

PEER REVIEW HISTORY

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ARTICLE DETAILS

TITLE (PROVISIONAL)	Prevalence and trends of sensitization to aeroallergens in allergic rhinitis patients in Guangzhou, China: A 10-year retrospective study
AUTHORS	Wang, Weihao; Huang, Xuekun; Chen, Zhuanggui; Zheng, Rui; Chen, Yulian; Zhang, Gehua; Yang, Qintai

VERSION 1 - REVIEW

REVIEWER	De Yun Wang National University of Singapore
REVIEW RETURNED	23-Jan-2016

GENERAL COMMENTS	<p>Although it is not completely novel in the international community, it is an interesting study in China with a representative and large sample size accumulated from the past 10 years. Allergic rhinitis (AR) together with other allergic diseases is a common chronic disease that causes major illness and disability worldwide. AR is frequently caused by exposure to perennial or seasonal allergens which exist in our living indoor and outdoor environment. A standard diagnostic approach, as recommended by almost all international consensus report or guidelines, is a careful medical history, a nasal examination and allergy tests (skin tests, in vitro tests or even nasal challenge) to confirm or exclude an allergic etiology. Therefore, understanding the prevalence and sensitization (IgE-mediated) profile is an important part of allergy management and prevention. It is indeed a need of such important data from different parts of China, which will help significantly in the improvement of allergy care.</p> <p>It is interesting to learn from this study that house dust mite (HDM) allergens are the most common aeroallergens in Guangzhou, one of the largest city in China, and there is an increasing trend of pet sensitization that is influenced by the change of lifestyle (having pets at home) in many developing and developed countries.</p> <p>This paper requires a major revision in the scientific content and also extensive English language improvement to make it acceptable.</p> <p>Specific comments:</p> <ol style="list-style-type: none">1. Abstract: Please reorganize the Abstract by following the given subtitles. Please see my suggestions below:<ol style="list-style-type: none">a. Participants: A review of the medical records was performed in all patients (n=5,486) with nasal hyper-reactivity symptoms who visited the Ear, Nose and Throat Outpatient Clinic between 2005 and 2014 were performed. Among them, 4,085 patients (2,269 males and 1,816
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	<p>females) have completed an allergy screen test by measuring allergen-specific IgE (sIgE) in serum and were recruited in this study.</p> <p>b. Outcome measures: Prevalence and trends of sensitization to different types of aeroallergens were assessed.</p> <p>c. Results: The overall prevalence of sIgE sensitization to (Please add sensitization). It is not clear for the sentence "For each additional 10 years, ...".</p> <p>2. Introduction: (The last two sentences) please change it to "However, there is a lack of such data in Guangzhou, the largest city in southern China. Thus, the aim of this study was to investigate the prevalence and trends of sensitization to aeroallergens in patients with allergic rhinitis (AR) in Guangzhou over the past decade.</p> <p>3. Materials and methods:</p> <p>a. Paragraph 1, line 6: please delete "of the 5,486 samples". Thus, 4,085 patients (2,269 males and 1,816 females) who have completed an allergy screen test by measuring allergen-specific IgE (sIgE) in serum and were recruited in this study.</p> <p>b. Any geographic difference between six districts? Are they all within the urban area?</p> <p>c. Ethics statement: The last sentence needs to be revised.</p> <p>4. Discussion: Please cut off at least one-third content by omitting redundant sentences.</p> <p>5. Table 1: Please include "sub- and total number" in the table.</p> <p>6. The last Figure is interesting. Please check if there is a mistake when plotting the number of pet allergens in "A" and "B".</p>
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REVIEWER	Katie M. Phillips and Ahmad R. Sedaghat Massachusetts Eye and Ear Infirmary Harvard Medical School USA
REVIEW RETURNED	24-Jan-2016

