PEER REVIEW HISTORY

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ARTICLE DETAILS

<table>
<thead>
<tr>
<th>TITLE (PROVISIONAL)</th>
<th>Is the Peripapillary Retinal Perfusion related with Myopia in Healthy Eyes?: a Prospective Comparative Study</th>
</tr>
</thead>
<tbody>
<tr>
<td>AUTHORS</td>
<td>Wang, Xiaolei; Kong, Xiangmei; Jiang, Chunhui; Li, Mengwei; Yu, Jian; Sun, Xinghuai</td>
</tr>
</tbody>
</table>

VERSION 1 - REVIEW

| REVIEWER | Young Hoon Hwang  
Department of Ophthalmology, Konyang University, Kim’s Eye Hospital, Myung-Gok Eye Research Institute, Seoul, South Korea |
<table>
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<tbody>
<tr>
<td>REVIEW RETURNED</td>
<td>30-Dec-2015</td>
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</table>

GENERAL COMMENTS

The present study evaluated the correlation between myopia and retinal circulation as determined by OCT angiography. Although the technique and study results provide new information, some issues are needed to be clarified.

1. Line 4. Please consider changing short title from “peripapillary perfusion in highly myopic eyes” to “peripapillary retinal perfusion in myopic eyes”.

2. Line 41 and elsewhere. Please change “optic coherence tomography” to “optical coherence tomography”.

3. Line 96. Please clarify in what condition blood flow was decreased.

4. Line 125. Please provide inclusion criteria of healthy myopic eyes. Currently, no criteria are provided regarding optic nerve head, retinal nerve fiber, and visual field test results. Line 146. How refractive status was measured? Line 147. Please change “intraocular pressure (IOP)” to “IOP”.

5. Line 266. Why c/d ratio and rim area were considered as confounding factors? How these parameters were measured? Among various optic nerve head parameters, why optic nerve head area was not considered as a confounding factor?

6. Line 267. Please change “paripapillary” to “peripapillary”.

7. Discussion section. Peripapillary atrophy (PPA) itself may play a role in the decreased peripapillary blood flow in myopic eyes. For instance, even in eyes with similar degrees of myopia, an eye with a greater PPA may have a lower blood flow. Thus, PPA area can be considered as a confounding factor. Please consider assessing the effect of PPA area on peripapillary blood flow.
8. Discussion section. Why only retinal blood flow is decreased in myopic eyes? The reason for non-significant difference in choroidal blood flow among the four groups needs to be explained.

9. Line 370. Previous studies reported that macular retinal layers (including ganglion cell layer) as well as peripapillary retinal nerve fiber layer are thinner in myopic eyes than non-myopic eyes. Therefore, it is difficult to understand the first point addressed by the authors (why retinal blood flow is decreased only in peripapillary area). In the present study, no significant difference in macular GCC thickness was found among the four groups. What would be the cause of this finding (previous studies reported thinner GCC in myopic eyes)? Why GCC thickness was compared? What about the results of macular total retinal thickness difference comparison among the four groups?

10. In tables, please provide host poc analysis results for values which showed significant differences in ANOVA.

11. In table 3 and 4, beta values seem to be too low. Please provide R2 values and standardized beta values.

REVIEWER
Gabor Hollo
Semmelweis University, Budapest, Hungary
I am an unpaid consultant of Optovue and Zeiss

REVIEW RETURNED
05-Jan-2016

GENERAL COMMENTS
In this cross sectional investigation the authors investigated the relationship of peripapillary retina and choroid thickness and the corresponding peripapillary Optovue-Angiovue OCT angioflow parameters; and the macular retina and choroid thickness and the corresponding OCT angioflow parameters in healthy emmetropic and increasingly myopic groups. They found a significant relationship between the OCT angiography parameters and peripapillary retinal thickness across the groups, and no relationship for the macular parameters across the same group.

The main result of the well-designed study is not the relationship revealed (since it has been shown using various other methods in earlier investigations) but the applicability of Optovue OCT angioflow measurement for this (and similar) purposes, since this is a new, promising and non-invasive method.

This reviewer has no major problem with the study design, methods, statistics and discussion, but has some points where the authors may improve/clarify their manuscript (see them below). Minor English style improvement is also suggested, but this can be made during technical editing if the manuscript is accepted for publication.

