Author's response to reviews

Title: Metabolic alteration of urinary steroids in pre- and post-menopausal women, and men with papillary thyroid carcinoma

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Author's response to reviews: see over
Thank you for your e-mail dated April 27, 2011 on the comments of reviewers about our manuscript (ref #: 1840262401525881) entitled; “Metabolic alteration of urinary steroids in pre- and post-menopausal women, and men with papillary thyroid carcinoma”. The some text of the manuscript was rewritten (red colored in the text) for the better understanding of our work based on reviewer’s comments.

I hope that the work will receive a fair and expedient review. Thank you in advance for all of your efforts.

Sincerely yours,

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Reviewer #1:

This is a well elaborated manuscript dealing with the urinary steroid profile in 3 groups (men, pre- and post-menopausal women) of patients with papillary thyroid carcinoma (PTC) compared to gender, age and menopausal status controls. The work is original as there are no previous reports on the urinary steroid metabolites in such patients. The main findings are increased excretion of some androgen, estrogen (except for 2-hydroxy-17β-estradiol that was reduced), glucocorticoid metabolites and cholesterol in patients compared to controls.

However,

Major Compulsory Revisions

1. The clinical significance of the results is not adequately discussed. The rational behind investigating a possible link between PTC relies on the clinical observation that PTC is more frequently encountered in women and on ex vivo data reporting up-regulation of both ERα and AR in PTC cells compared to normal thyroid cells. The rational behind studying the mineralocorticoid and glucocorticoid metabolites in the clinical setting of PTC is not given. Moreover the urinary metabolite excretion is expected to differ between the three PTC groups (pre-menopausal women, postmenopausal women and men) because of the intrinsic differences in the hormonal profile among men and women and among pre- and postmenopausal women. Therefore this analysis does not add information regarding PTC-related alterations.

<Reply>
To reveal the menopausal and gender differences, all quantitative data obtained from the patients were normalized by the mean values of the corresponding control subjects.

2. It should be emphasized that this is a cross-sectional study and no conclusions regarding possible pathogenetic mechanisms can be extracted. This is not clearly given in the paper and any statement about possible pathogenesis should be made with great caution.

<Reply>
We have tried to explain our results on the possible pathogenesis with great caution as you pointed out.
3. The abstract’s conclusion “… examining the effect of various treatments for PTC regardless of gender and menopausal conditions” is not supported, as no data on effect of treatment is given. Also, since there is no prospective evaluation of the patients, the authors’ conclusion statement: “the thyroid cancer risk was found to be associated with…” is not substantiated by these data.

<Reply>
The two sentences indicated have been removed.

4. The laboratory methods are well described, important clinical data, however, is lacking. The authors state that the diagnosis in all patients was based on pathologic examination, this means that they were all operated? If so, no data regarding the histology of PTC, staging, previous treatment, time from treatment to enrolling and current burden of disease in the study population are given. This information is important because one would expect that if a link between altered steroid metabolism and PTC existed, an association between the severity of PTC and the steroid profile ought also to be observed. Moreover, patients with a history of PTC are usually on thyroid hormone suppressive treatment and it is possible that this excess of thyroid hormones could influence the urinary metabolite profiling. Therefore adjustment for thyroid hormone status is also required (patients are expected to have higher levels of thyroid hormones compared to normal controls) and a comment on how thyroid hormone over-replacement potentially influences the urinary steroid profile should be added. Furthermore, it is known that some the studied metabolites (e.g. the androgen, estrogen, glucocorticoid and mineralocorticoid metabolites) are also influenced by BMI and adiposity. Are the groups matched for BMI?

<Reply>
We appreciate your great comments on the sampling. The information of the clinical subjects has been prepared in detail. As you can see, all groups were matched for BMI.

5. The results are given in a rather complicated manner. They should be presented in a more meaningful way, probably summarizing the main findings according to steroid hormone groups. Moreover it is not clear which enzyme’s activity the urinary steroid ratios reflect and what the observed differences really mean. There seems to be confusion between mineralocorticoid and glucocorticoid precursors and metabolites. For example corticosterone is a precursor of aldosterone in humans whereas tetrahydrocortisol, a metabolite of cortisol.
Their metabolic pathways, actions and regulation of secretion are different. Therefore they should not be evaluated together as “corticoids”. The discussion section needs to be revised so that the main findings and their possible clinical implications are more clearly presented. Any conclusions regarding pathogenetic mechanisms are not adequately supported by the given data.

<Reply>
We have rewritten many sentences for better understanding in the whole text, and especially it has been explained by two different types of corticoids as your comments.

