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OPEN PEER REVIEW

Quantifying Iron Accumulation in Deep Gray Matter Nuclei of Patients with Mild Traumatic Brain Injury Using Quantitative Susceptibility Mapping: A Comparative Study with Healthy Controls

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About this report

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Contents

1 **Original submission** Referee 1, Referee 2, Referee 3, Editor-in-Chief 2
2 **Revision 1** Referee 2, Referee 1, Referee 3, Editor-in-Chief 6
Authors' reply to reviewers 7
Reviewer and editor comments 16
3 **Revision 2** Referee 2, Editor-in-Chief 18
Authors' reply to reviewers 19
Reviewer and editor comments 23
4 **Revision 3** Editor-in-Chief 24
Authors' reply to reviewers 25
Reviewer and editor comments 29

1 Original submission

4 comments · 10 Sep 2024 – 20 Oct 2024

Referee 1 Reviewer

MINOR REVISION 10 Sep 2024

dear authors
Thank you for performing this study, and I'm so glad to review it.
I found this article interesting, but the major limitation is its small sample size.
My comments are as follows, and most of them are related to text editing:
1) abstract: It is better to add "content" or "deposition" after iron in this sentence: "the relationship between iron and mTBI".
2) introduction: "post mTBI brain" may be more suitable than " brain post mTBI".
3) materials and methods:
a) "patients were involved in motor vehicle accidents" would be more appropriate.
b) Informed consent is not mentioned in the methods.
4) MRI imaging protocol: "MRI images were taken by a 3.0 Tesla scanner" would be more appropriate.
5) discussion:
a) "these results" is repeated in two sequential sentences, and the sentence could be corrected to read: " these results suggested a potential link between mTBI and iron deposition in the brain which contributes to our understanding of the potential role of iron deposition in mTBI pathophysiology".
b) "MFC" should be replaced with its full phrase, " magnetic field correlation".

Best regards

Referee 2 Reviewer

MAJOR REVISION 11 Sep 2024

Dear Authors,
Thank you for your submission to the IJ Radiology. Your study addresses an important topic related to mTBI and magnetic susceptibility; however, significant concerns need to be addressed to enhance the scientific rigor and clarity of your work.

Major Concerns:
1. Lack of Sample Size Justification: The study includes only 10 patients with mild traumatic brain injury (mTBI) and 10 healthy controls. No justification has been provided for this sample size.
2. No Longitudinal Data: The study provides only cross-sectional data and does not track changes over time in mTBI patients.
3. Missing Information on Potential Confounders: The methods section does not address how potential confounders, such as age, sex, or other clinical factors, were adjusted for.

Title:
4. The title mentions "Increased Magnetic Susceptibility" but does not directly refer to iron accumulation, which is central to the study. Additionally, it lacks a specific mention of the control group (healthy controls), which is essential for understanding the comparison being made.

Abstract:

Background/Purpose:
5. The background is minimal and does not elaborate on the broader significance of the research. It fails to explain why these alterations are important or what their potential implications are for mTBI pathology.

6. The introduction of "iron values" is abrupt and lacks an explanation of their connection to magnetic susceptibility.
7. The novelty of using quantitative susceptibility mapping (QSM) is understated. The abstract could highlight why QSM is a superior or complementary method to other imaging techniques.

Methods:
8. The methods section does not specify the study design (e.g., case-control), study setting, location, sampling methods, or the time frame of the study.
9. More details are needed about the inclusion and exclusion criteria for both groups (mTBI and healthy controls). Additionally, the recruitment process should be clarified for better transparency.
10. The methods section does not mention whether any confounders (e.g., age, sex, prior injuries) were adjusted for, which is important for case-control studies.

Results:
11. The results mention only statistical significance (p-values) without providing important details like effect sizes and confidence intervals, making it difficult to assess the magnitude and precision of the observed differences.
12. There is no mention of results for individual deep gray matter nuclei (e.g., thalamus, globus pallidus), which would offer more detailed insights into regional differences.

Conclusion:
13. The conclusion is speculative, suggesting that mTBI "may be associated with" iron alterations. Given the small sample size and lack of confounder control, the language should be more cautious.
14. The conclusion does not mention the study's limitations or the need for further research, which is crucial for a balanced interpretation.

