PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	The Relationships among Shift Work, Hair Cortisol Concentration and Sleep Disorders: A Cross-sectional Study in China
AUTHORS	Zhang, Yu; Shen, Jiayang; Zhou, Ziqi; Sang, Lingli; Zhuang, Xun; Chu, Minjie; Tian, Tian; Xiao, Jing; Lian, Yulong

VERSION 1 – REVIEW

REVIEWER	Kristiina Rajaleid Stress Research Institute, Stockholm University, Sweden
REVIEW RETURNED	15-Apr-2020

GENERAL COMMENTS	Comments on the ms "The Relationships among Shift Work, Hair Cortisol Concentration and Sleep Disorders: A Cross-sectional Study in China" Overall, I think the statistics in this paper is quite straightforward and relatively well described. I however suggest adding some further clarifications as well as have more focus on interpretation of the results. More specifically: 1. P. 6, from line 31. You selected 6 teams and included 10 teams, an explanation is needed. 2. P. 8, line 45. I think the sentence gives an impression that the authors themselves developed the KHB method. 3. P. 9 from line 11, please specify what the numbers and ranges refer to (SD, IQR). 4. OR's with three digits after decimal point is far too much, giving a false impression of high level of precision. One digit is enough, please round the numbers. 5. P. 9 line 51 (and other places with the same information), specify the unit of predictor variable for the OR's. 6. I don't think you name in the paper how many individuals you had in the different shift work categories. This information could e.g. be added in Table 2 as an additional column. 7. More information is needed wrt the KHB modelling: a. How was the shift work variable treated in these models, dichotomised? b. What is the interpretation of parameters a, b and c? c. How do the results from KHB models relate to the results from the pairwise analyses between the three components in the models presented previously? Assuming you dichotomised shift work, (transformed) HCC is continuous and sleep disorders are dichotomised, the results on Figure 1 imply 0.4 units higher HCC in shift workers compared to fixed day workers (much higher than crude difference as seen e.g. in Table 2); OR=exp(1.9) for sleep disorder per unit increase in HCC (much higher than the OR's seen in Table 3) etc. What is the meaning of parameter c? To conclude, I

am not sure the two parts of analyses tell us the same story as the estimates seem to have substantially different magnitudes.
d. What is the interpretation of the mediating effect you found? Can you interpret it quantitatively (percent mediated)? Qualitatively?
e. What are the assumptions related to the method and did your data meet the assumptions?
8. Your results of the KHB modelling seem to suggest that the whole association between shift work and sleep disorders is explained by / mediated through HCC-related mechanisms. Is it realistic and reasonable?

REVIEWER	Ryan Burns University of Utah
REVIEW RETURNED	16-Apr-2020

GENERAL COMMENTS	Abstract: -ORs and 95% CIs can be expressed to two decimal places within the abstract and throughout the manuscript. -Please calculate and present effect sizes for each pairwise comparison. -The mediating effect should also have with it a 95% CI. Methods: -Was a power analysis conducted to justify the obtained sample size? -Please indicate the referent levels within the Methods section of the manuscript. Statistical Analysis: -Please communicate how variables were entered into the logistic regression model. -The data structure appears to be nested, was this accounted for within the analyses? -Effect sizes should also be calculated for the t-tests. -% of effect mediated should be calculated from the mediation analysis. -Please expand on the STATA "KHB" command as many will not be familiar with this method. Results: -Effect sizes should be reported for pair-wise comparisons. -95% CIs for the mediation effect should be reported within the text as well. -% of total effect that was mediated by HCC should be reported. -Table 4: ORs and 95% CIs for all covariates should be reported.

REVIEWER	SAFEERA KHAN California Institute of Behavioral Neurosciences and Psychology, USA
REVIEW RETURNED	21-Jul-2020

GENERAL COMMENTS	This study addressed an important topic, as an association of shift work with sleep disorders can affect the health of the shift workers. The association of Hair Cortisol Concentration with shift work and Insomnia can help in the timely identification of any future potential health issues in shift workers. The article is well-written overall. However, if the authors can expand a little more, and further highlight in their article as to why this specific study design was chosen.

