Association of self-reported allergic rhinitis with dengue severity: a case-control study

Running title: Allergy and severe dengue

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Highlights

- Several factors are involved in the development of severe dengue.
- Allergic rhinitis and reinfection with dengue virus could be risk factors for severe dengue.
- Higher levels of dengue-specific IgE were not associated with worse outcomes in patients with allergies or allergic rhinitis.

Abbreviations

ADE: antibody-dependent enhancement

cfDNA: cell-free DNA

DENV: dengue virus

DF: dengue fever

DHF: dengue hemorrhagic fever

DSS: dengue shock syndrome

ELISA: enzyme-linked immunosorbent assay

HRP: Horseradish Peroxidase

IgE: immunoglobulin E

IRB: Institutional Review Board

STROBE: Strengthening the Reporting of Observational Studies in Epidemiology

TMB: 3,3',5,5'-Tetramethylbenzidine

WHO: World Health Organization

Declarations

Conflict of interest

All authors certify that they have no affiliations with or involvement in any organization or entity with any financial interest (such as honoraria; educational grants; participation in speakers' bureaus; membership, employment, consultancies, stock ownership, or other equity interest; and expert testimony or patent-licensing arrangements), or non-financial interest (such as personal or professional relationships, affiliations, knowledge, or beliefs) in the subject matter or materials discussed in this manuscript.

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Ethical approval

Ethical approval was obtained from the Institutional Review Board (IRB) of the Hospital for Tropical Diseases in Ho Chi Minh City (IRB Number: 2 – signed on January 18, 2017). The study was conducted according to the World Medical Association Declaration of Helsinki.

Consent to participate

Written informed consent was obtained from each participant and/or parent/guardian.

Consent to publish

All authors confirm that they have contributed to the development of this manuscript, and they all agreed to the manuscript version submitted.

Data availability statement

The datasets generated and/or analyzed during the current study are not publicly available because we have not obtained consent from the participants to share the raw material. However, the data can be provided by any of the corresponding authors (Nguyen Tien Huy; tienhuy@nagasaki-u.ac.jp or Nguyen Thi Cam Huong; dr_camhuong@ump.edu.vn) upon the provision of a reasonable request.

Authors' contribution

NTH and NTCH developed the idea. NTCH, NTH, NTN, AR, VD, DTHT, VTT, and KH developed the protocol and applied for ethical approval. NTCH and NTN collected the data using the hospital case reporting system. NTCH, NTH, NTN, AR, VD, DTHT, VTT, AMM, SPD, and KH performed the data analysis and interpreted the results. All authors wrote the manuscript and approved the final version of the manuscript before submission for publication.

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Abstract

Background: The severity of dengue infection has been reportedly associated with patients' allergic reactions. To further elucidate the role of allergy in dengue severity, we conducted a matched case-control study to assess the association between allergic background and dengue shock syndrome.

Methods: This is a matched case-control study that was carried out in the Hospital for Tropical Diseases, Ho Chi Minh City, Vietnam from January to December 2017. Dengue infection was determined by non-structure protein 1 (NS1) diagnostic quick test or anti-dengue antibodies (IgM). The total and dengue-specific IgE levels were measured using ELISA. Patients' demographics, clinical, and allergic profiles were collected using a structured questionnaire.

Results: A total of 572 dengue patients with positive NS1 (92.7%) or IgM antibodies (7.3%) results were included in this study. Of these patients, 143 patients developed dengue shock syndrome (case group) while the other 429 patients did not (control group). None of the baseline characteristics including age, sex, or being overweight was significantly different between the two groups ($p>0.05$). In multivariable analysis, having a history of dengue infection (OR=3.35, 95% CI: 1.8-6.17, $p<0.001$) and allergic rhinitis (OR=1.95, 95% CI: 1.11-3.4, $p=0.019$) were found to be associated with dengue shock syndrome. Higher levels of dengue-specific IgE were not associated with worse outcomes in patients with allergies ($p=0.204$) or allergic rhinitis ($p=0.284$).

Conclusion: Dengue patients presenting with a history of a previous dengue infection or allergic rhinitis should be considered high-risk patients for the development of dengue shock syndrome.

