PEER REVIEW HISTORY

BMJ Open publishes all reviews undertaken for accepted manuscripts. Reviewers are asked to complete a checklist review form and are provided with free text boxes to elaborate on their assessment. These free text comments are reproduced below.

ARTICLE DETAILS

Title (Provisional)

Association of helicobacter pylori infection and white blood cell counts: A cross-sectional study

Authors

Jiao, Rui; Ma, Xiaojuan; Guo, Xiaoqing; Zhu, Yanli; Wu, Xue; Wang, Haiying; Zhang, Shaifei; Wang, Yahong; Yang, Yang; Wang, Qiang

---

VERSION 1 - REVIEW

Reviewer	**1**
Name	**Görlich, Dennis**
Affiliation	**Westfälische Wilhelms-Universität Münster, Institute of Biostatistics and Clinical Research**
Date	**14-Dec-2023**
COI	**None**

The authors presented results from a cross sectional cohort study designed from retrospectively collected data.

The main aim of the manuscript is to elucidate the association of H.pylori infection in a chinese population sample with white blood cells counts.

While the overall setup of the analysis seems to be sound there are several points/issues I would like to raise here:

1. Representativity: The title refers to the analysis to be representative for the general (chinese) population. I cannot follow this argument from the data presented. The sample size (N=864) is of course not small, but also not in itself a reason to assume representativty per se. The authors did not provide any (supplementary) analyses to show that e.g. distributions on major variable (age, sex, etc) justify a generalizability to the general population (in china). Also data was collected from a single center from participants who "underwent health examinations". This would indicate a cause (an there may be many) that makes a health examination necessary, which may bias the cohort to, at least, being selected away from the general population. I would strongly suggest to tackle this point, and either

better explain in the manuscript and/or provide more information and analyses (e.g. in supplementary material).

2. More transparent reporting on the selection of the cohort:

The manuscript reports several steps where participant exclusions may have happended. I would suggest to a patient flow chart as a figure to clearly describe the screening steps and the number of exclusions giving reasons.

3. Improvement of reporting: Please consider the STROBE guideline (https://www.strobe-statement.org/; Elm E et al. BMJ. 2007 Oct 20;335(7624):806-8. PMID: 17947786) and submit the STROBE checklist with a potential revision of the manuscript for clarity.

4. Please move the following sentence to the discussion section: "Therefore, these data suggested that H. pylori exposure and

colonization directly or indirectly alters the hematological components and immunological microenvironment" it somehow opens discussion already in the middle of the results part.

5. Figure 1: Seems to come from a different work and does show the correct data. Please resubmitt the correct figure.

6. Statistical issues:

6.1 Correlation analyses: The manuscript, beside comparing Hp+ and Hp- groups, also reports correlations between continous variables for many comparisons. The manuscript reports these as Pearson correlations. Since no information on the distribution of the variable was given (Figure 1 would be probably helpful but is incorrect) it cannot be assessed if this is a proper choice. From experience I would expect some or many factors not to be normally distributed and then Pearson correlation is not applicable. Please reconsider to analyse correlation by the non-parametric Spearman correlation coefficient. and reinterprete the data.

6.2. The same issue arises with linear regression models. The authors list "generalized" linear models as their choice, but never defined the chosen generalization (distributional assumptions and link function). So it remains unclear whether a linear model for probably non-linear data was applied, or if the generalization was really used. In the latter case the interpretation of regression coefficients (betas) might change, so this information is crucial.

The same applied to the General additive model. Please provide more information in the methods section.

6.3 Power calculation. The methods section reports that "The statistical power of this study was calculated using Stata SE 12.0 software based on the difference of means and standard deviation of WBC counts between Hp+ and Hp- groups" (l.164ff) but no result of the calculation nor information about actual planning parameters where given. Also if this was done a-priori of a-posteriori was not reported.

6.4 reporting of p-values: please recheck you p-values reported as 0.000. This seems to be directly copied from SPSS where this kind of "leaving away" the decimals is implemented. Please check numbers and consider reporting as "<0.0001" is applicabel and correct.

6.5. Reporting of betas: In the text it is, at some occassions, hard ot assign the beta values to the factor/variable. Consider labeling the betas (eg with a subscript) to make it clear to which variable it belongs.

6.6 Analysis of subgroups: Table 5 and the text report subgroup analyses. It seems the analysis was conducted by filtering the data and perform the linear regession on a smaller (filtered) dataset, i.e. within the subgroup. This also reduces the power to detect effects in the subgroup and may lead to larger p-values. Reconsider if a linear regression model of the whole cohort with an interaction term between DMP and e.g. gender might be a better methodological choice to estimate associations within the subgroups.