REVIEWER	debora rosa IRCCS istituto Auxologico Italiano, Milan, Italy
REVIEW RETURNED	26-Aug-2020

GENERAL COMMENTS	There is a lack of indications for future research and proposals for possible interventions on the problem.

REVIEWER	Kyoung Sook Jeong Wonju Severance Christian Hospital, Wonju, Republic of Korea Shift work Occupational health Firefighter Occupational health Environmental Health Birth cohort
REVIEW RETURNED	01-Sep-2020

GENERAL COMMENTS	Introduction Add the meaning of Hair cortisol. For example, hair cortisol reflects the cumulative secretion of cortisol or chronic cortisol secretion. Method Statistical analysis HCC and mean ± SD of the transformed variable.=> HCC and geometric mean ± GSD Results Please add the description of abbreviation at footnote in all tables. Transformed HCC Mean±SD should be changed to HCC GM±GSD in Table 1 & 2. In Table 1, Please describe the prevalence of sleep disorder at each row. I suggest to display the percent for row, not column at each variable. In Table 3, I suggest the caption as followings to consider the meaning of table. Odds ratios of sleep disorder by shift type' Model 3 additionally adjusted for ~ with covariates in Model 2

Please clarify the meaning of table 4.

Do you want to show the odds ratio for sleep disorder of Q4 compared to Q1 of HCC?

The description is obscure. It is better to display the results like Table 3 and the caption as I suggest for Table 3.

The notation was wrong as Table 3 at the line 46-51, page 9.

I suggest to add the Table 5 to Figure 1 as following. (Jeong et al, J Trace Elem Med Biol 2015;29:336-41)
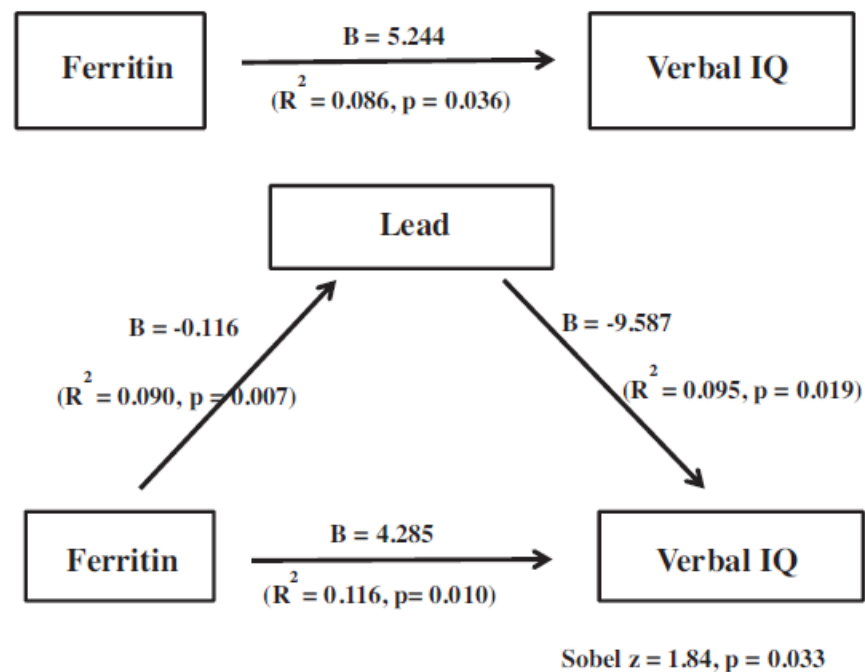

Fig. 1. Regression path analyses showing that blood lead accounts for approximately 20.7% of the relationship between iron deficiency and verbal IQ. The *P* values shown are for one-tailed probability, following adjustment for sex, educational level of both parents, family income, and CRP concentrations.

