MICROSTRUCTURAL ANALYSIS OF RETINAL MACULAR THICKNESS AND VASCULATURE IN HEALTHY INDIVIDUALS USING OPTICAL COHERENCE TOMOGRAPHY (OCT) AND OCT-ANGIOGRAPHY

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РЕЗЮМЕ

Цел: Да се изследват дебелината на слоя на ганглийните клетки и на вътрешния плексиформен слой (GCL+IPL) в макулата, дебелината на ретината от вътрешната гранична мембрана до ретиналния пигментен епител (ILM-RPE) в макулата, както и параметрите на фовеалната аваскуларна зона (FAZ), включително съдова/перфузионна плътност (FAZ Vessel Density, FAZ Perfusion density) при здрави индивиди. Да се оцени влиянието на пола и възрастта върху тези параметри.

Материали и методи: В проучването са включени 122 очи на 63 души, без очно заболяване, на възраст от 6 до 78 г., със зрителна острота без или със корекция, равна на 1.0. Изследването е направено с апарата ZEISS CIRRUS HD-OCT. Използван са протоколите за „Анализ на ганглийните клетки”, „Анализ на макулната дебелина” и системата AngioPlex™ OCT ангиография.

Резултати: Установената средна дебелина на комплекса ганглийни клетки и вътрешния плексиформен слой (GCL+IPL) в макулата е 81,80±7,81 µм. Средната дебелина на ретината от вътрешната гранична мембрана до ретиналния пигментен епител (ILM-RPE) в областта на макулата е 283,13±13,04 µм.

Средната и минималната GCL+IPL дебелини в макулата, дебелината на ILM-RPE в макулата и средният обем на макулния куб показват статистически значима отрицателна корелация с възрастта. При женския пол средната GCL+IPL дебелина в макулата е статистически значимо по-висока. Не се откриха статистически значими връзки между половете и средната дебелина на ILM-RPE в макулата и средния обем на макулния куб. Средната дебелина на ILM-RPE в централното подполе на макулната област показва статистически значима положителна корелация с женския пол.

Установената средна площ на фовеалната аваскуларна зона (FAZ) е 0,245±0,1 мм², а средната съдова плътност в централната FAZ е 9,36±3,12 мм⁻¹, като средно в цялата фовеална аваскуларна зона е 17,34±2,18 мм⁻¹. Средната перфузионна плътност във FAZ е 42±5%. Площта на FAZ и периметърът на FAZ показват отрицателна, но не статистически значима корелация с възрастта и положителна статистически значима връзка с женския пол.
**ABSTRACT**

**Aim:** The aim of this article is to generate the normative data of macular ganglion cell layer-inner plexiform layer (GCL+IPL) thickness, macular internal limiting membrane-retinal pigment epithelium (ILM-RPE) thickness, as well as foveal avascular zone (FAZ) metrics, including FAZ vessel density and FAZ perfusion density in healthy individuals. It aimed to evaluate the influence of gender and age over these parameters.

**Materials and Methods:** A total of 122 eyes of 63 healthy individuals aged between 6 and 78 years, with no ocular disease and best corrected visual acuity of 20/20, were scanned using ZEISS CIRRUS HD-OCT. The protocols used in the study were macular thickness analysis, ganglion cell analysis and the AngioPlex OCT angiography scan, which allows visualization of retinal and choroidal vasculature without the need for contrast dye injection.

**Results:** The mean thickness of the complex GCL+IPL in the macula was 81.80±7.81µm. The mean thickness of the retina from ILM-RPE in the macular area was 283.13±13.04 µm. The mean and the minimum GCL+IPL thickness in the macula, ILM-RPE thickness in the macular area and the average volume of the macular cube showed significant negative correlation with age. In females the mean GCL+IPL thickness in the macula was significantly higher. We have not found significant correlation between genders and the mean ILM-RPE thickness in the macular area and the average volume of the macular cube. ILM-RPE mean thickness in the central subfield of the macular area showed significant positive correlation with male gender.

The average area of FAZ was 0.245±0.1mm². The mean vascular density in the central FAZ was 9.36±3.12 mm⁻¹, and the average of the entire FAZ was 17.34±2.18 mm⁻¹. The average perfusion density in FAZ was 42±5%. FAZ area and FAZ perimeter showed negative but not significant correlation with age and significant positive correlation with female gender.

**Conclusion:** Our findings provide normative database for macular parameters in healthy individuals, which is important for early diagnosis and evaluation of pathological changes in various retinal diseases.

