

## Peer Review File

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### **Reviewer A**

Abstract:

**1. I'm not sure you add relevant information with the sentence "After surgery, the patient was informed of a black thyroid, and the final diagnosis was papillary thyroid cancer with multiple nodal metastases". I would consider erasing this sentence.**

→ We would like to thank Reviewer A for the time and effort in reviewing our manuscript and providing comments and suggestions, which have considerably helped us improve our manuscript. We have answered each of your points below and hope that our responses and revisions sufficiently address all your comments.

In accordance with your comment, we have removed the above-mentioned sentence. We focused on the pathological examination of the black thyroid revealing brown pigmentation in the thyroid parenchyma in the Case presentation section of the Abstract, page 2, line 36-38.

### **Revised:**

(a) 'Case presentation' section in the Abstract

During level IV dissection, we noticed a similar black discoloration in the adipose tissue of the lower neck. **Pathological examination revealed brown pigmentation with few macrophages on several foci of the thyroid parenchyma.** Brown pigmentation was not identified in the thyroid tumor, metastatic and normal lymph nodes, or background adipose tissue.

**2. "We report a case of papillary thyroid cancer in a black thyroid with cervical nodal metastases." consider rephrasing in "We report on a case of papillary thyroid microcarcinoma with cervical lymph node metastases in a black thyroid".**

→ Following your comment, we have rephrased the above-mentioned sentence to "We report on a case of papillary thyroid microcarcinoma with cervical lymph node metastases in a black

thyroid." in the Conclusion section of the Abstract, page 2, line 40-41.

**Revised:**

(a) 'Conclusion' section in the Abstract

We report on a case of papillary thyroid microcarcinoma with cervical lymph node metastases in a black thyroid. The clinical findings were consistent with those of previous reports. Based on the literature, it remains unclear if this minocycline triggered finding is associated with an increased frequency and/or aggressiveness of thyroid carcinomas. In the absence of adequate evidence, prolonged minocycline users should ideally undergo routine thyroid assessment to identify possible malignancy.

**3. I would suggest for a conclusion: "We report on a case of papillary thyroid microcarcinoma with cervical lymph node metastases in a black thyroid. Based on the literature, it remains unclear if this minocycline triggered finding is associated with an increased frequency and/or aggressiveness of thyroid carcinomas. In the lack of adequate evidence, prolonged minocycline users should probably undergo routinely thyroid assessment".**

→ Thank you for suggestion. In accordance with your comment, we have modified the above-mentioned sentences to "We report on a case of papillary thyroid microcarcinoma with cervical lymph node metastases in a black thyroid. The clinical findings were consistent with those of previous reports. Based on the literature, it remains unclear if this minocycline triggered finding is associated with an increased frequency and/or aggressiveness of thyroid. In the absence of adequate evidence, prolonged minocycline users should ideally undergo routine thyroid assessment to identify possible malignancy." in the Conclusion section of the Abstract, page 2, line 40-45.

**Revised:**

(a) 'Conclusion' section in the Abstract

We report on a case of papillary thyroid microcarcinoma with cervical lymph node metastases in a black thyroid. The clinical findings were consistent with those of previous reports. **Based on the literature, it remains unclear if this minocycline triggered finding is associated with an increased frequency and/or aggressiveness of thyroid carcinomas. In the absence of adequate**

evidence, prolonged minocycline users should ideally undergo routine thyroid assessment to identify possible malignancy.

**4. Line 75: please explain here "LN" not at line 78.**

→ Thank you for your comment. We have mentioned the full term 'lymph node' and its abbreviation 'LN' in the Patient section of the Case presentation, page 5, line 78-80. Thereafter, we have used only the abbreviation 'LN'.

**Revised:**

(a) 'Patient' section in the Case presentation

A 29-year-old woman presented to a hospital after noticing a palpable lymph node (LN) at the left neck level V and was referred to our hospital because of a suspicious nodule in the left thyroid with cervical lymphadenopathy. Fine needle aspiration biopsies (FNAB) were performed in the thyroid nodule and the largest suspicious LN in the left neck level IV, which were confirmed as 'atypia of undetermined significance (Bethesda category III)' with focal features suggestive of papillary thyroid cancer (PTC) and 'metastatic PTC', respectively.

