Peer Review File

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Reviewer A

This is a prospective study (brief report) aiming to identify changes in GM3 ganglioside in BPH patients before and after laser therapy. Some critical points were listed below as follows:

Title

Comment 1: The word "monosialodihexosylganglioside" should be in the title instead of GM3 abbreviation alone.

Reply 1: Thank you for the careful review and relevant comments. We agree with your feedback and have changed the title, accordingly.

Changes in the text: Title "Perioperative changes in ganglioside monosialodihexosylganglioside (GM3) molecular species for benign prostatic hyperplasia: a preliminary report"

Abstract

Comment 2: The text should be all in the past: "samples are obtained", patients are included" and etc….

Reply 2: Thank you for raising this issue. We have revised the text accordingly.

Changes in the text: the preoperative and postoperative serum samples were obtained from patients who underwent holmium laser enucleation of the prostate (HoLEP) for BPH. (page 3, lines 47–49)

Twenty-three patients were included in the study. (page 3, line 51)

Comment 3: What is the meaning of “enucleated prostate” and how the authors have measured that?

Reply 3: We apologize for the lack of clarity. The enucleated prostate is the prostate that was surgically removed and weighed with a mass meter after removal from the body. The term “enucleated prostate” is difficult to understand so we have replaced it with the expression “resected prostate”.

Changes in the text: The average weight of the surgically resected prostate tissue was 49 g. (page 3, lines 52-53)

Comment 4: It is not clear for me the meaning of “d18:1-17:0”. Please, clarify in the text.

Reply 4: Thank you for raising this question. We have revised the text to improve clarity.

Changes in the text: Six GM3 species such as d18:1-17:0 (C17 acyl chain (-17:0) linked to a C18 sphingosine base with a double bond (d18:1-) by an amide linkage), were significantly reduced. (page 3, lines 55-57)

Comment 5: The authors should indicate in the text the exact tissue that has been used to perform the protocols. For instance: lines 53, 54 and 55, the GM3 measurement was performed in prostatic tissue or blood?

Reply 5: We have clarified this point in the text.

Changes in the text: Preoperative and postoperative measurements of serum GM3 species were performed one month before and three months after HoLEP. (page 3, lines 49–51)

At three months after HoLEP, the serum concentration of GM3 species was found to have decreased after HoLEP compared with the preoperative concentration of GM3 species. (page 3, lines 53–55)

Comment 6: It is missing a conclusion.

Reply 6: Thank you for raising this issue, we have added a concluding sentence in the abstract.

Changes in the text: This study showed the serum concentrations of several GM3 species, which indicate chronic inflammation, may be significantly reduced after BPH surgery. (page 3, lines 59–61)

Introduction

Comment 7: I'm not sure if really there is no medication to treat BPH. What about alfa1 adrenergic antagonists, PDE5 inhibitors and 5alpha-reductase inhibitors?

Reply 7: Thank you for this comment, we have deleted this sentence.

Comment 8: This is a quite small introduction section. The authors should give a background for the readers about GM3 and the role of inflammation in BPH genesis and progression. I suggest the authors go deeper on the above-mentioned points, including more information and studies to this section.

Reply 8: Thank you for raising this issue. Following your suggestion, we have added the relevant information.

Changes in the text: The number of male patients undergoing BPH treatment, including drug therapy and surgery, is increasing (2). BPH progresses with age and is associated with chronic

inflammation (3-7). Male patients with chronic inflammation of the prostate had a seven-fold higher risk of BPH than those without inflammation (4). Also, there was a significant correlation between the degree of prostate inflammation, prostate volume, and dysuria after BPH surgery (5,6). It is important to identify the relationship between BPH and chronic inflammation to determine the risk of BPH progression and understand its underlying mechanism, in order to achieve an impact on the course and treatment of BPH (7).

Gangliosides constitute one class of glycosphingolipid which contain one or more sialic acid residues (8). Gangliosides can modulate receptor mediated signal transduction within the plasma membrane. Ganglioside monosialodihexosylganglioside (GM3) species act as pro- and anti-inflammatory endogenous toll-like receptor 4 modulators, and potentially serve as diagnostic and therapeutic targets of chronic inflammation diseases (9,10). (page 4, lines 67–81).

Comment 9: It is missing a hypothesis for this study

Reply 9: Thank you for raising this point. We have added a hypothesis, as suggested.