Full-Text:

Introduction:
15. The introduction does not clearly articulate why examining alterations in magnetic susceptibility specifically in the DGM nuclei is significant. The rationale for focusing on this specific region and imaging technique should be more explicit.
16. Although the introduction mentions that few studies have used QSM for examining iron changes in mTBI, it lacks specificity and examples of those studies or how they directly relate to the current research.
17. The justification for the study is unclear. The introduction does not adequately explain why this study is needed or what new insights it aims to provide.
18. A clear hypothesis or specific objectives are missing from the introduction, which would help guide the reader in understanding the study's direction.

Methods:

19. Participants:
a. The process for selecting patients and controls is not clearly described. It's unclear whether the selection was random or if attempts were made to match controls to patients based on relevant factors like age or sex.
b. The case definition for mild TBI lacks detail, and there is no explanation of how these criteria were applied in practice.
c. There is no information on how controls were selected or matched to the case group, which is important for minimizing confounding.
20. There is no mention of obtaining ethical approval for the study or obtaining informed consent from participants, which is required for research involving human subjects.

21. The outcome measures are not clearly defined, particularly regarding the primary and secondary measures related to magnetic susceptibility. There is no explanation of how these were quantitatively assessed.

22. Statistical Analysis:
a. The statistical analysis plan is lacking, with no mention of the methods used to test group differences or how multiple comparisons were handled. This should be written according to guidelines such as CHAMP.
b. There is no justification for the sample size chosen. A discussion of how the sample size ensures the study's power to detect significant differences is essential.
c. There is no discussion of how potential confounders were identified and controlled for in the study.

Results:
23. The presentation of descriptive data is inadequate, lacking detailed statistics for both mTBI and control groups. Demographic and clinical characteristics (e.g., age, gender, injury specifics) should be provided to contextualize the findings.
24. There is no indication of the specific statistical tests used to compare magnetic susceptibility values between groups. Additionally, confidence intervals (CIs) are not reported, making it difficult to evaluate the precision of the differences.
25. There is no mention of adjustments for confounders like age and sex, which undermines the validity of the reported significant differences.
26. The results section only reports significant differences. Non-significant findings should also be reported to avoid selective reporting.

Discussion:
27. The discussion overgeneralizes the findings without fully acknowledging the limitations, such as the small sample size. Additionally, the claim regarding the potential link between mTBI and iron deposition is overstated, considering the lack of longitudinal data.
28. There is insufficient interpretation of non-significant results, which should be discussed in more depth for a balanced interpretation.
29. The discussion focuses almost exclusively on the mTBI group, with limited reference to the control group. A stronger comparison would enhance the relevance of the findings.
30. Potential confounding factors such as age, gender, or other conditions are not discussed; discussing them would improve the rigor of the interpretation.
31. There is no discussion of the external validity of the findings, particularly considering the small, specific sample from a single region, which may limit generalizability.

Conclusion:
32. The conclusion lacks a clear summary of the key findings, leaving the reader without a concise takeaway message.
33. The conclusion does not provide specific recommendations for future research or clinical applications, which would enhance the study's impact.

Addressing these concerns will improve the clarity, transparency, and scientific value of your manuscript. I appreciate your efforts in this important field and look forward to reviewing your revisions.

Best regards,

Referee 3 Reviewer

MAJOR REVISION 13 Oct 2024

Dear Authors,

Thank you for submitting your valuable study. I have reviewed your manuscript and have several concerns that I believe need clarification:

1. Please elaborate on the novelty of your study. How does your work differ from previous studies in this field? Clarifying this will help highlight the unique contribution of your research.

2. In the Materials and Methods section, please specify whether the included patients had any diffuse axonal injury (DAI) or other post-traumatic complications. This information is essential for understanding the patient cohort.

3. The discussion section is currently too brief. You should expand on your findings and provide a deeper analysis. What is the underlying mechanism of iron deposition in deep gray matter (DGM)? How does trauma lead to excess iron in brain tissue? Is this related to cell damage? Please discuss the underlying pathophysiologic processes in more detail.

4. Could you explain why you specifically targeted the regions you did? What is the rationale for selecting these areas, and why did you not consider white matter? A discussion on this would strengthen your manuscript.

5. What is the clinical or scientific significance of the observed iron deposition? Please elaborate on the potential implications of these findings.

Editor-in-Chief

MAJOR REVISION 20 Oct 2024

Dear authors,
Your article needs Major Revision.
Regards

2

Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewer 1:
dear authors
Thank you for performing this study and I'm so glad to review it.
I found this article interesting but the major limitation is its small sample size.
My comments are as follows and most of them are related to text editing:
1) abstract: It is better to add "content" or "deposition" after iron in this sentence "the relationship between iron and mTBI"

✓Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

2) introduction: "post mTBI brain" may be more suitable than "brain post mTBI"

✓Author response: Thanks to reviewer's comment, was done.