GENERAL COMMENTS	<p>Summary:</p> <p>This study is a retrospective study, which calculates the prevalence and trends of prevalence of common allergen sensitivities by measuring specific allergen IgE concentrations in patients with allergic rhinitis over a ten year period in Guangzhou, China. The significant findings include (1) house dust mite sensitivity is the most prevalent allergen among the study population, (2) the 10-19 year old age group has the highest prevalence of sensitivities to common allergens among the study population and (3) the prevalence of sensitivity to pet allergens is increasing over the studied 10 year period. With this information, more targeted therapy can be applied to specific allergens with higher prevalence among the study population. However, we have some concerns related to the specific methodology used by the authors that makes interpretation of these results confusing.</p> <p>Major:</p>
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	<p>1. The study population for each analysis that is performed needs to be more clearly defined in the methods sections. For example, the authors state that inclusion criteria include a positive allergy test. However, in this context, Figure 2 is unclear. Based on the authors' inclusion criteria, the prevalence of sensitivity should be 100% across all age groups. If the results in figure 2 represent prevalence data from the larger pool of 5486 patients, then authors should state that. But they should also state that this represents the prevalence of allergen sensitivities in the population of patients **with nasal hypersensitivity symptoms**. Related to this point – are the results in Table 1 and Figure 3 limited to the 4085 sensitized patients or all 5486 patients?</p> <p>2. Did the testing include IgE levels to each of the allergens specified in the “sIgE measurements” section of the methods? And the authors lumped the results into groups specified (e.g. “molds”)? Or did the assay measure sIgE level to any mold that is specified?</p> <p>3. Related to #2 above, if sIgE was measured to each allergen, no comment is made regarding specific concentration of specific allergen IgE although the IgE measurements were subdivided based on concentration. Did this information have any interesting trends? Were there concentrations of IgE significantly higher for any particular allergen?</p> <p>4. Can you comment on why skin testing results were not also included? Do the authors not use skin testing in their institution? If skin testing is performed, then how is it decided whether patients undergo serological vs. skin testing?</p> <p>5. Methodology: were these patients enrolled as a part of a different study? The authors state that consent was obtained but also state that this was a retrospective study. Did the authors obtain consent from patients once the authors decided to perform this study? Or were the patients consented when they underwent allergy testing?</p> <p>6. Do the results in Table 1 include data points from across all time points (years 2005 – 2014)?</p> <p>7. Discussion – Although it is unclear from the text currently (as I have already pointed out) I believe that your prevalence of allergen sensitivity results is from your overall 5486 patient population. You therefore need to clarify that your prevalence results are in the population of patients with **nasal hypersensitivity**. These prevalence results are not applicable to the general population in Guangzhou.</p> <p>8. Discussion: I disagree with the authors' statement that SIT is the only treatment for AR. There are , of course, antihistamines, intranasal steroids and leukotriene modifiers that can and should be used for AR. Many consensus guideline statements suggest that these medical therapies should be trialed before SIT.</p> <p>9. Are there any published data that have described how allergen exposure/prevalence has changed in Guangzhou over the last 10 years? If this data exists somewhere, it would be a very interesting to include this information in your discussion.</p> <p>Minor:</p>
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	<p>1. The Results section of the Abstract is unclear. First, does the “overall prevalence” include data points from all years (2005-2014)? Second, without reading the manuscript, it is not immediately obvious that “For each additional 10 years, occurrence...” refers to the age groups studied by the authors. should mention age group because “additional 10 years” does not specify the variable “age group” which is referred to in this result.</p> <p>2. Please be consistent with the use of significant digits for reporting P-values. For example, in the Results section of the Abstract, you report $P < 0.01$ and $P < 0.001$.</p> <p>3. The authors use the term “allergen” and “aeroallergen” interchangeably in the manuscript. I’d suggest picking one term and being consistent.</p> <p>4. For “cockroaches” allergy, what allergens did the authors test for? German cockroach? American cockroach? Some other species? Please specify.</p> <p>5. Figure 1 is confusing. Does this add anything more than the results that the authors have already described in the text?</p> <p>6. What is the difference between p^* and P_{delta} at the bottom of Table 1?</p> <p>7. Second sentence of the Discussion – this is inaccurate—you collected sIgE results from 5486 patients.</p> <p>8. Discussion – you do not need to include such explicit restatement of the results in the discussion. For example, stating that the prevalence of sIgE decreases by 5.13% (95%CI: -7.28 – -2.98, $P < 0.01$) for each additional 10 years is unnecessary. Citing your 5.13% finding is enough since you already described these findings in the Results.</p>
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REVIEWER	Shokrollah Farrokhi Bushehr university of medical sciences, Iran
REVIEW RETURNED	09-Feb-2016

GENERAL COMMENTS	<p>This study is interested and it declare the trends of allergic rhinitis with age.</p> <p>The reviewer also provided a marked copy with additional comments. Please contact the publisher for full details.</p>
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VERSION 1 – AUTHOR RESPONSE