Changes in the text: We hypothesized that there would be a difference in the levels of GM3, a marker of chronic inflammation, between pre- and post-treatment for BPH. (page 4, lines 84–86)

Material and methods

Comment 10: Have the authors evaluated other parameters such as obesity, diabetes and hypertension on these patients? I suggest to include these other patient's parameters in the manuscript.

Reply 10: We are grateful for this suggestion. We have revised the text accordingly.

Changes in the text: The median body mass index was 23.3, 35% of patients had diabetes, and 65% had hypertension. (page 5, lines 110-111)

Comment 11: The number of patients should be indicated here instead of results section.

Reply 11: Thank you for your suggestion, we have revised the text accordingly.

Changes in the text: Twenty-three patients were included in the study. (page 5, line 110)

Comment 12: I suggest to clarify which symptoms were used to include the patients on this study.

Reply 12: Thank you for your suggestions, we have revised the inclusion criteria accordingly.

Changes in the text: Patients aged 50-90 years with symptoms of BPH for a duration of three months or more were included in the study. Indications for surgery were lower urinary tract symptoms refractory to medical therapy, maximum urinary flow rate ≤15 mL/s, International Prostate Symptom Score (IPSS) ≥8, and prostate volume ≥30 mL. Consecutive patients who met the inclusion criteria and consented to participate in the study were included. Patients who could not be followed up at 3 months after surgery, such as patients referred from distant locations, were excluded. (page 5, lines 100–107)

Comment 13: Considering that some patients exhibit BPH-induced LUTS even with small prostate, have the authors determined a minimum prostate weight value to include the patients in the study?

Reply 13: Thank you for raising this important question. We have added this to the inclusion criteria.

Changes in the text: Indications for surgery were lower urinary tract symptoms refractory to medical therapy, maximum urinary flow rate ≤15 mL/s, International Prostate Symptom Score (IPSS) ≥8, and prostate volume ≥30 mL. (page 5, lines 102-104)

Results

Comment 14: I suggest to include a table containing all the results presented in the first paragraph of "Results" section (lines 108-115).

Reply 14: Thank you for this suggestion. We have added revised Table 1 accordingly.

Discussion

Comment 15: Line 134: ….to identify changes in chronic inflammation….Which parameters the authors have used to measure chronic inflammation in the manuscript?

Reply 15: Thank you for raising this important question. We have revised the text to clarify this point.

Changes in the text: This study examined changes in GM3 species levels, one of the markers of chronic inflammation, between the pre-operative and post-operative period to identify changes in chronic inflammation due to prostate volume reduction and improvement of urinary tract obstruction. (page 8, lines 163–166)

Comment 16: I understand that there is no study addressing the role of GM3 on BPH patients. So, to improve this section, I suggest the authors to compare the results obtained here with other prostatic conditions, such as prostatitis and cancer.

Reply 16: Thank you for this suggestion. We have added this information.

Changes in the text: The relationship between GM3 and BPH has not been previously reported; furthermore, there are no previous reports on the relationship of GM3 and prostatitis or prostate cancer. (page 8, lines 160–162)

Conclusion

Comment 17: This is not a conclusion but a summary of the results obtained in the study.

Reply 17: Thank you for your comment. We have revised the text to address this issue.

Changes in the text: This study found that the serum concentrations of several GM3 species, which indicate chronic inflammation, may be significantly reduced after surgery for BPH. Further studies are required to establish the pathology underlying the changes in each GM3 species. Future research is needed to identify the GM3 species involved in BPH and examine whether there is a significant correlation with symptoms and course of treatment. (page 9, lines 186-191)

References

Comment 18: Eleven references are too little.

Reply 18: Thank you for your feedback. We have added a further five references.

Reviewer B

I extend my congratulations to the authors for their research.

Comment 1: The study, while promising, is limited by a small sample size (n=23), which potentially introduces bias and restricts the power to detect significant differences. Conducting a power analysis to ascertain an appropriate sample size for future research is recommended to substantiate the findings presented here.

Reply 1: We thank the reviewer for the thorough review of our manuscript and the important comments. We have added this point to the section on limitations.

Changes in the text: This study had a limited sample size; further research should be conducted using an appropriate sample size. (page 8, lines 178-179).

