Retinal nerve fiber layer thickness by optical coherence tomography in sample of normal Iraqi population

Mohammed Hussein Oudah¹, Qasim Kadhim Farhood², Suzan Amana Rattan³

¹Al_Habobi Teaching Hospital/Thi-Qar Health Office /Iraq
²Department of Ophthalmology, College of medicine/University of Babylon/Iraq
³Department of surgery, Al-Kindy College of Medicine, University of Baghdad, Iraq

*Corresponding Author: Suzan Amana Rattan, Address: Baghdad /Iraq.
E-mail address: suzanamana@kmc.uobaghdad.edu.iq

Abstract
Background: retinal nerve fiber layer assessment had become a standard of care in diagnosing and following up patients with glaucoma, at the same time it is crucial to determine the normal range in healthy Iraqi population by the available spectral domain OCT in order to anchor a strong foothold for studying it in different diseases. The aim of the current study: To quantitatively assess the normative values for retinal nerve fiber layer thickness by spectral domain optical coherence tomography in sample of normal Iraqi population.

Methods: across sectional study involved 130 healthy subjects (260 eyes). The assessment included: Visual acuity (VA) testing, slit-lamp examination, routine fundus examination, gonioscopy, and IOP measured by air puff tonometer corrected by central corneal thickness. Optical coherence tomography was done by using spectral domain technology via the Cirrus HD-OCT 5000 (Carl Zeiss Meditec, Inc.), for the optic nerve and the neighboring four quadrants retinal nerve fiber layer thickness, namely Temporal, Superior, Nasal, and Inferior using circular scans of 3.4 mm centered on the optic nerve head.

Results and conclusion: The study showed that the mean thickness was 91.73 ± 8.61 µm, it was higher in younger subjects compared to older subjects (p-value = 0.043). The linear regression module revealed a statistically significant regression of age with mean and temporal RNFL thickness, and it predicted a mean decrease of 0.167 µm per year, highest superiorly (-0.211 µm/year) and lowest inferiorly (-0.039 µm/year). The thickest sector was the inferior with 120.27 ± 14.23 µm, followed by the superior 120.27 ± 14.23 µm, then the nasal 70.44 ± 11.36 µm, and thinnest in the temporal 62.3 ± 8.56 µm. There was a significant statistical negative correlation between RNFL thickness and IOP (r=-0.225), which meant there was a decrement in thickness with increasing IOP. The analysis concludes that the retinal nerve fiber layer thickness was lower in healthy Iraqi subjects compared to other ethnic groups.

Keywords: retinal nerve fiber thickness, optic nerve head assessment, glaucoma

measurement of RNFL thickness with OCT in normal subjects. It is highly likely that these factors affect the RNFL thickness differently, and hence affect diagnosis and monitoring in diseased conditions.\(^{[6]}\) Currently, each OCT machine has its own database of age-matched normal values used to interpret RNFL thickness measurements. However, most normative databases comprise measurements from subjects of European descent. Therefore, it is important that researchers ascertain normal RNFL thickness values in other races, in our case, the Iraqis. By doing so, clinicians will be able to distinguish normal from pathological changes more clearly.\(^{[7]}\)

Subjects and methods
Across sectional study that involved the data collected from healthy medical staff and relatives of patients attending Ibn Al-Haitham Teaching Eye Hospital from 130 healthy subjects (260 eyes) by the researcher for 9 months from August/2018 to April/2019. The assessment included: Visual acuity (VA) testing, slit-lamp examination, routine fundus examination, gonioscopy, and IOP measured by air puff tonometer corrected by central corneal thickness. Optical coherence tomography was done by using spectral domain technology via the Carus HD-OCT 5000 (Carl Zeiss Meditec, Inc.), for the optic nerve and the neighboring four quadrants retinal nerve fiber layer thickness, namely Temporal, Superior, Nasal, and Inferior using circular scans of 3.4 mm centered on the optic nerve head.

Inclusion criteria:
1. Adult subjects older than 18 years.
2. Normal IOP (10–21 mm Hg).
3. Anterior chamber angle width >20 degree (Shaffer grade 3 and 4).

Exclusion criteria:
1. Previous ophthalmic surgeries.
2. Refractive errors in excess of -1 to +1 diopters sphere or cylinder.
3. History of ocular trauma.
4. Cup/disc ratio ≥ 0.3 or difference of 0.2 between the two eyes.
5. Any ocular disease like diabetic retinopathy, age related macular degeneration, optic neuropathy, retinal vascular disease, macular dystrophies, or ocular inflammatory diseases.
6. Medical condition like hypertension, diabetes mellitus dementia, retinal dystrophies, and CNS diseases.

