Author’s response to reviews

Title: Epithelial Changes with Corneal Punctate Epitheliopathy in Type 2 Diabetes Mellitus and Their Correlation with Time to Healing

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Author’s response to reviews:

Dear Editor,

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “Epithelial Changes with Corneal Punctate Epitheliopathy in Type 2 Diabetes Mellitus and Their Correlation with Time to Healing” (MANUSCRIPT ID: BOPH-D-16-00467). We have studied their comments carefully and have made correction which we hope meet with their approval. Following was the list of actions and responses to reviewers.

List of Actions (LOA):

LOA1: The red words were modified by AJE language editor. The blue words were modified according to reviewers’ comments.

LOA2: The statement in Abstract section was deleted the “BEC nucleus reflection was enhanced in patients with T2DM”. (Abstract section, line 36-37, page 2).

LOA3: The statement in Background section was revised to emphasize the coordination (Background section, line 73-91, page 3).
LOA4: The statement in method section was added the measurement of SBN density (Method section, line 154-155, page 5).

LOA5: The statement in Results section was added the duration of T2DM (Results, line 179-180, page 6).

LOA6: The statement in Results section was added Figure 2 (Results, line 179, page 6).

LOA7: The statement in Discussion section was revised to emphasize the coordination (Discussion section, line 240-277, page 8-9).

Reviewer reports:

Cirous Deghani (Reviewer 1):

Abstract, Page 2

1- Paragraph 2 (Methods), line 26: What do you mean by "T2DM patients diagnosed as CPE due to a single cause"? In next paragraph you are referring to "CPE with different etiologies in the T2DM". This is confusing.

A: T2DM patients with CPE caused by one single factor, such as: Dry eye related; MGD related; Cataract extraction related; Drug related; Exposure related.

2- Paragraph 2 (Methods), line 27: Numbers should be spelled-out in the beginning of sentences. Consider this throughout the document.

A: I'm very sorry for this negligence. Numbers have already spelled-out in the beginning of sentences.

3- Paragraph 2 (Methods), line 31: Revise to "Density of LCs, SBN and BEC were compared between two groups".

A: In accordance with reviewer's request has been amended.

4- Paragraph 3 (Results): Consider consistency of the numbers in terms of decimal points. Suggest to round them up to two decimal points.

A: In accordance with reviewer’s request has been amended.
5- Paragraph 3 (Results), line 43-44: As I will refer to this later in Methods, you have not conducted an appropriate qualitative and/or quantitative technique in order to evaluate this. So I would recommend to exclude this finding.

A: we had deleted this finding.

6- Paragraph 3 (Results), line 45-49: Suggest presenting R2 in decimal format.
A: In accordance with reviewer’s request has been amended.

7- Paragraph 4 (Conclusion):

a) "…leading to lower BEC density and nuclei enhanced reflection." From your findings, such a conclusion cannot be drawn, you have only shown some association not cause and effect. Revise this section.

b) -What's more? Change to "Furthermore"

c) …the decreased BEC density finally may cause corneal epithelium..

d) "The potential immune mediated may play a role in delayed wound closure in T2DM patients". You need to elaborate on this in your discussion section in more details.

A: In accordance with reviewer’s request has been amended.

Background

1- Page 3, First paragraph: For updated prevalence of diabetes go to this link: http://www.diabetesatlas.org/

A: In accordance with reviewer’s request has been amended.

2- Page 3, 2nd paragraph (lines 19-20): The provided citation (#6) only refer to changes in relation to diabetic neuropathy. You need to provide proper references for basement membrane abnormalities of skin, kidneys, retina and cornea.
A: In accordance with reviewer’s request has been amended.

3- Page 3, 2nd paragraph (line 21-28): Langerhans cells (LCs), Dendritic cells (DCs) and presumably dendritic cells, are they functionally and morphologically different in cornea? You need to adopt one definition and then review the previous studies and their finding.

A: The dominant antigen presenting cells in the cornea and ocular surface are Langerhans cell (LC) and Dendritic cells (DCs). Using Cornea confocal microscopy (CCM) had shown an increase in the number of highly reflective cells ‘presumably dendritic cells’ was LC.

4- Page 3, 2nd paragraph (line 29): Reduction means something that happens over time, I recommend using "reduced" instead which is more suitable for cross-sectional studies.

A: In accordance with reviewer’s request has been amended.

5- Page 3, 2nd paragraph (30-31): Reference required for "T2DM effect on epithelial basal cells, epithelial basement membrane...."

A: In accordance with reviewer’s request has been amended.

Overall the Background is poorly constructed and you have not provided a proper sequence of information which would let the reader to understand why your report is important and also to justify your aims.

Methods

1- Page 3, last paragraph: Does not make sense, "928 were eligible for inclusion" then you note that "160 were eligible for inclusion"? Revise this section.

A: 928 patients who received a diagnosis of CPE during the study period from November 2010 to October 2015.