**5. Line 76-78 : was it level V or IV ?.**

→ We appreciate your comment. In fact, the patient noticed a palpable LN at the neck level V, which was located in the superficial neck. Cervical ultrasonography (US) showed multiple suspicious LNs in the left lateral neck. Fine needle aspiration biopsy (FNAB) was done in the largest LN at the neck level IV. We have modified the sentence to clarify the situation in the Patient section of the Case presentation, page 5, line 78-83.

**Revised:**

(a) 'Patient' section in the Case presentation

A 29-year-old woman presented to a hospital after noticing a palpable lymph node (LN) at the left neck level V and was referred to our hospital because of a suspicious nodule in the left thyroid with cervical lymphadenopathy. Fine needle aspiration biopsies (FNAB) were

performed in the thyroid nodule and the largest suspicious LN in the left neck level IV, which were confirmed as ‘atypia of undetermined significance (Bethesda category III)’ with focal features suggestive of papillary thyroid cancer (PTC) and ‘metastatic PTC’, respectively.

**6. Line 79 : papillary thyroid cancer (Bethesda V), right ?**

→ We appreciate your comment on the FNAB result of the thyroid. However, the thyroid nodule was confirmed of “atypia of undetermined significance (Bethesda category III)”. A pathologist additionally commented on ‘the focal suspicious features of PTC’. We have added the comment on the FNAB result of the thyroid nodule (focal suspicious features of PTC) to indicate the primary malignancy in the Patient section of the Case presentation, page 5, line 82-83.

**Added:**

(a) ‘Patient’ section in the Case presentation

...Fine needle aspiration biopsies (FNAB) were performed in the thyroid nodule and the largest suspicious LN in the left neck level IV, which were confirmed as ‘atypia of undetermined significance (Bethesda category III)’ with focal features suggestive of papillary thyroid cancer (PTC) and ‘metastatic PTC’, respectively.

**7. Line 86: did palpation really reveal a LN of 5mm? or was it ultrasound?**

→ We appreciate you pointing this out. We acknowledge that it is not relevant to describe the specific diameter of the LN through palpation. Cervical US revealed the diameter of the LN as 7 mm. We have corrected the description to a hard LN on palpation and removed its diameter in the Patient section of the Case presentation, page 5, line 89.

**Revised:**

(a) ‘Patient’ section in the Case presentation

Physical examination of the neck revealed a hard LN at the left level V.

**8. Line 136 : please quote the previous similar report, such as BMJ Case Rep. 2021 Jun 21;14(6):e242133. ; Ear Nose Throat J. 2016 Mar;95(3):E28-31 for instance.**

→ We appreciate your suggestion. Following your comment, we have added the citation of the above-mentioned case report in the Discussion, page 8, line 152.

**Added:**

(a) Discussion

The black thyroid is an unusual and incidental finding during surgery or autopsy. Diagnosing a black thyroid using FNAB is unreliable and extremely rare. Black or brown pigmentation have been scarcely reported, and mimicking factors and other differential diagnoses should also be considered (12,13). Our patient's FNAB result for the thyroid nodule was atypia of undetermined significance, with no evidence of pigmentation. In our patient, black thyroid was found incidentally during surgery, similar to previous reports (14).

(b) Reference

14. Nishimoto K, Kumai Y, Murakami D, et al. A case of minocycline-induced black thyroid associated with papillary carcinoma. *Ear Nose Throat J* 2016;95:E28-31.

**9. Line 159: in my opinion you need to mention that the high prevalence of malignancy might be explained by the fact that black thyroid is mostly diagnosed during surgery triggered by the suspicion of thyroid cancer. There is as far as I know no evidence for an association of minocycline with an increased risk of malignancy or more aggressive cancer (Ref 10 in your Reference List).**

→ We appreciate your suggestion. In accordance with your comment, we have commented on the possible association between higher incidence of PTC in black thyroid with the incidental finding of black thyroid during cancer surgery. We have added comments after the unclear clinical relevance of black thyroid in thyroid cancer in the Discussion, page 9, line 188-190.

