>et al.,</i> 2020)	March-May 2020	186	42 (19-92)	Ankara, Turkey
12	(Khalil et al., 2020)	February-June 2020	146	Control: NA, Case: 41.9 ± 18.52	Jnah, Lebanon
13	(Kibler et al., 2020)	NA	702	82 ± 6.9	Strasbourg, France
14	(Levi <i>et al.</i> , 2021)	June 2020	6 457	NA	Dasa, Brazil
15	(Li <i>et al.,</i> 2020)	February-March 2020	265	NA	 Wuhan Jinyintan Hospital Renmin Hospital of Wuhan University, Wuhan, China
16	(Muñiz-Diaz et al., 2021)	NA	130 273	Control: 45 (32-53), 72.1 (58.2-82.5), Case: 45 (36-53), 69.0 (59.0-77.0)	Catalonia, Spain
17	(Rahim et al., 2021)	April-July 2020	1 935	20-30, 31-40,41-50, above 50	Peshawar, Pakistan
18	(Ray et al., 2021)	January-June 2020	225 556	Mean age 54	Ontario, Canada
19	(Solmaz & Araç, 2021)	March-July 2020	1 667	NR	Diyarbakır Gazi Yaşargil Training and Research Hospital, Kayapınar/Diyarbakır, Turkey
20	(Wu et al., 2020)	January-March 2020	187	NA	The First Hospital of Changsha, Changsha, China
21	(Zhao <i>et al.,</i> 2020) ^a	NA	1 775	NR	 Wuhan Jinyintan Hospital Renmin Hospital of Wuhan University Shenzhen Third People's Hospital, Guangdong province, China
22	(Zhang et al., 2020)	December 2019- February 2020	3 824	60.78 ± 12.98 years	Wuhan Jinyintan Hospital
23	(Zietz <i>et al.,</i> 2020)	NA	14 112	NA	New York-Presbyterian/Columbia University Irving Medical Center, New York, USA

^a three data presented in the study were combined, to avoid false publication bias.

b mean that is calculated by combining mean and standard deviations of control and case group.

Figure 2 shows the association between ABO blood group and Rhesus factor with COVID-19 susceptibility populations, with blood A is more likely to be infected with COVID-19, compared to other blood groups (Figure 2, p < 0.05). Whereas populations with blood group O are least likely to be infected with COVID-19 (Figure 2, p < 0.05). No significant difference was found when subgroup analysis according to age and gender matched, was carried out (number of studies = 5).

Figure 3 shows the association between blood group antigens and COVID-19 susceptibility. The presence of antigen (blood group A, B and AB) or particularly the presence of antigen A (blood group A and AB) showed significant difference in its association with susceptibility to COVID-19 (Figure 3, p < 0.05). When subgroup analysis of rhesus positive and rhesus negative of each blood group is carried out, blood group AB+ showed significant difference in positive association with COVID-19 susceptibility, whereas blood

group O+, A-, O- and AB- showed significant difference in negative association with COVID-19 susceptibility (Figure 3, p < 0.05). However, the number of studies in this subgroup analysis is low; with only 4 studies that provide the data (Figure 3).

Publication bias and QUADAS-2

Publication bias analyses indicated the presence of publication bias for blood group A and O. The robustness of this analysis was supported by a high number of studies (n = 10~23) but its power of analysis remains questionable with the presence of high heterogeneity (I² > 75%) (Field & Gillett, 2010; Rücker *et al.*, 2011). Besides, some studies were suspected to cause publication bias due to overlap in coauthor, study duration, study location, or using the same healthy controls. These studies are listed in Table 2. To test whether the publication bias has caused the significant

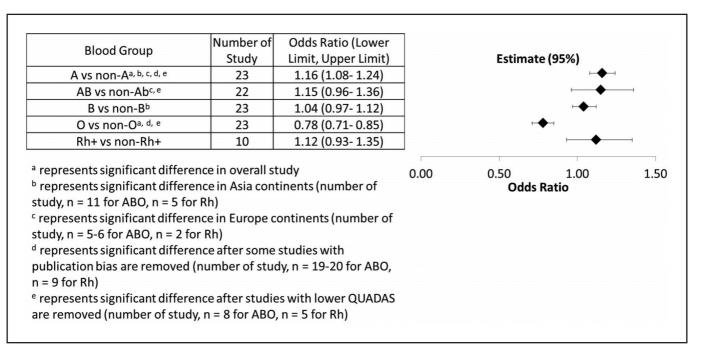


Figure 2. Odds ratio of blood group ABO and Rhesus factor.

