Author’s response to reviews

Title: Choroidal Structural Changes Correlate with Severity of Diabetic Retinopathy in Diabetes Mellitus

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Author’s response to reviews:

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “Choroidal Structural Changes Correlate with Severity of Diabetic Retinopathy in Diabetes Mellitus” (ID: BOPH-D-19-00132R1). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to us.

We have studied comments carefully and have made correction which we hope meet with approval. I uploaded two manuscript versions in the formats of WORD. The modified place in revised version is marked in red in form of word document and uploaded in the “Optional Supplementary Materials” part, the finished revised clean version is upload in Word format in the “Manuscript File” part. The main corrections in the paper and the responds to the reviewer’s comments are as flowing:

→ Comments from Reviewer 1(Jee Taek Kim, M.D., Ph.D.):

1. CVI in patients with diabetes was previously reported by Kim et al. (Sci Rep. 2018 Jan 8;8(1):70. Please clarify the difference with this previous study in Introduction and add discussion about the differences in Discussion.

My answer: Thank you for your kind advice. It was my carelessness.
First, the indicators evaluated are different. In the study of Mirinae Kim, they only assessed choroidal changes in diabetic patients by measuring choroidal vascularity index (CVI) and choroidal thickness (CT) in conjunction with DR stage. But in our study, we divided the patients into 4 groups: Healthy controls, DM with no DR eyes, non-proliferative DR (NPDR) eyes, PDR eyes and assessed retinal nerve fiber layer (RNFL), retinal thickness, subfoveal choroidal thickness (SCT). At the same time, the total choroidal area (TCA), stromal area (SA), the luminal areas (LA) and CVI were compared.

Second, according to different evaluation indicators, the conclusions are different. In the study of Mirinae Kim, they only evaluated CVI and got the conclusion “These findings carefully suggest that changes in choroidal vasculature could be the primary event in diabetes even where there is no DR”. But in our study, we compared CVI, LA, TCA. We showed significantly smaller CVI and bigger SA in patients with DM, compared to normal controls, regardless of the presence of DR. In our study, we also observed the choroidal structure indicators with different stages of DR, SA and TCA were significantly lower in DM with no DR eyes, followed by NPDR eyes and the PDR eyes (P <0.001). CVIs were decreased in eyes of DM with no DR patients, NPDR and PDR patients (65.30 ± 2.75, 63.35 ± 5.21 and 61.48 ± 4.35, P=0.019). But LA were not change with the progression of DR. Conclusion: “This may because the thickening of the choroid in the different stages of DR is stromal thickening, not vascular change”.

In the “Introduction” part, I have added “In the study of Kim, they assessed choroidal changes in diabetic patients by measuring CVI and CT in conjunction with DR stage. But they did not assessed the other indicators, such as TCA, SA and LA.” And in the “Discussion” part, I have added some discussion about the differences: “We used the ratio of the luminal to choroidal area to describe the change of choroid structure, which has the same meaning of CVI. In the study of Kim[14], they assessed choroidal changes in diabetic patients by measuring choroidal vascularity index (CVI) and choroidal thickness (CT) and found that the PDR eyes exhibited a significantly lower CVI value than the healthy control, no DR, and NPDR eyes; the CVI in DM eyes was significantly lower than those of healthy controls even without DR. So they came to the conclusion that changes in choroidal vasculature could be the primary event in diabetes eyes even with no DR. Our research is different from they, we compared LA, TCA and the ratio of the luminal to choroidal area in this study……” by using bold and red text as the follow screenshot.
2. In this study, DME patients were not included. Why the eyes with DME were not included?

My answer: Thank you for your kind comments.

In some studies, it has been confirmed that macular edema and choroidal thickness changes have a certain relationship (what is the cause of ME). The reduced choroidal blood flow in the choriocapillaris is also a possible factor of CME.

In the study of Fleissig, Efrat, they studied the choroidal thickness in clinically significant pseudophakic cystoid macular edema (CME), and found the choroidal thickness of the affected eye was significantly (P < 0.01) thinner than that of the fellow eye. The thinner choroid in eyes with CME than in fellow eyes may suggest that the reduced choroidal blood flow in the choriocapillaris is also a possible factor of CME. They also investigated choroidal thickness in eyes with clinically significant pseudophakic cystoid macular edema (PCME) during the acute phase and following resolution of the edema, and found that mean SCT in eyes with PCME measured 258 ± 83 μm at baseline and decreased to 215 ± 79 μm after CME resolution (CHANGES IN CHOROIDAL THICKNESS IN CLINICALLY SIGNIFICANT PSEUDOPHAKIC CYSTOID MACULAR EDEMA). Eliwa Tamer F also evaluated CT change in DME patients and found some differences. (Choroidal Thickness Change in Patients With Diabetic Macular Edema)

The causal relationship between macular edema and choroidal thickness changes is not well defined. In order to rule out the effect of macular edema on choroidal thickness, making the results more reliable, and to investigate the changes in the fundus microstructure of diabetic retinopathy patients with or without macular edema in the further study, so the DME patients were not include. We only investigated the choroidal structure changes in diabetic retinopathy patients with and without macular edema. Next, we will focus on the changes in the fundus microstructure of diabetic retinopathy patients with or without macular edema.