Response to Reviewer 1:

1. Answer: We are appreciated for your instructive advice and the Abstract section has been edited in the revised manuscript. The sentence “For each additional 10 years” refers to the age groups, which has been changed into “with 10 years old increasing” for better understanding in revised manuscript.
2. Answer: Thank you for your comment and we have edited the sentence and highlighted the changes in red color.
- 3a. Answer: After examining the other reviewer’s comment carefully, we decided to delete the sentence that you were concerned about to avoid confusion about the study population for each

analysis. The inclusion criteria in the previous manuscript had been edited and all the patients with nasal hypersensitivity symptoms who completed the aeroallergen sIgE test were recruited in our study. We are sorry for the unclear statement in this section. Anyway, thank you for your arduous work and instructive advice.

3b. Answer: There exists no major geographic difference between those six districts. Tianhe, Yuexiu, Haizhu, and Liwan are within the urban area, while Baiyun, Conghua and Zengcheng districts are rural area.

3c. Answer: We have revised the sentence and highlighted the changes in red color.

Discussion: Answer: Thank you for your suggestion and we have cut off the redundant sentences in Discussion section.

4. Answer: We edited the Table 1 and highlighted the amendments in red color.

5. Answer: The Figure 3A represent the trends in the prevalence of sensitization to 6 types of aeroallergens respectively over the past decade. Figure 3B represent the upward trend in the prevalence of sensitization to pet allergen separately. We are sorry for causing confusion about the difference of Figure 3A and Figure 3B. Therefore, we have decided to delete the Figure 3A but retain the Figure 3B in the revised manuscript.

Response to Reviewer 2:

Major:

1. Answer: After examining the reviewer's comments carefully, we must admit that we have not expressed our ideas precisely in the previous manuscript. We apologize for this confusion. But before responding to the comments above, we should clarify the rearrangement of the figures in the revised manuscript again which was suggested by the other reviewer. We have deleted original Figure 1 and Figure 3A. Thus, the original Figure 2 as well as Figure 3 have been relabeled to Figure 1 and Figure 2 respectively in the revised manuscript.

According to the comments of the reviewers, we found that the description of inclusion criteria in previous manuscript caused confusion when understanding each analysis. Therefore, we edited the inclusion criteria in the revised manuscript. The accurate inclusion criteria for enrolled patients should be as follows: (1) presence of nasal hyper-reactivity symptoms and (2) completed the serum aeroallergen sIgE. Thus 5,486 patients with nasal hyperreactivity symptoms were recruited in our study. The study population for each analysis were not limited to AR patients (such as the results of original Figure 2), whose sIgE tests results were positive to at least one aeroallergen. Original Figure 2 (Figure 1 in revised manuscript) represents the results of age difference in the prevalence of sensitization to at least one aeroallergen among the patients who underwent the allergy test. By another words, this analysis can be understood to study the age difference in the proportion of the numbers of AR patients among patients with nasal hyperreactivity symptoms. Thus, the prevalence results of the analysis above were from 5486 patients with nasal hypersensitivity symptoms. However the original Figure 3 (Figure 2 in revised manuscript) represents the results of the trends of prevalence of sensitization to each aeroallergen among AR patients over the past 10 years. The study population of this analysis were 4085 AR patients. Similarly Table 1 represents the results of age difference in the prevalence of sensitization to each aeroallergen among AR patients, which means that the study population of this analysis were 4085 AR patients. We have stated the sources of study population for each analysis in Results section in revised manuscript.

2. Answer: As what we have stated in method section, German AllergyScreen (Mediwiss Analytic GmbH, Moers, Germany) was used to measure serum allergen sIgE levels. According to the manufacturer's protocols, both of the serum sIgE levels of single allergen and mixed allergens can be measured. The mixed allergens component means that the allergens which are classified to the same species are lumped into one group. The serum sIgE results of mixed allergens are displayed as the total sIgE levels in one group. However, the German AllergyScreen system does not provide further information about the serum sIgE levels of each allergen that is specified in the mixed group. For example, the mold allergens we measured were mixed allergen component and the serum sIgE level of *Penicillium notatum*, one specified mold in this mixed mold group, was not available. In addition,

the cockroach allergen, pet allergen, weed pollens, and tree pollens we measured in the present study were mixed allergen component too. All in all, according to the manufacturer's protocols of German AllergyScreen system, the serum sIgE levels of each allergen specified in the mixed allergen component were not available and the total sIgE levels of mixed allergens were not lump into groups by us artificially.