Ethical considerations: consent was taken from all the subjects about their enrollment in the study and for their data to be published.

Statistical analysis
The collected data was handled and analyzed by IBM© SPSS© (Statistical Package for the Social Sciences) Statistics Version 23. Chi-square test was used for categorical data. Univariate ANOVA test and independent samples T-test and was used for numerical and normally distributed data. Pearson Correlation was used to identify the possible linear correlation between the study variables. All analyses were done with 95% confidence intervals (CI). Linear regression module was done to investigate the changes in RNFL thickness in correlation to patients’ age P-values less than 0.05 were considered statistically significant throughout this study.

Results
From the results of table (1) among 260 eyes of 130 subjects, the commonest age group was 30-39 years with 42 (32.3%) subjects, followed by 40-49 years with 32 (24.6%) subjects, and the mean age of subjects was 41.31±12.99 years. There were very close male/female ratio, as there were 67 males and 63 females enrolled in the study.

Table (1): basic characteristics of study group.
The mean thickness of the fiber layer was higher in younger subjects compared to older subjects, and this decrease was statistically significant (p-value = 0.043), but the analysis of each quadrant revealed an insignificant statistical difference between age groups. There was a significant statistical inverse correlation between age and RNFL thickness, with a weak correlation, as the correlation coefficient was -0.210 (as shown in table 2).

Table (2): distribution of RNFL thickness of 260 eyes according to age.

<table>
<thead>
<tr>
<th>Age group</th>
<th>Number (% )</th>
<th>Mean thickness± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>20-29</td>
<td>42 (16.2)</td>
<td>94.04 ± 10.05</td>
</tr>
<tr>
<td>30-39</td>
<td>84 (32.3)</td>
<td>92.68 ± 7.22</td>
</tr>
<tr>
<td>40-49</td>
<td>64 (24.6)</td>
<td>92.48 ± 9.02</td>
</tr>
<tr>
<td>50-59</td>
<td>44 (16.9)</td>
<td>89.56 ± 9.37</td>
</tr>
<tr>
<td>60-69</td>
<td>26 (10.0)</td>
<td>85.88 ± 4.98</td>
</tr>
<tr>
<td>Total</td>
<td>260</td>
<td>91.73 ± 8.61</td>
</tr>
</tbody>
</table>

The linear regression module revealed a statistically significant regression of patients ages in years with mean and temporal RNFL thickness, and it predicted a mean decrease of 0.167 µm per year, highest superiorly (-0.211 µm/ year) and lowest inferiorly (-0.039 µm/ year). As shown in table (3).

Table (3): linear regression module for predicting RNFL thickness by age.

<table>
<thead>
<tr>
<th>Sector</th>
<th>Loss of RNFL thickness (µm/ year)</th>
<th>SE</th>
<th>95% confidence interval</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>-0.167</td>
<td>0.057</td>
<td>-0.279 -0.055</td>
<td>0.004</td>
</tr>
<tr>
<td>Inferior</td>
<td>-0.039</td>
<td>0.097</td>
<td>-0.230 0.153</td>
<td>0.688</td>
</tr>
<tr>
<td>Superior</td>
<td>-0.211</td>
<td>0.167</td>
<td>-0.429 0.007</td>
<td>0.058</td>
</tr>
<tr>
<td>Nasal</td>
<td>-0.069</td>
<td>0.077</td>
<td>-0.221 0.084</td>
<td>0.372</td>
</tr>
<tr>
<td>Temporal</td>
<td>-0.116</td>
<td>0.057</td>
<td>-0.229 -0.002</td>
<td>0.045</td>
</tr>
</tbody>
</table>

There was a highly significant difference in mean RNFL thickness between sectors and mean RNFL thickness (p <0.001). The thickest sector was the inferior with 120.27 ± 14.23 µm, followed by the superior 120.27 ± 14.23 µm, then the nasal 70.44 ± 11.36 µm, and thinnest in the temporal 62.3 ± 8.56 µm as in table 4.

Table (4): Retinal nerve fiber layer thickness according to sectors.