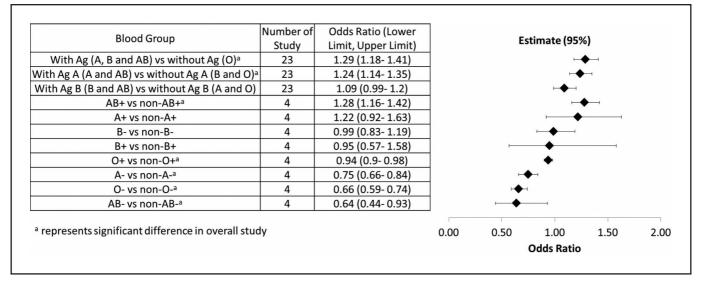


Figure 3. Subgroup analysis of blood group antigens and Rhesus positive or negative of each blood group.

Table 2. Studies with overlap co-authors, study duration or study location. These studies were suspected to have contributed to publication bias. Studies with smaller sample size were removed while studies with larger sample size were remained

No.	Studies	Number of overlap co-authors	Overlap in study duration	Overlap in study location	Remark	Studies that were removed
1	Arac <i>et al</i> . (2020); Solmaz & Araç (2021)	1	Yes	Yes	Both studies used the same healthy controls.	Study done by Arac <i>et al.</i> (2020) (sample: 392) was removed from meta-analysis of blood group A, B, AB, O and Rhesus. Studies done by Solmaz & Araç, (2021) (sample: 1 667) was remained.
2	Li <i>et al.</i> (2020); Zhang <i>et al.</i> (2020); Zhao <i>et al.</i> (2020)	-	-	Yes	All three studies used the same healthy controls in Wuhan.	Studies done by Li et al., (2020) (sample: 265); Zhang et al. (2020) (sample: 3 824) were removed from meta-analysis of blood group A, B, AB and O. Studies done by Zhao et al., (2020) (sample: 1 775) was remained.

Table 3. Summary of quality of each included study. Risk of bias and applicability concerns of each included studies were assessed by using QUADAS-2

		Risk	of bias		Applicability concerns			
Rating of QUADAS-2 items	Patient selection	Index Test	Reference Standard	Flow and Timing	Patient Selection	Index Test	Reference Standard	Total
(Abdollahi et al., 2020)	_	_	+	+	+	+	+	5
(Anderson et al., 2021)	_	_	+	_	+	_	+	3
(Arac et al., 2020)	_	_	+	_	+	_	+	3
(Barnkob et al., 2020)	+	_	+	+	+	_	+	5
(Bhandari et al., 2020)	_	_	+	_	+	_	+	3
(Boudin et al., 2020)	+	_	+	+	+	_	+	5
(Deleers et al., 2021)	_	_	+	_	+	_	+	3
(Dzik et al., 2020)	+	_	+	+	+	_	+	5
(Fan et al., 2020)	_	_	+	_	+	_	+	3
(Gallian et al., 2020)	-	_	+	_	-	-	_	1
(GÖKER <i>et al.,</i> 2020)	-	_	+	_	+	-	+	3
(Khalil et al., 2020)	_	_	+	_	+	_	+	3
(Kibler et al., 2020)	-	_	+	_	-	-		1
(Levi <i>et al.,</i> 2021)	-	_	+	_	+	-	+	3
(Li et al., 2020)	+	-	_	+	+	-	+	4
(Muñiz-Diaz et al., 2021)	-	_	+	-	+	-	+	3
(Rahim et al., 2021)	-	_	+	+	+	-	+	4
(Ray et al., 2021)	+	_	+	+	+	-	+	5
(Solmaz & Araç 2021)	+	_	+	+	+	-	+	5
(Wu et al., 2020)	_	_	_	_	+	_	+	2
(Zhang et al., 2020)	_	-	+	_	_	-	_	1
(Zhao et al., 2020)	_	-	+	_	+	-	+	3
(Zietz, et al., 2020)	-	_	_	_	+	-	+	2

⁺ represents low risk of bias.

difference in the results of blood group A and O, some studies that were suspected to be the cause of publication bias (Arac et~al., 2020; Li et~al., 2020; Zhang et~al., 2020) were removed, and meta-analysis was performed again. Study done by Arac et~al. (2020) was removed and study done by Solmaz and Araç (2021) was maintained because it has larger sample size. In the same principle, Li et~al. (2020) and Zhang et~al. (2020) were removed and the study done by Zhao et~al. (2020) was maintained. It was found that blood group A and blood group O still showed significant difference in their association with COVID-19 susceptibility (Figure 2, p < 0.05).

