Reviewer’s report

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

Version: 0 Date: 28 Nov 2016

Reviewer: Cirous Deghani

Reviewer's report:

General comments

Although the investigated topic by Jing-hao Qu et al. is interesting, there are a number of concerns and shortcomings with this manuscript. Below I present my main comments followed by some examples of specific comments through manuscript.

First and most important, this manuscript reads like an initial draft rather than a manuscript that has undergone some degree of editing and internal review before being sent out to this journal. Therefore, the manuscript needs language editing with focus on the scientific issues. The authors are encouraged to seek the assistance to help improve the clarity and readability of the paper. Secondly, the methodology, in particular the statistical analysis and results need major clarifications and improvements. The discussion is also short with limited compare and contrast with previous studies and explaining the possible involved mechanisms.

Specific comments

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 aetiologies in the T2DM". This is confusing.

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

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

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

6- Paragraph 3 (Results), line 45-49: Suggest presenting R2 in decimal format.

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.

Background

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

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.

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.

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.

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

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.

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 aetiologies including dry eye, MGD, cataract extraction. Make it clear.

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 epithelipathy)? 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".

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


Figure 1 should be illustrated in colour.

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

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.

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.

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.

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.

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

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?

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

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.

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.

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

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

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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Please indicate the quality of language in the manuscript:
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