2- Did you mean "Patients were excluded if the CPE was attributed to multiple factors (a single cause could not be established)." As I mentioned this before, what do you mean by "single cause"? You are later referring to different etiologies including dry eye, MGD, cataract extraction. Make it clear.
A: Patients where the CPE was attributed to multiple factors (a single cause could not be established) were excluded.

3- Page 4, last paragraph: Were the images selected from volume or section scans? Did you select three images for each of them? The second sentence reads as you have only selected one image for each. Was the examiner masked to the subjects' condition (diabetic, control, type of epithelopathy)? Revise this properly. I would also say "three quality images of subbasal nerve plexus layer were used to". Which software did you use to count the LC? Change to "BEC was measured manually using HRT III proprietary software".

A: we used section scans to get the images. LCs density was counted (without software) then divided the image area (0.16mm2).

4- Page 4, Line 57 : use the below citation for "Erik Meijering".


Figure 1 should be illustrated in colour.

A: In accordance with reviewer’s request has been amended.

5- Pge 5, Lines 15-19: did you check for the normality of the data to use appropriate tests, for example the Pearson correlation?

A: I had checked the normality of the data. The data was normally distributed.

6- Please also define the SBN density clearly here. Many pioneers in this field has defined nerve density as total number of main nerves in unit of no/mm2. For example see (Tavakoli M, Quattrini C, Abbott C, Kallinikos P, Marshall A, Finnigan J, et al. Corneal confocal microscopy: A novel noninvasive test to diagnose and stratify the severity of human diabetic neuropathy. Diabetes Care. 2010;33:1792-7.). If you are following a particular definition, provide a proper reference as well.

A: SBN density was defined as the total nerves lengths in units of mm/mm2.
7- Something that was worth exploring here was the extent or the grading (1+ to 4+) of the CPE in all subjects. Obviously, the larger the size of the CPE is, the longer time require to heal. Furthermore, including other variables such as diabetes duration and patients glycaemic control would explain some of your findings.

A: It was quiet pity that patients’ glycaemic control situation was not collected.

Results

1- Page 5, the first paragraph of results (lines 21-27) again very confusing. You are reporting your findings and I am still struggling to work out what your sample size is! Please clearly and simply say how many T2DM subjects and controls were assessed, and how many met your exclusion and inclusion criteria. Perhaps a simple diagram works better.

2- Page 5, Lines (29-35). Firstly, you repeated the same sentence twice. Furthermore, you better tabulate these proportions and include the diabetic group as well not just reporting the controls. You also indicate that "The control group was matched for gender, age and etiology". What tests did you use and what are the statistics? Again these can be included in the above mentioned Table.

3- Based on the information provided in Table 1 both sample sizes are identical 160 subjects in T2DM and 160 controls! While you refer to 160 in T2DM and 149 in controls. Rectify this discrepancy.

A: I’m very sorry for this negligence. Numbers have already double checked.

4- "Cell nucleus reflection was enhanced and cell borders were..." As noted before, you have not conducted an appropriate qualitative and/or quantitative assessment for these criteria (reflection and border irregularities). If you want to include, you need to clearly explain it in the methods section.

A: This result has already deleted.

5- Page 5, Lines 53-60: No need to present the regression equations and also change the R2 to decimal.

A: In accordance with reviewer’s request has been amended.
Discussion

1- Page 6, lines 32-40. Apart from the first sentence, this paragraph is poorly constructed. How do you explain the lack of relationship between BEC and healing time in controls? What do you think drive the epithelial healing in those people?

A: Corneal sensory nerves supply trophic neuropeptides, such as substance P and calcitonin gene-related peptide (CGRP); these increase the rate of mitosis of corneal epithelial cells in vitro and may also modulate other aspects of epithelial cell behavior such as differentiation and migration[1]. Those dysfunction of BEC could lead to prolonged in healing time. The density of BEC showed no relationship with healing time in control group, because the SBN density was normal in control group.

2- Page 6, lines 49-53: Do you think presence of epitheliopathy could have an effect on increased LC density?

A: SBN along with LCs may play a role in delayed wound closure in T2DM patients. This delayed may be develop into epithelopathy.

3- "because HRT 3 have higher resolution ratio to observer LCs compared with Tomey Confoscan corneal confocal microscope". I understand what you trying to say but this sentence does not make sense. Revise this section.

A: In accordance with reviewer’s request has been amended.

4- Page 7, lines 3-4: "Quadrado et al[11]and Chang et al[28] who reported lower BEC density in T2DM patients." These studies also reported the correlation between sub-basal nerve structure and epithelial cells. Compare your finding with these reports as well.

A: In accordance with reviewer’s request has been amended.

5- Page 7, lines 5-6: "As the corneal epithelial metabolism depends on appropriate corneal innervation". This sentence requires citation.

A: In accordance with reviewer’s request has been amended.
6- Page 7, lines 13-21: "All patients were recruited from the corneal clinic of a single department of ophthalmology and are unlikely to be representative of patients seen in a general clinic or of the population." I do not think this is a limitation to this study.

"Also, the numbers were too small to adjust for severity of CPE in the assessment of time to healing." I think you have decent numbers to look at a lot of other variables including the severity of CPE, diabetes duration and age. You could run a multiple linear regression and include those as predictors.

