Peer Review File

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Reviewer A

Summary:

Kim and colleagues report a case of a 29-year-old female who presented with chest pain, headache, and acute brain infarcts that led to the discovery of a left 2.8 cm pheochromocytoma. Postoperatively, it was noted the patient had skin pigmentation and laboratory values consistent with adrenal insufficiency. She was monitored without steroid supplementation and her adrenal insufficiency resolved without intervention.

Page 3, line 55, Introduction. A unilateral adrenalectomy is not generally considered a factor for adrenal insufficiency in a patient with a non-functional tumor. The sentence should be re-written to be more specific to that condition, since a unilateral adrenalectomy in a patient with Cushing's or subclinical Cushing's syndrome is a factor in the postoperative development of adrenal insufficiency.

***Author's response**: We appreciate the reviewer's comment. The meaning of the non-functional tumor we defined in the text means the cortisol independent tumor. It is described except for the cortisol dependent tumor such as the Cushing's or subclinical Cushing's syndrome that you commented on. Because patients with cortisol-producing tumors (Cushing's or subclinical Cushing's syndrome) are considered at risk for adrenal insufficiency after adrenalectomy. Cases of cortisol-producing tumors should be excluded. To make it more clear, we have modified our text as advised (see Page 3, line 47-50)".*

Unilateral adrenalectomy is not generally considered a factor that increases the risk of adrenal insufficiency in patients with normal adrenocortical function, because the reserve function of the remaining adrenal gland maintains homeostasis.

Page 3, line 58, Introduction. This sentence regarding adrenal insufficiency after unilateral adrenalectomy is rare is misleading with the second part. Eller-Vainicher and colleagues reported that one out of three patients (33%) with normal preoperative HPA axis function developed postoperative hypocortisolism. In addition 'five out of nine patients showing only one altered parameter of HPA axis function before surgery developed post-operative hypocortisolism.' It is not as rare as believed per the citation. The caveat is that with only three patients with normal HPA axis, the incidence of postoperative hypocortisolism after a unilateral adrenalectomy for a normal HPA axis is not known.

***Author's response**: We appreciate the reviewer's comment. We wholly agree with the reviewer's opinion. The expression that the "incidence of adrenal insufficiency after*

unilateral adrenalectomy is rare" is not appropriate and may cause misunderstanding, so it was deleted. In addition, it was newly described by adding a new reference to indicate that the incidence of adrenal insufficiency is not rare when unilateral adrenalectomy is performed in patients without preoperative cortisol hypersecretion.

we have modified our text as advised (see Page 3, line 50-52).

Postoperative adrenal insufficiency after unilateral adrenalectomy has been reported up to 22-33% of patients without preoperative HPA axis imbalance, which is unexpectedly not uncommon.[1]

Page 3, line 71, Case presentation. For the preoperative hormonal work-up, did this include a 1 mg dexamethasone suppression test? This is the standard screening test for an adrenal incidentaloma. Were the basal ACTH and cortisol levels drawn in the morning between 8 and 9 am? 24 hour urinary free cortisol? Midnight salivary cortisol? Even one abnormality of the HPA axis may indicate a risk for postoperative hypocortisolism, albeit low.

***Author's response** : We appreciate the reviewer's comment.*
We conducted sufficient tests to evaluate HPA axis, but it was not described in the case text.
During the final diagnosis of pheochromocytoma, a hormonal study was conducted to differentiate adrenal incidentaloma. Results related to Cushing's syndrome, primary aldosteronism, and adrenal carcinoma were normal. The 1mg dexamethasone suppression test mentioned in the comment was performed, and the cortisol level was 3.22 ug/dL, which was negative. The basal ACTH and cortisol levels did not drop further when compared to those measured at 8:00 am and 4:00 pm.
ACTH am 8 : 15.73pg/mL, pm 4 : 14.42pg/mL (normal range : 10-60 pg/mL)
cortisol am 8 : 15.63ug/dL, pm 4 : 11.35ug/dL (normal range : 9.41-26.06ug/dL)
24hr urinary free Cortisol(24h) level is 24.34 ug/L/24hrs (reference range : 13.7-75.3).
Midnight salivary cortisol was not performed, because sufficient tests were performed, including a confirmatory test to rule out.