<table>
<thead>
<tr>
<th>Sector</th>
<th>Mean ± SD</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nasal</td>
<td>70.44 ± 11.36</td>
<td>68.47 – 72.41</td>
</tr>
<tr>
<td>Superior</td>
<td>111.12 ± 16.41</td>
<td>108.67 – 114.36</td>
</tr>
<tr>
<td>Temporal</td>
<td>62.3 ± 8.56</td>
<td>60.81 – 63.79</td>
</tr>
<tr>
<td>Inferior</td>
<td>120.27 ± 14.23</td>
<td>117.28 – 122.74</td>
</tr>
<tr>
<td>Total</td>
<td>91.13 ± 8.67</td>
<td>89.63 – 92.34</td>
</tr>
</tbody>
</table>

There were statistically insignificant differences in RNFL thickness between genders, whether mean or according to quadrants, being slightly higher in males 91.27 ± 8.91 µm compared to females 90.98 ± 8.48 µm. As shown in table (5).

Table (5): Retinal nerve fiber layer thickness according to gender.
There was a significant statistical negative correlation between RNFL thickness and IOP ($r = -0.225$), which meant there was a decrement in thickness with increasing IOP as in figure 1.

**Figure (1):** Scatter plot showing the relationship between IOP and retinal nerve fiber layer thickness ($r = -0.225$, $p = 0.010$)

**Table (6):** Retinal nerve fiber layer thickness by age and ethnicity.

<table>
<thead>
<tr>
<th>Investigators</th>
<th>Mean age ± SD</th>
<th>Ethnic group</th>
<th>RNFL thickness ± SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Choi</td>
<td>50.22 ± 14.50</td>
<td>Korean</td>
<td>106.1 ± 7.6</td>
</tr>
<tr>
<td>Ha</td>
<td>60.6 ± 13.6</td>
<td>Korean</td>
<td>110.0 ± 7.9</td>
</tr>
<tr>
<td>Celebi</td>
<td>38.89 ± 11.19</td>
<td>Turkish</td>
<td>97.01 ± 7.42</td>
</tr>
<tr>
<td>Cubuk</td>
<td>35.1 ± 9.6</td>
<td>Turkish</td>
<td>111.5 ± 9.3</td>
</tr>
<tr>
<td>Al-Sa’ad</td>
<td>60 ± 12</td>
<td>Jordanian</td>
<td>99 ± 11</td>
</tr>
<tr>
<td>Alasail</td>
<td>55.2±16</td>
<td>Caucasian</td>
<td>96.0±9.2</td>
</tr>
<tr>
<td></td>
<td>51±19</td>
<td>Hispanic</td>
<td>102.9±11</td>
</tr>
<tr>
<td></td>
<td>52±16</td>
<td>African American</td>
<td>99.2±10.2</td>
</tr>
<tr>
<td></td>
<td>51±17</td>
<td>Asian</td>
<td>100.7±8.5</td>
</tr>
<tr>
<td>Mehboob</td>
<td>44.48±4.34</td>
<td>Pakistani</td>
<td>126.98±10.07</td>
</tr>
<tr>
<td>Bendschneider</td>
<td>20 to 78</td>
<td>Caucasian</td>
<td>97.2±9.7</td>
</tr>
<tr>
<td>Kudhair and Kareem</td>
<td>47.1± 14.8</td>
<td>Iraqi</td>
<td>92.46±10.83</td>
</tr>
</tbody>
</table>

Discussion:
In this study we measured RNFL thickness of 260 eyes for 130 healthy adult subjects by using spectral domain OCT and we compared its differences between different age groups, gender and its relation to IOP. In the current study, the mean age was 41.31 ± 12.99 years and retinal nerve fiber layer thickness was 91.73 ± 8.61 μm, and there were statistically significant differences in RNFL thickness between age groups. Table (6) showed the different values reported by different investigators throughout the world\cite{8,9}. This showed that the Iraqi thickness was lower than other studies, although the mean age was comparatively lower than those studies, this could be due to different devices with different techniques (swept source or spectral domain), and also there is evidence for differences between ethnic groups (lower for Caucasian)\cite{8}. Some factors related to the eyes itself could affect the thickness like myopia and longer axial length\cite{7}, another study that support our results in Iraq was done by Kudhair (2017)\cite{10}, which could mean that there might be ethnic or local environmental factors that put Iraqi in lower thickness of RNFL.