**Added:**

(a) Discussion

Owing to the relative scarcity of data, the clinical relevance of black thyroid in thyroid

cancer remains unclear. Previous reports on higher incidence of PTC in black thyroid can be a comprehensible finding, since the recognition of black thyroid predominantly occurs during surgery. Spared pigmentation in malignant tumors compared to surrounding tissue and unproven carcinogenic activity of minocycline still raise questions on their relationships (9,21). Thus, proper treatment strategy has not yet been established. Despite the possible selection bias and different methodologies, the higher incidence of thyroid cancer in black thyroid warrants precise evaluation of the effect of minocycline-induced black thyroid on thyroid malignancy.

**10. Line 160: what do you mean by "The result was remarkable for PTC, which was predominantly observed in black thyroids"? Did you mean that malignancy was most often PTC? Consider reformulating this sentence. PTC makes out 80% of all thyroid malignancy. I don't think it's remarkable that it is the most frequent carcinoma in black thyroid, it's also the most frequent carcinoma in non-black thyroid.**

→ We have revised the above-mentioned sentence to "PTC was most frequently observed in black thyroids." in the Discussion, page 9, line 181.

**Revised:**

(a) Discussion

Although the causal relationship between minocycline-induced black thyroid and thyroid cancer has not been proven, few studies have identified a significantly higher incidence of thyroid cancer in black thyroid glands than in non-black thyroid glands (11,20). PTC was most frequently observed in black thyroids. Other thyroid malignancies, including follicular and Hürthle cell carcinomas, were less frequently found in black thyroids.

**11. Line 165: perhaps you can mention that this was a pT1a with lateral lymph node metastasis and negative BRAF status, thus more aggressive than usual microPTCs. How about the literature? Are pT1a PTCs more aggressive in black thyroid ?**

→ We appreciate your comment. We have reviewed the previous literature reporting on PTC in black thyroid. However, there has been no current information on the association between

black thyroids and LN metastasis of PTC. Only the characteristics of the tumor (tumor size and multifocality of PTC) with black thyroid have been evaluated, which are mentioned in the original manuscript. We have added comments stating that no data on black thyroid and the LN metastases of PTC is available in the Discussion, page 9, line 185-186.

**Added:**

(a) Discussion

When evaluating the correlation between black thyroid and PTC aggressiveness, these studies yielded diverse results regarding the tumor size. No significant differences were found in tumor multifocality (11,20). **None of the previous studies have yet evaluated the association between black thyroid and LN metastasis of PTC.**

**12. Line 182 : has the black discoloration of the surrounding adipose tissue of the neck level and/or lymph node been reported in the previous literature as well ?**

→ Thank you for the comment. We have mentioned in the original manuscript that we recognized the black thyroid and the similar black discoloration in the adipose tissue of the lower neck level IV specimens. However, pathological examination revealed the pigmentation only in the thyroid parenchyma, but not in the thyroid tumor, the metastatic LNs, normal LNs, or background adipose tissue. There has been no report on black discoloration of the surrounding adipose tissue of the neck level and/or LN in association with black thyroid.

As a different angle, the black-colored LNs from PTC metastasis has been mentioned, which have a greater degree of cystic degeneration and hemosiderin deposition leading to discoloration, but not in association with black thyroid (Reference. Crist HS, Evans JJ, Mani H, et al. Do black (dark) lymph-node metastases in papillary thyroid carcinoma suggest more advanced or aggressive disease? *Thyroid* 2013;23:977-81. doi: 10.1089/thy.2012.0321. PMID: 23343222.)

To clarify the meaning, we modified the description as ‘similar’ black discoloration in the adipose tissue in the Discussion, page 10, line 207.

**Revised:**

(a) Discussion

Herein, we report a case of PTC with cervical lymph node metastases in a black thyroid. Consistent with previous reports, our patient's minocycline use was the most plausible cause of black thyroid. It was discovered incidentally during surgery for thyroid cancer. In our case, black discoloration was **also seemingly** observed in the surrounding adipose tissue of the neck level IV. Pathologic examination identified brown pigmentation in the thyroid parenchyma, but not in the thyroid tumor, LNs, or surrounding adipose tissues.