Page 3, line 74, Case presentation. Was it enquired if the patient had a family history of early heart attacks, sudden deaths, or strokes? Patient's may have a family history, but have not been diagnosed yet.

***Author's response**: As you mentioned, I agree with the statement that there is a possibility that there may be a family history that has not yet been diagnosed. However, in history taking, there was no family history of heart attacks, sudden deaths, or strokes.*

Page 3, line 74, Case presentation. Was genetic testing performed on the patient preoperatively, given the nearly 40% rate of germline mutations (whether familial or de novo)?

***Author's response**: We appreciate the reviewer's comment. In particular, it is more likely if the onset occurs at a young age like our case.*
Pheochromocytoma can present as a family syndrome, such as multiple endocrine neoplasia (MEN2), von Hippel-Lindau disease(VHL), and type 1 neurofibromatosis(NF1). [2] Among them, a RET gene mutation test corresponding to MEN2 was performed before surgery, and the result was negative.

Page 4, line 86, Case presentation. Was an ACTH stimulation test performed when the skin pigmentation occurred? Or when the cortisol levels were deemed low?

***Author's response**: We appreciate the reviewer's comment. The ACTH stimulation test, also known as the Synacthen test, is a standard screening test for diagnosing adrenal insufficiency.*
On the third day after surgery, the day of pigmentation occurred, ACTH and cortisol were collected, respectively. Cortisol level was normal (8.62ug/dL) and ACTH level was mildly elevated (75.70pg/mL) (table 2). Except for pigmentation, there were no other symptoms to suspect adrenal insufficiency or adrenal crisis. ACTH stimulation test was performed on the first month after surgery, and it was reported that there was no adrenal insufficiency (table 3).

Page 5, line 117, Discussion. The authors should discuss the risk of co-secretion from a pheochromocytoma, as this may be another reason that the patient had postoperative hypocortisolism. This may be less likely in this case given the normal ACTH levels, though.
***Author's response**: As the reviewer pointed out, there may be co-secretion in case of pheochromocytoma. I'll mention it in Discussion. (see Page 6, line 124-127).*

Although very rare, dual hormone secreting pheochromocytoma with ACTH should also be considered. We will also consider the ACTH immunohistochemistry whether the phenomenon is caused by co-secretion of pheochromocytoma.

Page 7, line 147, Discussion. The authors should discuss routine cosyntropin testing postoperatively as steroid replacement can be dictated based on those test results.

***Author's response**: We appreciate the reviewer's comment. Postoperative adrenal insufficiency mostly occurs in the adrenal tumor that secretes cortisol, but it can even occur in the nonfunctional tumor and can be potentially fatal. A diagnostic tool after surgery is important. The most well-known standard method is the Cosyntropin test.*
According to a recent study by Shamalia Zama and colleagues, cortisol cut offs used in the cosyntropin test for predicting adrenal insufficiency after unilateral

adrenalectomy for non-cortisol secreting adrenal tumors can be unaccurately. [3]
In addition, postoperative adrenal insufficiency can be defined based on cortisol routinely measured at AM 8 on the pod 1 day in patients who underwent unilateral adrenalectomy for nonfunctional adrenal lesions, and glucocorticoid replacement was performed based on cortisol level. [4]

We delete the sentence "therefore, there are no accepted guidelines regarding its diagnosis and treatment." in Page 7, line 151-158, Discussion, and we have changed the "Discussion" section accordingly.

Therefore, a diagnostic tool of postoperative adrenal insufficiency is important. The most well-known standard method is the Cosyntropin test. However, according to a recent study by Shamalia Zama and colleagues, cortisol cut offs used in the cosyntropin test for predicting adrenal insufficiency after unilateral adrenalectomy for non-cortisol secreting adrenal tumors can be unaccurately. Mitchell et al. showed that routine cortisol testing at 8:00 a.m. after surgery was helpful in diagnosing postoperative adrenal insufficiency and determining steroid replacement.

Page 7, line 167, Discussion. If the patient is going to be observed without steroid replacement, what would trigger the authors to provide steroids? Clinical symptoms? Cortisol level less than…?

