Peer Review File

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Reviewer A

The article is reasonably well written but need some minor language corrections.

1. The authors mention that HT occurs mainly in early PTC patients with 130 smaller primary tumor size, suggesting that HT may limits the growth of PTC. This has been shown in other studies as well. To suggest that HT limits the size of cancer growth is not proven, and hence the statement may be modified.

Reply 1: Thank you for your comment. After careful consideration and review of the literature, we have revised the statement of this conclusion.
The revised contents as follows and is highlighted in yellow in the revised manuscript.
Changes on Page 9, Line 147-150 in the text: Additionally, pathological HT predominantly occurs in early-stage PTC, … These findings may imply that pre-existing HT was associated with early-stage PTC risk.

2. On histology, how many thyroidectomy specimen had focal thyroiditis versus diffuse thyroiditis?

Reply 2: We are grateful for your professional commentary. Given the multicenter retrospective design of this study, it is unfortunate that our data did not provide additional insights to distinguish between localized and diffuse forms of Hashimoto's thyroiditis.

3. Line 72. …extracted some data, not dates as mentioned in the manuscript

Reply 3: Thank you for your kind reminder. Because of our carelessness, we mistakenly wrote dates instead of data. We have revised this error.
The revised contents as follows and is highlighted in yellow in the revised manuscript.
Changes on Page 5, Line 72 in the text: We reviewed medical records and extracted some data including…

4. Table 3 & 4 Odd ratio should read as Odds ratio

Reply 4: Thank you for your kind reminder. We have check for typos and revised our manuscript.
The revised contents as follows and is highlighted in yellow in the revised manuscript.

Reviewer B

In this study, authors conducted a multicenter case-control study to investigate the association of HT with clinicopathological features of PTC in the central provinces of China. The study showed 22.9% of overall prevalence of HT in PTC patients. Pathological HT was a potential protective factor for some aggressive characteristics of PTC. The data provided are of interest and the manuscript lucid and the figures clear. This study has important implications for further understanding of thyroid cancer and provides clinical insights into the association between HT and PTC.
Several critical issues are noted below:
In the Abstract,
Include the objectives of your study in your abstract.
Methods need to be specified, including specimens, measurements, and analysis methods involved.
Basic information of a total of 15305 PTC patients participating in this study, such as age, number (percentage) of females and males, should be provided.

Reply 1: Thank you for your thoughtful comments. The suggestions are very professional to our manuscript, and the section in question has been modified.
The revised contents as follows and is highlighted in yellow in the revised manuscript.
Changes on Page 1, Line 6-9 in the text: We conducted a multicenter case-control study to…, by logistic analysis.
Changes on Page 1, Line 11-12 in the text: A total of 15305 patients…, including 11 465 women (74.9%) and 3840 men (25.1%).

In the Methods,

How was the coexistence of HT and PTC determined, and how was BRAFV600E mutation status detected?
Reply 2: We appreciate the comments and would like to clarify that in our study, HT and PTC were diagnosed through postoperative paraffin-embedded tissue specimens' histologic examination. The BRAFV600E mutation status was also determined using these specimens. This information has been revised and elaborated in the Methods section. Thank you again for your reminder.
The revised contents as follows and is highlighted in yellow in the revised manuscript.
Changes on Page 5-6, Line 78-80 in the text: Pathological HT and PTC…. … determined using these specimens.

In the Results,
Move the "Characteristics and baseline of participants" section to the first section, followed by the "Prevalence of Hashimoto's thyroiditis in papillary thyroid carcinoma" section.
Reply 3: We would like to express our gratitude for your notification. We have carefully considered your suggestions and adjusted the order of this section.

In lines 123-125, the authors present the differences in the aggressive features of PTC between pathological HT and clinical HT in Table 4. How many cases were classified as both pathological HT and clinical HT? How many cases were classified as only pathological HT or only clinical HT? Would it be more appropriate to use pathological HT in the future?
Reply 4: We appreciate your insightful commentary. In the current study, we included a total of 3,505 patients diagnosed with pathological hypertension (HT), of which 677 cases presented exclusively with pathological HT. In contrast, 2,390 patients were identified as exhibiting solely clinical HT. The pertinent data have been updated in Table 4 and are highlighted in yellow in the revised manuscript. Based on the conclusions derived from this study, it can be inferred that there may be a correlation PTC and HT. The presence of pathological HT appears to serve as a potential protective factor against certain aggressive characteristics associated with PTC. However, this protective effect was not evident in cases of clinical HT.