Keywords: Dengue fever; Severe dengue; Dengue shock syndrome; Allergy, IgE

Introduction

Dengue infection, an arthropod-borne viral disease, constitutes a major worldwide burden in terms of morbidity and mortality [1], where it affects around 50-100 million humans yearly [2]. It affects humans mainly in tropical and subtropical regions [3], through the Aedes mosquitoes [4, 5]. Subsequently, we witnessed 291,964 dengue cases till 2016, most of them in the Western Pacific region, followed by the Americas [2]. Moreover, the cases in the last two decades were much higher than cases reported in the twentieth century [6].

In 1997, ~~it~~ the WHO ~~was~~ classified the severity of dengue infection into dengue fever (DF), dengue hemorrhagic fever (DHF), and dengue shock syndrome (DSS) [7, 8]. However, there are limitations to using this classification. For instance, the requirement for four specific criteria to distinguish DHF from DF does not allow the classification of severe cases with atypical manifestations that do not comply with DF/DHF [9]. Therefore, WHO in 2009 recommended re-classifying dengue cases into dengue with and without warning signs and severe dengue [10, 11]. Although there have been multiple historic papers discussing possible factors (e.g., circulating non-neutralizing dengue virus antibodies ~~prior to~~before a dengue infection) [12-14] to be associated with worse dengue outcomes, the mechanisms of progression to more severe dengue are only partly understood with the absence of valid prediction models preventing better clinical planning and prognosis for dengue patients [15, 16].

The classical description of DSS was first published by Cohen and Halstead in 1966 [17]. This was followed by two reports of an association between DSS and a second heterotypic dengue virus infection between 1966 and 1969 [18, 19]. In 1969, there was also some evidence of the first dengue virus infection in infants with circulating maternal dengue antibodies [12]. In 2002, Halstead *et al*. established a theory of possible different serotypes of dengue virus (DENV-1, to -4) to explain the DHF/DSS underlying mechanisms by which abnormal Fc-receptor signaling suppresses the innate immunity and other related mediators [20], which was later described to be antibody-dependent enhancement (ADE) [21]. This leads to a substantial increase in the number of infected cells, and increased vascular permeability, explaining the capillary leakage seen in DHF/DSS [22]. However, in some endemic areas (and also globally) and even during epidemics, in which populations have high levels of circulating antibodies, the cumulative incidence of DHF/DSS remains small (less than 4%) [13, 14, 23-27]. Therefore, other factors such as genetic background, other viral strains, and serotypes could be considered to influence the DHF/DSS progression [28].

Additionally, chronic comorbidities such as asthma, diabetes, and hypertension have been also reported to be associated with the severity of dengue infection [29, 30]. Moreover, some plasma markers like cell-free DNA (cfDNA) were regarded as prognostic biomarkers for severe dengue [31]. Figueiredo *et al.* [24] also found that patients with diabetes and allergies are more likely to develop DHF. Besides, in a recent cohort study, DENV-specific IgE was found to show elevated levels during the disease, and the specific IgE/total IgE was highly correlated to disease severity [28]. This was explained by the release of large amounts of mast cell mediators if the virus was not locally cleared up by the immune system. However, not many studies have investigated the correlation between patients' allergy profiles and the development of severe dengue. Therefore, to further elucidate the role of allergy in dengue severity, we conducted a matched case-control study to assess the association between allergic background and dengue shock syndrome.

Methods

Ethics statement

Ethical approval was granted by the Institutional Review Board (IRB) of the Hospital for Tropical Diseases (IRB number: 2 – signed on January 18, 2017). The study was conducted according to the World Medical Association Declaration of Helsinki with written informed consent being obtained from each participant and/or parent/guardian.

Study design and participants

This was a matched case-control study (3 controls per case) carried out at the Hospital for Tropical Diseases, Ho Chi Minh City, from January to December 2017. The usage of a 1:3 ratio is based on the following as elaborated by Hennessy et al. [32]: this is a matched case-control study with a relatively small percentage of ~~the~~ exposure in the control group. The reporting of this study conformed with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement [33] – the case-control version of the checklist (Supplementary Table 1).