**Keywords:** macula, FAZ, OCT-A
Целта на настоящото проучване е да се създаде прогресивна база данни за нормативни стойности на тъкани и за да се анализира влиянието на пола и възраст на пациентите върху тези показатели. За целта са използвани OCT-А изображения на фовеоларната аваскуларна зона (FAZ) и измерването на дебелината на макулата от вътрешна гранична мембрана (GCL) и вътрешен плексиформен слой (IPL). Наред с това се анализира и плътността на съдовете във FAZ и перфузионната плътност във фовеоларната зона.

**МATERIALS AND METHODS**

Целта на настоящото проучване е да се създаде прогресивна база данни за нормативни стойности на тъкани и за да се анализира влиянието на пола и възраст на пациентите върху тези показатели. За целта са използвани OCT-А изображения на фовеоларната аваскуларна зона (FAZ) и измерването на дебелината на макулата от вътрешна гранична мембрана (GCL) и вътрешен плексиформен слой (IPL). Наред с това се анализира и плътността на съдовете във FAZ и перфузионната плътност във фовеоларната зона.

**МАТЕРИАЛ И МЕТОДИ**

В проучването са изследвани 122 очи на 63 души между октомври 2018 г. и август 2019 г. Тридесет и шест от тях са здрави зрители, а останалите са с различни диабетични и други увреждания на ретината. Всички участници са бележени с нормална визия без корекция. Изследването е съгласувано с местните медицински институции и е със санкциите на комитета по защита на хората в медицинските изследвания. Всички участници са информирани за целите, процедурите и рисковете на проучването и са дадени волuntaryни разрешения. OCT-А изображенията на вътрешна гранична мембрана (GCL) и вътрешен плексиформен слой (IPL) са измервани и анализирани със специална алгоритмика. Плътността на съдовете във FAZ е измерена чрез анализ на OCT-А спектър.
Microstructural analysis of retinal macular thickness and vasculature in healthy individuals using optical coherence tomography (OCT) and OCT-Angiography

Incident participants are women (70 eyes) and 27 are men (52 eyes) between 6 and 78 years. The participants are thoroughly informed about the study and have signed an informed consent form. After obtaining medical and family history, all subjects underwent a comprehensive ophthalmic examination, including corrected visual acuity (BCVA), slit-lamp biomicroscopy, fundus examination, optical coherence tomography, and OCT-Angiography. All healthy participants had transparent ocular media with the best corrected visual acuity of 20/20. The examined participants had no history of glaucoma over 21 mmHg, no eye disease. The exclusion criteria were current eye disease, high myopia or hyperopia, clinically significant vitreous opacities, or images of low quality due to unstable fixation.

The patients were examined with a Zeiss Cirrus 5000 HD-OCT (Carl Zeiss Meditec, Dublin, California, USA) device. All OCT protocols, software. In all OCT maps, macular layer thickness detection was performed automatically by device software analysis without manual intervention of the person who operates the machine.

GCL+IPL thickness was measured using the ganglion cell analysis protocol with 6.0 x 6.0 mm volume cube scans, 512x128 pixel scan resolution, presenting quantitative and qualitative evaluation of them. An image of the posterior segment of the eye displayed maps for GCL+IPL thickness and the elliptical measurement annulus was centered about the fovea. The thickness chart showed median and minimal thickness within the elliptical annulus.

Based on the 6 mm x 6 mm data cube captured by the Macular Cube 512x128 pixel scan resolution, the macular thickness analysis permitted analysis concerning retinal qualities as well as retinal quantitative metrics. The slice navigator enabled simultaneous representation of a chosen spot on line scanning ophthalmoscope (LSO) image, OCT fundus

<table>
<thead>
<tr>
<th>Name:</th>
<th>ID:</th>
<th>Exam Date:</th>
<th>Examin Time:</th>
<th>Gender:</th>
<th>Female</th>
<th>Senior Number:</th>
<th>5000-8924 5000-8924</th>
<th>Technician:</th>
<th>Cirrus, Operator</th>
<th>Signal Strength:</th>
</tr>
</thead>
</table>

**Ganglion Cell OU Analysis: Macular Cube 512x128**

**OD Thickness Map**

**OS Thickness Map**

**OD Deviation Map**

**OS Deviation Map**

**OD Horizontal B-Scan**

**OS Horizontal B-Scan**

**Doctor's Signature**

**Fig. 1. Ganglion cell OU analysis protocol**
исползвани в изследването, са „Анализ на макулната дебелина“, „Анализ на ганглийните клетки“ и системата AngioPlex™ OCT-A. При OCT сканирането дебелината на слоя ганглийни клетки и на вътрешния плексиформен слой в макулата (GCL+IPL), както и дебелината на ретината от вътрешна гранична мембрана до ретинален пигментен епител в областта на макулата (ILM-RPE) са определени автоматично и анализирани от OCT софтуер. Във всички OCT карти измерването на дебелината на макулните слоеве се извършва автоматично чрез софтуерен анализ, без ръчна настройка от оператора.

