·Comment and Response·

Comment on *in vivo* corneal confocal microscopic analysis in patients with keratoconus

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Dear Editor,

The article by Bitirgen *et al* [1] published in the journal presents an interesting analysis of keratoconus patients and controls by *in vivo* corneal confocal microscopy. However, addressing the following observations regarding the study design used by the authors may help add another dimension to the discussion.

The age range of the patient group has been stated as 18-41y and for controls as 18-37y. Although the mean age is similar in the two groups, were the cases and controls matched with each other on a one to one basis? We assume this may not have been done as there are 78 patients and 36 controls. Also, age matching has not been performed before subgroup (mild, moderate and severe keratoconus) analysis. This implies that, theoretically, we may have younger patients with mild keratoconus and older patients with severe keratoconus. This may falsely influence the analysis of certain parameters by confocal microscopy, for example, endothelial cell density. The difference in endothelial cell density between the subgroups may theoretically be due to different age of the participants rather than due to different disease severity. Thus age may be an important confounding factor. This may partly explain the variable results regarding endothelial cell density in keratoconus patients, referred to by the authors under the Discussion section of the article. The cases and controls have not been sex-matched on a one to one basis as well (female: male ratio is 46:32 for cases, 17:19 for controls).

For patients with bilateral keratoconus, one eye was randomly chosen. How was this choice made? Most patients with keratoconus usually have different disease severity in the two eyes. Analyzing both eyes of bilateral cases could have yielded more cases with mild keratoconus which, the authors stated, were in short supply. Also one eye of a patient could have been compared with the other (in unilateral cases for keratoconus versus no keratoconus, in bilateral cases for mild versus moderate versus severe keratoconus) to determine differences in confocal microscopy parameters while minimizing confounding factors.

Also, the authors stated that none of the patients had any history of any ocular procedure or contact lens use. It is somewhat surprising to note that patients even up to 41y of age and having even severe keratoconus sought/received no treatment for their condition till present time.

The authors have also mentioned that a single unmasked observer was used for image analysis, which may have led to observer bias.

In conclusion, although the authors have presented an excellent analysis on the use of confocal microscopy in keratoconus, some modifications in study design would probably have further enhanced the impact of the study.

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Conflicts of Interest: Bhambhwani V, None.

REFERENCES

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Author Reply to the Letter

Dear Editor,

We are grateful to Dr. Bhambhwani for his interest in our recent published study [1] and appreciate his thoughtful comments about the study design. We would like to clarify the points that he got confused as detailed as possible.

In order to create an age-matched control group, the frequency matching method was performed instead of an individual matching protocol (*e.g.* If 15% of cases were under age 20, fifteen percent of the controls were also under age 20). We totally agree with Dr. Bhambhwani that age may be a compounding factor when comparing endothelial cell density among mild, moderate, and severe keratoconus groups but, in our study, there were no significant differences between the three subgroups in terms of age (the mean age was 25.0 ±5.7y in mild keratoconus group, 24.9 ±4.8y in moderate keratoconus group, and 26.9 ±5.9y in severe

keratoconus group; ANOVA test, P=0.274). We have not stated in the article that control subjects were sex-matched to the patients with keratoconus. Instead, we mentioned that there was no significant difference between the two groups in terms of gender (P=0.241).

It is known that both eyes are usually affected in patients with keratoconus, which may result in between-eye correlations. If such correlations are not taken into account in statistical analyses, there may be errors in the results obtained, usually resulting in falsely precise confidence intervals and falsely small P values [2]. Thus, we used a computer-generated process (random number method, odd number-right eye and even number-left eye) to randomly select one eye of each patient.

Another subject that Dr. Bhambhwani pointed out is the exclusion criteria. We excluded the patients who are currently using and/or who previously used contact lenses to avoid the potential confounding effects of the contact lens related alterations, which we have recently reported in patients with keratoconus[3]. The reasons of our study subjects for not using contact lenses were recent diagnosis, poor compliance, or patient preference.

We have stated in the limitations paragraph of the discussion section that the quantitative analysis of the confocal microscopy images were performed by an observer who was unmasked about the images belonging to a keratoconus or a control subject, but was masked about the severity of the keratoconus.

We hope we clarified the points related to Dr. Bhambhwani's comments and again thank him for the valuable contribution.

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