In this study, the overall prevalence of BRAFV600E was 85.6% (75.7% in HT PTC and 88.4% in non-HT PTC), which is much higher than 53.0% in PTC in a recent study (JAMA Netw Open. 2023;6(7):e2323500. doi:10.1001/jamanetworkopen.2023.23500). Are Chinese people more likely to carry this variant?
Reply 5: We appreciate your feedback. A comprehensive review of the available data has confirmed a significant BRAF mutation rate within the designated cohort. This finding aligns with our previously published studies, which also reported a similarly elevated BRAF mutation rate (doi: 10.1155/2021/6621067; doi: 10.3389/fendo.2022.987906). Additionally, a literature review indicates that the reported BRAF mutation rates in PTC in both China and South Korea are notably high, ranging from 68% to 78% (doi: 10.1007/s40291-024-00721-1; doi:10.4143/crt.2018.612; doi: 10.1245/s10434-015-4765-z; doi: 10.3390/cancers15225395). This observation may be linked to the regional characteristics of the study population.

At lines 95-96, "HT occurs mainly in early PTC patients with small primary tumor size, …". Since 58.2% of PTCs are microPTCs and 75.7% of HT PTCs carry BRAFV600E mutations, what is the BRAF V600E mutation profile in these microtumors? What is the trend of BRAFV600E in microtumors and HT?
Reply 4: Thank you for your insightful feedback. In our research, we observed that the frequency of the BRAFV600E mutation in micro-PTC was 87.4% (6276/7176). Specifically, the mutation frequency was 80.3% (1271/1581) within the HT cohort and 89.5% (5005/5595) in the non-HT cohort, with a P-value of <0.001. These findings indicate that a substantial proportion of micro-PTC cases may be attributed to HT rather than the BRAFV600E mutation, a conclusion that aligns with previous studies (doi:10.1089/thy.2024.0142; doi:10.3389/fendo.2023.1273498).

Minor Comments:
"none-HT group" should be "non-HT group"
Reply 5: Thank you for your professional comments. We have thoroughly reviewed our manuscript for any typos and made the necessary revisions.

Reviewer C

2.2. Study design: Would the authors consider add ultrasound findings to diagnose HT?
Reply 1: Thank you for your comments. Serum thyroid autoantibodies represent the primary non-invasive approach for the clinical diagnosis of HT. In clinical practice, we often encounter patients with autoimmune thyroiditis who test positive for thyroid autoantibodies but present with negative pathological findings. This study seeks to assess the discrepancies between pathologically diagnosed HT and cases diagnosed clinically based solely on serological positivity, as well as their association with the invasive characteristics of thyroid cancer. Additionally, the ultrasound diagnosis of Hashimoto's thyroiditis may be influenced by the echogenicity of thyroid tumors. Consequently, we considered only cases that were serologically positive for thyroid autoantibodies as clinically HT.

When considering the positive diagnosis of HT, did the author refer to pathological or clinical findings or both?
Reply 2: Thank you for your feedback. In this study, we categorized Hashimoto's Thyroiditis (HT) into two distinct classifications: clinically diagnosed HT and pathologically diagnosed HT. Clinically diagnosed HT is characterized by a positive result for thyroid autoantibodies, primarily targeting thyroperoxidase and thyroglobulin, while exhibiting negative results in pathological assessments. Conversely, pathologically diagnosed HT is confirmed through histological examination of postoperative paraffin-embedded tissue specimens. Detailed methodologies are outlined in the manuscript on Page 5-6, Lines 76-80.

Lines 111 / 112: “These results were consistent with some previous reports (Table 2)” is a statement that does not fit with the Results.
Reply 3: Thank you for your thoughtful reminder. We acknowledge that there may have been some ambiguity in our description of the results. Our findings indicate that, in comparison to the non-HT group, PTC patients with HT exhibited a lower prevalence of aggressive characteristics. These characteristics include primary tumor size, BRAFV600E mutation status, bilateral tumor presence, extrathyroidal extension, primary tumor stage, lymph node stage, and the number of metastatic lymph nodes ($P<0.05$ for all). We have thoroughly reviewed this issue and made the necessary revisions to our manuscript.
The revised contents as follows and is highlighted in yellow in the revised manuscript.
Changes on Page 7, Line 111-115 in the text: The findings indicated that PTC patients with pathological HT exhibited a lower prevalence of aggressive characteristics when compared to the non-pathological HT group. These characteristics included …, with all comparisons yielding a significance level of $P <0.05$ (refer to Table 2).