Discussion

Please add the explanation for difference by shift type. (Ref. Lim et al, Int J Environ Res Public Health 2020; 17, 4760)

The risk of sleep disorder by shift pattern was as expected. But the HCC was highest at four shift. You consider both the working time and adaptation time to working schedule by shift pattern to compare HCC.

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VERSION 1 – AUTHOR RESPONSE

Reviewer: 1

Overall, I think the statistics in this paper is quite straightforward and relatively well described. I however suggest adding some further clarifications as well as have more focus on interpretation of the results. More specifically:

1. P. 6, from line 31. You selected 6 teams and included 10 teams, an explanation is needed.

Response: We apologized for the editing error and the correct sampling method has been prepared in the revised manuscript.

2. P. 8, line 45. I think the sentence gives an impression that the authors themselves developed the KHB method.

Response: We were very sorry for the confusion and have already added the developers of the method in the revised manuscript.

3. P. 9 from line 11, please specify what the numbers and ranges refer to (SD, IQR).

Response: We apologized for the confusion and they have been modified in the paper.

4. OR's with three digits after decimal point is far too much, giving a false impression of high level of precision. One digit is enough, please round the numbers.

Response: ORs and 95%CIs have been expressed to two decimal places followed the opinion of reviewer 2 as we still want to present a higher accuracy.

5. P. 9 line 51 (and other places with the same information), specify the unit of predictor variable for the OR's.

Response: We have changed the description in the revised manuscript.

6. I don't think you name in the paper how many individuals you had in the different shift work categories. This information could e.g. be added in Table 2 as an additional column.

Response: The numbers of individuals in different shift patterns have been added in Table 2 as a new column.

7. More information is needed wrt the KHB modelling:
a. How was the shift work variable treated in these models, dichotomised?

Response: Shift work was divided into two groups in the KHB model: "day workers" and "shift workers".

b. What is the interpretation of parameters a, b and c?

Response: Coefficient a is the effect of "shift work" on "HCC"; coefficient b is the effect of "HCC" on "sleep disorders"; coefficient c is the total effect of "shift work" on "sleep disorders" without the mediator HCC. But in the revised manuscript, we have changed the description of the parameters into "coefficient B" according to the opinion of one reviewer.

c. How do the results from KHB models relate to the results from the pairwise analyses between the three components in the models presented previously? Assuming you dichotomized shift work, (transformed) HCC is continuous and sleep disorders are dichotomized, the results on Figure 1 imply 0.4 units higher HCC in shift workers compared to fixed day workers (much higher than crude difference as seen e.g. in Table 2); OR=exp(1.9) for sleep disorder per unit increase in HCC (much higher than the OR's seen in Table 3) etc. What is the meaning of parameter c? To conclude, I am not sure the two parts of analyses tell us the same story as the estimates seem to have substantially different magnitudes.

Response: In the revised manuscript, "shift work", "HCC" and "sleep disorders" were dichotomized when the KHB command was conducted, and the new results were reported in Table 5 and Figure 1. Parameters in Table 5 and Figure 1 corresponded to coefficients of the relationships but not the ORs reported in Table 3 and Table 4.

d. What is the interpretation of the mediating effect you found? Can you interpret it quantitatively (percent mediated)? Qualitatively?

Response: HCC acts as a part mediator between shift work and sleep disorders in this study. The mediating effect accounted for 24.38% of the total effect.

e. What are the assumptions related to the method and did your data meet the assumptions?

Response: We proposed an assumption like "HCC is a mediating effect between shift work and sleep disorders in the Introduction section of our manuscript and the data showed that the result was consistent with the assumption.

8. Your results of the KHB modelling seem to suggest that the whole association between shift work and sleep disorders is explained by / mediated through HCC-related mechanisms. Is it realistic and reasonable?
Response: We felt sorry for the confusion and we regrouped "shift work" and "HCCs", The results showed that HCC acts as a part mediator between shift work and sleep disorders and the mediating effect accounted for 24.38% of the total effect.