Comment 2: Furthermore, the manuscript could provide a more detailed depiction of the participant selection criteria, including exclusion factors and the exact process of selection to improve the external validity of the study. A more detailed account of the analytical method used for GM3 species identification and quantification, especially in terms of the LC-MS/MS analysis process, is suggested to bolster the methods section.

Reply 2: Thank you for your comment. We have added this information, as suggested.

Changes in the text: Patients aged 50-90 years with symptoms of BPH for a duration of three months or more were included in the study. Indications for surgery were lower urinary tract symptoms refractory to medical therapy, maximum urinary flow rate ≤15 mL/s, International Prostate Symptom Score (IPSS) ≥8, and prostate volume ≥30 mL. Consecutive patients who met the inclusion criteria and consented to participate in the study were included. Patients who could not be followed up at 3 months after surgery, such as patients referred from distant locations, were excluded. (page 5, lines 100–107)

Quantification was conducted by LC-MS/MS analysis of serum GM3 species with differing acyl-chain structures. Each GM3 species were separated using Develosil carbon 30 column (C30-UG, 3μm, 1×50 mm, Nomura Co. Ltd, Japan). (page 6, lines 121–123)

Comment 3: While the statistical analysis utilizes a paired sample t-test, integrating other statistical

tests or methods for a more comprehensive data analysis could be beneficial. Additionally, expanding upon the data distribution details and checks for assumptions of the employed statistical tests would strengthen the study.

Reply 3: Thank you for your suggestions. However, because of the small sample size, it was determined that the use of other statistical methods would be difficult; therefore, we reported these preliminary results in this paper.

Comment 4: In the results and discussion sections, a deeper analysis could be facilitated through a more comprehensive data presentation, possibly with additional figures or tables showcasing individual or sub-group data. The discussion section could benefit from a more thorough exploration of existing literature on GM3 species' involvement in chronic inflammation and its potential connection to BPH. Also, a more extensive discussion on the acknowledged limitations of the study and how these could influence the results and be addressed in future research would be beneficial.

Reply 4: Thank you for your insightful comments. We have added Table 1.

Changes in the text: The relationship between GM3 and BPH has not been previously reported; furthermore, there are no previous reports on the relationship of GM3 and prostatitis or prostate cancer. In HoLEP, the prostatic adenoma is completely enucleated, resulting in significant prostate volume reduction. This study examined changes in GM3 species levels, one of the markers of chronic inflammation, between the pre-operative and post-operative period to identify changes in chronic inflammation due to prostate volume reduction and improvement of urinary tract obstruction. (page 8, lines 160-166)

Comment 5: The conclusion section should ideally outline a detailed roadmap for future research,

pinpointing specific research queries to be addressed and prospective methodologies to be employed.

Reply 5: Thank you for your comments. We have revised the text accordingly.

Changes in the text: This study found that the serum concentrations of several GM3 species, which indicate chronic inflammation, may be significantly reduced after surgery for BPH. Further studies are required to establish the pathology underlying the changes in each GM3 species. Future research is needed to identify the GM3 species involved in BPH and examine whether there is a significant correlation with symptoms and course of treatment.

(page 9, lines 186-191)

Comment 6: While the manuscript is generally well-written, a round of meticulous proofreading could enhance the clarity and coherence in certain sections. Nevertheless, the existing sections on funding, conflict of interest, and ethical considerations are commendably presented.

Reply 6: Thank you for your supportive comments. The manuscript has undergone English proofreading.

Comment 7: Overall, this preliminary study sets a promising trajectory for future research into GM3 species' role in BPH, with potential enhancements as noted above likely to augment its impact and rigor.

Reply 7: Thank you for the careful review and supportive comments.

Reviewer C

The authors investigated the relation between an inflammatory serum marker (GM3) and removal of BPH adenoma. It is an interesting study exploring the concept of BPH chronic inflammation, however some major issues should be addressed before publication:

Introduction:

Comment 1: The sentence in lines 66-67 "BPH causes dysuria and frequent urination, leading to lower urinary tract symptoms 66 (LUTS) and reduced quality of life (QOL)" is not relevant and should be removed.

Reply 1: Thank you for this comment, we have deleted the sentence, as suggested.

Comment 2: lines 71-72 - instead of "understand the mechanism underlying its symptoms" please change to "understand its underlying mechanism"

Reply 2: Thank you for your suggestion, we have revised the text accordingly.

Changes in the text: It is important to identify the relationship between BPH and chronic inflammation to determine the risk of BPH progression and understand its underlying mechanism, in order to achieve an impact on the course and treatment of BPH (7).