Detailed comments:
1. The pts' age was around 16-y, which (since pathological myopic degenerations were excluded) suggests that retinal damage/perfusion impairment etc may have not yet developed (this comment also fits to Introduction, line 94). This needs to be discussed.
2. Methods: A very problematic point: no information on BCVA and VF is available. We really do not know what we are dealing with. Please provide data. It is interesting that several perfusion parameters were investigated (these parameters are expected not to be informative in healthy 16-y old persons, and in fact they were not
informative), but the most important functional data are missing.

3. Methods: The authors probably used the Optovue Angiovue instrument, which is built-in the RTVue-XR OCT, but they indicate "RTVue OCT" which is incorrect or "RTVue_XR Avanti". Please clarify and use the same terminology throughout the whole manuscript.

4. Methods: page 10, line 178. The authors write: "...SSI<45, and if a scan contains severe artifacts, it will be excluded from analysis." This is an important point, and the authors need to correctly discuss the influence of their "non-severe" (?) artifacts on the segmentation and the results. With increasing severity of myopia vitreoretinal artifacts appear with increasing frequency. Using the authors' OCT methods it was recently shown that even if TRVue-XR OCT reduces peripapillary and macular segmentation errors in myopia compared to RTVue-100 OCT, they are still there in many cases. This issue needs to be discussed in the discussion. For reference: Hollo G, Hsu SW, Naghizadeh F: Evaluation of a new software version of the RTVue optical coherence tomograph for image segmentation and detection of glaucoma in high myopia. J Glaucoma 2015 published online

5. Repeatability study (Methods and Results): the authors use the term "normal subjects", but this is unclear since all eyes were normal by definition, and refractive error alone is not a disease. Please use the term "emmetropic subjects". Though this reviewer thinks that the results (CV and ICC) are informative, it remains unclear why repeatability was not evaluated for the other groups (or all study eyes). In the current form it is suggestive for "hiding" data. Please add data from the other groups, or explain why myopic eyes were not involved in the repeatability study. In the table add "%" symbol to CV.

6. Discussion: to this reviewer some parts on the reason of reduced blood flow in myopia are speculative. The current study is not an investigation where the reasons and pathomechanisms are investigated. I suggest to remove or shorten those parts. An important point was not even raised: the arterial blood supply is different for the peripapillary retina and the macula, which may also represent a reason for the different relationships.

7. Discussion: the authors make a statement which is not true as it is. Please either modify the statement or use different wording to avoid confusion.

"To our knowledge, the peripapillary or parafoveal perfusion had not been measured in eyes with high myopia using quantitative techniques previously." (page 15, lines 310-312). Here is just one paper in which I investigated this problem 20 years ago: Evaluation of the peripapillary circulation in healthy and glaucoma eyes with scanning laser Doppler flowmetry. Holló G, Greve EL, van den Berg TJ, Vargha P. Int Ophthalmol. 1996-1997;20(1-3):71-7.

8. Introduction and Discussion: since the number of publications addressing the relationship of peripapillary retinal thickness and perfusion as measured with RTVue-XR OCT/ Angiovue OCT angiography is very limited use of all published data is recommended. A recently published study (Holló G: Vessel density calculated from OCT angiography in three peripapillary sectors in normal, ocular hypertensive and glaucoma eyes. Eur J Ophthalmol 2015, published online first, DOI: 10.5301/ejo.5000717) may even provide information on the changes for different peripapillary sectors (this is important to the current submission since myopia-related alterations are more pronounced in the temporal sectors) and on the relationship between retinal tissue loss and vessel loss in the peripapillary area.
The present study evaluated the correlation between myopia and retinal circulation as determined by OCT angiography. Although the technique and study results provide new information, some issues are needed to be clarified.

1. Line 4. Please consider changing short title from “peripapillary perfusion in highly myopic eyes” to “peripapillary retinal perfusion in myopic eyes”.

   Thanks. We have changed them in Line 4 in the marked copy.

2. Line 41 and elsewhere. Please change “optic coherence tomography” to “optical coherence tomography”.

   Thanks. We have changed them in Line 41, 69, 105 and 536 in the marked copy.

3. Line 96. Please clarify in what condition blood flow was decreased.

   Thanks. It was that the retinal or choroidal blood flow in myopic eyes was decreased. We have changed them in Line 96 in the marked copy.

4. Line 125. Please provide inclusion criteria of healthy myopic eyes. Currently, no criteria are provided regarding optic nerve head, retinal nerve fiber, and visual field test results.

   Thanks. We have changed them in Line 127-130 in the marked copy. We did not perform the test of visual field test. However, the participants have the normal retinal nerve fiber layer and ganglion cell complex thickness. Therefore, the participant did have healthy eyes.