**13. Line 183-4 : May the fact that "pathologic examination identified brown pigmentation in the thyroid parenchyma, but not in the thyroid tumor, LNs, or surrounding adipose tissues" mean that the tumor was not triggered by minocycline induced pigmentation ?**

→ Pigmentation found only in the thyroid parenchyma and not in the thyroid tumor is a common finding, according to the previous reports. (Reference: 1>Bann DV, Goyal N, Crist H, et al. Black thyroid. Ear Nose Throat J 2014;93:E54-5., 2> Yu NY, Gamez ME. Black Thyroid. Int J Surg Pathol. 2019;27:401-402. doi: 10.1177/ 1066896918800773.) The findings were similar in our case. However, there has been no explained mechanism for this phenomenon. Nevertheless, this can be another reason for nonsignificant relationships between minocycline and black thyroid with PTC.

Following your comment, we have added descriptions on the pathological aspect of black thyroid with PTC in the Discussion, page 9, line 173-177. We have also added comments on this phenomenon as a possible reason for nonsignificant relationship between minocycline and black thyroid with PTC in the Discussion, page 9, line 190-192.

**Added:**

(a) Discussion

In the pathological aspect, decreased pigmentation of malignant cells compared to the surrounding tissue is interestingly common in cases of black thyroid with PTC (7). In our case, the pigmentation was found only in the thyroid parenchyma, but not in the thyroid tumor. Pigmentation was also not found in the metastatic LNs, normal LNs, or background adipose tissue. This is consistent with the previous reports.

(b) Discussion



Owing to the relative scarcity of data, the clinical relevance of black thyroid in thyroid cancer remains unclear. Previous reports on higher incidence of PTC in black thyroid can be a comprehensible finding, since the recognition of black thyroid predominantly occurs during surgery. **Spared pigmentation in malignant tumors compared to surrounding tissue and unproven carcinogenic activity of minocycline still raise questions on their relationships (9,21).** Thus, proper treatment strategy has not yet been established.

**14. Line 185: Please specify what you mean by "prolonged use of minocycline". How many months ? Are there recommendation based on the literature ?**

→ We appreciate your comments. Previous literature report that minocycline-induced pigmentation occurs more frequently in patients who have taken minocycline for more than a year at cumulative doses of >100 g (Reference: Birkedal C, Tapscott WJ, Giadrosich K, et al. Minocycline-induced black thyroid gland: Medical curiosity or a marker for papillary cancer? *Curr Surg* 2001;58:470-1.) However, the duration of minocycline use prior to the discovery of black thyroid varies from 27 days to 20 years (Reference: Kondo T, Nakatsugawa M, Hirai Y, et al. Minocycline-induced black thyroid with black pigmentation of the thyroid cartilage, cricoid cartilage, and trachea found during reconstructive surgery for hypopharyngeal cancer. *Auris Nasus Larynx* 2022;49:299-303.)

Minocycline-induced black thyroid has been commonly mentioned in association with minocycline ingestion “for one year or more” in the literature (Reference: Kandil E, Khalek MA, Ibrahim WG, et al. Papillary thyroid carcinoma in black thyroids. *Head Neck* 2011;33:1735-8). Following your comment, we have specified the term for prolonged use of minocycline for ‘more than one year’ in the Discussion, page 10, line 210-212. We have also mentioned on the specified term of minocycline use based on the literature in the Introduction, page 3, line 61-64.

**Revised:**

(a) Discussion

Even with obscure clinical implications of minocycline-induced black thyroid in PTC, **higher incidence of PTC in black thyroid can warrant the avoidance of prolonged (> one year) minocycline use.** Further evaluation and treatment strategies for thyroid cancer should be

investigated in chronic minocycline users.

(b) Introduction

.. It has been reported to cause pigmentation in various tissues, including the skin, nails, oral mucosa, teeth, eyes, bones, cardiac valves, coronary vessels, substantia nigra, and atherosclerotic plaques (5). **Pigmentation can occur more frequently in patients who have taken minocycline for more than one year at cumulative doses of >100 g, although the duration of minocycline use prior to black thyroid discovery varies from 27 days to 20 years (6,7).**

**15. Is your conclusion that dermatologists and/or other physician prescribing minocycline should consider thyroid assessment (to rule out malignancy) of every minocycline treated patient? Please formulate a concrete conclusion.**

→ We appreciate your comments and suggestions. Following your comments, we have formulated a conclusion: “Even with obscure clinical implications of minocycline-induced black thyroid in PTC, higher incidence of PTC in black thyroid can warrant the avoidance of prolonged (> one year) minocycline use. In the absence of adequate evidence, prolonged minocycline users should ideally undergo routine thyroid assessment to identify possible malignancy.” in the Discussion, page 10, line 210-213).