Result of QUADAS-2 can be found in Table 3. Case control studies (n = 15) were ranked as having high risk of bias and lower QUADAS-2 score. To test whether the quality of the studies has affected the results, studies with lower QUADAS score (1, 2, 3) were removed and meta-analysis was performed again. It was found that blood group AB and O showed significant difference in their association with COVID-19 susceptibility (Figure 2, p < 0.05) and the heterogeneity reduces (63%).

 $[\]boldsymbol{-}$ represents high risk of bias.

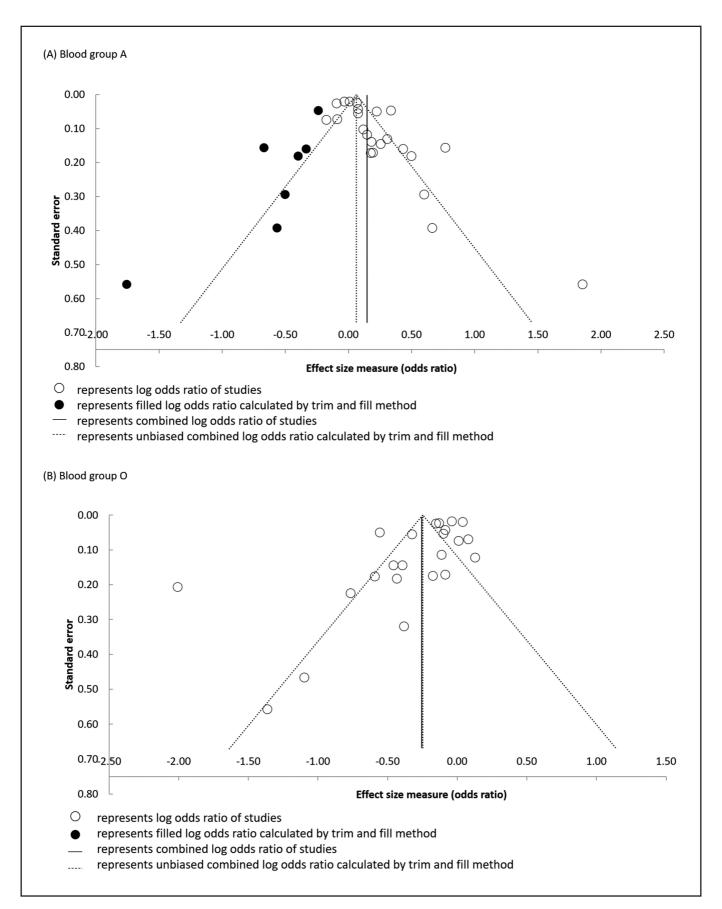


Figure 4. Funnel plot of blood group A and O.

DISCUSSION

Based on the results, population with blood group A was found to be the most susceptible to COVID-19 infection whereas blood group O was found to be the least susceptible. This observation could be explained by the absence of anti-A antibody in the population with blood group A and the presence of anti-A antibody in blood group O. Study done by Guillon et al. (Guillon et al. 2008) has shown that anti-A antibody could inhibit the adhesion of SARS-CoV S protein to angiotensin-converting enzyme related carboxypeptidase (ACE2) expressing cell line (Fan et al., 2020; Göker et al., 2020). SARS-CoV is closely related to SARS-CoV-2. Both show similarity in nucleic acid sequence as well as the binding of the ACE2 receptor to enter the cell. Therefore, anti-A antibody may also inhibit adhesion of SARS-CoV-2 thereby reducing the probability of infection in population with blood group O (Fan et al., 2020; Zhao et al., 2020). In addition, it was also found that patients with COVID-19 had significantly lower IgM anti-A and/or B antibody compared to controls. This further support the role of anti-A antibody in the pathogenesis of COVID-19 infection (Deleers et al., 2021).