7. Overall merit and recommendation:

Currently the manuscript contains a number of issues that needs to addressed before reconsideration. The title of this manuscript needs to be adjusted to better reflect the study design and the (restricted) generalizability due to it. Also, as the authors themself reported, the main observation that H.pylori infection is associated with elevated WBC counts is known and published within a larger cohort (Yu YY et al. 2018). Nevertheless, the authors add additional information analyses from an independent cohort to this.

Reviewer	**2**
Name	**Nasif, Wesam A**
Affiliation	**Umm Al-Qura University, biochemistry department**
Date	**11-Jan-2024**
COI	**None**

Strengths:

Observational study: Useful for investigating associations between exposures (H. pylori infection) and outcomes (WBC counts) in large populations.

Cross-sectional design: Efficient for collecting data on a large number of participants at a single point in time.

Standardized measurements: Consistent methodology for UBT, blood tests, and other measurements minimizes bias.

Weaknesses:

Causality limitations: Cannot establish cause-and-effect relationships due to the observational nature. Other factors might influence both H. pylori infection and WBC counts.

Temporal relationship: Doesn't capture the temporal sequence of events. It's unclear if H. pylori infection precedes the increase in WBC counts or vice versa.

Generalizability: Limited to participants who underwent health examinations at the specific hospital, potentially not representative of the general population.

Overall, the study design provides valuable insights into the association between H. pylori infection and WBC counts, but it cannot definitively prove causation.

Method Description Sufficiency

The methods described are sufficiently detailed to allow the study to be repeated, with some minor reservations:

Strengths:

Clear inclusion and exclusion criteria for participants.

Detailed description of UBT procedure, analyzer used, and DPM interpretation criteria.

Precise measurements of various anthropometric, biochemical, and hematological parameters with specified instruments.

Areas for improvement:

Lack of information about sample size calculation and justification.

Vague mention of "general medical examination" – specifying details about medical history or potential confounding factors would strengthen the study.

Consider mentioning the specific statistical tests used for comparing WBC counts between groups and analyzing correlations.

Overall, the provided methods description allows for replication of the study with some additional details for better clarity and completeness.

Reviewer   **3**

Name   **Izhari, Mohammad Asrar**

Affiliation   **Al-Baha University**

Date   **22-Jan-2024**

COI   **Nil**

Dear authors,

The research on "Helicobacter pylori" is needed to understand the pathogenesis of the disease caused by it. Your effort to carry out the research on this topic is really significant. I hope the review report will be helpful during manuscript revision.

Thanks, and regard.

Review reports/Comments for the authors

Review checklist Please elaborate on any 'No' answers in the free text section below.	Yes	NO	NA
1. Is the research question or study objective clearly defined? **Major Comments:** Regarding the objectives of the research, In line number 26: the author says the study was aimed to elucidate the effects H. pylori on hematological parameters in the general population. While In lines number 101-13, the author talks about the objective of the research. The study aimed to explore the relationship between *H. pylori* infection and WBC count qualitatively and quantitatively. The objective of the study is not delineated by the authors whether they want to evaluate only WBC or various other hematological parameters is not clear as a result section lipid profile, and various other metabolic parameters have been summarized.		NO	
2. Is the abstract accurate, balanced, and complete? **Major Comments:** In line 33, DPM appears for the first time, therefore, it should be written in full form at the first place. Results are not completely summarized in the abstract section. The conclusion is generalized, and not based on the evidence of the present research. **Minor comment:** The English language needs extensive revision.		NO	
3. Is the study design appropriate to answer the research question? **Comments:** The statistical test was applied according to the standard; however, the case and control were not elaborated on clearly,howeve, the exposure was defined.		NO	
4. Are the methods described sufficiently to allow the study to be repeated?	Yes		
5. Are research ethics (e.g. participant consent, ethics approval) addressed appropriately? **Comment:** **I would suggest the authors to add the date of approval and the approval number in the method section**	yes		
6. Are the outcomes clearly defined? **Comment:**		NO	