**Revised:**

(a) Discussion

Even with obscure clinical implications of minocycline-induced black thyroid in PTC, **higher incidence of PTC in black thyroid can warrant the avoidance of prolonged (> one year) minocycline use. In the absence of adequate evidence, prolonged minocycline users should ideally undergo routine thyroid assessment to identify possible malignancy.**

**Reviewer B**

**1. This paper is a case report of a patient with a black thyroid found during thyroidectomy.**

**This condition, based in the literature existent, does not have clinical implication in the thyroid diseases. There is no correlation in the cancer development or in the prognosis of the patient. Therefore the presence of this findings do not interfere in the management of**

**the patient, been a curious finding during surgery.**

**It is an interesting case report because of the visual aspect of the gland. Unfortunately, the picture was taken after excision of the thyroid. Usually the gland tend to became darker after excision and the difference in coloration in the author's picture does not appear so well. I suggest in future events, the reminder to take a picture in vivo, if possible.**

**It was a well written report with a good review of the subject. Besides the care taking the picture I don't have further corrections to make. If more pictures are available, I suggest to send them for better illustration of the case.**

→ We deeply appreciate your thoughtful review and comments. Unfortunately, we did not take the picture of the black thyroid before thyroid excision during surgery. The provided figures are from the best pictures that we have. In future events, we will ensure to take pictures in vivo, following your comment. We have also asked the pathologists to take better pictures of the pathological specimens. However, please note that the provided figures were from the best pictures that they could obtain. Thank you.

### **Reviewer C**

**This case report describes the incidental finding of a black thyroid gland with papillary microcarcinoma in a patient with minocycline medication. In addition, the discussion gives an overview of the current literature. The case is well worked up and also well documented histopathological. With only very few case reports in the literature, no conclusive statement is possible.**

**I have a few questions:**

**1. Were there any known thyroid nodules in the patient's history?**

→ We deeply appreciate your thoughtful review and comments. As mentioned in the original manuscript, the patient did not have any history of thyroid disease or irradiation exposure and had no family history of thyroid cancer. This was the first incident of thyroid diseases. After revision, the sentence can be found in the Patient section of the Case presentation, page 5, line 87-88 (revised manuscript).

**Previously mentioned:**

(a) 'Patient' section in the Case presentation

She had a medical history of anxiety disorder and depression that was treated with benzodiazepines and a selective serotonin reuptake inhibitor for 10 years. She had been suffering from severe acne and rosacea for the past two years and had been taking isotretinoin and minocycline for 19 months. She did not have any history of thyroid disease or irradiation exposure, and had no family history of thyroid cancer.

**2. What was the indication of the CT scan? The cytological findings confirmed a thyroid carcinoma, so I see no clear advantage of a CT. In addition, the administration of the contrast agent must be evaluated critically.**

→ We appreciate your comment. Similar to our previous reports (Reference: 1>Kim MJ, Jung H, Park CS. Papillary thyroid cancer with suspicious uterine cervix metastasis: a case report and literature review. *Gland Surg* 2022;11:1270-1278. doi: 10.21037/gs-22-210. 2> Kim MJ, Jung H, Park CS. Incidental paratracheal air cyst in papillary thyroid cancer patient: a case report. *Gland Surg* 2021;10:2334-2339. doi: 10.21037/gs-21-139.) We perform cervical ultrasonography (US) and thoracocervical CT scan as a “routine” assessment in patients with thyroid cancer for accurate clinical staging. We also take non-contrast CT scan in patients who have allergic reactions to certain contrast agents.

In this case, extensive cervical LN metastases on US have led us to take thoracocervical CT scan to check for possible metastases in the retropharyngeal or superior mediastinal regions, and the lung. Fortunately, the patients had no allergy to the contrast agents. Following your comments, we have commented on the purpose of CT evaluation in the Patient section of the Case presentation, page 5, line 97-99.