Blood group AB shared the same characteristic as blood group A in that it also has antigen A and the absence of antibody A. Based on Figure 3, when blood group A and AB were grouped together, they showed significant difference in the association with COVID-19 susceptibility. This further supported the hypothesis that the presence of antigen A or the absence of antibody A may be the cause of susceptibility to COVID-19. However, blood group AB alone did not show significant difference in its association with COVID-19 susceptibility. This blood group showed significant difference when studies with lower QUADAS-2 score were removed. Possible explanation to this condition could be that most of the lower quality studies were case control studies. Case control studies are susceptible to bias because of the retrospective nature of the data and the resulting lack of control the investigator has over the many items of interest (Sutton-Tyrrell, 1991). As a result, it has caused high variance in the percentage of blood group AB in COVID-19 patients in lower quality studies, as compared to higher quality group (80.8 vs 8.4), as well as high heterogeneity in the metaanalysis result (92%). When these studies are removed, heterogeneity reduces (63%) and blood group AB showed significant difference.

Based on previous studies, other than SARS-CoV and SARS-CoV-2, blood group also displays similar association with rotavirus gastroenteritis, where population with blood group A and AB is more prone to rotavirus infection, while blood group O is less prone to rotavirus infection (Pérez-Ortín *et al.*, 2019). Possible explanation to this association is that P[9], P[14] and P[25] strains of rotavirus interact with A antigen and therefore, enhances the infection of the cells (Barbé *et al.*, 2018).

There is no association found between Rhesus blood group and COVID-19 susceptibility. Previous studies have shown the association between Rhesus negative blood group and risk of West Nile virus infection (Arac *et al.*, 2020). However, in the context of COVID-19, even though some studies have suggested that Rhesus negative might have a protective effect towards COVID-19 infection (Arac *et al.*, 2020; Ray *et al.*, 2021); others have shown that Rhesus negative COVID-19 patients have a higher susceptibility (GÖKER *et al.*, 2020; Rahim *et al.*, 2021). Contradictory results of the individual studies may have cancelled off the effect of each other and caused the combined results to have no significant difference.

The results showed that the publication bias could be due to the unavoidable selection bias of patients from the same populations, evidenced by the overlap in co-author, study duration, and locations in studies as well as using the same data of healthy control. Multiple publication of the same data is a kind of publication bias which may lead to intervention effect (Tramèr et al., 1997). Therefore, we have removed some studies that were involved in the publication bias and redo the meta-analysis and publication bias analysis. The results showed that the blood group A and O still showed significant difference in combined odds ratio (Figure 2, p < 0.05). Whereas for publication bias, it still exists but has reduced, with Begg's test no longer show significant difference. Since the removal of the studies has reduced publication bias, this suggests that these studies are involved in publication bias; however, they do not disprove the association between blood group ABO and susceptibility to COVID-19, because blood group A and O still show significant difference.

LIMITATIONS

Subgroup analysis according to continents, gender, and age match studies, as well as the subgroup analysis of Rhesus factor of each blood group in this study have low number of studies (n = 4-6); therefore, their results are inconclusive, and requires more study to confirm. Only one study provides the information concerning the ethnicity of patients and controls; therefore, no subgroup analysis according to ethnicity was carried out. Moreover, even though factors such as medical conditions and genetics have been reported to associate with the risk of developing COVID-19 infection or causes severe symptoms (Fakhroo et al., 2020; Hou et al., 2020), these details were not recorded in the studies. This may become a confounding factor for our meta-analysis. Further studies that consider these factors are necessary.

CONCLUSION

Blood group A showed significant difference in positive association with COVID-19 infection, whereas blood group O showed negative association with COVID-19 infection. The absence of anti-A antibody in people with blood group AB may also increase their susceptibility to COVID-19. Therefore, blood group ABO maybe considered together with many other factors in COVID-19 prevention or treatment strategies. People with blood group A and AB might need to strengthen their personal protection and receive more surveillance.

Conflict of interests

The authors declare that they have no conflict of interests.

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SUPPLEMENTARY DATA

https://msptm.org/files/Vol39No1/tb-39-1-015-Soo-K-M-supplementary-data.pdf