***Author's response**: We appreciate the reviewer's comment. In response to your comment, both clinical symptoms and cortisol level will be the answer. Our patient had no symptoms other than mild fatigue and pigmentation as evidence to suspect adrenal insufficiency.*
If other symptoms such as hypotension, hypoglycemia or dehydration were present, appropriate steroid replacement would have been performed.
It can also be considered when the cortisol level is reduced, and in the reference paper, when the cortisol level measured at 8:00 am on postoperative day 1 is less than 3.4ug/dl, steroid replacement is recommended. [4]

Minor Comment
Page 7, line 157, Discussion. "synaptic" is misspelled.
***Author's response**: We appreciate for finding our mistake. We corrected it to "synaptic" > "synacthen". Thank you for the opportunity to correct the mistake according to the reviewer's point.*

FOR EDITOR
The initial hormonal work-up is not thorough enough to exclude subclinical hypercortisolism or cyclical subclinical hypercortisolism. Therefore, it is difficult to draw conclusions as to whether this is a case of a pheochromocytoma status post unilateral adrenalectomy without sufficient adrenal reserve on the right side versus a case of co-secretion of pheochromocytoma plus subclinical hypercortisolism.

Reviewer B

Line 35, 72: “serum ACTH and cortisol levels were within normal ranges”. Can the authors provide some additional details about the preoperative ACTH/cortisol testing? Were these random ACTH/cortisol levels? Morning levels? Obtained after a dexamethasone suppression test?

***Author's response**: We appreciate the reviewer's comment. We conducted sufficient tests to evaluate HPA axis, but it was not described in the case text. During the final diagnosis of pheochromocytoma, a hormonal study was conducted to differentiate adrenal incidentaloma. Results related to Cushing's syndrome, primary aldosteronism, and adrenal carcinoma were normal. The 1mg dexamethasone suppression test mentioned in the comment was performed, and the cortisol level was 3.22 ug/dL, which was negative. The basal ACTH and cortisol levels did not drop further when compared to those measured at 8:00 am and 4:00 pm.*
ACTH am 8 : 15.73pg/mL, pm 4 : 14.42pg/mL (normal range : 10-60 pg/mL)
cortisol am 8 : 15.63ug/dL, pm 4 : 11.35ug/dL (normal range : 9.41-26.06ug/dL)
24hr urinary free Cortisol(24h) level is 24.34 ug/L/24hrs (reference range : 13.7-75.3).

Line 72: I see the reference ranges for cortisol and ACTH in the tables, but can the authors include ranges for normal reference ranges for ACTH and cortisol in the text as well?

***Author's response** : We appreciate the reviewer's comment. We changed sentences to include ranges for normal reference ranges to avoid confusion. (see Page 4, line 82,84)*

Line 82: Is there a protocol used at your institution for postoperative biochemical testing after adrenalectomy? What are the discharge criteria and typical length of stay? Would be helpful to add so that readers can have an understanding of whether these levels were obtained standardly or as a result of patient symptomatology.

***Author's response**: We appreciate the reviewer's comment. In our hospital, in the case of non-functional tumor, patients are usually discharged on the 3rd day after surgery. Basic CBC, electrolytes are measured every day after surgery, and discharge is decided when recovery of general condition and no abnormalities are found in the lab. We added this in case presentation. (page 4, line 74)*

Line 74: When there were no abnormalities in clinical symptoms, complete blood count(CBC), and electrolytes, it was decided to be discharged on the 3rd day after surgery, and this patient also prepared for discharge with normal course.

Line 82: Were there any other signs or symptoms of adrenal insufficiency other than hyperpigmentation? Were mineralocorticoid levels checked postoperatively?

***Author's response**: We appreciate the reviewer's comment. Our patient had no symptoms other than mild fatigue and pigmentation as evidence to suspect adrenal insufficiency. We measured cortisol levels every day after surgery. The results are shown in table 2. However, the mineralocorticoid was not measured after surgery.*

Line 61, 163-164, 171: I would reconsider statements declaring this patient had adrenal insufficiency. For the most part, (1) her adrenals were largely able to maintain normal cortisol levels postoperatively, (2) no other symptoms of adrenal insufficiency other than hyperpigmentation (see comment above) were reported, (3) her ACTH stimulation test was normal, and (4) she never required steroid replacement therapy. It appears the authors may have considered this as the language in the abstract does not declare the patient was adrenally insufficient (lines 27-29, 44-45). I would consider revising the statements in the main body of the manuscript to mirror those in the abstract for the sake of precision of language/terminology.