**Added:**

(a) 'Patient' section in the Case presentation

To further evaluate metastases in the retropharyngeal, or superior mediastinal regions, and the lung, a thoracocervical 64-slice computed tomography (CT) scan with intravenous contrast was performed. CT scan showed an approximately 0.8 cm-sized, ill-defined, low-

density lesion in the left thyroid and multiple enlarged LNs with contrast enhancement in neck levels III, IV, V, and VI. None of the radiologic findings suggested pulmonary metastasis.

### **3. Did the patients undergo a radioiodine treatment?**

→ We appreciate your comment. Recently, the patients underwent 150mCi radioactive iodine (RAI) ablation treatment owing to the lymphatic invasion of the tumor, and N1 disease with 1.6 cm in largest dimension, which were intermediate risk factors for disease recurrence according to the 2015 American Thyroid Association (ATA) risk stratification system. The follow up studies have not been yet performed.

Following your comment, we have added the ‘Radioactive iodine treatments after surgery’ section in the Case presentation, page 7, line 129-137. We have commented on the indication, the process, and the post-therapeutic results of RAI ablation.

#### **Added:**

(a) ‘Radioactive iodine treatments after surgery’ section in the Case presentation

Adjuvant radioactive iodine (RAI) ablation treatment was indicated for the patient owing to the lymphatic invasion of the tumor, and N1 disease with 1.6 cm in largest dimension, which were intermediate risk factors for disease recurrence according to the 2015 American Thyroid Association (ATA) risk stratification system. One month after cessation of levothyroxine medication and the exogenous stimulation with recombinant human TSH, she received RAI ablation treatment by administration of 150 mCi I-131 with no adverse events. A whole-body scan (WBS), following therapeutic RAI ablation, showed residual uptake in the thyroid bed alone.

### **4. Did other studies report a possible detection of hyperpigmentation in fine needle aspiration in the case of a black thyroid?**

→ We appreciate your comment. As mentioned in the original manuscript, diagnosing a black thyroid using fine needle aspiration biopsy (FNAB) is unreliable and extremely rare. There is

only one report which has diagnosed the black thyroid through FNAB. The findings showed thyroid follicles with a chromatic pattern in thick lumps as well as abundant ceroid cytoplasmic pigment with inflammatory cells and homochromatic cytoplasm, consistent with black thyroid. However, the patient had no history of minocycline medication. This report was quoted in the Discussion of the original manuscript. After revision, the related sentences with the reference are located in the Discussion, page 8, line 147-149 (revised manuscript).

### **Previously mentioned:**

#### (a) Discussion

The black thyroid is an unusual and incidental finding during surgery or autopsy. Diagnosing a black thyroid using FNAB is unreliable and rarely done. Black or brown pigmentation have been scarcely reported, and mimicking factors and other differential diagnoses should also be considered (12,13). Our patient's FNAB result for the thyroid nodule was atypia of undetermined significance, with no evidence of pigmentation.

#### (b) Reference

13. Stoopen-Margain E, Aroesty SV, Montesinos LS, et al. Diagnosing Microscopic Black Thyroid with Fine Needle Aspiration: Rare but Possible. World Journal of Endocrine Surgery 2012;4:13-4.

### **Minor comments:**

#### **5. Line 60: in various tissues.**

→ We have revised the phrase 'in a variety of tissues' to "in various tissues" in the Introduction, page 3, line 60.

### **Revised:**

#### (a) Introduction

It has been reported to cause pigmentation in **various** tissues, including the skin, nails, oral mucosa, teeth, eyes, bones, cardiac valves, coronary vessels, substantia nigra, and atherosclerotic plaques (5).

**6. Line 67: thyroids.**

→ Following your comment, we have corrected the word thyroid to “thyroids” in the Introduction, page 3, line 70.

Revised:

(a) Introduction

Meanwhile, several studies have reported cases of carcinoma in minocycline-induced black thyroids. However, a causal relationship between them has not yet been demonstrated (6,11).

**7. Line 145: to treat acne.**

→ We have corrected the phrase ‘for treating’ to “to treat acne” in the Discussion, page 8, line 161.

Revised:

(a) Discussion

We recognized that the patient was on minocycline for 19 months, to treat acne and rosacea, which is a drug highly associated with the production of black pigmentation in the thyroid.