We enrolled children and adults diagnosed with dengue before being discharged by dengue NS1 antigen assay (SD Bioline Dengue NS1 Ag Rapid Test, Alere, USA) and/or dengue virus IgM enzyme-linked immunosorbent assay (ELISA) (NovaLisa, NovaTec Immundiagnostica GmbH, Dietzenbach, Germany). There were no restrictions to the inclusion of patients based

on age, sex, ethnicity, religion, educational background, socioeconomic status, comorbidities, or coinfections. Dengue infection was classified according to the 2009 WHO criteria [11]. Other causes of fever were further investigated to confirm dengue infection. The case group included patients with dengue shock syndrome (severe dengue) while the control group included dengue patients with or without warning signs (non-severe dengue). Both cases and controls were matched based on sex and age groups (of every 5 years of age) with a ratio of one case per three control patients. Total IgE was estimated using the Human IgE ELISA Quantitation Kit (Bethyl Laboratories, Inc., Montgomery, USA). Following Inokuchi et al., dengue-specific IgE was measured in 155 patients on day 6 or 7 post-infection by an in-house ELISA as dengue-specific IgE ~~were~~ was likely to peak on one of those days [28]. Briefly, the ELISA plate was coated with goat anti-human IgE antibody (Bethyl Laboratories, Inc., Montgomery, USA, Cat.no: A80-108A). Ten times diluted serum was then added. Subsequently, dengue antigens of all four serotypes were added followed by an anti-dengue antibody conjugated with horseradish peroxidase (HRP). After the substrate (tetramethylbenzidine (TMB)), the reaction was stopped by 1N H_2SO_4 and the absorbance was measured at 450nm/620nm.

Data collection

Cases and controls were interviewed before being discharged using a standardized questionnaire to collect baseline demographic data; age, sex, address, history of previous dengue infection, and specific information concerning the history of allergic diseases (if patients had symptoms that fit at least one type of allergy in the last 12 months). The questionnaire asked about the following:

1) Allergic conjunctivitis: itching, redness, tearing, and photophobia [34].
2) Allergic rhinitis: congestion, rhinorrhea, nasal and ocular itching, tearing, and sneezing without having a cold or flu [35].
3) Food allergy: having skin manifestations, gastrointestinal, respiratory, or anaphylaxis after contact with suspicious foods [36].
4) Atopic eczema: having dry depigmented patches (abnormally dry skin and a lowered threshold for itching). Common eczema sites include flexural areas (cubital fossae, neck, wrists, and ankles), the nape, and the dorsum of the hands and feet [37, 38].
5) Urticaria: the development of wheals (hives) which can occur anywhere on the body and exist from a few hours to a maximum of 24 hours [39].

6) Angioedema: deeper swellings of skin and mucous membranes which last up to 72 hours, regardless of the occurrence of another allergic reaction [39, 40].
7) Contact dermatitis: the presence of rash or skin lesion at the site of exposure. Oozing, draining, or crusting can happen, in addition to the skin becoming raw, scaled, or thickened. This results in itching, skin redness, and/or inflammation [41].
8) Asthma: wheezing or whistling chest sounds. The history of asthma medications was also a hint of the presence of bronchial asthma [42].

Finally, all participants were assessed for their body mass index (BMI) by obtaining their height and weight. Participants were considered overweight if their BMI exceeded 24.9 Kg/m^2 for adults or if the weight-for-height is greater than two standard deviations above the WHO Child Growth Standard median for children [43].

Study outcomes and data analysis

For matching cases and controls, we used the "Matching" package for the R programming language [44], which balances the final pairing by weighing all variables. Categorical variables for the baseline characteristics were described with frequencies and percentages while ordinal, non-normally distributed variables were described with the median and interquartile range. To assess the crude association/ odds ratio (OR and 95% CI) of allergic background and shock syndrome in dengue patients, we firstly compared the rates of patients with allergic backgrounds between severe and non-severe dengue cases. Then, we compared the rate of each specific type of allergy in the same comparative groups. It is worth pointing out that this study only reports dengue shock syndrome from the types of severe dengue. Our outcomes were investigated using cross-tabulation based on the Chi-squared ($\chi 2$) test (or Fischer's exact test as appropriate). An adjustment was done for the history of potential confounding variables (history of previous dengue). $P<0.05$ was considered significant. Statistical analysis was conducted using the IBM SPSS software program, version 25.0 [45].

Multivariable analysis was also done to identify the possible factors that were associated with dengue shock syndrome. It was established by comparing the case and control groups by the $\chi 2$ test, by which variables with $p<0.2$ were put in multivariable analysis to find risk factors for dengue shock syndrome.