***Author's response**: We appreciate the reviewer's comment. 1)As described, the cortisol level decreased mildly on the first day after surgery and returned to normal on the day of discharge, there was a temporary decrease in cortisol level after surgery (Case presentation line 81-82).*
2)As mentioned in the statement (Case presentation line 78), mild general weakness was accompanied when pigmentation occurred. Fatigue, one of the symptoms of adrenal insufficiency can be considered to have appeared. 3)The ACTH stimulation test was normal, but it was performed one month after surgery, and skin pigmentation, a clinical manifestation of adrenal insufficiency, was improved at that time. Although the ACTH stimulation test was not performed at the time pigmentation appeared, Cortisol level was normal (8.62ug/dL) and ACTH level was mildly elevated (75.70pg/mL) (table 2). Therefore, this case can be considered as temporary adrenal insufficiency after surgery. 4)There are few guidelines for the selection criteria for patients who require the precisely replacement of perioperative steroids. As there is a study suggested that postoperative steroid replacement is not required for patients receiving adrenalectomy for a unilateral non-Cushing adrenal tumor. [5] Therefore, it cannot be concluded that there is not adrenal insufficiency just because a steroid replacement is not required.
In the abstract, the terminology of adrenal insufficiency was not used in the background (lines 6-8, 23-24) because the phenomenon had to be explained, but in the conclusion part, adrenal insufficiency, which coincided with the main body, was described (line 25). The principle of transient adrenal insufficiency after surgery in our patient can be understood as an increase in ACTH secretion from the pituitary gland to compensate for adrenal insufficiency in patients with postoperative adrenal reserve function decline.

Minor comments:
Line 38: Would recommend changing "hypochondriac" to "subcostal". At least in American English, hypochondriac more commonly refers to the psychiatric condition

of worrying about one's health condition rather than an anatomical location.

***Author's response**: We appreciate for finding our mistake. We changed "hypochondriac" to "subcostal". Thank you for the opportunity to correct the mistake according to the reviewer's point.*

Overall this is a well-written and well-presented case report describing a unique phenomenon that I believe would be educational to the readership.

Reviewer C

The authors present a case of Unusual skin pigmentation after unilateral adrenalectomy due to pheochromocytoma and hypothesise that the skin pigmentation appeared due to an imbalance of the hypothalamic–pituitary–adrenal (HPA) axis after resection of PCC. There are some grammatical errors that needs to be addressed in the manuscript.

There are some questions for the authors:

1. What anticoagulation was started for the patient and was it continued perioperatively?

***Author's response**: We appreciate the reviewer's comments. The anticoagulant was warfarin, and heparin bridging protocol was performed 1 week before surgery. Before surgery, echocardiography confirmed no intracardiac thrombus. After consultation with the cardiologist, no additional anticoagulant was administered.*

2. In the discussion the authors mention of synaptic stimulation test. Should read as synacthen.

***Author's response** : We appreciate for finding our mistake. We corrected it to "synaptic" > "synacthen". Thank you for the opportunity to correct the mistake according to the reviewer's point.*

3. There are reports of PCC's secreting ACTH and CRH which may explain the marginal increase in the hormone profile post surgery (refer to Zhang X, Lian P, Su M, Ji Z, Deng J, Zheng G, Wang W, Ren X, Jiang T, Zhang P, Li H. Single-cell transcriptome analysis identifies a unique tumor cell type producing multiple hormones in ectopic ACTH and CRH secreting pheochromocytoma. Elife. 2021 Dec 14;10:e68436. doi: 10.7554/eLife.68436. PMID: 34905486). Perhaps the authors can consider doing ACTH immunohistochemistry to ascertain if this is the case and would support the hypothesis.