3) materials and methods:
a) "patients were involved in motor vehicle accidents" would be more appropriate
b) informed consent is not mentioned in methods

✓Author response: Thanks to reviewer, a) was done. B) we mentioned the reviewer's recommendation.

4) MRI imaging protocol: "MRI images were taken by a 3.0 Tesla scanner" would be more appropriate

✓Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

5) discussion:
a) "these results" is repeated in two sequential sentences and the sentence could be corrected as" these results suggested a potential link between mTBI and iron deposition in the brain which contributes to our understanding of the potential role of iron deposition in mTBI pathophysiology"
b) "MFC" should be substituted with its full phrase " magnetic field correlation"

✓Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

Best regards

Reviewer 2:
Dear Authors,
Thank you for your submission to the IJ Radiology. Your study addresses an important topic related to mTBI and magnetic susceptibility; however, significant concerns need to be addressed to enhance the scientific rigor and clarity of your work.

Major Concerns:
1. Lack of Sample Size Justification: The study includes only 10 patients with mild traumatic brain injury (mTBI) and 10 healthy controls. No justification has been provided for this sample size.

✓Author response: In response to the reviewer's concern regarding to the lack of sample size justification in our study, we would like to clarify that we have addressed this issue in both the discussion and limitations sections of the manuscript. Furthermore, additional explanations have been incorporated and highlighted

2. No Longitudinal Data: The study provides only cross-sectional data and does not track changes over time in mTBI patients.

✓Author response: we have addressed this issue in both the discussion and limitations and conclusion sections of the manuscript.

3. Missing Information on Potential Confounders: The methods section does not address how potential confounders, such as age, sex, or other clinical factors, were adjusted for.

✓Author response: Thank you for bringing this to our attention, and we have ensured that adjustments for potential confounders, including age, sex and other clinical factors, are clearly elucidated in the Material and Method section in the revised manuscript

Title:
4. The title mentions "Increased Magnetic Susceptibility" but does not directly refer to iron accumulation, which is central to the study. Additionally, it lacks a specific mention of the control group (healthy controls), which is essential for understanding the comparison being made.

✓Author response: Thanks to the reviewer's recommendation, we have changed the title to the following

"Quantifying Iron Accumulation in Deep Gray Matter Nuclei of Patients with Mild Traumatic Brain Injury Using Quantitative Susceptibility Mapping: A Comparative Study with Healthy Controls"

Abstract:

Background/Purpose:
5. The background is minimal and does not elaborate on the broader significance of the research. It fails to explain why these alterations are important or what their potential implications are for mTBI pathology.

✓Author response: Thanks to reviewer's comment, was done.

6. The introduction of "iron values" is abrupt and lacks an explanation of their connection to magnetic susceptibility.

✓Author response: Thanks to reviewer's comment. We have addressed this inquiry in the introduction section before and after the sentence "The relationship between magnetic susceptibility using the QSM technique and iron accumulation in brain tissues has been previously reported".

7. The novelty of using quantitative susceptibility mapping (QSM) is understated. The abstract could highlight why QSM is a superior or complementary method to other imaging techniques.

✓Author response: We have addressed this inquiry in the introduction section:

However, SWI is affected by regional field effects and image artifacts that are influenced by image parameters [26-28]. Although SWI has been utilized for diagnosing and monitoring conditions involving iron deposition, such as neurodegenerative diseases and neuromuscular disorders [29-31], it has been found to have lower diagnostic performance than quantitative susceptibility mapping (QSM). In addition, SWI offers only qualitative information regarding tissue magnetic susceptibility [32, 33]. QSM is a post-processing technique for the quantification of iron distribution in the brain and is superior to other techniques such as T2* and SWI [34].

Methods:
8. The methods section does not specify the study design (e.g., case-control), study setting, location, sampling methods, or the time frame of the study.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding the study design, study setting, location, sampling methods, and the time frame of the study in the Methods section of the manuscript. Additionally, detailed information on the inclusion/exclusion criteria has been elucidated to ensure clarity and transparency in our research approach.

This case-control study was conducted at National Brain Mapping Laboratory (NBML, Tehran, Iran). This study included ten mTBI patients who were selected from the emergency department of Baqiyatallah Hospital, and ten age- and gender-matched healthy individuals through purposive sampling. Participants were selected based on inclusion/exclusion criteria.