Results

Patients' characteristics

We included 572 patients in this study with 143 cases in the dengue shock syndrome group and 429 in the control group (non-severe dengue). Most patients (92.7%) were NS1 positive while anti-dengue antibodies (IgM) for dengue virus were detected in 7.3% of patients only. We found that 55.2% of patients were under 15 years old, 50.3% were males, 32.0% of patients were overweight, and 8.2% of patients had previous dengue infections. Due to the matching nature of the study, we found no statistically significant differences between the two groups regarding age, sex, or overweight status. Contrastingly, the rate of having a history of dengue infection was statistically significant between the dengue shock syndrome and non-severe dengue patients (16.1% vs 8.2%, respectively, $p<0.001$) (Table 1).

Allergic manifestations of participants

Among the study population, 17.1% of patients had an allergy condition and nine patients (1.5%) had ≥ 2 types of allergies. Among the different types of allergies that were noticed in the study participants: allergic rhinitis (11.0%), urticaria (4.0%), asthma (1.7%), and food allergy (1.4%) were the most prevalent forms. Other forms are reported in Table 2. Moreover, we found a statistically significant difference between dengue shock syndrome and non-severe groups in the rate of patients having allergic rhinitis (16.1% vs 9.3%, respectively, $p=0.025$). No differences between the two groups were found statistically significant (Table 2).

Factors related to severe dengue

Of all variables, we only included four with $p<0.2$ in the final multivariable analysis: age, history of having dengue infection, allergic rhinitis, and asthma. Among these, two variables were found to be associated with dengue shock syndrome: having a history of dengue infection (OR=3.35, 95% CI: 1.8-6.17, $p<0.001$), and having allergic rhinitis (OR=1.95, 95% CI: 1.11-3.4, $p=0.019$) (Table 3).

IgE levels and assessed outcomes

Among the 155 patients (93 with dengue shock syndrome and 62 non-severe) whose total and dengue-specific IgE were measured, the median (IQR) of total and dengue-specific IgE levels were 2708 (1809-3885) and 0.294 (0.209-0.429) ng/mL, respectively. No significant difference in the levels of total and dengue-specific IgE was found between patients with and without dengue shock syndrome. Interestingly, 21.9% (34/155) of patients had an allergy. However,

the total and dengue-specific IgE levels were not significantly higher in patients with ~~allergy~~ allergies compared to other patients without ~~allergy~~ allergies (p=0.083 and p=0.204, respectively). Allergic rhinitis was found in 16.8% of the patients that were assessed for total and dengue-specific IgE levels. Although the median total and dengue-specific IgE levels were higher in patients with allergic rhinitis, no significant difference was observed (p=0.284 and p=0.096, respectively) (Table 4).

Discussion

In this study, we aimed at finding the relationship between having a background of allergy and the development of dengue shock syndrome. There is no specific treatment for dengue, and the vaccine has not been proven very effective in primary prevention [46-48]. Moreover, the management of severe dengue is still complex due to several factors including the incomplete definition of risk factors [49]. So, finding the most predictive factors (biomarkers) or related factors for severe dengue is the right answer for the clinical management of patients who would be at risk of developing severe dengue.

The results from our study support the current literature which identifies an association between having a history of previous dengue and severe dengue infection [50, 51]. Reinfection may increase the risk of developing warning signs leading to severe dengue [52]. Increased risk of developing severe dengue in patients with a history of infection may be due to the suboptimal stimulation of the immune system or immune system exaggeration which produces heterotypic antibodies against certain serotypes of the DENV when reinfection occurs [30, 53]. A meta-analysis showed that secondary infection with different DENV serotypes is positively associated with dengue shock syndrome (OR=1.75, 95% CI: 1.26-2.42) [54]. This may be due to antibody-dependent enhancement (ADE) phenomena in which non-neutralizing reactive antibodies from the primary infection bind to DENV in the secondary infection, enhancing its ability to enter Fc-receptor positive cells, such as mast cells [52]. The same meta-analysis also showed that younger age is more likely to be associated with DHF [54]. A Nicaraguan study also found severe dengue to be associated with ~~the~~ infancy of 4 to 9 months, and childhood of 5 to 9 years [55]. Besides, this study also found that secondary infection was also a risk factor for severity in children. However, we found no significant difference between the two groups in terms of age, which was also reported by Figueiredo et al. [24].

In this study, we did not find any correlation between urticaria, food allergy, or asthma and developing severe dengue. In concordance with our findings, a meta-analysis by Kien *et al*.