3. Answer: As what we have responded to #2 above, we did not measure the serum sIgE levels of each particular allergen in the mixed allergen group. Therefore, the information about the sIgE levels of particular allergen were not available.

4. Answer: We agree that it will be interesting if the results of skin prick test (SPT) were included in our study. However, we should admit honestly that we do not use SPT in our institution. We prefer to use the allergen sIgE test, a measurement with a high specificity and sensitivity for the diagnosis of common allergen sensitization. Therefore, it is difficult for us to provide and analyze the SPT results in our study.

5. Answer: The medical records we collected were not a part of a different study. We apologize to make you confuse of the consent forms in our study. Regarding the consent forms you are concerned about we feel a little confusing that whether you refer to the BMJ consent form. If so, we must admit that we do not need to complete BMJ consent form because our study does not include any information about an identifiable living individual. However, the patients we study have signed the consent forms made by our hospital which are legal in mainland China. Actually, our institution is characterized by the Grade-A hospital, which is affiliated to Sun Yat-sen University, a famous university in China. According to the related laws and regulations, all the patients or the guardians should sign the consent forms before undergoing any clinical test in such hospitals. Moreover, those consent forms are kept and managed by hospitals. All the enrolled patients in the present study signed informed consent when they underwent the allergy test and the consent forms were kept by our hospital. Therefore, we did not obtain consent forms from patients once we decided to perform this study. Instead, we can collect the consent forms efficiently and conveniently which profits from the systemic storage and management by our hospital.

6. Answer: Yes.

7. Answer: Thank you for your critical comments. We have stated the prevalence results are from the population of patients with nasal hypersensitivity, which are not applicable to the general population in Guangzhou in the last paragraph in Discussion section.

8. Answer: We apologize for this mistake in writing. What we wanted to state actually in the previous manuscript was that SIT was the only disease-modifying treatment for AR, however we left out the important word of disease-modifying. The reference 25 in previous manuscript listed below can prove our originally accurate statement. We have revised our paper and hope that this mistake in writing would not negatively affect the acceptance of the publication.

Reference:25 Pfaar O, Barth C, Jaschke C, et al. Sublingual allergen-specific immunotherapy adjuvanted with monophosphoryl lipid a: a phase i/ii study *Int Arch Allergy Immunol*. 2011;154:336-344.

9. Answer: Thank you for your critical comments and we totally agree with your suggestions which might be of great help to improve the quality of our discussion section. However there is a lack of such data in Guangzhou over the last 10 years. The present study is the first investigation on the prevalence and trends of aeroallergen sensitization in patients with AR in Guangzhou over the last 10 years. Although there exists several limitations in our study, we believe our study results can offer useful information for future study in related field.

Minor:

1. Answer: We are sorry that we have not expressed our ideas clearly in the previous manuscript. First, the overall prevalence in the results section of the Abstract indeed includes data points from all years (2005-2014). Second, in order to avoid misunderstanding, we have changed the sentence "for each additional 10 years" into "with 10 years old increasing" and highlighted the change in red color in revised manuscript.

2. Answer: Thank you for your suggestion and we have edited the significant digits for reporting P-values in the section of Abstract and Results and highlighted the changes in red color.
3. Answer: Thank you for your suggestion and we decided that the term “allergen” should be changed into “aeroallergen” to keep the term consistent.
4. Answer: As what we have responded to the question 2 in the Major section, the cockroach allergen was mixed allergens component. The German AllergyScreen system does not specify the species of cockroach we measured. Detailed information about cockroach allergen (numbered as i6 at the website page) can be found at the website of Mediwiss Analytic GmbH:
<http://www.mediwiss-analytic.de/products/allergen-directory.html?allergens=venom>
5. Answer: After examining the reviewer’s comment carefully, we must admit that there were no differences between Figure1 and the description in the results section. Therefore, we decided to delete the Figure1 but retain the description in the revised manuscript.
6. Answer: We sorry for causing confusion about the difference between p* and P delta at the bottom of Table 1. We have added the explanation for both of the P-value in the revised manuscript.
7. Answer: Thank you for your comment and we have edited the sentence and highlighted the changes in red color.
8. Answer: We appreciate for your helpful comment. The redundant statements of the results in the discussion section have been deleted.