Table S2. Multivariate Regression Models of Axial Length Affecting Peripapillary Perfusion in Subjects.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Model Retinal Flow Index</th>
<th>Retinal Vessel Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Standardized β(SE) Adjusted R2 P value</td>
<td>Standardized β(SE) Adjusted R2 P value</td>
<td></td>
</tr>
<tr>
<td>Axial length 1a</td>
<td>-0.376(0.001) 0.130 0.001</td>
<td>-0.39(0.47) 0.144 &lt;0.001</td>
</tr>
<tr>
<td>Axial length 4</td>
<td>-0.241(0.001) 0.197 0.043</td>
<td>-0.23(0.51) 0.252 0.046</td>
</tr>
</tbody>
</table>

Line 146. How refractive status was measured?

   Thanks. We have changed them in Line 148-149 in the marked copy.

Line 147. Please change “intraocular pressure (IOP)” to “IOP”.

   Thanks. We have changed them in Line 150 in the marked copy.

5. Line 266. Why c/d ratio and rim area were considered as confounding factors? How these parameters were measured? Among various optic nerve head parameters, why optic nerve head area was not considered as a confounding factor?

   Thanks. The c/d ratio and rim area are the important parameters of the disc. What's more, the parameters can be easily obtained from the OCT report. We also analyzed the optic nerve head area in the model 4 (Table S2 and S3). The results were similarly with that of c/d ratio and rim area in the model 4 (Table 3 and 4). Therefore, the c/d ratio and rim area were still considered as confounding factors in the line 271—272 in the marked copy.
6. Line 267. Please change “paripapillary” to “peripapillary”.
Thanks. We have changed them in Line 272 in the marked copy.

7. Discussion section. Peripapillary atrophy (PPA) itself may play a role in the decreased peripapillary blood flow in myopic eyes. For instance, even in eyes with similar degrees of myopia, an eye with a greater PPA may have a lower blood flow. Thus, PPA area can be considered as a confounding factor. Please consider assessing the effect of PPA area on peripapillary blood flow.
Thank you very much for your advice. The PPA itself may play a role in the decreased peripapillary blood flow in myopic eyes. We have discussed them in the limitation (Line 396-398 in the marked copy).

8. Discussion section. Why only retinal blood flow is decreased in myopic eyes? The reason for non-significant difference in choroidal blood flow among the four groups needs to be explained.
Thanks. In the present study, the choroidal blood flow of the parafoveal area was defined as being from RPE reference with an offset of 29 microns to 59 microns using the OCT measurement (Fig. 1f). So, the blood flow of choroid in the present study was just a part of choroid, and not the whole choroid. The previous studies reported that the choroidal blood flow was decreased in patients with myopic eyes using Color Doppler Imaging (CDI), which measure the posterior ciliary artery mean peak systolic and peak diastolic blood flow velocity values. This may be the reason.

9. Line 370. Previous studies reported that macular retinal layers (including ganglion cell layer) as well as peripapillary retinal nerve fiber layer are thinner in myopic eyes than non-myopic eyes. Therefore, it is difficult to understand the first point addressed by the authors (why retinal blood flow is decreased only in peripapillary area). In the present study, no significant difference in macular GCC thickness was found among the four groups. What would be the cause of this finding (previous studies reported thinner GCC in myopic eyes)? Why GCC thickness was compared? What about the results of macular total retinal thickness difference comparison among the four groups?
Thanks. The reason why the peripapillary and parafoveal areas have different changes in microvasculations is still not fully understood, but the following points might provide some insights. First, the arterial blood supply is different for the peripapillary and parafoveal retina, which may represent a reason for the different relationships. Second, the parafoveal area is a belt 0.95mm in width that surrounds the foveal margin. Only 4–6 layers of ganglion cells and 7–11 layers of bipolar cells were found in this area, while the peripapillary area was found to have all 10 layers of the retina. Third, the participants in our study were very young, and their myopic changes were mainly located in the peripapillary area instead of the parafovea area. What's more, the subjects in our study did not present as pathological myopia.

In our study, the GCC thickness was lower in myopic eyes than that of emmetropic eyes, but the difference was of no significance. The reasons may be that the participants in our study were very...
young, and their myopic changes were mainly located in the peripapillary area instead of the parafoveal area. The macular total retinal thickness was not compared among the four groups in present study. The blood flow of the superficial layer in the parafoveal area was measured, which was defined as being from the inner limiting membrane with an offset of 3 microns to the inner plexiform layer with an offset of 29 microns (Fig. 1e), and the area was similar with the define of the GCC. Therefore, the GCC thickness was compared in this study.