The relevance of the outcome depends on the clarity of the objective of the research. But the objective of this research is not delineated therefore all other components were not clearly articulated.			
7. If statistics are used are they appropriate and described fully?	yes		
8. Are the references up-to-date and appropriate?	yes		
9. Do the results address the research question or objective? **Comment:** **The objective of the research is not clearly defined.**		NO	
10. Are they presented clearly? **Comments:** Figure 1 is in the form of panel, therefore, a brief explanation of each component of the figure such as A, B, C ……should added below the figures. The table should be written in place of Tab. Footnotes should be mentioned for table Table 2 should be added. Table 4 is not clearly outlined. Table 3 needs footnotes to be added for all the short forms mentioned as parameters.		NO	
11. Are the discussion and conclusions justified by the results **Comments:** In line 362-364 the authors say "Collectively, our study found that in the general population, participants with H. pylori infection 363 are more likely to have higher WBC counts, and the prevalence of H. pylori infection gradually increases with the increase of WBC quartile". The authors are describing here the prevalence of the infection with WBC count. To the best of my knowledge, prevalence is another concept that is not part of this research objective. Prevalence study should not be mixed with this objective. However, the authors have elaborated discussed the result of the manuscript.		NO	
12. Are the study limitations discussed adequately? **Comments:** **Although the authors have mentioned the limitation in line 53 and 54 as** "Our study is limited to its retrospective nature. Information on individual disease history and 54 comorbidity are not available" However, Exposure assessment has been done only by urea breath test, which does not give clear perspective, therefore, endoscopy, histopathological examination and molecular evaluation could be key limitations of the study.		NO	
13. Is the supplementary reporting complete (e.g. trial registration; funding details; CONSORT, STROBE or PRISMA checklist)?			NA
14. To the best of your knowledge is the paper free from concerns over publication ethics (e.g. plagiarism, redundant publication, undeclared conflicts of interest)? **Comments:** I have not checked due lack of resources and I do not bear any such responsibility. It's the journals' responsibility to ensure this.			NA

15. Is the standard of written English acceptable for publication?	Yes

---

VERSION 1 - AUTHOR RESPONSE

Reviewer: 1

The authors presented results from a cross sectional cohort study designed from retrospectively collected data. The main aim of the manuscript is to elucidate the association of H.pylori infection in a chinese population sample with white blood cells counts. While the overall setup of the analysis seems to be sound there are several points/issues I would like to raise here:

Response: Thanks very much for your time, effort and very helpful comments, which greatly helped us to improve our manuscript. The following is a list of responses to each of Reviewers' major concerns.

Comment 1: Representativity: The title refers to the analysis to be representative for the general (chinese) population. I cannot follow this argument from the data presented. The sample size (N=864) is of course not small, but also not in itself a reason to assume representativty per se. The authors did not provide any (supplementary) analyses to show that e.g. distributions on major variable (age, sex, etc) justify a generalizability to the general population (in china). Also data was collected from a single center from participants who "underwent health examinations". This would indicate a cause (an there may be many) that makes a health examination necessary, which may bias the cohort to, at least, being selected away from the general population. I would strongly suggest to tackle this point, and either better explain in the manuscript and/or provide more information and analyses (e.g. in supplementary material).

Response 1: We are sorry for the unreasonable extrapolation and expansion of the research conclusions due to the problems we expressed, and we deleted the expression "in general population" in the revised manuscript. We also revised the title to "Association of helicobacter pylori infection and white blood cell counts: A cross-sectional study".

Comment 2: More transparent reporting on the selection of the cohort: The manuscript reports several steps where participant exclusions may have happended. I would suggest to a patient flow chart as a figure to clearly describe the screening steps and the number of exclusions giving reasons.

Response 2: Thanks for your professional suggestions. Indeed, when we conducted volunteer recruitment, it was explicitly stated that the volunteers taking proton pump inhibitors in the preceding 15 days or any antibiotics within 30 days before the examination were excluded, so the 864 subjects who ultimately qualified for recruitment were included in this study for retrospective analysis.

Comment 3: Improvement of reporting: Please consider the STROBE guideline (https://www.strobe-statement.org/; Elm E et al. BMJ. 2007 Oct 20;335(7624):806-8. PMID: 17947786) and submit the STROBE checklist with a potential revision of the manuscript for clarity.

Response 3: According to your comments, we supplemented the STROBE checklist and revised the manuscript. Please refer to the attachment, the STROBE checklist.

Comment 4: Please move the following sentence to the discussion section: "Therefore, these data suggested that H. pylori exposure and colonization directly or indirectly alters the hematological components and immunological microenvironment" it somehow opens discussion already in the middle of the results part.

Response 4: We have made correction according to the Reviewer's comments. The statement has been moved to the Line 261-262, the Section Discussion.

Comment 5: Figure 1: Seems to come from a different work and does show the correct data. Please resubmit the correct figure.