***Author's response**: We appreciate the reviewer's comment. Thank you for recommending high-quality references. In this paper, an extremely rare type of pheochromocytoma that secretes both ACTH and CRH is reported and emphasizes the importance of proper diagnosis. As the reviewer pointed out, there may be*

co-secretion in case of pheochromocytoma. I'll mention it in Discussion. In our case, we will also consider the ACTH immunohistochemistry as recommended by the reviewer to confirm whether the phenomenon is caused by co-secretion of pheochromocytoma.

Page 6 line 124-127 Although very rare, dual hormone secreting pheochromocytoma with ACTH should also be considered. We will also consider the ACTH immunohistochemistry whether the phenomenon is caused by co-secretion of pheochromocytoma.

Reviewer D

This case report is somewhat worth considering its contribution to expanding our current knowledge.
So, I would recommend it for acceptance after some points listed below.

・In the present case, although pigmentation is present, it has not led to adrenal insufficiency. Are there any reports of adrenal insufficiency after pheochromocytoma surgery?

***Author's response**: We appreciate the reviewer's comment. As mentioned, pigmentation is a minor manifestation to be seen as evidence of adrenal insufficiency, but adrenal insufficiency can be diagnosed due to the increase in ACTH level along with pigmentation. There are case reports of adrenal insufficiency that occurred after unilateral adrenalectomy for adrenal non-functional tumor, adrenocortical carcinoma or renal cell carcinoma.*
Adrenal insufficiency after unilateral adrenalectomy for pheochromocytoma was reported as a dual hormone secreting pheochromocytoma, and the prevalence is less than 1%. [6]

・Skin pigmentation is also observed in neurofibromatosis, so the author should mention about this.

***Author's response**: We appreciate the reviewer's comment. Neurofibromatosis type 1 (NF1) is an autosomal dominant disease associated with gene mutations, and skin lesions such as café-au-lait macules play a key role in this diagnosis. When cafe-au-lait skin pigmentation occurs in a patient, we should be considered including neurofibromatosis, which you commented on, as a differential disease for diagnosis. However, the typical cafe-au-lait skin pigmentation seen in NF1 is round or oval shape, which is different from our patient's crescent shape. Also, most of the skin pigmentation of NF1 occurred at a young age, but in our case, patient's skin pigmentation occurred after the event of surgery and was normalized and disappeared, NF1 can be clearly excluded as a causative disease. [7]*

We added some sentences about neurofibromatosis type 1 (NF1). (see Page 6, line 135-139)

When cafe-au-lait skin pigmentation occurs in a patient, should be considered including neurofibromatosis type 1 (NF1). The typical cafe-au-lait skin pigmentation seen in NF1 is round or oval shape, which is different from our patient's crescent shape. Also, most of the skin pigmentation of NF1 occurred at a young age, but in our case, pigmentation occurred after the event of surgery and was disappeared, NF1 can be clearly excluded as a causative disease.

Reference

1. Mitchell, J., et al., *Unrecognized adrenal insufficiency in patients undergoing laparoscopic adrenalectomy.* Surg Endosc, 2009. **23**(2): p. 248-54.
2. van Berkel, A., J.W. Lenders, and H.J. Timmers, *Diagnosis of endocrine disease: Biochemical diagnosis of phaeochromocytoma and paraganglioma.* Eur J Endocrinol, 2014. **170**(3): p. R109-19.
3. Zaman, S., et al., *Synacthen Stimulation Test Following Unilateral Adrenalectomy Needs to Be Interpreted With Caution.* Front Endocrinol (Lausanne), 2021. **12**: p. 654600.
4. Ortiz, D.I., et al., *Cosyntropin stimulation testing on postoperative day 1 allows for selective glucocorticoid replacement therapy after adrenalectomy for hypercortisolism: Results of a novel, multidisciplinary institutional protocol.* Surgery, 2016. **159**(1): p. 259-65.
5. Shen, W.T., et al., *Selective use of steroid replacement after adrenalectomy: lessons from 331 consecutive cases.* Arch Surg, 2006. **141**(8): p. 771-4; discussion 774-6.
6. Sjoeholm, A., et al., *Adrenal insufficiency in a child following unilateral excision of a dual-hormone secreting phaeochromocytoma.* Endocrinol Diabetes Metab Case Rep, 2015. **2015**: p. 150041.
7. Ozarslan, B., et al., *Cutaneous Findings in Neurofibromatosis Type 1.* Cancers (Basel), 2021. **13**(3).