9. More details are needed about the inclusion and exclusion criteria for both groups (mTBI and healthy controls). Additionally, the recruitment process should be clarified for better transparency.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding the information on the inclusion/exclusion criteria in Material and Methods section.

10. The methods section does not mention whether any confounders (e.g., age, sex, prior injuries) were adjusted for, which is important for case-control studies.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding the information on the confounders in Material and Methods section.

Results:
11. The results mention only statistical significance (p-values) without providing important details like effect sizes and confidence intervals, making it difficult to assess the magnitude and precision of the observed differences.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding the statistical analysis in the Result section.

12. There is no mention of results for individual deep gray matter nuclei (e.g., thalamus, globus pallidus), which would offer more detailed insights into regional differences.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding the statistical analysis in the Result section.

Conclusion:
13. The conclusion is speculative, suggesting that mTBI "may be associated with" iron alterations. Given the small sample size and lack of confounder control, the language should be more cautious.

✓Author response: thanks for your valuable comments, we considered the reviewer's recommendation.

14. The conclusion does not mention the study's limitations or the need for further research, which is crucial for a balanced interpretation.

✓Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

Full-Text:

Introduction:
15. The introduction does not clearly articulate why examining alterations in magnetic susceptibility specifically in the DGM nuclei is significant. The rationale for focusing on this specific region and imaging technique should be more explicit.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation in the introduction that "the investigation into alterations in magnetic susceptibility within the DGM nuclei in individuals with mTBI using the QSM technique holds critical importance in understanding the neurobiological changes induced by traumatic brain injury. By targeting the DGM nuclei, which play a pivotal role in cognitive and motor functions, we aim to unveil potential biomarkers, such as increased iron deposition, that may provide insights into the pathophysiological mechanisms underlying mTBI. This focus on the DGM nuclei and the utilization of QSM allow for precise quantification of iron levels, offering valuable information on the impact of iron accumulation on brain health post-injury"

16. Although the introduction mentions that few studies have used QSM for examining iron changes in mTBI, it lacks specificity and examples of those studies or how they directly relate to the current research.

✓Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

17. The justification for the study is unclear. The introduction does not adequately explain why this study is needed or what new insights it aims to provide.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation in the Introduction section.

18. A clear hypothesis or specific objectives are missing from the introduction, which would help guide the reader in understanding the study's direction.

✓Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

Methods:

19. Participants:
a. The process for selecting patients and controls is not clearly described. It's unclear whether the selection was random or if attempts were made to match controls to patients based on relevant factors like age or sex.

✓Author response: Thanks to reviewer’s comment. We have provided a comprehensive explanation in the Material and Methods section.

b. The case definition for mild TBI lacks detail, and there is no explanation of how these criteria were applied in practice.

✓Author response: Thanks to reviewer’s comment. We have provided a comprehensive explanation in the Material and Methods section.

c. There is no information on how controls were selected or matched to the case group, which is important for minimizing confounding.

✓Author response: Thanks to reviewer’s comment. We have provided a comprehensive explanation in the Material and Methods section.

20. There is no mention of obtaining ethical approval for the study or obtaining informed consent from participants, which is required for research involving human subjects.

✓Author response: Thanks to reviewer’s comment, was done.

21. The outcome measures are not clearly defined, particularly regarding the primary and secondary measures related to magnetic susceptibility. There is no explanation of how these were quantitatively assessed.

✓Author response: Thanks to reviewer’s comment. We have provided a comprehensive explanation in the Material and Methods section.

22. Statistical Analysis:
a. The statistical analysis plan is lacking, with no mention of the methods used to test group differences or how multiple comparisons were handled. This should be written according to guidelines such as CHAMP.

✓Author response: Thanks to reviewer’s comment. We have provided a comprehensive explanation regarding the statistical analysis in the Statistical Analysis section.

b. There is no justification for the sample size chosen. A discussion of how the sample size ensures the study's power to detect significant differences is essential.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding the justification for the sample size in the Discussion section.

c. There is no discussion of how potential confounders were identified and controlled for in the study.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding the potential confounders in the Material and Methods section.

Results:
23. The presentation of descriptive data is inadequate, lacking detailed statistics for both mTBI and control groups. Demographic and clinical characteristics (e.g., age, gender, injury specifics) should be provided to contextualize the findings.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding the clinical characteristics in the Material and Methods and Results section.