Your suggestion is very pertinent and helpful for our next research. Thanks very much.
3. The major result of this study is the changes of CVI. Please clearly present using graph the changes of choroidal thickness and CVI that was presented in Table 2 and 3.

My answer: We are very grateful for your questions, thank you very much~! I have added the graphs in the article named Fig 3 and Fig 4, describe the parameter changes between different groups intuitively.

I have added this 2 graphs as the follow screenshot.

4. The changes of choroidal thickness in eyes with diabetic retinopathy in the previous reports have been going on debates. Considering the debates, the number of enrolled eyes were relatively small. Add this on limitation of the study.

My answer: Thank you for your kind advice. I have already added the limitation “However, the sample size of this study was relatively small, which may have limited the statistical strength of the analysis and reduced our ability to perform correlational analyses for DM and the fundus microstructure. Future studies should be performed with larger cohorts and longer follow-up periods to determine the fundus microstructure changes in patients with different degrees of DR” in the “Discussion” part and highlight the changes by using bold and red text as the follow screenshots.

5. There are no subsection as to limitation. Please describe the other potential limitations

My answer: Thank you for your kind question. I have already described other limitations and added them in the “Discussion” part: “The other limitation was that our image processing technology clearly displays and quantifies vascular tissue in the choroidal cross section, but it can only reflect the choroidal vasculature change in a certain part, not a wide range of fundus choroidal change. Therefore, future research can analyze choroidal vascular density in a wide range of fundus with OCTA simultaneously. did not show clearly the structural of choroidal vessels, there were measurement errors in choroidal vascular density. Later studies require OCT-A devices with better choroidal imaging quality to analyze choroidal vascular changes”.

I highlight the changes by using bold and red text as the follow screenshots.
6. The authors described possible reasons for debates on choroid. However, authors have to add previous reported variables as to choroid with reference as follows: age, diurnal variation, refractive error, systemic factors, hypertension or blood pressure, the status of kidney status.

My answer: Thank you for your kind question. Many systemic factors are known to affect choroidal structural. Previous research has also added these variables in their articles. In the course of research, we have done statistics and analysis on these systemic factors in each group, this is my negligence, not including this information in this article. Based on your suggestion, I add these factors “age, diurnal variation, refractive error, systemic factors, hypertension or blood pressure” in Table 1. But I did not provided “the status of kidney” in Table 1. Because I think that diabetic nephropathy has little effect on the choroidal measurement results. At present, there is not much research in this area. But our research does record this part. If you feel it is necessary to put this in Table 1, please let me know. I will improve it in the next modified version.

7. Please rewrite the discussion with intensive literature review.

My answer: Thank you for your kind question. Based on your suggestion, I have rewrite the discussion part and added 10 more references using bold and red text. A more comprehensive and detailed discussion of the results of this study, as well as previous research results had been conducted.

First, we have added some studies on choroidal index and fundus changes in other diseases, such as AMD “Koh et al.[31] found CVI was lower in age-related macular degeneration (AMD) eyes as compared to normal controls, suggested that possible reduction in choroidal vascularity in eyes with AMD”(Koh LHL, Agrawal R, Khandelwal N, Sai Charan L, Chhablani J. Choroidal vascular changes in age-related macular degeneration. Acta Ophthalmol (Copenh). 2017. doi:10.1111/aos.13399.).

Second, we added some basic study about pathologic change in animal DR model: “Animal model showed that choroidal blood flow deficit can be an early pathologic change in DR[32]”(Muir ER, Rentería RC, Duong TQ. Reduced ocular blood flow as an early indicator of diabetic retinopathy in a mouse model of diabetes. Invest Ophthalmol Vis Sci. 2012;53:6488–94.).

…and so on.

If you feel that the discussion part should be improved, please let me know, I will seriously modify it again in the next revision.
8. Inclusion criteria: The choroid in eyes with DR was affected by intravitreal injection or laser treatment. Please clarify whether the eyes were treatment-naïve eye or treated eyes.

My answer: Thank you for your kind question. I am sorry that I did not describe it in detail in this article.