Our study agreed with classic ISNT rule (Table 4), which was also showed by Chen et al (2018)\cite{7}, and Alasil et al (2013)\cite{8}, which is related to optic nerve dimensions (wider vertically than horizontally) because the optic nerve is the collection of retinal nerve fibers, although Poon et al (2017)\cite{11}in their study to investigate the validity of this rule concluded that this rule applied for less than half of normal subjects, but when they depended only upon "inferior > superior", it applied to > 70% of them.

In the current study, there was a statistically significant inverse correlation between age and RNFL thickness which meant that with advancing in age there was a linear decrease in its thickness ($r=-0.210$). This was comparable to results of Hondur et al (2018) in Columbia\cite{12}, who studies the effect of aging on the nerve fiber layer in 49 healthy subjects and they measured peripheral and the peripheral and the peri-papillary RNFL thickness and reported that both of them had inverse correlation with age. Also Choi et al (2017) in South Korea\cite{13}, who studied the peri-papillary RNFL thickness in 309 eyes of healthy subjects, and reported that the mean thickness decreased with age with a negative correlation coefficient ($r$) of -0.172. Older studies using spectral domain OCT had reported similar results regarding the negative correlation between age and nerve fiber layer thickness\cite{8,14,16}. It is important to identify normal age-related loss of thickness because ophthalmologists are using OCT increasingly for glaucoma treatment follow up, so this "physiological decline" should be differentiated from the loss associated with glaucomatous damage to the nerve fibers.

In the current study, it was found that with each year difference between patients there was a predicted loss of 0.167 μm, highest in superior and temporal quadrants (Table3), this was comparable to results of Mansoori and Balakrishna (2017) in India\cite{17}, who studied fifty eyes of healthy subjects by spectral domain OCT, and they reported that the thickness decreased with increasing age, with a predicted decrement of ~0.54 μm per year highest in the inferior and nasal quadrants. Also the original research done by Hondur et al (2018) in Colombia\cite{12}, who used a swept source OCT, and reported that the peripheral and the ratio of the peripheral to peripapillary nerve fiber thicknesses were negatively correlated with age. In similar manner, Chen et al who studied 143 eyes with swept source OCT and reported negative correlation with age ($r=0.210$) that with every decade the mean thickness decreased 4.97 μm highest in superotemporal and superonasal segments\cite{7}. The decrease in nerve fiber layer thickness could be attributed mainly for the age related decrease in ganglion cells per year\cite{18}, in addition to that there is diminishing in retinal vascularity of the inner retinal layers\cite{19}, all might reduce RNFL thickness.

Our study showed no significant association between gender and RNFL thickness was observed in any quadrant (Table 5), this agreed with the results of Mansoori et al (2012) in India\cite{20}, but Dhami et al (2016) also in India\cite{21} revealed that women had thicker RNFL in the temporal quadrants, while Khawaja et al (2013)\cite{22}, in large population based study of 11,030 eyes and reported that male have thinner mean RNFL thickness by approximately ~0.44 μm. These conflicting reports might be attributed to external factors like age difference, baseline difference between genders, or anatomical variances.

In the current study, there was a statistically significant negative correlation between IOP and RNFL thickness (Figure 1), loss of nerve fibers(amount and speed) was linked to higher IOP in glaucomatous patients\cite{23}, in healthy individuals Zhao et al (2014) in China\cite{24} in a large population-based study that enrolled 3468 subjects, and they reported significant negative correlation between IOP and thickness, also El-Hifnawy et al (2017) in Egypt\cite{25} who studied 100 eyes of healthy subjects and reported the same negative correlation. In glaucoma, the high IOP causes damage on RNFL either via ischemia or...
direct trauma, but in healthy subjects with IOP within the normal range, these mechanisms does not explain the negative correlation, which opens the door for searching for another causes, or confirming this relationship.

Conclusions
1. The retinal nerve fiber layer thickness was lower in healthy Iraqi subjects compared to other ethnic groups.
2. There was a negative correlation between thickness and age and IOP, with no gender effect.

Recommendations
1. Further studies should be done regarding studying retinal nerve fiber layer thickness in large population based studies.
2. Studying the effect of aging on thickness on the same subjects following them throughout different age intervals.

Ethical clearance: The ethical committee of the Iraqi Board for Medical Specializations had approved this study.

Conflict of interest: The authors declare that they don’t have conflict of interest

Funding: self-funding.

References:
3. Jones-Odeh E, Hammond C. How strong is the relationship between glaucoma, the retinal nerve fibre layer, and diseases such as Alzheimer’s disease and multiple sclerosis? Eye. 2015;29(10):1270.