Response to Reviewer 3

1. Answer: The concentrations of serum sIgE were quantitatively ranked strictly based on the manufacturer’s protocols of German AllergyScreen, the measurement system we used in our study. If necessary, we can provide the text file of the manufacturer’s protocols for you to make our response more convincing.
2. Answer: We apologize for the unclear statement about the age difference in the prevalence of sensitization to pet allergen. Each aeroallergen sensitization has its own distribution among age groups. The prevalence of sensitization to pet allergens was higher in younger AR patients, which was not paradoxical to what we have showed that the highest prevalence of allergic sensitization to HDM was in the younger AR patients. We edited the description of the prevalence results about the pet allergen sensitization in the revised manuscript to make it better understand and highlighted the amendments in red color.
3. Answer: The paragraph the reviewer mentioned was one part of the Results section. All of the prevalence results in this paragraph were from our study originally. Therefore we believe that we do not need mention the references. Anyway, thank you for your arduous work and comments.

VERSION 2 – REVIEW

REVIEWER	Professor Wang De Yun National University of Singapore, Singapore
REVIEW RETURNED	19-Mar-2016

GENERAL COMMENTS	The paper has been revised with my satisfactory.
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REVIEWER	Katie M. Phillips MD and Ahmad R. Sedaghat MD PhD Harvard Medical School Massachusetts Eye and Ear Infirmary USA
REVIEW RETURNED	01-Apr-2016

GENERAL COMMENTS	We thank the authors for their revisions and the clarifications that
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	<p>were made. However, we would recommend some additional work to clarify when you are describing results from the overall population vs. from the AR cohort:</p> <p>1) The Results section of the Abstract is still unclear. The first sentence is related to prevalence of sensitivity in the patients with AR while the last two sentences are related to the overall patient population. Please clarify. Also, the phrase “with 10 years old increasing” is also unclear. We would recommend: “When all patients with nasal hyper-reactivity were stratified by decade of age, increasing age was associated with a decrease in sIgE positivity...”</p> <p>2) As the first sentence of the “allergen sensitization by age group” subsection, we would recommend removing “in AR patients” to make it more clear that these numbers are from the overall cohort of patients with nasal hyper-reactivity.</p> <p>3) In the “Participants” section of the Abstract, sentence that begins with “All the patients met inclusion criteria...” should be changed to “All patients who presented with nasal hyper-reactive symptoms with completed serological allergy testing, measuring soluble IgE in the serum (sIgE) were included.”</p>
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VERSION 2 – AUTHOR RESPONSE

Response to Reviewer 1:

Answer: We appreciated the reviewer’s evaluation.

Response to Reviewer 2:

1. Answer: We apologized for the unclear statement on the Results section of the Abstract which may be responsible to our poor writing and expression in English. We agreed with your comment that the first sentence is related to the prevalence of sensitivity in AR patients. However the last two sentences may be still unclear to describe the sources of the results, thus we edited the unclear sentences to make it more understanding. The explanations are listed below:

First, the last second sentence is related to the results of the positive rate of sIgE in the overall patient population. We have changed the original sentences into “When all patients with nasal hyper-reactivity were stratified by...” , which was suggested by you. The recommended sentences accurately show that the results are from the overall patient population. We appreciated your helpful comment.

Second, the last sentence is related to the trend in the prevalence of pet allergen sensitivity, which was the further study that based on the results which were showed in the first sentence in the Results section in Abstract. Thus the main study population were patients with AR instead of the overall patient population. Therefore we added the phrase “in AR patients” in the last sentence to make it clearer to show our ideas.

All in all, we apologized for the unclear sentences in Abstract section, and we hope these revisions and explanations are satisfactory. Thank you very much for your work concerning our paper.

2. Answer: Thank you for your comment and we have edited the sentence.

3. Answer: Thank you for your critical comments and we totally agree with your suggestions which might be of great help to make it clearer to express our ideas. We have revised the sentence and highlighted the changes in red color.

VERSION 3 - REVIEW

REVIEWER	Katie M Phillips MD and Ahmad R Sedaghat MD PhD Harvard Medical School Massachusetts Eye and Ear Infirmary USA
REVIEW RETURNED	20-Apr-2016

GENERAL COMMENTS	I thank the authors for addressing our comments.
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