(page 4, lines 72-75)

Comment 3: lines 72-76 - please re-arrange and re-phrase the aim of the study to be more concise and accurate.

Reply 3: Thank you for the careful review and relevant comments. We agree with your feedback and have added the sentences.

Changes in the text: Gangliosides constitute one class of glycosphingolipid which contain one or more sialic acid residues (8). Gangliosides can modulate receptor mediated signal transduction

within the plasma membrane. Ganglioside monosialodihexosylganglioside (GM3) species act as pro- and anti-inflammatory endogenous toll-like receptor 4 modulators, and potentially serve as diagnostic and therapeutic targets of chronic inflammation diseases (9,10). This study focused on the relationship between BPH and ganglioside GM3, a sialic acid-containing glycosphingolipid involved in chronic inflammation. Patients with chronic inflammatory diseases have increased pro-inflammatory serum GM3 molecular species levels (10,11). We hypothesized that there would be a difference in the levels of GM3, a marker of chronic inflammation, between pre- and post-treatment for BPH. (page 4, lines 76–86)

Comment 4: Lines 76-77 - "Changes in the GM3 species were analyzed before and after BPH treatment " - this is part of methods not the introduction.

Reply 4: Thank you for the careful review and relevant comments. We agree with your feedback and have revised the text.

Changes in the text: This study aimed to characterize the changes in the levels of GM3 species before and after BPH treatment. (page 4, lines 86–87)

Methods:

Comment 5: Statements about ethics should be placed at the beginning of this section.

Reply 5: Thank you for this suggestion. We moved the ethics section, as recommended.

Changes in the text: This study was approved by the Ethics Committee of the Tohoku Medical and Pharmaceutical University Hospital School of Medicine, Japan (2017–2-120), and conforms with the tenets of the Declaration of Helsinki. Written informed consent was obtained from all patients. (page 5, lines 90–93)

Comment 6: Please elaborate more about the inclusion and exclusion criteria of the patients: how diagnosis of BPH was performed, tests, imaging, flowmetry, and for which indications was surgery preformed (LUTS? Urinary retention? Infections? Hematuria?)

Reply 6: Thank you for raising these questions. We have revised the text accordingly.

Changes in the text: Patients aged 50-90 years with symptoms of BPH for a duration of three months or more were included in the study. Indications for surgery were lower urinary tract symptoms refractory to medical therapy, maximum urinary flow rate ≤15 mL/s, International Prostate Symptom Score (IPSS) ≥8, and prostate volume ≥30 mL. Consecutive patients who met the inclusion criteria and consented to participate in the study were included. Patients who could not be followed up at 3 months after surgery, such as patients referred from distant locations, were excluded. (page 5, lines 100–107)

Comment 7: Were systematic and local inflammation markers (CRP, WBC, nitrites, cultures) evaluated before HOLEP? since if they are elevated, they may be responsible for some changes. please specify if yes or not. if yes - include the results. if not - mention in the limitations.

Reply 7: Thank you for raising these questions. We have revised the text accordingly.

Changes in the text: Preoperative median white blood cell was 5580 /μL and CRP was 0.2. Preoperative urine culture was performed in all patients. Five patients (22%) had positive cultures and were treated preoperatively with appropriate oral antibiotics. (page 7, lines 140–143)

Comment 8: Add briefly information about the surgical technique of the HOLEP.

Reply 8: Thank you for this suggestion. We have added the relevant information.

Changes in the text: The HoLEP procedure involves the removal of the line between the outer and inner glands of the prostate tissue and the removal of the inner gland. Therefore, HoLEP is one of the radical resection procedures for BPH. The enucleation procedure was performed following the anteroposterior dissection HoLEP method, as previously reported (12,13).

(page 5, lines 96-100)

Comment 9: Since this is a small sample, you should use medians and IQRs for all variables (clinical preoperative and also GM3 results), and compare results of before and after using non-parametric matched test (e.g. wilcoxon signed rank test).

Reply 9: Thank you for your suggestions. However, because of the small sample size, it was determined that the use of other statistical methods would be difficult; therefore, this study reported only preliminary results.

Results:

Comment 10: The results section lack information and should be more elaborated about the amount of total GM3 and also its species.

Reply 10: Thank you for this feedback. We have revised the text accordingly.

