Reviewer’s report

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

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Reviewer: DIMITRIOS HADJIDAKIS

Reviewer’s report:

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.

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.

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.

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?

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.

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

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.

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 in 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?

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**

'I declare that I have no competing interests'