Response 5: Thank you for pointing out the error. After verification, due to our negligence, Figure 1 comes from other work. The correct Figure 1 has been resubmitted.

Comment 6: Correlation analyses: The manuscript, beside comparing Hp+ and Hp- groups, also reports correlations between continous variables for many comparisons. The manuscript reports these as Pearson correlations. Since no information on the distribution of the variable was given (Figure 1 would be probably helpful but is incorrect) it cannot be assessed if this is a proper choice. From experience I would expect some or many factors not to be normally distributed and then Pearson correlation is not applicable. Please reconsider to analyse correlation by the non-parametric Spearman correlation coefficient. and reinterprete the data.

Response 6: Indeed, some of our data did not conform to the normal distribution, and Spearman's correlation coefficient analysis was re-used for the correlation analysis of this part data, which was corrected in the statistical analysis of the Section method and Table 2.

Comment 7: The same issue arises with linear regression models. The authors list "generalized" linear models as their choice, but never defined the chosen generalization (distributional assumptions and link function). So it remains unclear whether a linear model for probably non-linear data was applied, or if the generalization was really used. In the latter case the interpretation of regression coefficients (betas) might change, so this information is crucial. The same applied to the General additive model. Please provide more information in the methods section.

Response 7: Sorry for the misunderstanding caused by our translation error. In fact, the model used to analyze and adjust the relationship between DPM and WBC is general linear regression, not Generalized linear regression. We have corrected this error in the Section Abstract, and the Section 3.4. In addition, the related information of the General additive model has also been added in the Section 2.4 Statistical analysis, as shown below: The generalized additive model (GAM) was performed to identify the non-linear relationship of DPM and WBC counts by the smoothing plot using package mgcv of R software. In the GAM model, WBC was set as dependent variable, DPM and age were independent variables (Family = Gaussian, Link function = identity.

Comment 8: Power calculation. The methods section reports that "The statistical power of this study was calculated using Stata SE 12.0 software based on the difference of means and standard deviation of WBC counts between Hp+ and Hp- groups" (l.164ff) but no result of the calculation nor information about actual planning parameters where given. Also if this was done a-priori of a-posteriori was not reported.

Response 8: Sorry, this part of statistical analysis is not complete. We calculated the statistical power of this study according to the posterior calculation. Please refer to Line 156-160, the Section 2.4 Statistical analysis.

Comment 9: reporting of p-values: please recheck you p-values reported as 0.000. This seems to be directly copied from SPSS where this kind of "leaving away" the decimals is implemented. Please check numbers and consider reporting as "<0.0001" is applicabel and correct.

Response 9: We are very sorry for our negligence. After verification, we changed P from 0.000 to "<0.0001". Please refer to Table 4.

Comment 10: Reporting of betas: In the text it is, at some occassions, hard ot assign the beta values to the factor/variable. Consider labeling the betas (eg with a subscript) to make it clear to which variable it belongs.

Response 10: According to your requirement, we labeled the betas with a subscript.

Comment 11: Analysis of subgroups: Table 5 and the text report subgroup analyses. It seems the analysis was conducted by filtering the data and perform the linear regession on a smaller (filtered) dataset, i.e. within the subgroup. This also reduces the power to detect effects in the subgroup and may lead to larger p-values. Reconsider if a linear regression model of the whole cohort with an interaction term between DMP and e.g. gender might be a better methodological choice to estimate associations within the subgroups.

Response 11: The interaction terms in the whole cohort population were examined according to the reviewer's suggestion. Age, sex, BMI and SBP were taken as covariables respectively, and the DPM×covariate interaction term was introduced into the general linear regression. WBC was taken as the dependent variable for analysis. The results showed that gender, BMI and SBP had no significant interaction with DPM, although age seemed to interact with DPM in the interaction diagram, but it was not significant. We removed the original Table 5 and the corresponding results and added the Figure 3. Please refer to Figure 3 and the Section 3.5 Analysis of the interactive effects of DPM and covariates on WBC counts.

Comment 12: Overall merit and recommendation:

Currently the manuscript contains a number of issues that needs to addressed before reconsideration. The title of this manuscript needs to be adjusted to better reflect the study design and the (restricted) generalizability due to it. Also, as the authors themself reported, the main observation that H.pylori infection is associated with elevated WBC counts is known and published within a larger cohort (Yu YY et al. 2018). Nevertheless, the authors add additional information analyses from an independent cohort to this.