10. In tables, please provide host poc analysis results for values which showed significant differences in ANOVA.
Thanks, we have provided the Post-Hoc multiple comparisons results in the table 1.

11. In table 3 and 4, beta values seem to be too low. Please provide R2 values and standardized beta values.
Thanks, we have provided the R2 values and standardized beta values in the table 3 and 4.

Reviewer: 2
Reviewer Name: Gabor Hollo
Institution and Country: Semmelweis University, Budapest, Hungary

Please state any competing interests or state 'None declared': I am an unpaid consultant of Optovue and Zeiss

Please leave your comments for the authors below
In this cross sectional investigation the authors investigated the relationship of peripapillary retina and choroid thickness and the corresponding peripapillary Optovue-Angiovue OCT angioflow parameters; and the macular retina and choroid thickness and the corresponding OCT angioflow parameters in healthy emmetropic and increasingly myopic groups. They found a significant relationship between the OCT angiography parameters and peripapillary retinal thickness across the groups, and no relationship for the macular parameters across the same group.
The main result of the well-designed study is not the relationship revealed (since it has been shown using various other methods in earlier investigations) but the applicability of Optovue OCT angioflow measurement for this (and similar) purposes, since this is a new, promising and non-invasive method.

This reviewer has no major problem with the study design, methods, statistics and discussion, but has some points where the authors may improve/clarify their manuscript (see them below). Minor English style improvement is also suggested, but this can be made during technical editing if the manuscript is accepted for publication.

Detailed comments:
1. The pts' age was around 16-y, which (since pathological myopic degenerations were excluded) suggests that retinal damage/perfusion impairment etc may have not yet developed (this comment also fits to Introduction, line 94). This needs to be discussed.
Thanks. The participants in our study were very young, and their retinal damage impairment may have not yet developed. In this study, the eyes with high myopia were found to have a decreased peripapillary retinal perfusion, including flow index and vessel density, compared with the emmetropic eyes using OCT angiography. This suggests that retinal perfusion impairment did exist in high myopic eyes. However, the present study was limited by its cross-sectional design, in that the age of the subjects was very young, so further long-term studies with larger samples and a greater age spectrum might tell us more about ocular perfusion in myopic eyes.

2. Methods: A very problematic point: no information on BCVA and VF is available. We really do not know what we are dealing with. Please provide data. It is interesting that several perfusion
parameters were investigated (these parameters are expected not to be informative in healthy 16-y old persons, and in fact they were not informative), but the most important functional data are missing. Thanks. We have provided the information of BCVA results in the table 1. We did not perform the test of visual field, however, all participants have normal retinal nerve fiber layer and ganglion cell complex thickness. Therefore, the participant did have healthy eyes.

3. Methods: The authors probably used the Optovue Angiovue instrument, which is built-in the RTVue-XR OCT, but they indicate "RTVue OCT" which is incorrect or "RTVue _XR Avanti". Please clarify and use the same terminology throughout the whole manuscript. Thanks, we have changed it in line 154 and line 167 in the marked copy.

4. Methods: page 10, line 178. The authors write: "...SSI<45, and if a scan contains severe artifacts, it will be excluded from analysis. " This is an important point, and the authors need to correctly discuss the influence of their "non-severe" (?) artifacts on the segmentation and the results. With increasing severity of myopia vitreoretinal artifacts appear with increasing frequency. Using the authors' OCT methods it was recently shown that even if TRVue-XR OCT reduces peripapillary and macular segmentation errors in myopia compared to RTVue-100 OCT, they are still there in many cases. This issue needs to be discussed in the discussion. For reference: Hollo G, Hsu SW, Naghizadeh F: Evaluation of a new software version of the RTVue optical coherence tomograph for image segmentation and detection of glaucoma in high myopia. J Glaucoma 2015 published online

Thanks. This point that the reviewer mentioned is very important. Those poor signal-quality scans should be excluded. In this study, the exclusion criterion for OCT angiography scans was set as a signal strength index (SSI) lower than 45. Bulk motion due to eye movement shows up as bright lines in the OCT image along the affected B-scans, which are removed in the OCT image after motion correction technology (MCT) merge artifact ( Kraus MF, Liu JJ, Fujimoto JG, et al. Quantitative 3D-OCT motion correction with tilt and illumination correction, robust similarity measure and regularization. Biomed Opt Express. 2014 Jul 11; 5(8):2591-613). If motion artifacts or image artifacts after MCT merge artifacts were still noted resulting from the eyelids during the examination, then the examination was repeated. We reviewed scans for blink artifacts and MCT merge artifacts; if a scan contains bright lines, it will be excluded from analysis. In total, there were 9 (9/87) cases excluded from this study.