[56] found no significant difference in patients with DHF in terms of skin allergy, food allergy, and asthma. However, the study reported significant heterogeneity among its included studies, and when removing the cause of heterogeneity, a significant correlation was reported in terms of asthma between DHF and dengue fever patients. Moreover, our analysis indicated that allergic rhinitis was the only allergy related to severe dengue (OR=1.95, CI: 1.11-3.4, p=0.015). The relationship between the severity of dengue infection (progression to severe dengue) and the frequency of ~~allergies~~ allergy occurrence was investigated due to the link between the increased mast cell release and allergy. This was further indicated by the presence of significantly higher levels of dengue-specific IgE in patients that developed allergies than in patients who did not. In the same context, patients with allergic rhinitis had higher levels of total and specific IgE [57, 58]; however, this correlation was not significant which may be attributable to the limited number of IgE tests that were obtained from our patients. While other studies have investigated the differences in IgE levels in primary and secondary dengue infection [59-63], to our knowledge, this is the first study to report the relationship between IgE levels in patients with and without allergic conditions.

Studies have shown that the development of severe dengue and DHF is primarily due to ~~the~~ plasma leakage because of increased vascular permeability (mediated by IgG activation of mast cells), leading to a great reduction in blood pressure [64-67]. Because antibody-enhanced infection of mast cells can activate endothelial cell activation, chemokines CCL3, CCL4, and CCL5 in addition to TNF-alpha can also play a role in ~~the~~ vascular leakage [68]. However, no permanent lesions have been observed suggesting that the process is transient secondary to the release of certain inflammatory mediators which flare up the condition [69, 70]. This is supported by a study done in 2018, associating dengue IgE and chymase with the severity of dengue [71]. It showed an increasing trend of dengue-specific IgE during the fourth to sixth days of illness. In addition, it was found that the ratio between dengue-specific IgE and total IgE correlated with the severity of the illness. In the present study, we didn't measure IgE levels in the early days of illness, the blood samples were taken only in the recovery phase (6^{th} or 7^{th} days). However, Inokuchi et al. have found that the dengue-specific IgE peaks (with an increasing trajectory) at day 6 since the onset of the disease [28]. We also found no statistical difference between severe and non-severe dengue groups. The other limitation of our study is that we were not able to test all patients for IgE. Contrastingly, this study is the first one to investigate the relationship between IgE levels in patients with allergic conditions and severe dengue. Furthermore, although this study had matching groups from the start of the recruitment

process, we adjusted for the matched variables in the analysis as well [72]. However, this matching would suffer from other shortcomings and further expanded studies may be necessary for the external validity of this study. Finally, due to ~~the~~ financial limitations, we were not able to test all patients for IgE. Therefore, we were only able to include the first patients who accepted the participation offer. Once the money has been used up, the recruitment process was stopped. Contrarily, the selection bias generated from this process may have been reduced to some extent through the matching nature of the study enrollment.

Conclusions

Through a matched case-control study, we found an association between having a previous history of dengue infection or having allergic rhinitis with the development of dengue shock syndrome while higher levels of ~~dengue~~ dengue-specific IgE were not associated with worse outcomes in patients with allergies (p=0.204) or allergic rhinitis (p=0.284). More studies are needed for a possible explanation of allergic reactions in severe dengue patients. A prospective study of using anti-allergic drugs as a specific treatment for dengue-infected patients may be considered. Finally, dengue patients presenting with a history of a previous dengue infection or allergic rhinitis should be considered high-risk patients for the development of dengue shock syndrome.

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Tables legends

Table 1. Baseline characteristics of the study population.

Table 2. Allergic manifestations as reported by the study participants.

Table 3. Multivariable analysis of the factors related to severe dengue.

Table 4. Assessment of patients' outcomes based on the available IgE test data.

Supplementary Table 1. STROBE Statement — Checklist of items that should be included in reports of ***case-control studies***.

572 **Table 1.** Baseline characteristics of the study population.

Characteristics	**Total** (n=572)	**Dengue shock syndrome** (n=143)	**Non-severe dengue** (n=429)	**P-value***
Age				
≤15	316 (55.2%)	71 (49.7%)	245 (57.1%)	0.120
>15	256 (44.8%)	72 (50.3%)	184 (42.9%)	
Sex				
Female	284 (49.7%)	77 (53.8%)	207(48.3%)	0.247
Male	288 (50.3%)	66 (46.2%)	222 (51.7%)	
Overweight				
Yes	183 (32%)	49 (34.3%)	134 (31.2%)	0.501
No	389 (68%)	94 (65.7%)	295 (68.8%)	
Previous dengue infection				
Yes	47 (8.2%)	23 (16.1%)	24 (5.6%)	**<0.001**
No	525 (91.8%)	120 (83.9%)	405 (94.4%)	

573 *Chi-square test; significant p-value (<0.05) is highlighted in bold format.