Reviewer 2:

Strengths: Observational study: Useful for investigating associations between exposures (H. pylori infection) and outcomes (WBC counts) in large populations. Cross-sectional design: Efficient for collecting data on a large number of participants at a single point in time. Standardized measurements: Consistent methodology for UBT, blood tests, and other measurements minimizes bias.

Response: Special thanks to you for your good comments.

Comment 1: Causality limitations: Cannot establish cause-and-effect relationships due to the observational nature. Other factors might influence both H. pylori infection and WBC counts.

Response 1: We totally agree with you. This is a retrospective observational study and our results qualitatively and quantitatively described the association between DPM and WBC counts. We found that participants with H. pylori active infection are more likely to have higher WBC counts in the general population. In addition, using piecewise linear regression, it was shown that DPM is not statistically correlated with WBC count as DPM below the infection point 40, while when DPM value increased to 155 from 40, a significant positive correlation was indicated. When DPM value was above 155, the WBC counts decreased and there was a significant adverse association with DPM. However, this study cannot directly prove a causal relationship between WBC and H. pylori infection, and their results can only serve as evidence of association, which needs to be verified by further randomized controlled trials. Nonetheless, this study proved that the independent contribution of positive infection and activities of H. pylori on the changes of WBC counts. The above limitation has been discussed in the Line 347-351, Paragraph 5, the Section 4 Discussion.

Comment 2: Temporal relationship: Doesn't capture the temporal sequence of events. It's unclear if H. pylori infection precedes the increase in WBC counts or vice versa.

Response 2: Thank you, we totally go along with you. Indeed, this is a retrospective observational study and we only proved that participants with H. pylori infection are more likely to have higher WBC counts, and the prevalence of H. pylori infection gradually increases with the increase of WBC quartiles. However, the temporal relationship between WBC and H. pylori infection is still unclear. The above limitation has been discussed in the Line 347-351, Paragraph 5, the Section 4. Discussion.

Comment 3: Generalizability: Limited to participants who underwent health examinations at the specific hospital, potentially not representative of the general population.

Response 3: We are sorry for the unreasonable extrapolation and expansion of the research conclusions due to the problems we expressed, and we deleted the expression "in general population" in the revised manuscript. We also revised the title to "Association of helicobacter pylori infection and white blood cell counts: A cross-sectional study".

Comment 4: Strengths: Clear inclusion and exclusion criteria for participants. Detailed description of UBT procedure, analyzer used, and DPM interpretation criteria. Precise measurements of various anthropometric, biochemical, and hematological parameters with specified instruments.

Response 4: We thank the reviewer for the helpful comments and appreciation of our work.

Comment 6: Lack of information about sample size calculation and justification.

Response 6: The calculation methods and results of sample size and statistical efficiency have been supplemented according to the comments of reviewers. Please refer to Line 156-160, the Section 2.4 Statistical analysis.

Comment 7: Vague mention of "general medical examination" – specifying details about medical history or potential confounding factors would strengthen the study.

Response 7: We are very Sorry. Since this is the patient and data collected by the physical examination center, we cannot contact the patient and obtain the patient's medical history.

Comment 8: Consider mentioning the specific statistical tests used for comparing WBC counts between groups and analyzing correlations.

Response 8: According to the Reviewer's suggestion, we footnoted the statistical test in Table 1 and Table 3.

Reviewer: 3

Comment 1: The research on "Helicobacter pylori" is needed to understand the pathogenesis of the disease caused by it. Your effort to carry out the research on this topic is really significant. I hope the review report will be helpful during manuscript revision.

Response 1: Thanks very much for your time, effort and very helpful comments.

We sincerely appreciate the suggestions provided by the Editors and Reviewers and will respond to any further questions and comments that you may have.

Yang Yang, MD., PhD., and Qiang Wang MD., PhD.

Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education Faculty of Life Sciences and Medicine, Northwest University

229 Taibai North Road Xi'an 710069, China

Telephone: +86 13379217366

Email address:

yang200214yy@163.com

(Yang Yang) and

qiangwangshenmu@163.com

(Qiang Wang)

VERSION 2 - REVIEW

Reviewer	**1**
Name	**Görlich, Dennis**
Affiliation	**Westfälische Wilhelms-Universität Münster, Institute of Biostatistics and Clinical Research**
Date	**03-Jun-2024**
COI	**NONE**

Dear authors,

thank you for your thorough revision of the manuscript.

The manuscript was improved a lot and but I still have final questions.

The participant inclusion (Section 2.1) still lacks a proper explanation on the number of screened and excluded volunteers. Could you add the requested flow chart which explains the number of initially screened volunteers and which also explained the number of exclusions giving reasons (including number of individuals for each exclusion reason).