More specific comments:
Some data (including p values) are given in the discussion section instead of the results section.

<Reply>
It has been corrected.

6. The discussion regarding TSH levels is inadequate, especially since they do not provide data on the subjects’ TSH levels and they do not adjust for TSH levels. The point about lipogenesis seems unrelated to this study. The limitations of the work are not clearly stated. Inadequate sampling is a major concern in 24-hr urinary collections. How did the authors ensure adequate 24-hr urinary collections? Other limitations include the lack of clinical data and especially of thyroid hormone status of the investigated subjects, the design of the study (observational, cross-sectional) that cannot provide information about causality.

<Reply>
A sentence related on TSH has been removed and the present study was conducted with first-morning urine when the subjects were checked up in the medical centers. Detailed was indicated in the section of method.

7. Some sentences are not well understood, specifically: “The presence of steroid metabolic enzymes suggests that the activation of steroids to potentially reactive metabolites may play a role in endocrine diseases along with sexual and menopausal dysfunction”. The recording of this sentence is irrelevant since it refers to sexual dysfunction in men and women with endocrine disorders.
“The incidence of PTC is significantly higher in pre-menopausal women than men [1,2] because of the higher activities of ER than AR in PTC cells”. The reason for the higher incidence of thyroid cancer in women rather than men is not so simple.

“The normalized androgens levels in PTC men were significantly higher than normalized pre- or post-menopausal PTC women (androstenediol, P<0.005; 16-hydroxy- DHEA, P<0.002) through an AR effect in PTC”. Men do have higher levels of androgens compared to women (through testosterone production by the testes, not through an AR effect in PTC!).

Minor Essential Revisions

1. What are the possible mechanisms for decreased 2-hydroxyestrone levels in PTC patients?

It has been added in the section of discussion.
Reviewer #2:

This paper by Man Ho Choi et al, supports that the variation in steroid levels explain the increased incidence of PTC in premenopausal compared to potmenopausal women and men. This is an interesting issue especially to scientists with closely related research interests and provides an important input on the explanation of the influence of gender differences and menopausal conditions in PTC incidence.

As the main point of the paper is the influence of steroid levels in the PTC incidence, the background knowledge and the discussion has to focus on the existing data about steroids and PTC. The receptors (Era-Erb, AR) have to be mentioned in brief as a possible pathogenetic mechanism. The main criticism is that the way the data are presented and discussed is a little chaotic and thus makes the reading and the understanding of the paper difficult.

<Reply>
The description related on the steroid receptors have been minimized in the text as you pointed out.

Instead of referring to the various estrogens, androgens, corticoids, in the various studied groups, I suggest one Table with the statistically significant differences of normalized for controls values in pre-, postmenopausal women, men. The rest non significant differences or the absolute values should be described in a dense way in the Results. Also, instead of referring to various steroids it helps to categorize in power or less active estrogens and in parentheses the individual steroids) especially in the Discussion.

<Reply>
A new table containing the statistically significant steroids with references is very good idea, but there was just a few references can be explained in PTC and steroid profiling studies, and Table has been omitted. We have explained more focused on active estrogens.

Try to make clear why estrogens and not androgens are responsible for the increased PTC incidence in the premenopausal women, as both are increased in this group.

<Reply>
It has been described in the section of conclusion.

Is a simple early morning urine sample enough to have accurate evaluation of excreted
steroids?

<Reply>
The urine samples were taken when the subjects came in medical center for checking up and it was not able to maintain as the 24-hr urine.

It would be helpful to have the staging of the PTC. Do the observed differences in steroid levels correlate with the staging? At which time point the patients were evaluated? Were they hypothyroid after thyroidectomy? Did they receive a suppressive dose of thyroxine and have subclinical hyperthyroidism? Does the thyroid status in terms of thyroid hormone deficiency or increased thyroxine dose bias the steroid levels, or in other words the steroid metabolism? Did the premenopausal women receive contraceptives or the postmenopausal women estrogen replacement therapy.? As you mentioned every medication has been stopped for a defined period What is that?

<Reply>
The sampling information have been modified for better understanding.

In the discussion, there is no need to repeat the results and their statistical significance. This has been done in the results. The discussion has to be organized based on the results but this has to be more dense trying to discuss the main points of the paper, 1. levels of estrogens, androgens, corticoids and relationship to PTC development or progression 2. bad and good estrogens and PTC 3. possible explanations

<Reply>
We have rewritten the sentences for better understanding as your comments.

Discussion about the corticoids, their significance and explanations for the observed or onobserved differences?

<Reply>
It was experimental results.

The conclusion is not representative of the work done.

<Reply>
The conclusions have been rewritten.