5. Repeatability study (Methods and Results): the authors use the term "normal subjects", but this is unclear since all eyes were normal by definition, and refractive error alone is not a disease. Please use the term "emmetropic subjects". Though this reviewer thinks that the results (CV and ICC) are informative, it remains unclear why repeatability was not evaluated for the other groups (or all study eyes). In the current form it is suggestive for "hiding" data. Please add data from the other groups, or explain why myopic eyes were not involved in the repeatability study. In the table add "%" symbol to CV.

Thanks. We have use the term "emmetropic subjects" instead of "normal subjects" in line 207 and 250. The present study was limited by its cross-sectional design, in that the repeatability was not evaluated for all study eyes. However, the results (CV and ICC) in the emmetropic subjects were offer great intra-visit repeatability and inter-visit reproducibility in the measurement of the disc flow and the macula. Therefore, the myopic eyes were not involved in the repeatability study. In the table S1 the "%" symbol has been added to CV.

6. Discussion: to this reviewer some parts on the reason of reduced blood flow in myopia are speculative. The current study is not an investigation where the reasons and pathomechanisms are investigated. I suggest to remove or shorten those parts. An important point was not even raised: the arterial blood supply is different for the peripapillary retina and the macula, which may also represent a reason for the different relationships.

Thanks. We have deleted the first reason in the discussion in line 376-381 in the marked copy, and
stated that the arterial blood supply is different for the peripapillary retina and the macula, which may also represent a reason for the different relationships in line 374-376 in the marked copy.

7. Discussion: the authors make a statement which is not true as it is. Please either modify the statement or use different wording to avoid confusion. "To our knowledge, the peripapillary or parafoveal perfusion had not been measured in eyes with high myopia using quantitative techniques previously." (page 15, lines 310-312). Here is just one paper in which I investigated this problem 20 years age: Evaluation of the peripapillary circulation in healthy and glaucoma eyes with scanning laser Doppler flowmetry. Holló G, Greve EL, van den Berg TJ, Vargha P. Int Ophthalmol. 1996-1997;20(1-3):71-7.

Thank you for your advice, we have changed the sentence “To our knowledge, the peripapillary or parafoveal perfusion had not been measured in eyes with high myopia using quantitative techniques previously." to" Despite this, the study of peripapillary and parafoveal perfusion in eyes with high myopia has been rare." in line 316-317 in the marked copy.

8. Introduction and Discussion: since the number of publications addressing the relationship of peripapillary retinal thickness and perfusion as measured with RTVue-XR OCT/ Angiovue OCT angiography is very limited use of all published data is recommended. A recently published study (Holló G: Vessel density calculated from OCT angiography in three peripapillary sectors in normal, ocular hypertensive and glaucoma eyes. Eur J Ophthalmol 2015, published online first, DOI: 10.5301/ejo.5000717) may even provide information on the changes for different peripapillary sectors (this is important to the current submission since myopia-related alterations are more pronounced in the temporal sectors) and on the relationship between retinal tissue loss and vessel loss in the peripapillary area.

Thanks. The aim of this study was to evaluate the peripapillary and parafoveal perfusion of young healthy myopic subjects with spectral domain optical coherence tomography (OCT) angiography. We focus on the average peripapillary perfusion parameter and their relationship with the retinal nerve layer thickness. The changes for different peripapillary sector will be important and it will be studied further.

VERSION 2 – REVIEW

<table>
<thead>
<tr>
<th>REVIEWER</th>
<th>Young Hoon Hwang</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Department of Ophthalmology, Konyang University, Kim’s Eye Hospital, Myung-Gok Eye Research Institute, Seoul, Korea</td>
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<tr>
<td>REVIEW RETURNED</td>
<td>10-Feb-2016</td>
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</table>

GENERAL COMMENTS

Thank you for your excellent revision.

<table>
<thead>
<tr>
<th>REVIEWER</th>
<th>Gabor Hollo</th>
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GENERAL COMMENTS

The reviewer completed the checklist but made no further comments.
Is the peripapillary retinal perfusion related to myopia in healthy eyes? A prospective comparative study

Xiaolei Wang, Xiangmei Kong, Chunhui Jiang, Mengwei Li, Jian Yu and Xinghuai Sun

*BMJ Open* 2016 6:
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