Also, can you confirm that the ethical review committee explicitly gave a positive vote for the decision to not collect written informed consents from the study participants (lines 108-110). From my understanding having "no individual identifiable information" still requires an informed consent by the participants/study subject to use the observed data in an pseudonymized (or anonymized) manner (not clear from the manuscript).

The reasoning "participants were from the health-check project" also raises another question related to my first comment. Could you clearly explain in your manuscript how participants have been recruited to the trial. It seems there was a larger project (the health check project?) which was the basis for this work, but this never got explained. A flow chart and some background information would be really helpfull.

VERSION 2 - AUTHOR RESPONSE

Reviewer: 1

Comment 1: The participant inclusion (Section 2.1) still lacks a proper explanation on the number of screened and excluded volunteers. Could you add the requested flow chart which explains the number of initially screened volunteers and which also explained the number of exclusions giving reasons (including number of individuals for each exclusion reason).

Response: Thank you for your suggestion. It is necessary for us to explain the inclusion and exclusion criteria. Participants who had taken proton pump inhibitors within 15 days prior to the examination or any antibiotics within 30 days prior to the examination were excluded from this study. Volunteers with malignant solid tumors, hematologic malignancies, severe immune disorders, and active bacterial and viral infections were also excluded. From January 2021 to June 2021, healthy volunteers were collected at the hospital medical examination center and excluded through verbal questioning by nurses about the history of the above diseases and medication history. As of the inclusion of volunteers, no volunteers with the above medication history and disease history were found in this study, so all 864 volunteers were included, which is the main reason why we did not provide a flow chart of the inclusion and exclusion criteria. Finally, we will provide the flowchart based on your suggestion and explain the above in detail in the manuscript. Please refer to Figure 1 and the Section 2.1 Participates.

Comment 2: Also, can you confirm that the ethical review committee explicitly gave a positive vote for the decision to not collect written informed consents from the study participants (lines 108-110). From my understanding having "no individual identifiable information" still requires an informed consent by the participants/study subject to use the observed data in an pseudonymized (or anonymized) manner (not clear from the manuscript).

Response: Thank you for your suggestion. Ethical approval for this study was obtained from the Human Ethics Review Committee (SM020) of the Shenmu Hospital affiliated with Northwestern University and the medical ethics committee of our hospital waived the written informed consent of the patients.

Comment 3: The reasoning "participants were from the health-check project" also raises another question related to my first comment. Could you clearly explain in your manuscript how participants have been recruited to the trial. It seems there was a larger project (the health check project?) which was the basis for this work, but this never got explained. A flow chart and some background information would be really helpfull.

Response: Thank you for your question, the study did not rely on a larger program (health screening program). The volunteers in this study just attended the medical checkups at our hospital's health checkup center on their own, and we collected and analyzed data from patients who underwent medical checkups from January 2021 to June 2021. We have further explained the inclusion and exclusion criteria and provided a flowchart of the inclusion and exclusion criteria.

We sincerely appreciate the suggestions provided by the Editors and Reviewers and will respond to any further questions and comments that you may have.

Yang Yang, MD., PhD., and Qiang Wang MD., PhD.

Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education Faculty of Life Sciences and Medicine, Northwest University

229 Taibai North Road Xi'an 710069, China

Telephone: +86 13379217366

Email address:

yang200214yy@163.com

(Yang Yang) and

qiangwangshenmu@163.com

(Qiang Wang)

VERSION 3 - AUTHOR RESPONSE

Comment 1: Regarding the objectives of the research, in line number 26: the author says the study was aimed to elucidate the effects H. pylori on hematological parameters in the general population. While in lines number 101-13, the author talks about the objective of the research. The study aimed to explore the relationship between *H. pylori* infection and WBC count qualitatively and quantitatively. The objective of the study is not delineated by the authors whether they want to evaluate only WBC or various other hematological parameters is not clear as a result section lipid profile, and various other metabolic parameters have been summarized.

Response: Thank you for your suggestion. 1) First, it is really important to clarify the aim of the study clearly, we have re-defined the objective of the research according to your comment. Please refer to section Abstract and introduction paragraph 3.

Abstract: Helicobacter pylori (*H. pylori*) is a kind of gram-negative micro aerobic bacteria that hosts in the gastric mucosal epithelium. It can cause various gastrointestinal diseases, including gastritis, peptic ulcer, and gastric cancer. White blood cells (WBC), a common immune cell, the increase of its count often indicates the existence of infection. Currently, the relationship between *H. pylori* and WBC counts remains full of

controversy. This study aims to further elucidate the effects *H. pylori* on WBC counts in the physical examination population.