Of all DM 66 patients, 42 eyes were treated eye, and 24 eyes were treatment-naïve eye (including 22 DM with no DR eyes, which were not need for any laser treatment, and another 2 NPDR eyes). 42 patients received fundus laser treatment (22 in NPDR eyes and 20 in PDR eyes). I have described this in the results section and Table 1.

Comments from Reviewer 2 (Taiji Sakamoto):

1. Introduction and through the text: Although Agrawal et al proposed choroidal vascular index (CVI) as choroidal vascular parameter, this nomenclature is not approved by all researchers. Therefore, it is strongly recommended to use the term, "ratio of cho-roidal luminal area". The systemic treatment should be described in each group. (eg number of cases using insulin )

My answer: Thank you for your kind advice. Your advice is very pertinent and very rigorous. First, I have changed all “choroidal vascular index (CVI)” into “ratio of the luminal to choroidal area” in this paper follow your advice. Thank you very much!

2. The systemic treatment should be described in each group. (eg number of cases using insulin)

My answer: Thank you for your kind advice. It was my carelessness for this.

Of all 66 DM patients in this study, 62 patients received insulin treatment (19 in DM with no DR eyes, 23 in NPDR eyes and 20 in PDR eyes). 42 DM patients received fundus laser treatment (19 in DM with no DR eyes, 23 in NPDR eyes and 20 in PDR eyes). I described this in the results section and Table 1, and highlight the changes by using bold and red text: “62 patients received insulin treatment (19 in DM with no DR eyes, 23 in NPDR eyes and 20 in PDR eyes) in all 66 DM patients. 42 DM patients received fundus laser treatment (19 DM with no DR eyes, 23 NPDR eyes and 20 PDR eyes). (Table 1)” in the result part.
3. History of ocular treatment in each group should be described as well. It is hard to believe that none of them was treated by laser photocoagulation.

My answer: Thank you for your kind advice. It was my carelessness that I did not described this in detail in this article.

Of all DM patients in this study, 42 DM patients received fundus laser treatment (19 in DM with no DR eyes, 23 in NPDR eyes and 20 in PDR eyes). I described this in Table 1.

Comments from Reviewer 3 (Joana Tavares Ferreira):

1. A study with high clinical relevance, however with some statistical failures. A multivariate analysis was not performed that should be included when we talk about choroidal thickness because several factors influence it. On the other hand we do not know if we have patients with clinical or subclinical diabetic macular edema. There are some more recent papers in the literature that were not mentioned.

My answer: Thank you for your kind advice. Your opinion is very pertinent, and at the same time, I am aware of some objective problems in this research.

1. A multivariate analysis was not performed that should be included when we talk about choroidal thickness because several factors influence it.

When it comes to the choroid thickness, there are indeed many factors that influence the change in choroidal thickness. Axial length of the globe, sleeping and exercising habits, alcohol or drinks consumption, BMI, blood pressure, daily curve, axial length, refractive status, age etc. may affect the choroidal thickness; even some errors in the measurement, the measurement level of the technician will also affect the accuracy of the results. I have list the Axial length data, BMI, age, systolic BP, diastolic BP, IOP in Table 1 and the difference between the two groups was analyzed, not significant different (P>0.05). Therefore, we believe that this can eliminate the influence of confounding factors on the choroidal thickness results, making the results relatively reliable. However, if we add a multivariate analysis in our research, these results will be more accurate and scientific.
2. If we have patients with clinical or subclinical diabetic macular edema

It was my carelessness for this. I have not shown this in my research. Patients with macular edema have not been included in this study for these reasons: many previous studies have shown changes in choroidal thickness in patients with diabetic macular edema, the specific mechanism is not clear. Some think that diabetes develops to a certain stage, the process of macular edema, the abnormalities of ocular hemodynamics, will cause insufficient blood supply to the fundus and affects choroidal structure changes, which is reflected in the thinning of the choroid. In the study of Fleissig, Efrat, they studied the choroidal thickness in clinically significant pseudophakic cystoid macular edema (CME), and found the choroidal thickness of the affected eye was significantly (P < 0.01) thinner than that of the fellow eye. The thinner choroid in eyes with CME than in fellow eyes may suggest that the reduced choroidal blood flow in the choriocapillaris is also a possible factor of CME. Eliwa Tamer F also evaluated CT change in DME patients. (Choroidal Thickness Change in Patients With Diabetic Macular Edema). The relationship between CME and choroidal thickness changes is not well defined. In order to rule out the effect of macular edema on choroidal thickness, macular edema have not been included in this study.

3. There are some more recent papers in the literature that were not mentioned.

Based on your suggestion, I have rewrite the discussion part and added 10 more references by using bold and red text. A more comprehensive and detailed discussion of the results of this study, as well as previous research results had been conducted.

Thank you and all the reviewers for the kind advice.

Sincerely,

Hui Wang