RE-Review Comments

Reviewer C

Dear authors

The manuscript requires some minor revisions.

Abstract

Line 26: change an to a case of..

Author's response *: We have revised the phrase 'an' to "a case of" in the abstract, page 1, line 28.*

Revised:
abstract, background
: We report a case of unusual transient hyperpigmentation that developed immediately after unilateral adrenalectomy for pheochromocytoma and spontaneously resolved without corticosteroid supplementation.

Line 41: delete was

Author's response *: We have deleted 'was'. page 2, line 43.*
Revised:
abstract, case description
: On day 15, the pigmentation clearly disappeared and serum ACTH decreased to within the normal range. A month later, ACTH and all adrenal hormones were within normal range.

Line 45: change a to the

Author's response *: We have corrected the phrase 'a' to "the" in the Conclusions, page 2, line 47.*
Revised:
abstract, Conclusions
: Skin pigmentation may be the first and early manifestation of adrenal insufficiency in patients who undergo unilateral adrenalectomy due to a non-Cushing's tumor.

Line 46: change underwent to undergo

Author's response *: We have revised the phrase 'underwent' to "undergo" in the abstract, page 2, line 48.*
Revised:
abstract, Conclusions
: Skin pigmentation may be the first and early manifestation of adrenal insufficiency in patients who undergo unilateral adrenalectomy due to a non-Cushing's tumor.

Introduction
Line 59: to read as …reported in up to

Author's response *: We have revised the phrase 'reported up to' to "*reported in up to*" in the Introduction, page 3, line 58.*
Revised:
Introduction
: Postoperative adrenal insufficiency after unilateral adrenalectomy has been reported in up to 22-33% of patients without preoperative HPA axis imbalance, which is not uncommon.

Line 60: delete unexpectedly

Author's response *: We have deleted* 'unexpectedly*'. page 3, line 59.*
Revised:
Introduction
: Postoperative adrenal insufficiency after unilateral adrenalectomy has been reported in up to 22-33% of patients without preoperative HPA axis imbalance, which is not uncommon.

Case presentation
Lines 91-93: When there were no abnormalities in clinical symptoms, complete blood count (CBC), and electrolytes, it was decided to be discharged on the 3rd day after surgery, and this patient also prepared for discharge with normal course.

Please change to something like patient was discharged on day 3 following surgery with no clinical symptoms and normal blood tests (CBS and renal panel).

Author's response *: We appreciate your comments and suggestions. Following your comments, we have formulated a case presentation, page 4, line 81-82.*
Revised:
Case presentation
: Patient was discharged on day 3 following surgery with no clinical symptoms and normal blood tests (Complete blood count and renal panel).

Line 177: change … that occurred… to that occurs

Author's response *: We have revised the phrase 'occurred' to "*occurs*" in the Discussion, page 7, line 156.*
Revised:
Discussion
: Because of compensatory action, most adrenal insufficiency that occurs after unilateral adrenalectomy is not obvious and is overlooked.

Line 182: change ..can be unaccurately to can be inaccurate

Author's response *: We have revised the phrase 'unaccurately' to "*inaccurate*" in the Discussion, page 7, line 162.*
Revised:
Discussion
: However, according to a recent study by Shamalia Zama and colleagues, cortisol cut offs used in the cosyntropin test for predicting adrenal insufficiency after unilateral adrenalectomy for non-cortisol secreting adrenal tumors can be inaccurate.

Line 184: Kindly change the sentence to: There are debates for steroid replacement after adrenalectomy.
The use of routine steroid replacement following adrenalectomy in non-Cushing's adenoma or subclinical Cushing's is controversial.

Author's response *: We have revised the sentence as your recommendation, in the Discussion, page 7, line 163-164.*
Revised:
Discussion
: The use of routine steroid replacement following adrenalectomy in non-Cushing's adenoma or subclinical Cushing's is controversial.