…

Introduction: Our study aims to qualitatively and quantitatively explore the relationship between *H. pylori* infection and total WBC counts, potentially providing clinical evidence for the understanding of pathogenesis and management of *H. pylori*.

2) Second, we introduced the lipid profile and other various metabolic parameters in the part of Results to more comprehensively describe the baseline characteristics of participates and explore the the potential covariates.

Comment 2: In line 33, DPM appears for the first time, therefore, it should be written in full form at the first place. Results are not completely summarized in the abstract section. The conclusion is generalized, and not based on the evidence of the present research. The English language needs extensive revision.

Response: Thank you for your suggestion. 1) First, we have written the full name of DPM as "disintegrations per minute" when it appears first time. 2) Second, we have re-summarized the results and conclusions according to your comments. Please refer to section Abstract.

Results: "Finally, 403 subjects were diagnosed with *H. pylori* infection. WBC counts and platelets (PLT) in the Hp+ group were significantly higher than those in the Hp- group. Besides, the incidence of *H. pylori* infection gradually elevated with WBC counts quartiles (38.89% and 54.67% in Q1 and Q4 subgroups, respectively). Spearman's correlation analysis showed that DPM value significantly correlated with WBC counts (r=0.089, *P*=0.009) and PLT (r=0.082, *P*=0.017). The linear model revealed the positively independent association of *H. pylori* infection and DPM with WBC counts (β_{Hp+} = 0.398 [95% CI: 0.170, 0.625], *P*<0.001; β_{DPM} = 0.002 [95% CI: 0.000, 0.003], *P*=0.018). The results of GAM and piecewise linear regression suggested that the threshold value of DPM effect on WBC counts was 40 and 155 of DPM, that is, the effect of DPM on WBC counts varied with the difference of DPM less than 40, 40-155 and greater than 155 (β_{DPM}= -0.005, 95% CI: -0.017, 0.007, *P*=0.423, β_{DPM}= 0.006, 95% CI: 0.002, 0.013, *P*=0.047, and β_{DPM}=-0.007, 95% CI: -0.012, -0.002, *P*=0.004, respectively)."

Conclusions: "*H. pylori* infection was independently and positively correlated with WBC counts, but the effect of DPM on WBC counts varied from different intervals, suggesting distinct immunological responses

at different stages of infection."

3) Third, we have improved the English language of the full text.

Comment 3: The statistical test was applied according to the standard; however, the case and control were not elaborated on clearly, however, the exposure was defined.

Response: Thank you for your suggestion. We have illustrated the case and control more clearly according to your comments. Please refer to section 2.2 ^{14}C urea breath test (14C-UBT): *H. pylori* infection was considered positive if DPM $\geq$ 50, negative if DPM $\leq$ 40, and DPM within the range of 40-50 were uncertain for *H. pylori* and these cases were excluded. Subsequently, the overall population was divided into *H. pylori*-negative (Hp-) and -positive (Hp+) groups according to whether DPM $\geq$ 50 or $\leq$ 40.

Comment 4: I would suggest the authors to add the date of approval and the approval number in the method section

Response: Thank you for your suggestion. The approval number was SM020, and we have added the date of approval in section 2.1 Participates. Ethical approval of this study was obtained from the Human Ethics Review Committee of Northwest University Affiliated Shenmu Hospital in October 2022 with approval number (SM020). After obtaining ethical approval, the study was formally carried out.

Comment 5: The relevance of the outcome depends on the clarity of the objective of the research. But the objective of this research is not delineated therefore all other components were not clearly articulated

Response: Thank you for your comment. We revised the objective of the study according to your suggestion, which makes the current research outcomes were closely related to this objective. Please refer to section Abstract and introduction paragraph 3.

Abstract: Helicobacter pylori (*H. pylori*) is a kind of gram-negative micro aerobic bacteria that hosts in the gastric mucosal epithelium. It can cause various gastrointestinal diseases, including gastritis, peptic ulcer, and gastric cancer. White blood cells (WBC), a common immune cell, the increase of its count often indicates the existence of infection. Currently, the relationship between *H. pylori* and WBC counts remains full of controversy. This study aims to further elucidate the effects *H. pylori* on WBC counts in the physical examination population.

…

Introduction: Our study aims to qualitatively and quantitatively explore the relationship between *H. pylori* infection and total WBC counts, potentially providing clinical evidence for the understanding of pathogenesis and management of *H. pylori*.

