Author's response to reviews

Title: DNA repair deficiency in peripheral blood lymphocytes of endometrial cancer patients with a family history of cancer

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Version: 2 Date: 16 May 2014

Author's response to reviews: see over
Dear Editor,

We are pleased to submit the revised version of MS: 1843428373114847 Research article “DNA repair deficiency in peripheral blood lymphocytes of endometrial cancer patients with a family history of cancer” to BMC Cancer.

We would like to thank the reviewers for careful and thorough reading of this manuscript and for the thoughtful comments and constructive suggestions, which help to improve the quality of this manuscript. Our responses follow (the reviewer’s comments are in italics).

**Editorial Requirement**

*Consent statement:*

Please state in the Methods section whether written informed consent for participation in the study was obtained from participants or, where participants are children, a parent or guardian.

Reply: We have stated in the Patients & Methods section that written informed consent for participation in the study was obtained from all patients and healthy individuals.

**Response to Referee #1 Comments**

1) *In the methods part (page 5) authors mentioned that EC patients underwent genetic consultation. Could authors explain what kind of genetic consultation patients underwent?*

Reply: We used a standardized questionnaire to collect information on personal and family history of cancer in first, second and third degree relatives including age of cancer diagnosis and peculiarities of the disease.

2) *In the same part authors say that they selected 30 % of the EC patients with a strong family history of cancers of female reproductive organs: Could authors clarify: is it 30% of all unselected EC patients have had strong family histories of cancers of female reproductive organs?*

Reply: The correction has been made. Twelve of the forty patients had a family history of cancer.

*What types of cancers of female reproductive organs?*

Reply: These were endometrial and ovarian cancers.

**Response to Referee #2 Comments**

*Major comments:*

1) *The number of healthy control donors is with only ten individuals 4.5-times lower than the number of endometrial cancer patients.*
This needs to be addressed in more detail in the discussion. I strongly recommend having a higher number of age-matched healthy individuals. If this is impossible it has to be at least shown by the authors that the sample size is sufficient to yield good statistical power.

Reply: We agree that a limitation of this study is that the numbers of patients and controls were relatively small. We’ve mentioned this in the discussion. Although the current study is based on a small sample of participants, the sample size was enough to obtain sufficient statistical power. We’ve stated this in the Patients & Methods section. P-values are shown for all statistically significant differences.

2) The authors need to address confounding factors such as life style, work environment, medication, alcohol consumption and smoking status of the blood donors by elaborating on the individuals’ questionnaire in the material and methods section. These variables may need to be addressed in the statistical analysis.

Reply: Each woman was asked to complete a questionnaire establishing family history of cancer and details of her lifestyle, including occupational exposure, diet, medical conditions, smoking status and alcohol intake. Only women with approximately the same lifestyle were included in the study.

3) Did the patients receive radio- and/or chemotherapy? If yes, was blood taken before the start of the treatment?

Reply: The lymphocytes were collected from peripheral blood of patients before they had received any chemotherapy or radiation therapy.

Minor comments:

1) Abstract:
- The authors should mention that the difference of the levels of spontaneous DNA damage (= baseline damage) in lymphocytes of patients and healthy individuals was indeed significant as stated in the results section.

Reply: The correction has been made.

2) Introduction:
- It should read “… increased frequencies of chromosomal aberrations and breaks at fragile sites …”

Cytogenetic damage generally includes numerical abnormalities (e.g. due to an aneugen) and chromosomal aberrations (e.g. due to a clastogen) and I assume the authors meant the latter.

There are more than 120 known fragile sites in the human genome. Did the authors intend to say that their actual number increases in lymphocytes of cancer patients?

Reply: We mean “… chromosomal aberrations and breaks at fragile sites…” and intend to say that number of breaks at fragile sites increases in lymphocytes of cancer patients.

3) Patients & Methods:
- State the exact number of patients with a familial history of cancer.
Reply: The correction has been made. Twelve of the forty patients had a family history of cancer.

- It should read “... low-melting point agarose ...”

Reply: The correction has been made.

- Write “... treated with bleomycin (20 µg/ml) in phosphate buffered saline (PBS), pH 7.4, for ...”

Reply: The correction has been made.

- It is not clear when the treatment occurred. The way it is written it indicates a treatment and recovery of the cells while being embedded in agarose. Is this correct?