Riewer: 2
Abstract:
1.ORs and 95% CIs can be expressed to two decimal places within the abstract and throughout the manuscript.

Response: We would like to thank the reviewer for the comment and we have modified it according to the opinion.

2.Please calculate and present effect sizes for each pairwise comparison.

Response: We have calculated and present effect sizes in the revised manuscript.

3.The mediating effect should also have with it a 95% CI.

Response: We have reported the 95%CI of the mediating effect in the revised manuscript.

Methods:
1. Was a power analysis conducted to justify the obtained sample size?

Response: We felt very sorry for the limitation of our study. As the sample size of this study didn't reach the minimum theoretical sample size of a cross-sectional study and the power didn't reach 0.8. This limitation has been mentioned in the Discussion section of the revised manuscript.

2.Please indicate the referent levels within the Methods section of the manuscript.

Response: We felt very sorry for the confusion and we have indicated the referent levels in the Methods section of the manuscript.

Statistical Analysis:
3. Please communicate how variables were entered into the logistic regression model.

Response: We would like to thank the reviewer of this suggestion and we have elaborated how variables been entered into the logistic regression model in the Methods section of the revised manuscript.

4. The data structure appears to be nested, was this accounted for within the analyses?

Response: We felt grateful for the suggestion and the KHB method can solve the problem of comparing effects between nested nonlinear regression models.

5. Effect sizes should also be calculated for the t-tests.

Response: We have added the effect sizes of t-tests and variance analysis in Table 1 of the revised manuscript already.

6. % of effect mediated should be calculated from the mediation analysis.

Response: We have calculated the percentage and have added it into Table 5 already.

7. Please expand on the STATA "KHB" command as many will not be familiar with this method.

Response: We have extended the introduction of the Causal steps approach and "KHB" command in the Methods section of the revised manuscript.

Results:
1. Effect sizes should be reported for pair-wise comparisons.

 Response: Effect sizes have been reported in Table 1 and Table 2 of the revised manuscript.

2. 95% CIs for the mediation effect should be reported within the text as well.

Response: We have reported the 95%CIs for the mediation effect in the text.

3. % of total effect that was mediated by HCC should be reported.

Response: Thanks for the comment and we have added the percentage of total effect mediated by HCC in the Results section already.

4. Table 4: ORs and 95% CIs for all covariates should be reported.

Response: ORs and 95%CIs for all covariates have been reported in Table 1 of the revised manuscript.

Reviewer: 3
This study addressed an important topic, as an association of shift work with sleep disorders can affect the health of the shift workers. The association of Hair Cortisol Concentration with shift work and Insomnia can help in the timely identification of any future potential health issues in shift workers. The article is well-written overall. However, if the authors can expand a little more, and further highlight in their article as to why this specific study design was chosen.
Response: We would like to thank the reviewer for the suggestion and we have expanded the reason why we chose the study design in the Introduction section of the revised manuscript.

Reviewer: 4
There is a lack of indications for future research and proposals for possible interventions on the problem.
Response: we have put forward relevant suggestion in the Discussion section of the revised manuscript.

Reviewer: 5
Introduction

1.Add the meaning of Hair cortisol. For example, hair cortisol reflects the cumulative secretion of cortisol or chronic cortisol secretion.

Response: The meaning of hair cortisol has been added into the Introduction section of the manuscript.

Method

1.Statistical analysis

HCC and mean ± SD of the transformed variable.=> HCC and geometric mean ± GSD

Response: It has been corrected in the revised manuscript.

Results

1.Please add the description of abbreviation at footnote in all tables.

Response: We have added the description of abbreviation at footnote in all tables.

2. Transformed HCC Mean±SD should be changed to HCC GM±GSD in Table 1 & 2.

Response: We have changed the mean±SD to GM±GSD in Table 1 and 2 of the revised manuscript.