Changes in the text: A total of 20 different blood GM3 molecular species were measured in this study. (page 6, lines 126-127)

Specifically, the concentration of six GM3 species, notably, d18:1-15:0, d18:1-17:0, d18:1-20:1, d18:2-20:0, d18:2-23:0, and d18:2-24:0 was significantly reduced (Table 1). (page 7, lines 145-146)

Comment 11: What are the variables of the table: medians? average? anyway, it should be the pre and post medians (IQRs) GM3 concentrations.

Reply 11: Thank you for raising these questions. We have unified the median and the IQRs.

Changes in the text: The median patient age was 75 years, and the median prostate volume was 66 mL. The median weight of the resected prostate tissue was 42 g. The median IPSS and QOL scores were 22 and 6, respectively. Based on preoperative uroflowmetry, the median maximal flow rate and postvoid residual urine were 7 mL/s and 100 mL, respectively. At three months after HoLEP, the median IPSS and QOL scores were 5 and 2, respectively, while the median maximal flow rate and postvoid residual urine were 16 mL/s and 20 mL, respectively.

(pages 6-7, lines 134-140)

Discussion

Comment 12: Lines 131-132 - "the intraprostatic gland is completely enucleated" - please change to "the prostatic adenoma is completely enucleated"

Reply 12: Thank you for the relevant comments. Following your suggestion, we have revised the text.

Changes in the text: In HoLEP, the prostatic adenoma is completely enucleated, resulting in significant prostate volume reduction. (page 8, lines 162–163)

Comment 13: Please arrange the second paragraph of the discussion (131-144) so for every specific GM3 that was reduced after the HOLEP there will be a mention about what we know about it in the literature.

Reply 13: Thank you for this suggestion. We have revised the text accordingly.

Changes in the text: The relationship between GM3 and BPH has not been previously reported; furthermore, there are no previous reports on the relationship of GM3 and prostatitis or prostate cancer. (page 8, lines 160–162)

Comment 14: Lines 145-149 may be removed.

Reply 10: Thank you for this suggestion we have deleted the text accordingly.

Reviewer D

This manuscript details a preliminary study evaluating the serum concentrations of GM3 ganglioside molecular species pre and post HoLEP procedures. The hypothesis as I understand it is that these species would be elevated in BPH, which is reasonable. However, I have many concerns about the study as presented and while this is a brief report,the findings at this time do not warrant publication. I recommend that additional data be obtained, and the authors then submit a full study here or elsewhere.

Comment 1: This study is incomplete. I understand that it is observational, but what has been found does not appear to indicate the basis for mechanistic studies. Only 6 of 23 patients had a significant change in GM3 levels. Yet, the authors seem to suggest that this means that GM3 gangliosides may actually have some role in BPH. Please justify this conclusion.

Reply 1: Thank you for the careful review. We apologize for the confusion. We measured 20 GM3 species before and after BPH surgery and found that six GM3 species, not six patients, were significantly lower after BPH surgery. We have revised the text in the conclusion.

Changes in the text: This study found that the serum concentrations of several GM3 species, which indicate chronic inflammation, may be significantly reduced after surgery for BPH. Further studies are required to establish the pathology underlying the changes in each GM3 species. Future

research is needed to identify the GM3 species involved in BPH and examine whether there is a significant correlation with symptoms and course of treatment.

(page 9, lines 186–191)

Comment 2: It is unclear whether the changes correlate with inflammation vs loss of tissue volume vs some other unknown factor vs the changes being a random finding. Had this been seen in much more than 25% of the patients, it would be more convincing that there is anything of note that can be taken from these results. It is my opinion that this is currently a negative study. There is nothing wrong with a negative study, but the results cannot be used to justify a claim of significance. Can the authors please explain why they think this finding is significant? What criteria have they used?

Reply 2: Thank you raising these issues. We apologize for the confusion. We measured 20 GM3 species before and after BPH surgery and found that six GM3 species, not six patients, were significantly lower after BPH surgery. We have revised the conclusion.

Changes in the text: A total of 20 different blood GM3 molecular species were measured in this study. (page 6, lines 126-127)

Specifically, the concentration of six GM3 species, notably, d18:1-15:0, d18:1-17:0, d18:1-20:1, d18:2-20:0, d18:2-23:0, and d18:2-24:0 was significantly reduced (Table 1). (page 7, lines 145-146)