Дебелината на слоя ганглийни клетки и на вътрешния плексиформен слой (GCL+IPL) се измери с помощта на протокол „Анализ на ганглийните клетки“ с куб на сканиране image, retinal thickness map, layer maps, and OCT image displays. The ETDRS grid (9 regions defined in the Early Treatment Diabetic Retinopathy Study) was automatically centered on the fovea with Fovea Finder™. Retinal thickness values, from ILM to RPE, in microns were displayed. AngioPlex OCT-A allows visualization of retinal and choroidal vasculature without the requirement for contrast dye introduction into the bloodstream. AngioPlex Metrix measurements for the superficial retinal plexus, including vessel/perfusion density and FAZ parameters, were done for 6 x 6 mm scans. Vessel density is the complete length of perfused vasculature per unit area in the measured region; perfusion density is the total area of perfused vasculature per unit area in the measured region.
Microstructural analysis of retinal macular thickness and vasculature in healthy individuals using optical coherence tomography (OCT) and OCT-Angiography

In a volume of 6x6 mm, the resolving power of the scan was 512x128 pixels. The GCL+IPL thickness maps were superimposed on an image of the retinal surface. An elliptical measuring ring was centered around the fovea. The table shows the mean and minimum thickness within the elliptical ring.

The mean thickness of GCL+IPL in the macula was 81.80±7.81 µm, and the minimum thickness was 78.97±10.05 µm. The mean thickness of the retina from ILM to RPE in the macular area was 283.13±13.04 µm, with an average volume of the macular cube of 10.19±0.47 mm³. The mean thickness of ILM-RPE in the central subfield of the macular area was 259.3±22.88 µm.

Associations between macular measurements and age were examined by Pearson’s correlation analysis. Age-stratified comparisons of all macular and retinal nerve measurements between genders were carried out using independent sample t-test or ANOVA. The results were considered to be statistically significant at p<0.05.

RESULTS

One hundred twenty-two eyes of sixty-three normal subjects at a mean age of 46±18 years (from 6 to 78 years old) were included in the study (27 men – 43%; 36 women – 57%).

Table 1. Mean values for the measured retinal thicknesses in different age groups

<table>
<thead>
<tr>
<th>Age Groups in Years</th>
<th>Average GCL+IPL Thickness in µm</th>
<th>Minimum GCL+IPL Thickness in µm</th>
<th>ILM-RPE Thickness Central Subfield in µm</th>
<th>Thickness Average Cube ILM-RPE in µm</th>
<th>Volume Cube in mm³</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>84.22</td>
<td>82.66</td>
<td>253</td>
<td>289.22</td>
<td>10.42</td>
</tr>
<tr>
<td>21-40</td>
<td>83.93</td>
<td>81.90</td>
<td>253.9</td>
<td>286.81</td>
<td>10.33</td>
</tr>
<tr>
<td>41-60</td>
<td>81.85</td>
<td>78.51</td>
<td>261.61</td>
<td>282.19</td>
<td>10.15</td>
</tr>
<tr>
<td>61-80</td>
<td>79.08</td>
<td>75.88</td>
<td>262</td>
<td>279.35</td>
<td>10.05</td>
</tr>
</tbody>
</table>

Table 2. Mean values for the measured OCT-A parameters in different age groups

<table>
<thead>
<tr>
<th>Age Groups in Years</th>
<th>FAZ Area in mm²</th>
<th>FAZ Perimeter in mm</th>
<th>Central FAZ Vessel Density in mm⁻¹</th>
<th>Full FAZ Vessel Density in mm⁻³</th>
<th>Central FAZ Perfusion Density in %</th>
<th>Full FAZ Perfusion Density in %</th>
</tr>
</thead>
<tbody>
<tr>
<td>0-20</td>
<td>0.31</td>
<td>2.3</td>
<td>7.61</td>
<td>16.91</td>
<td>17</td>
<td>41</td>
</tr>
<tr>
<td>21-40</td>
<td>0.25</td>
<td>2.05</td>
<td>10.15</td>
<td>18</td>
<td>23</td>
<td>44</td>
</tr>
<tr>
<td>41-60</td>
<td>0.23</td>
<td>2.02</td>
<td>9.49</td>
<td>17.29</td>
<td>22</td>
<td>42</td>
</tr>
<tr>
<td>61-80</td>
<td>0.23</td>
<td>1.91</td>
<td>8.83</td>
<td>16.9</td>
<td>19</td>
<td>41</td>
</tr>
</tbody>
</table>
**DISCUSSION**

The purpose of this study was to report the normal reference limits for macular layers thickness measurements and vasculature measured by ZEISS CIRRUS 5000 HD-OCT. The measurements included various parameters of full retinal thickness in the macula, GCL+IPL thickness and FAZ metrics for healthy eyes.