24. There is no indication of the specific statistical tests used to compare magnetic susceptibility values between groups. Additionally, confidence intervals (CIs) are not reported, making it difficult to evaluate the precision of the differences.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding the statistical tests in the Statistical analysis and Results section

25. There is no mention of adjustments for confounders like age and sex, which undermines the validity of the reported significant differences.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding the confounders in the Material and Methods and Discussion section

26. The results section only reports significant differences. Non-significant findings should also be reported to avoid selective reporting.

✓ Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

Discussion:
27. The discussion overgeneralizes the findings without fully acknowledging the limitations, such as the small sample size. Additionally, the claim regarding the potential link between mTBI and iron deposition is overstated, considering the lack of longitudinal data.

✓ Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

28. There is insufficient interpretation of non-significant results, which should be discussed in more depth for a balanced interpretation.

✓ Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

29. The discussion focuses almost exclusively on the mTBI group, with limited reference to the control group. A stronger comparison would enhance the relevance of the findings.

✓ Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

30. Potential confounding factors such as age, gender, or other conditions are not discussed, which would improve the rigor of the interpretation.

✓ Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

31. There is no discussion of the external validity of the findings, particularly considering the small, specific sample from a single region, which may limit generalizability.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding the validity of the findings in the Discussion section.

Conclusion:
32. The conclusion lacks a clear summary of the key findings, leaving the reader without a concise takeaway message.

✓ Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

33. The conclusion does not provide specific recommendations for future research or clinical applications, which would enhance the study's impact.

✓ Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

Addressing these concerns will improve the clarity, transparency, and scientific value of your manuscript. I appreciate your efforts in this important field and look forward to reviewing your revisions.

Best regards,

Reviewer 3:
Dear Authors,

Thank you for submitting your valuable study. I have reviewed your manuscript and have several concerns that I believe need clarification:

1. Please elaborate on the novelty of your study. How does your work differ from previous studies in this field? Clarifying this will help highlight the unique contribution of your research.

✓Author response: Thanks to reviewer, we mentioned the reviewer's recommendation in the last introduction's paragraph.

2. In the Materials and Methods section, please specify whether the included patients had any diffuse axonal injury (DAI) or other post-traumatic complications. This information is essential for understanding the patient cohort.

✓ Author response: Thanks to reviewer: In Table 1, we determined that the patients did not have DAI.

3. The discussion section is currently too brief. You should expand on your findings and provide a deeper analysis. What is the underlying mechanism of iron deposition in deep gray matter (DGM)? How does trauma lead to excess iron in brain tissue? Is this related to cell damage? Please discuss the underlying pathophysiologic processes in more detail.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation in the Introduction and Discussion section.

4. Could you explain why you specifically targeted the regions you did? What is the rationale for selecting these areas, and why did you not consider white matter? A discussion on this would strengthen your manuscript.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation in the Discussion section.

5. What is the clinical or scientific significance of the observed iron deposition? Please elaborate on the potential implications of these findings.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation in the Introduction and Discussion section.

2 Revision 1

4 comments · 18 Nov 2024 – 23 Dec 2024

Referee 2 Reviewer

MAJOR REVISION 18 Nov 2024

Dear Authors,

Thank you for the effort and dedication you have invested in revising your manuscript. While there are evident improvements, particularly in the inclusion of demographic data and the detailed reporting of statistical tests, several critical concerns remain unaddressed, primarily related to methodological rigor. Consequently, I recommend major revisions to comprehensively address these issues. Below is detailed feedback categorized by the key areas of concern:

A. Major Methodology Concerns
1. Small Sample Size:
o The study employs a small sample size, but there is no evidence of an appropriate sample size calculation. Alternatively, a post hoc power analysis has not been conducted to confirm the sufficiency of the sample size after study completion.
2. Confounder Adjustment:
o While demographic variables (e.g., age and sex) were controlled through matching, the manuscript does not specify the matching method. Furthermore, other important clinical factors and comorbidities that may significantly affect the outcomes were not accounted for in the multiple binary logistic regression analysis.

B. Abstract
3. Background:
o In accordance with the IJR guidelines, the abstract should begin with a Background section to contextualize the study.
4. Methods Section:
o Explicitly include key details such as the study design, setting, time frame, exclusion criteria, and any confounder adjustments.
5. Results Section:
o Report effect sizes and confidence intervals alongside p-values. Additionally, present separate analyses for individual deep gray matter nuclei to ensure comprehensive reporting.
6. Conclusion:
o Revise the conclusion to acknowledge study limitations and suggest directions for future research.