3. In Table 1, Please describe the prevalence of sleep disorder at each row. I suggest to display the percent for row, not column at each variable.

Response: We felt grateful for the comment and we have made this change in Table 1 of the revised manuscript.

4. In Table 3, I suggest the caption as followings to consider the meaning of table.

Odds ratios of sleep disorder by shift type'

Model 3 additionally adjusted for ~ with covariates in Model 2

Response: We have changed the title of the table and the footnote according to the suggestions of the reviewer in the revised manuscript.

5. Please clarify the meaning of table 4.

Do you want to show the odds ratio for sleep disorder of Q4 compared to Q1 of HCC?

The description is obscure. It is better to display the results like Table 3 and the caption as I suggest for Table 3.

Response: We felt sorry for causing the confusion. We showed the ORs for sleep disorders of "High HCC" to "Low and intermediate HCC" with or without controlling the covariates, and we've changed the title of the table.

6. The notation was wrong as Table 3 at the line 46-51, page 9.

Response: The notation has been corrected according to the reviewer's suggestion in the revised manuscript.
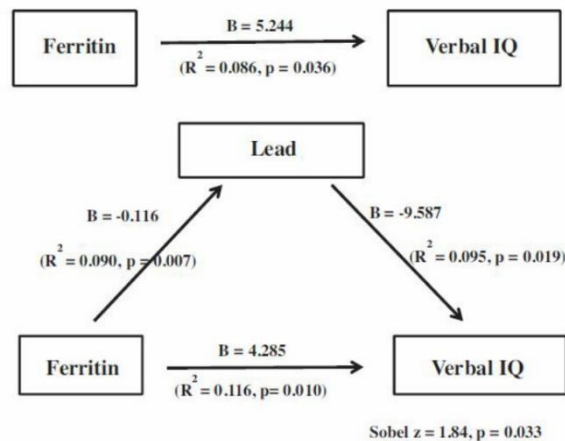
7. I suggest to add the Table 5 to Figure 1 as following.

Response: We would like to thank the reviewer for the suggestion and the Table 5 has been added into Figure 1 in the Results section of the revised manuscript.

Discussion

1. Please add the explanation for difference by shift type. (Ref. Lim et al, Int J Environ Res Public Health 2020; 17, 4760)

Response: We have added the explanation in the Discussion section of the revised manuscript.

2.The risk of sleep disorder by shift pattern was as expected. But the HCC was highest at four shift. You consider both the working time and adaptation time to working schedule by shift pattern to compare HCC.

Response: We felt grateful for the suggestion and have expanded the explanation in the Discussion section of the revised manuscript.

VERSION 2 – REVIEW

REVIEWER	Kristiina Rajaleid Stress Research Institute, Stockholm University, Sweden
REVIEW RETURNED	07-Oct-2020

GENERAL COMMENTS	I would like to thank the authors for the thorough revision of the paper. I would however appreciate some further clarifications, see comments below. P 5 line 29, cortisol levels of 26 or 49 (i.e. around 30) pg/mg hair (result in the article you refer to) and around 3 ng/g hair (your own results) seem to be of different magnitude. What is your comment on this? P 5 line 29-31 and 40-41, if you introduce the idea of different associations between shift-work and hair cortisol levels by age, you

should also follow it up with your analyses. It could be done by e.g stratifying the data as age <40 vs >40, or adding an interaction term in the model. Currently you show in Table 1 (plus comment in the Discussion) that the average HCC levels do not differ by age but that does not eliminate the possibility that the association between shift work type and levels of hair cortisol is different in different age groups.

As the KHB method is not a generally known method I suggest showing the exact command that you used, in the text or as an Appendix, thus making it easier for others to replicate your study.

In Table 2, in the column "n/N", two cells have obviously been mixed up.