574 **Table 2.** Allergic manifestations as reported by the study participants.

Types of allergy	**Total** (n=572)	**Dengue shock syndrome** (n=143)	**Non-severe dengue** (n=429)	**P-value***
Allergic conjunctivitis	1 (0.2%)	0	1	NA
Allergic rhinitis	63 (11%)	23 (16.1%)	40 (9.3%)	**0.025**
Food allergy	8 (1.4%)	2 (1.4)	6 (1.4)	1.000
Atopic eczema	1 (0.2%)	0	1	NA
Urticaria	23 (4%)	8 (5.6%)	15 (3.5%)	0.269
Contact dermatitis	2 (0.3%)	1	1	0.438
Asthma	10 (1.7%)	0	10 (2.3%)	0.065

575 *Chi-square test; significant p-value (<0.05) is highlighted in bold format; NA (not applicable).

576 **Table 3.** Multivariable analysis of the factors related to dengue shock syndrome.

Parameters	**Multivariable analysis**		
	OR	**95% CI**	**P-value**
≤15 years old	0.78	0.53-1.15	0.201
History of having dengue infection	3.35	1.8-6.17	**<0.001**
Allergic rhinitis	1.95	1.11 - 3.4	**0.019**

577 A significant p-value (<0.05) is highlighted in bold format.

578 **Table 4.** Assessment of patients' outcomes based on the available IgE test data.

Characteristics	IgE tests				
	Total n (%)	**Total IgE (ng/mL)**	**P-value***	**Specific IgE**	**P-value***
Level of IgE	155 (100)	2708 (1809-3885)	NA	0.294 (0.209-0.429)	NA
Dengue shock syndrome					
Yes	93 (60)	2780 (1999-3722)	0.182	0.295 (0.205-0.409)	0.539
No	62 (40)	2347 (1494-4477)		0.291 (0.218-0.474)	
History of DHF					
Yes	15 (9.7)	2488 (1999-3646)	0.952	0.263 (0.194-0.390)	0.767
No	140 (90.3)	2708 (1797-3932)		0.295 (0.211-0.439)	
Allergy					
Yes	34 (21.9)	3208 (2135-5642)	0.083	0.332 (0.247-0.549)	0.204
No	121 (78.1)	2476 (1809-3484)		0.291 (0.205-0.406)	
Allergic rhinitis					
Yes	26 (16.8)	3208 (2135-6068)	0.096	0.326 (0.247-0.584)	0.284
No	129 (83.2)	2507 (1809-3524)		0.293 (0.205-0.409)	

579 *Man-Whitney U test; significant p-value (<0.05) is highlighted in bold format; DHF (dengue hemorrhagic fever); NA (not applicable).

580 **Supplementary Table 1.** STROBE Statement — Checklist of items that should be included in reports of ***case-control studies***.

	Item No	Recommendation	Page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1, 5
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	5
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	6
Objectives	3	State specific objectives, including any prespecified hypotheses	6
Methods			
Study design	4	Present key elements of study design early in the paper	7
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	7
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls	7
		(*b*) For matched studies, give matching criteria and the number of controls per case	NA

Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	7, 8
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	8
Bias	9	Describe any efforts to address potential sources of bias	7, 8
Study size	10	Explain how the study size was arrived at	7
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	9
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	9
		(*b*) Describe any methods used to examine subgroups and interactions	9
		(*c*) Explain how missing data were addressed	NA
		(*d*) If applicable, explain how matching of cases and controls was addressed	NA
		(*e*) Describe any sensitivity analyses	NA
Results			

Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	9
		(b) Give reasons for non-participation at each stage	NA
		(c) Consider use of a flow diagram	NA
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	9
		(b) Indicate number of participants with missing data for each variable of interest	NA
Outcome data	15*	Report numbers in each exposure category, or summary measures of exposure	9
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	9
		(*b*) Report category boundaries when continuous variables were categorized	9
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	NA

Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	NA
Discussion			
Key results	18	Summarise key results with reference to study objectives	10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11, 12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	12, 13
Generalisability	21	Discuss the generalisability (external validity) of the study results	12, 13
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	3

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