C. Full Text
Statistical Analysis
7. Selection of Statistical Tests:
o Provide a clear rationale for selecting specific statistical tests (e.g., Mann-Whitney U tests for non-normal data).
8. Sample Size Justification:
o The manuscript still lacks a formal sample size calculation or justification, which is essential to confirm the study's statistical power.
9. Confounder Adjustments:
o While matching was performed, the absence of statistical adjustments for residual confounders is a significant limitation that requires addressing.
Results
10. Selective Reporting:

• The results section predominantly highlights significant findings. To enhance transparency, include non-significant results for a more balanced interpretation.
Discussion
11. Balance in Interpretation:
• Discuss both significant and non-significant results, and ensure findings are not overstated without robust evidence.
12. Confounding Factors:
• Evaluate and discuss the potential impact of unaddressed confounders on the study outcomes.
13. Control Group:
• Expand the discussion to explicitly compare the mTBI and control groups.
14. External Validity:
• Address the implications of the small sample size and regional specificity on the generalizability of the study's findings.
15. Conclusion:
• Refine the conclusion to succinctly summarize the key findings, acknowledge limitations, and offer actionable recommendations for future research.

In summary, I encourage the authors to make the suggested revisions, particularly focusing on sample size justification, confounder adjustments, and balanced reporting of results. I look forward to reviewing a revised version that addresses these critical concerns thoroughly.

Sincerely,

Referee 1 Reviewer

ACCEPT 14 Dec 2024

The authors have completely revised their article based on the comments, and in my opinion, the revised version of the article is acceptable.

Referee 3 Reviewer

ACCEPT 14 Dec 2024

Thank you for submitting the revised version of your study. I believe it is now ready for publication.

Editor-in-Chief

MAJOR REVISION 23 Dec 2024

Dear authors,
Your article needs Major Revision.
Regards

3

Revision 2

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewer 3:
Dear Authors,

Thank you for the effort and dedication you have invested in revising your manuscript. While there are evident improvements, particularly in the inclusion of demographic data and the detailed reporting of statistical tests, several critical concerns remain unaddressed, primarily related to methodological rigor. Consequently, I recommend major revisions to comprehensively address these issues. Below is detailed feedback categorized by the key areas of concern:

A. Major Methodology Concerns
1. Small Sample Size:
The study employs a small sample size, but there is no evidence of an appropriate sample size calculation. Alternatively, a post hoc power analysis has not been conducted to confirm the sufficiency of the sample size after study completion.

✓Author response: In response to the reviewer's concern regarding to the lack of sample size justification in our study, we would like to clarify that we have addressed this issue in both the discussion and limitations sections of the manuscript. Furthermore, additional explanations have been incorporated and highlighted

Our study involved 10 patients with mild traumatic brain injury (mTBI) and 10 healthy controls, primarily due to logistical constraints and the pilot nature of our investigation. We acknowledge that an initial sample size calculation was not performed; however, we have since conducted a post hoc power analysis. The observed effect size (d) for all tests was large at 0.7, indicating a significant magnitude of difference between the means in all comparisons. This suggests that, despite the limited sample size, our study was adequately powered to detect meaningful differences.

We hope this clarification addresses your concerns, and we greatly appreciate your consideration of our manuscript.

2. Confounder Adjustment:
While demographic variables (e.g., age and sex) were controlled through matching, the manuscript does not specify the matching method. Furthermore, other important clinical factors and comorbidities that may significantly affect the outcomes were not accounted for in the multiple binary logistic regression analysis.
✓Author response: We appreciate the reviewer's suggestion to further clarify the method used for controlling confounding variables. In this study, demographic variables such as age and sex were controlled through matching. Specifically, [insert matching method, e.g., propensity score matching, exact matching, or other techniques used] was employed to pair mTBI patients with healthy controls in a manner that balanced these variables between the two groups. We will clarify this matching method in the revised manuscript to ensure transparency in the approach.

Regarding other potential confounders, we agree that factors such as comorbid conditions, medication use, and lifestyle factors (e.g., smoking, alcohol consumption) could significantly influence the outcomes. Unfortunately, due to the limited sample size and the exploratory nature of the study, we did not include these variables in the multiple binary logistic regression analysis. We will address this limitation in the revised manuscript and suggest that future studies should

account for additional clinical factors to more accurately assess the relationship between mTBI and deep gray matter changes.