Regarding my previous comment e) about assumptions. I think you mixed the meaning of words assumption and expectation. Assumptions are usually a quite critical aspect in the context of mediation analysis (e.g the results are typically only unbiased if the assumption of no unmeasured confounding between the mediator and outcome is met). I am however not familiar with this specific method. That is why I ask you to elaborate on this issue.

I am still not satisfied with your comment on the interpretation of the coefficients from the mediation analysis. Yes, I understand between which variables the coefficients a, b, c (or currently B, B and B) are placed in the figure. The question is: what is the meaning of the values 1.04, 1.07 etc. You call it "coefficients" but the interpretation of the coefficients depends on whether you used regression or logit (or some other alternative) as the model type in the KHB analysis. If it was logit (and it probably should have been as you had three dichotomised variables in the model), the difference 0.25 is measured on log-odds scale and not easy to grasp. Also, in this case all the coefficients are on the log-odds scale and would become easier to grasp and interpret if you presented them as odds ratios (try adding the option "or" in the end of your khb command line in Stata). If you instead used linear regression, what is your motivation for this choice and how would you then interpret the values of the coefficients? What do the R2's in Figure 1 show?

The coefficients a, b and c in the previous version of the manuscript, and the coefficients B, B and B in the current version have dramatically different values even between variables that you probably did not change in the current analysis. How do you explain this?

Minor comments:

P 4 line 6, the half sentence about Switzerland is not relevant in the context and could be removed.

Your reference 5 is most probably not from year 1900.

REVIEWER	Ryan Burns University of Utah, USA
REVIEW RETURNED	07-Oct-2020

GENERAL COMMENTS	Thank you for addressing my previous comments.

REVIEWER	Debora Rosa
	IRCCS Istituto Auxologico Italiano, Italy
REVIEW RETURNED	08-Oct-2020

| GENERAL COMMENTS | Thank you for the opportunity to review the paper. Comparing with the previous version I can say that the authors have responded adequately to the reviewers' notes. I would recommend the publication |

REVIEWER	Kyoung Sook Jeong
	Wonju Severance Christian Hospital, Republic of Korea
REVIEW RETURNED	22-Sep-2020

| GENERAL COMMENTS | In table 4, correct 95% CI of β. |

VERSION 2 – AUTHOR RESPONSE

Reviewer: 5
Reviewer Name
Kyoung Sook Jeong
Institution and Country
Wonju Severance Christian Hospital, Republic of Korea

1.In table 4, correct 95% CI of β.
Response: We have checked the results of 95%CI of β, but found no errors. In addition, we added 95%CIs of ORs in Table 4.

Reviewer: 2
Reviewer Name
Ryan Burns
Institution and Country
University of Utah, USA

Thank you for addressing my previous comments.

Reviewer: 1
Reviewer Name
Kristiina Rajaleid
Institution and Country
Stress Research Institute, Stockholm University, Sweden

I would like to thank the authors for the thorough revision of the paper. I would however appreciate some further clarifications, see comments below.

1.P 5 line 29, cortisol levels of 26 or 49 (i.e. around 30) pg/mg hair (result in the article you refer to) and around 3 ng/g hair (your own results) seem to be of different magnitude. What is your comment on this?
Response: The possible reasons for the phenomenon were as follows: (1) the detection methods for HCC were different in two studies and we don't think the salivary ELISA cortisol kit used in the previous study (reference 11) to detect HCC was absolutely reasonable; (2) the sample size of the previous study was 122, which was far smaller than our study, so a lager sampling error would be generated; (3)the previous study was conducted in the Netherland and some researches showed that cortisol levels may be related to race. In addition, we have consulted a lot of relevant literatures and found that our results on HCC are on the same order of magnitudes as those in most literatures.

2.P5 line29-31 and 40-41, if you introduce the idea of different associations between shift-work and hair cortisol levels by age, you should also follow it up with your analyses. It could be done by e.g stratifying the data as age <40 vs >40, or adding an interaction term in the model. Currently you show in Table 1 (plus comment in the Discussion) that the average HCC levels do not differ by age but that does not eliminate the possibility that the association between shift work type and levels of hair cortisol is different in different age groups.
Response: We stratified the data as age <40 vs ≥40, and analyzed the association between shift work and HCC in different age groups, and the results were consistent with those without grouping. We have added the analysis results to the revised manuscript already.