I would suggest to start with something like: "These data provide support for the role of IVCM in microstructural evaluation of corneal epithelium and the associated factors with EPC."

I think the last sentence is also requires revision.

A: These data provide support for the role of IVCM in microstructural evaluation of corneal epithelium and the associated factors with CPE. There are also several limitations to our study. IVCM images only in the first visit of patients and do not images after treatment to compare; The glycemic control data was not collected in T2DM patients.

Overall, the discussion is very limited and most of the findings, comparison with previous studies and possible involved mechanisms have not been fully discussed.

Dimitra Makrynioti (Reviewer 2):

1. p.3, Line 05: Delete "in 2013" after (T2DM)

A: In accordance with reviewer’s request has been amended.

2. p.3, Lines 05 & 06: Rephrase please - maybe "to increase to 592 by 2035, an increase of 55%"

A: In accordance with reviewer’s request has been amended.


A: In accordance with reviewer’s request has been amended.
4. p.4, Lines 1-3: Give more details on inclusion and exclusion criteria.

A: Patients where the CPE was attributed to multiple factors (a single cause could not be established) and had undergone previous corneal or ocular surgery, had any ocular pathology other than diabetic retinopathy and keratopathy were excluded.

5. p4, Lines 1-3: How did you choose which eye? Give details. You mention that in line 49-50, but add more details In lines 1-3 as well.

A: In accordance with reviewer’s request has been amended.

6. p.4, Lines 1-3: Any previous power analysis? Please give details.

A: We had done the power analysis, all the results were good.

7. p.4, Lines 11-14: Onset of T2DM? For how many years they had T2DM? Was it under consideration?

A: The duration of T2DM was 13.4±8.30 year (from 1 to 30 years).

8. p.4, Lines 16-17: Was the categorization of CPE used for all patients, with & without T2DM? Give details.

A: Yes, all patients used same categorization.

9. p.4, Lines 51-52: The three images for the analysis were selected and analyzed by the same examiner? Was the procedure done in masked way or not? Give details.

A: A single experienced and masked examiner performed all scans


A: In accordance with reviewer’s request has been amended.
11. p.5, Lines 6-7: (1) Shouldn't all patients be examined within the same time limit after the initiation of the treatment? Please explain. (2) Also give details on the examination procedure of the follow up visits. (3) Did you take IVCM images and after treatment to compare, or only in the first visit?

A: (1) All patients were from outpatient clinical, so the reexamination time was 3-7 days.

(2) All patients underwent visual acuity testing as well as slit lamp biomicroscopic examination with fluorescein staining.

(3) All ICVM images were took by the first visit, it would be one of limitation in our work.


A: In accordance with reviewer’s request has been amended.

13. p.5, Lines 41-51, IVCM results, p.6, Lines 18-23, Figure 3: Based on my IVCM experience, I am afraid that in your Figure 3 you have: (A) Wing cells in A and most of the dendritic formations to be artefacts due to anaesthetic/fluorescein/gel being introduced onto the cornea before IVCM and sits in-between the wing cells, (B) Basal cells invading in your Bowman's layer in B, (C) Wing cells in C, (D) Basal cells in D. Given that you selected the clearer of your images to present, on which you based your analysis and results, I am doubtful on the validity of your results and following discussion. Please explain and give details.

A: As we all known the wing cells: The cells of the intermediate layers are characterized by bright cell borders and a dark cytoplasm. The cell nucleus can be distinguished only with difficulty (Figure 5.6). The basal cells are located immediately above Bowman’s membrane. They present as brightly bordered cells in which the cell nucleus is not visible. Between-cell comparison reveals inhomogeneous reflectivity of the cytoplasm. Like the wing cells above them, the basal cells displaya only minimal variation in shape and size (Figure 5.7). LCs present as bright corpuscular particles with dendritic cell morphology and a diameter of up to 15 mm. LC distribution follows a gradient from low numbers in the center to higher cell densities in the periphery of the cornea (Figure 5.8)[2].

(A) First of all, it’s not wing cells, according to the depth of images. Second, there was no chance to see wing cells nucleus reflection was enhanced. Third, the dendritic formations were LCs, according to morphology and a diameter. Fourth, we found out the BEC nucleus reflection was enhanced in CPE patients with T2DM.
(B) Images of Bowman’s layer were used to quantify both langerhan cell density and nerve fibre morphology. It’s not basal cells invading in your Bowman's layer, only the junction part of basal cells and Bowman’s layer.

(C) BEC nucleus reflection was enhanced in CPE patients with T2DM. There was no chance to be the wing cells, because we could see nerve fibre.

14. p.7, Line 2: "We too show" - Rephrase please.
A: In accordance with reviewer’s request has been amended.

A: In accordance with reviewer’s request has been amended.

A: In accordance with reviewer’s request has been amended.

A: In accordance with reviewer’s request has been amended.

18. p.8, Lines 3-5: Consent form - mention that in methods.
A: In accordance with reviewer’s request has been amended.

References: