Reviewer's report

Title: Endogenous melatonin and oxidative guanine DNA base damage

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Reviewer: Eva S Schernhammer

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General comments:

This is a cross-sectional study to evaluate associations between overnight urinary aMT6s levels and markers for oxidative damage (8-oxodG and 8-oxoGua). The reasoning behind choosing these two markers for oxidative damage has been well justified. However, there are several important flaws in the design, methods, and presentation of this work.

Major compulsory revision:

The summary of results in the abstract is unclear and not well structured.

Background: Much of what is said in the lengthy background section appears more appropriate for the discussion section. Moreover, no clear case has been laid out for this study and its specific design. For example, what was the rational to choosing family pairs in this context? A sentence that synthesizes why the two chosen markers of repair / oxidative damage were important in conjunction with melatonin, and a hypothesis (i.e., which direction do the authors expect these associations to go?) are warranted at the end of the introduction.

Methods: Basic information is missing. For example: where (in which country) is the study performed at all? How were these family pairs recruited (advertisement, hospital, etc?), and, as queried above, - what was the rational for recruiting family pairs as opposed to say random volunteers? Much questionnaire based information is collected and should be made us of in the analyses. Height and weight are collected, but weight, rather than more appropriately, BMI, has been used in the analyses. What is the exact time frame for overnight urine collection (i.e., 7pm until 8am, etc?). Is urinary creatinine correlated with age, BMI, etc? What lab was used to conduct aMT6s and oxidative damage marker assays? To compare three means (mother, father, daughter groups), not the paired t-test but ANOVA should be used.

Results: The authors state that current smoking was not associated with levels of 8-oxodG or 8-oxoGua, but they fail to report on the association between smoking and urinary melatonin. They say that there were some assay problems (n=XX?), levels below the detection limit of the assay (n=XX?), and outliers (n=XX?), but they lead to the exclusion of >1/3 of the entire sample, so this warrants further explanation and consideration in terms of how this may have biased their results.
The statement “there were a few subjects who were obese” should be replaced by the average BMI for each of the three groups and their SD/range. Multivariate adjustments (most importantly, adjustment for age and BMI, given their strong association with aMT6s), and not univariate results, should be presented. And BMI, not weight, should be adjusted for. Also, how does menstrual cycle variation impact oxidation levels? Is it possible that for the presumably mostly premenopausal daughters their aMT6s levels were not comparable because not collected at the same day within their menstrual cycle (i.e., luteal or follicular phase)? The single daughter with a weight of 358 pounds should not be treated as an “outlier”, given such a weight is in the range of plausibility. Thus, her exclusion appears not warranted, and BMI should be used instead. The presentation of results in bullet points is not appropriate.

Discussion: No discussion of their results in the context of prior literature is provided. Are their findings in line with the authors’ hypotheses and supported by other, even if indirect evidence?

Table 1 should display BMI rather than weight. It should also show smoking rates, reproductive history (parity), and other covariates that were collected on their questionnaire. Table 2 should display results after adjustment for age and BMI. Also, there is no prior hypothesis as to why the mother-father-daughter pairs were chosen and what this would add, as well as why one would want to compare them with each other. So I would deleted Table 2 and consider presenting the data for all three groups combined, adjusting for age, gender, and BMI (and in secondary analyses, also for smoking status).

Table 3 shows p for trend; it would be useful to show this in the form of geometric means, by quantile of DNA repair product. Table 4 should show partially adjusted correlations (adjusted for age and BMI).

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

**Quality of written English:** Acceptable

**Statistical review:** Yes, and I have assessed the statistics in my report.

**Declaration of competing interests:**

I declare that I have no competing interests