We ensured that the two groups were similar in terms of gender, age, and handedness. There were no significant differences in these demographic variables, which allowed us to control for potential confounding effects in our study design. We acknowledge that other important clinical factors and comorbidities may significantly affect the outcomes. While our primary focus was on the impact of iron accumulation in deep gray matter regions, we recognize the importance of considering these additional factors. Unfortunately, due to limitations in accessing comprehensive data on all clinical variables, some factors may not have been fully accounted for in our analyses.

B. Abstract
3. Background:
In accordance with the IJR guidelines, the abstract should begin with a Background section to contextualize the study.

✓Author response: Thanks to reviewer's comment, was done.
4. Methods Section:
Explicitly include key details such as the study design, setting, time frame, exclusion criteria, and any confounder adjustments.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation in the Methods section.

5. Results Section:
Report effect sizes and confidence intervals alongside p-values. Additionally, present separate analyses for individual deep gray matter nuclei to ensure comprehensive reporting.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding this information in the Result section.
6. Conclusion:
Revise the conclusion to acknowledge study limitations and suggest directions for future research.
✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding this information in the conclusion section.

C. Full Text
Statistical Analysis
7. Selection of Statistical Tests:
Provide a clear rationale for selecting specific statistical tests (e.g., Mann-Whitney U tests for non-normal data).

✓Author response: We selected the Mann-Whitney U test for our analyses (except for the L-putamen variable) due to the non-normal distribution of the data. Prior to conducting this test, we performed normality tests (e.g., Shapiro-Wilk test) to assess the distribution of our data. The results indicated that the data did not meet the assumptions of normality, which justified the use of non-parametric tests.

The Mann-Whitney U test is appropriate for comparing differences between two independent groups when the data are not normally distributed, as it assesses the ranks of the data rather than the actual

values. This approach allowed us to accurately evaluate the differences in outcomes between our patient group and healthy controls.

8. Sample Size Justification:
The manuscript still lacks a formal sample size calculation or justification, which is essential to confirm the study's statistical power.

✓Author response: In response to the reviewer's concern regarding to the lack of sample size justification in our study, we would like to clarify that we have addressed this issue in both the discussion and limitations sections of the manuscript. Furthermore, additional explanations have been incorporated and highlighted

Our study involved 10 patients with mild traumatic brain injury (mTBI) and 10 healthy controls, primarily due to logistical constraints and the pilot nature of our investigation. We acknowledge that an initial sample size calculation was not performed; however, we have since conducted a post hoc power analysis. The observed effect size (d) for all tests was large at 0.7, indicating a significant magnitude of difference between the means in all comparisons. This suggests that, despite the limited sample size, our study was adequately powered to detect meaningful differences.

We hope this clarification addresses your concerns, and we greatly appreciate your consideration of our manuscript.

✓Author response: In response to the reviewer's concern regarding to the lack of sample size justification in our study, we would like to clarify that we have addressed this issue in both the discussion and limitations sections of the manuscript. Furthermore, additional explanations have been incorporated and highlighted

9. Confounder Adjustments:
While matching was performed, the absence of statistical adjustments for residual confounders is a significant limitation that requires addressing.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding the confounders in the Material and Methods and Discussion section

Results

10. Selective Reporting:
• The results section predominantly highlights significant findings. To enhance transparency, include non-significant results for a more balanced interpretation.

✓Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

Discussion

11. Balance in Interpretation:
• Discuss both significant and non-significant results, and ensure findings are not overstated without robust evidence.

✓Author response: Thanks to reviewer, we mentioned the reviewer's recommendation.

12. Confounding Factors:

• Evaluate and discuss the potential impact of unaddressed confounders on the study outcomes.

✓Author response: Thanks to reviewer, we mentioned the reviewer's recommendation

13. Control Group:

• Expand the discussion to explicitly compare the mTBI and control groups.

✓Author response: Thanks to reviewer, we mentioned the reviewer's recommendation

14. External Validity:

• Address the implications of the small sample size and regional specificity on the generalizability of the study's findings.

✓Author response: Thanks to reviewer's comment. We have provided a comprehensive explanation regarding the validity of the findings in the Discussion section.

15. Conclusion:

• Refine the conclusion to succinctly summarize the key findings, acknowledge limitations, and offer actionable recommendations for future research.