Comment 6: The objective of the research is not clearly defined.

Response: Thank you for your comment. We have re-defined the objective of the research clearly according to your suggestion. Please refer to section Abstract and introduction paragraph 3.

Abstract: Helicobacter pylori (*H. pylori*) is a kind of gram-negative micro aerobic bacteria that hosts in the gastric mucosal epithelium. It can cause various gastrointestinal diseases, including gastritis, peptic ulcer, and gastric cancer. White blood cells (WBC), a common immune cell, the increase of its count often indicates the existence of infection. Currently, the relationship between *H. pylori* and WBC counts remains full of controversy. This study aims to further elucidate the effects *H. pylori* on WBC counts in the physical examination population.

…

Introduction: Our study aims to qualitatively and quantitatively explore the relationship between *H. pylori* infection and total WBC counts, potentially providing clinical evidence for the understanding of pathogenesis and management of *H. pylori*.

Comment 7: Figure 1 is in the form of panel, therefore, a brief explanation of each component of the figure such as A, B, C ……should added below the figures. The table should be written in place of Tab. Footnotes should be mentioned for table Table 2 should be added. Table 4 is not clearly outlined. Table 3 needs footnotes to be added for all the short forms mentioned as parameters.

Response: Thank you for your comment. 1) First, the figure 1 is a complete flow chart, which illustrated the process of inclusion and exclusion criteria of the study. The figure showed a total of 864 eligible participates were retrospectively enrolled in this cross-sectional study in Health Care center from January 2021 to June 2021, no volunteers were excluded due to malignant solid tumors hematologic malignancies, severe immunological diseases, and active bacterial and virus infections. Besides, no volunteers taking proton pump inhibitors in the preceding 15 days or any antibiotics within 30 days before the examination. Based on the

above, 864 eligible volunteers were retrospectively collected in the study and then divided into *H. pylori*-negative (Hp-) and positive (Hp+) groups.

2) Second, we have replaced the Tab of the full text with Table.

3) We have added footnotes and full names of parameters to Table 2, Table 3, and Table 4. Please refer to Table 2, Table 3, and Table 4.

Comment 8: In line 362-364 the authors say "Collectively, our study found that in the general population, participants with H. pylori infection 363 are more likely to have higher WBC counts, and the prevalence of H. pylori infection gradually increases with the increase of WBC quartile". The authors are describing here the prevalence of the infection with WBC count. To the best of my knowledge, prevalence is another concept that is not part of this research objective. Prevalence study should not be mixed with this objective. However, the authors have elaborated discussed the result of the manuscript.

Response: Thank you for your comment. The brief description of prevalence was just to discuss the proportion of volunteers infected with H. pylori infection over January 2021 to June 2021 in our Health Care center. The detailed description of results (i.e., 3.1 Baseline characteristics of the participates, 3.2 Correlations between UBT test DPM and hematological parameters, 3.3 Potential factors contributing to the increase of WBC counts, 3.4 Linear regression analysis of the association between H. pylori infection and WBC counts, 3.5 Analysis of the interactive effects of DPM and covariates on WBC counts, and 3.6 Non-linear model of dose-response relationship between DPM and WBC counts) were closely related to the purpose of this study and appropriately answered the research question.

Comment 9: Although the authors have mentioned the limitation in line 53 and 54 as "Our study is limited to its retrospective nature. Information on individual disease history and 54 comorbidity are not available" However, Exposure assessment has been done only by urea breath test, which does not give clear perspective, therefore, endoscopy, histopathological examination and molecular evaluation could be key limitations of the study.

Response: Thank you for your comment. Because our study participants were volunteers in Health Care center from January 2021 to June 2021, only urea breath test was used to assess whether H. pylori infection or not. According to your suggestion, we regarded endoscopy, histopathological examination, and molecular

evaluation as one of the limitations of our study. Please refer to section Strengths and limitations of this study: Our study is limited for its retrospective nature and incomplete various examinations. Information on individual disease history, comorbidity, endoscopy, and histopathological examinations are not available.

We sincerely appreciate the suggestions provided by the Editors and Reviewers and will respond to any further questions and comments that you may have.

Yang Yang, MD., PhD., and Qiang Wang MD., PhD.

Key Laboratory of Resource Biology and Biotechnology in Western China, Ministry of Education Faculty of Life Sciences and Medicine, Northwest University

229 Taibai North Road Xi'an 710069, China

Telephone: +86 13379217366

Email address: yang200214yy@163.com (Yang Yang) and qiangwangshenmu@163.com (Qiang Wang)