The average macular GCL+IPL thicknesses measured using SD-OCT and ranging between 68 and 74.8 µm have been reported in normal eyes (12,13). The limitations of these studies are that they included only a small number of eyes and did not explore the relationship between macular GCL+IPL thickness and demographic parameters like sex or age. Our result for the mean thickness of GCL + IPL in the macula was 81.80±7.81 µm, which is higher than that in the cited studies. The difference in the results may be explained with the fact that the scans were made with different SD-OCT models. In another study Jean-Claude Mwanza et al. published an average GCL-IPL thickness of 82.1±6.2 µm (range, 68–101), whereas the minimum GCL+IPL thickness was 80.4±6.4 µm (range, 64–98) (14). The minimum GCL+IPL thickness in our study was 78.97±10.05 µm. The published paper of Jean-Claude Mwanza et al. has results close to ours for GCL-IPL mean and minimum thicknesses. The authors also stratified participants by age groups and found that the GCL-IPL thickness was stable between 18 and 49 years of age and then decreased progressively (14), which corresponds to our finding that the mean and the minimum GCL+IPL thickness in the macula have a significant negative correlation with age. Jean-Claude Mwanza et al. found in their study that the average GCL-IPL did not differ between the sexes, which is in contrast to our results that in females the mean GCL+IPL thickness in the macula is significantly higher.

In our study the mean thickness of ILM-RPE in the central subfield of the macular area was 259.3±22.88 µm, thicker than the results in previous studies (200.58±19.22 µm) (15). T. Buehren et al. reported mean central subfield thickness (ILM - RPE) based on CirrusTM HD-OCT scan of 263.2 microns, which is closer to our results (16). Sruhti Aregnali et al. found that the mean retinal thickness (ILM-RPE) tends to measure slightly higher in younger patients than in older patients. In their study in males the mean central ILM to RPE thickness was slightly higher than in females (15). We found in our study that the mean ILM-RPE thickness in the macular area and the average volume of the macular cube show significant negative correlation with age. We
have not found significant correlation between genders and the mean ILM-RPE thickness in the macular area and the average volume of the macular cube, but ILM-RPE mean thickness in the central subfield of the macular area showed significant positive correlation with male gender.

Part of the purpose of our study was to measure the FAZ area and vessel/perfusion density using OCT-A in patients with normal healthy eyes and to establish a database with normative data for these parameters.

As it can be seen on Fig 3, the mean values for the FAZ area in the superficial capillary retinal plexus that we have collected are slightly lower than those published in three previous studies. Guo J et al. found an average area of the FAZ of 0.373±0.109 mm², Zivkovic et al. - 0.292±0.048 mm², and the closer to our results were those of Magrath et al. - 0.2657 mm² (17,18, 19).

There is a potential role of the microvasculature and blood flow in the pathophysiology of various retinal diseases. In our study the average vessel density of the entire FAZ was 17.34 ± 2.18 mm⁻¹. In their study Mayss Al-Sheikh et al. determined the repeatability of OCT-A-derived automated vessel density measurements in the superficial retinal layer (SRL) and deep retinal layer (DRL) in healthy individuals. They found a result in the first and second session of 19.43±3.10 mm⁻¹ and 19.72±3.78 mm⁻¹ for the SRL (20).

We also measured the average perfusion density in FAZ. As described by Durbin et al., perfusion density is a variable picturing the total area of perfused vasculature per unit in a region of interest. Within the desired region of interest, the average of the binarized slab is taken for average perfusion density in FAZ. They published a result of 42% desired region of interest, the average of the binarized slab is taken for average perfusion density in FAZ. Within the desired region of interest, the average perfusion density in FAZ was 42% of the desired region of interest. In their study, Atsushi Fujiwara et al. did not find significant correlation with age. In contrast to our result (21).

CONCLUSION

In this study we evaluated the microstructure of retinal macular thickness and vasculature in healthy human subjects with optical coherence tomography and OCT-A. Our findings provide normative
REFERENCES


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