Discussion, first paragraph: there is a lack of literature references.
I appreciated the last paragraph of the Discussion in which the authors mentioned their limitations.
Reply 3: Thank you for your thoughtful reminder and acknowledgment of our efforts. We have reviewed our manuscript and revised the discussion of the manuscript.

Reviewer D

The paper is interesting, but I would have developed the underlying hypotheses in a more extensive manner.
Are there substantial differences between the characteristics of aggressiveness and the varying levels of thyroid autoantibodies?
Do you think you could provide a more detailed description of the data derived from the tables?
Reply 3: We sincerely appreciate the insightful feedback provided by the reviewer. The suggestions offered are highly valuable to our manuscript. We acknowledge certain limitations within our study that warrant consideration. Our future research will focus on investigating the relationship between thyroid autoantibodies in relation to thyroid cancer. While the datasets generated and/or analyzed during this study are not publicly accessible due to participant privacy concerns, they can be obtained from the corresponding author upon reasonable request. We thank you once again for your constructive comments.

Reviewer E

1- It would have been better if a correlation was made with s=thyroglobulin level not only being positive or negative

Reply 1: Thank you for your comments. We intend to gather additional data to conduct a more thorough investigation into the potential association between HT and PTC.

2- While all the patients undergo surgical intervention, how could you explain your findings with clinical HT? Did you have patients who were clinically HT but pathologically not and vice versa?

Reply 2: We would like to express our sincere appreciation for the insightful commentary provided. In this study, we categorized Hashimoto's Thyroiditis (HT) into two distinct classifications: clinically diagnosed HT and pathologically diagnosed HT. Clinically diagnosed HT was defined as the presence of thyroid autoantibodies, primarily thyroperoxidase and thyroglobulin, while being negative for pathological criteria. The cohort included a total of 2,390 patients. The histopathological diagnosis of HT was established through a thorough examination of postoperative paraffin-embedded tissue samples, which involved a total of 3,505 patients. Among these, 677 cases were classified as negative for thyroid autoantibodies, while 2,828 cases were identified as positive.

3-Also, while all the patients were PTC, clinically, HT means that markers for HT were performed preoperatively. What were their indications in known patients of PTC?

Reply 3: We appreciate your comments and would like to respond as follows. Indeed, previous studies have demonstrated a correlation between preexisting thyroid autoimmunity and an increased risk of papillary thyroid cancer (doi: 10.1200/JCO.21.02618). Moreover, coexisting HT offer the negative effect on an indeterminate response to therapy of thyroid cancer (doi:10.3390/diagnostics14020166). Additionally, our prior research has shown that Hashimoto's thyroiditis (HT) may complicate preoperative ultrasound assessments of thyroid nodules and cervical lymph node status (doi: 10.3389/fendo.2022.987906). Consequently, in this cohort, all patients underwent testing for thyroid hormone and thyroid autoantibodies prior to surgery to evaluate the presence of coexisting HT.

4- Most of the discussion is just a repetition of the results

Reply 4: We sincerely appreciate the reviewers for their invaluable professional insights. Their suggestions have significantly improved the quality of our manuscript. We have made the necessary revisions in this manuscript.

5- While the article is reported to be a multicenter study, 97.7% of the patients belonged to one center. This should be explained, discussed, and added to the limitations

Reply 5: We sincerely appreciate your constructive feedback, which has significantly contributed to the enhancement of our manuscript's professionalism. It is essential to recognize the limitations of the current study, which have been thoroughly documented and integrated into the discussion section, thereby addressing the constraints of the research.

The revised contents as follows and is highlighted in yellow in the revised manuscript.

Changes on Page 9, Line 165-167 in the text: Finally, despite being a multi-center study, …. Consequently, it is imperative that future research endeavors strive to incorporate a larger number of cases from diverse centers.