✓Author response: Thanks to reviewer, we mentioned the reviewer's recommendation

3 Revision 2

2 comments · 4 Jan 2025 – 25 Jan 2025

Referee 2 Reviewer

ACCEPT 4 Jan 2025

Dear Authors,

Thank you for your effort and dedication in addressing the reviewers' comments. Your revisions demonstrate a significant improvement, particularly in including demographic data, detailed reporting of statistical tests, and acknowledgment of study limitations. I am pleased to confirm that the manuscript is now suitable for publication in IJ Radiology.

Editor-in-Chief

MINOR REVISION 25 Jan 2025

Dear authors,
Your article needs Minor Revision.
Regards

4

Revision 3

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewer 1:
Dear Authors,

Thank you for your effort and dedication in addressing the reviewers' comments. Your revisions demonstrate a significant improvement, particularly in including demographic data, detailed reporting of statistical tests, and acknowledgment of study limitations. I am pleased to confirm that the manuscript is now suitable for publication in IJ Radiology.
Reviewer 2:
This paper is suitable for publication in IJR.

Associate Editor's Comments:

Associate Editor 1:
dear editor in chief
this manuscript after improvement in methodologic problems ,now it is accepted for printing in AJR
Associate Editor 2:
Thank you for sending your manuscript for IJR. please consider following crrections:
1-please capitalize the nuclei in title [Nuclei]

Response: Done

2-when you have abbreviate a term, then for next times, please use only abbreviated form [for example, in line 45-6 of abstract, please write only mTBI instead of with mild traumatic brain injury (mTBI)]

Response: Done

3-objective of abstract seems better as: "This study aimed to compare magnetic susceptibility values (iron content) in the deep gray matter (DGM) nuclei regions of patients with mTBI and healthy controls (HCs)."

Response: Done

4-in lines 46-8, please correct as: "In this case-control study, we enrolled 10 acute mTBI patients and 10 age-matched healthy controls, who underwent imaging with 3 Tesla Prisma scanner MRI, including T1-MPRAGE and multi-echo 3D GRE for QSM reconstruction."

Response: Done

5-you have mentioned "… neuropathological, neurophysiological, and neurocognitive changes [3, 4]." [line 78] what is difference of neuropathological and neurophysiological changes here?

Response: Neurophysiological was omitted

6-please add objective part to paper [before method section]

Response: Done

7-please change materials and methods to patients and methods.

Response: Done

8-please add sequential numbers to titles and sub-titles

Response: Done

9-line 136, please correct: "…. of MRI, were at least 16 years of age…."

Response: Done

10-please capitalize first characters of sentences. For example, "To prevent the …." [line 136]

Response: Done

11-please add full term of abbreviations for the first time for example STAR, SHARP, ….

Response: Done

12-this part is wrong: "p Value values" [two value is not appropriate. Please correct in text and table legend]

Response: Done

13-line 188, please correct as "the right caudate"

Response: Done

14-line 192, please correct as "….(p = 0.02) and left hippocampus…"

Response: Done

15-I think globus pallidus is better than palidus [line 191 and table 3 and figure 2]

Response: Done

16-table 1 legend, please change involved to enrolled

Response: Done

17-what is the meaning of a p < 0.05. in table 1 footnote?

Response: considered significant. Corrected.

18-table 2 legend, please correct as: Table 2 Shapiro-wilk test results for assessment of normality of the variables. [please don't use the SPSS output for presenting tables. Table 2 is SPSS output pasted here]

Response: Done

19-table 3 legend is better as: "Comparison of Mean Magnetic susceptibility of the Subcortical Brain Nuclei Between the mTBI and Healthy Controls."

Response: Done

20-please correct Discussions title to discussion

Response: Done

21-in line 230, please correct nucleus to nuclei.

Response: Done

22-what is the meaning of ferritin H chain? If it is ferritin heavy chain, please write it as ferritin heavy chain.

Response: Done

23-please delete Limitations and Recommendations title.

Response: Done

24-please move the paragraph: “Financial and time limitations and …….. nature of this study.” After this sentence: “Our study had some limitations. Further research is needed due to the relatively small sample size used to confirm these findings”

Response: Done

25-please delete Conclusions title and begin paragraph with: In conclusion, ….

Response: Done

26-please add manufacturer data of SPSS.

Response: Done

27-it is better you mention full term of abbreviations for the first time [for example GRE, ….]

Response: Done

4 Revision 3

1 comment · 2 Feb 2025

Editor-in-Chief

ACCEPT 2 Feb 2025

Dear authors,
Your article is acceptable for publication.
Regards