3.As the KHB method is not a generally known method I suggest showing the exact command that you used, in the text or as an Appendix, thus making it easier for others to replicate your study.
Response: We have uploaded the exact commands we used in this study as a supplementary material.

4.In Table 2, in the column "n/N", two cells have obviously been mixed up.
Response: We felt sorry for the error and we have made the correction.

5.Regarding my previous comment e) about assumptions. I think you mixed the meaning of words assumption and expectation. Assumptions are usually a quite critical aspect in the context of mediation analysis (e.g the results are typically only unbiased if the assumption of no unmeasured confounding between the mediator and outcome is met). I am however not familiar with this specific method. That is why I ask you to elaborate on this issue.
Response: The assumption of KHB method is that scales do not differ. However, this assumption cannot be tested and indirect effects cannot be meaningfully compared without credible exclusion restrictions. The scales in our data kept the same.

6.I am still not satisfied with your comment on the interpretation of the coefficients from the mediation analysis. Yes, I understand between which variables the coefficients a, b, c (or currently B, B and B) are placed in the figure. The question is: what is the meaning of the values 1.04, 1.07 etc. You call it "coefficients" but the interpretation of the coefficients depends on whether you used regression or logit (or some other alternative) as the model type in the KHB analysis. If it was logit (and it probably should have been as you had three dichotomised variables in the model), the difference 0.25 is measured on log-odds scale and not easy to grasp. Also, in this case all the coefficients are on the log-odds scale and would become easier to grasp and interpret if you presented them as odds ratios (try adding the option "or" in the end of your khb command line in Stata). If you instead used linear regression, what is your motivation for this choice and how would you then interpret the values of the coefficients? What do the R^2's in Figure 1 show?
Response: We felt grateful for the comment of the reviewer. In this study, we used "logit" as the model type in the KHB analysis, and we added the ORs in Table 4 and Figure 1 to make the results easier to understand. The R^2s in Figure 1 evaluated the goodness-of-fit of logistic models, ranging from 0 to 1. And we added R^2s in Figure 1 according to another reviewer's comment.

7.The coefficients a, b and c in the previous version of the manuscript, and the coefficients B, B and B in the current version have dramatically different values even between variables that you probably did not change in the current analysis. How do you explain this?
Response: In the previous version of the manuscript, shift work was divided into 5 groups and HCC was taken as a continuous variable, and we found that HCC plays a complete mediating role in the relationship between shift work and sleep disorders. Considering that the result may not be realistic, we tried to analyzed the mediating effect by different classification methods according to your comment. As we didn't find the difference in HCC of workers with different shift patterns, we divided shift work into two categories named "day workers" (referent level) and "shift workers". HCCs were divided into "low and intermediate HCC" (referent level) and "high HCC" at the Q_3 threshold. Finally, we figured out current results.

Minor comments:

8.P 4 line 6, the half sentence about Switzerland is not relevant in the context and could be removed.
Response: We have removed the half sentence about Switzerland.

9.Your reference 5 is most probably not from year 1900.
Response: We felt very sorry for the editing error and we have corrected it in the revised manuscript.

Reviewer: 4
Reviewer Name
Debora Rosa
Institution and Country
IRCCS Istituto Auxologico Italiano, Italy

Thank you for the opportunity to review the paper. Comparing with the previous version I can say that the authors have responded adequately to the reviewers' notes. I would recommend the publication

VERSION 3 – REVIEW

REVIEWER	Kristiina Rajaleid Stress Research Institute, Sweden
REVIEW RETURNED	12-Oct-2020

GENERAL COMMENTS	I would like to thank the authors for the possibility to study their interesting work. I don't have any further comments or questions.