Reply: Yes. Treatment with bleomycin and recovery were conducted while lymphocytes were embedded in agarose. Such approach is possible for cell treatment with different agents (for example, Fpg and hOGG1 enzymes, peroxide) for comet assay.

- Please state the exact conditions of the recovery phase in PBS such as temperature etc.

Reply: The correction has been made. DNA repair capacity was evaluated as the extent of removal of damage after immersion slides into PBS without bleomycin and incubation for 15 min, 37°C.

- Write “...with a significance level of p < 0.05.”

Reply: The correction has been made.

4) Results:

- Give p-values when showing significances.

Reply: P-values are given.

- The authors wrote “... we found differences after we removed bleomycin and studied DNA damage in a time course sampling ...” Do you mean DNA damage repair?

Reply: We do mean DNA damage repair. We have specified this.

- Is there any significance between the repair capacity of lymphocytes from patients with and without a familial history of cancer? If yes, state it with p-value.

Reply: Yes, there is significance. P-value is given.

5) Discussion:

- Exchange “depressed” with “suppressed” or “reduced”

Reply: The correction has been made.

- Two recent articles are addressing the sensitivity of lymphocytes from cancer patients. The authors should include the following articles in their discussion:

  Kurzawa-Zegota et al. (2012; in Food Chem Toxicol 50(2):124-129) which stated that lymphocytes from colon cancer patients had greater baseline DNA damage compared to those from healthy individuals and this higher level of damage was also observed throughout in vitro treatment with genotoxins.
Najafzadeh et al. (2012; in Mutagenesis 27(3):351-357) which identified peripheral lymphocytes from patients with cancers (malignant melanoma and colorectal cancer) or their precancerous states to be more sensitive to a generic mutagen than lymphocytes from healthy individuals.

Reply: Articles are included in the discussion.

6) Tables 1&2:

- Add an extra column before the “Removed DNA damage” column indicating the measured DNA damage in % tail DNA after the 15 min recovery. I assume this value has been compared to the baseline damage for statistics.

Reply: DNA repair capacity can be equally reflected in removed DNA damage and residual DNA damage. We consider that the amounts of damage removed more adequately reflect the repair capacity compared to residual DNA damage.

- In the footnote it must read p<0.05.

Reply: The correction has been made.

- Please use decimal points and not commas.

Reply: The correction has been made.

- The number n of patients should be stated in the description of the rows.

Reply: The correction has been made.

- Please use the wording “healthy individuals” instead of “control”.

Reply: The correction has been made.

Response to Referee #3 Comments

Reject

The paper is focused on the determination of the DNA background damage, the bleomycin-induced DNA damage (to check cellular susceptibility) and the DNA repair capacity of bleomycin-treated lymphocytes from endometrial cancer (EC) patients and controls using the comet assay. The idea is very interesting but unfortunately there is a technical problem that invalidates a big part of the study. The concentration of the bleomycin used is so high that the comet assay is saturated so the real DNA damage cannot be determined (they obtain about 87.61 % DNA in tail in controls and 98.66 % DNA in tail in EC patients when the comet assay is saturated at about 70 or 80% DNA in tail). Authors should have performed a concentration-response curve with bleomycin and lymphocytes and should have chosen some concentration in the linear part of the curve. This is also very important for the DNA repair determinations since authors cannot know the real level of DNA damage they start with (it can be that the level of the DNA damage in patients is much higher than in controls) and this is important since the cell is not going to repair with the same efficiency very different amounts of damage.

Reply: We followed the method described by Schmezer et al. (2001; in Mutagenesis Jan;16(1):25-30) in which, as an index of repair capacity, DNA damage was assessed at 15 min after termination of the incubation with 20 µg/ml bleomycin. Unfortunately, since in that paper tail moment rather than % tail DNA was given as the measure of DNA damage, it was not
apparent that the assay was saturated at this concentration. Nevertheless, we believe that our approach gives meaningful results: first, according to the dose-response curve in Schmezer et al., 20 µg/ml is not a 'super-saturating' dose; second, there is no reason to think that the damage inflicted by this dose of bleomycin would differ significantly between healthy subjects and cancer patients; third, it is clear from figure 2 that there is a great difference between controls and patients in the levels of removed damage after 15 min incubation.