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Glaucoma is a major disease that potentially results in irreversible blindness.

It is characterized by characteristic changes of;

- ONH
- RNFL
- Visual field.

Basma Gamal, Ophthalmology Department, Tanta University, Thesis for MSc, August 2018
Intraocular pressure is a major risk factor and was recognized as the only cause of neural tissue loss at the ONH. Many studies revealed that IOP reduction alone cannot prevent VF loss progression in all patients.

So, it was suggested that vascular factors play a critical role in glaucoma development.

However, till now it remains unclear whether the decrease in blood flow in glaucoma is the cause or the result of GON.
Review of literature

Blood Supply of the ONH

- Mainly from the short posterior ciliary arteries.
- Branches in the superficial layer that arise from CRA.
- CRV is the sole significant route of venous drainage.

Factors Influencing the ONH Blood Flow

Many factors that finally determine the state of the ONH circulation.

- Ocular Perfusion pressure (OPP).
- Auto regulation.
- Arterial Blood Pressure (ABP).
- Endothelial Derived Vasoactive Agents.
- Calcium Channel Blockers (CCBs)
- Intraocular Pressure.
Mechanisms of glaucomatous optic neuropathy

1. Neural tissue loss:
   - RGCs and higher neural cell loss:
     - a- Apoptosis
     - b- Oxidative stress
     - c- Glutamate mediated toxicity

2. Glial cells activation

3. Tissue remodeling

4. Vascular ischemic factor

Optical coherence tomography angiography (OCTA)

OCTA is a new imaging technology that provides:

- A non-invasive.
- High resolution.
- Three dimensional images of the fundus microcirculation.
Optical coherence tomography angiography (OCTA)

# Technical principle

OCTA images are essentially motion contrast images based on that in a static eye the only moving structure is blood cells inside the vessels. This requires repeated consecutive B-scans at the same section.

OCTA role in POAG

OCTA is a characteristic technique giving:

- 3D visualization of ONH vasculature.
- near-automated quantification of disc perfusion and vessel density.

This allows better understanding of the pathophysiological processes in glaucoma.
Attenuated micro vascular net work ONH

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Focal capillary dropout in the infra-temporal area PPCP

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OCTA versus FA and ICGA

<table>
<thead>
<tr>
<th>Differences</th>
<th>OCTA</th>
<th>FA &amp; ICGA</th>
</tr>
</thead>
<tbody>
<tr>
<td>Invasiveness</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Dye dependence</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Image dimensions</td>
<td>3D</td>
<td>2D</td>
</tr>
<tr>
<td>Field of view</td>
<td>Small</td>
<td>Wide</td>
</tr>
<tr>
<td>Time sequence</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Staining &amp; leakage</td>
<td>No</td>
<td>Yes</td>
</tr>
<tr>
<td>Segmentation</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Contraindications</td>
<td>No</td>
<td>Yes</td>
</tr>
</tbody>
</table>

OCTA disadvantages or Limitations

- **small field of view** (but now there is extended field OCTA).
- Inability to show leakage and staining.

**Artifacts**: 
- Image artifact
- Motion artifact
- Projection artifact
Aim of the work

This study aimed to investigate optic disc perfusion differences between normal and primary open angle glaucoma eyes using optical coherence tomography angiography.
It was carried out on 45 glaucomatous eyes of 45 patients attending Tanta University Hospital and 30 eyes of 30 subjects of age matched normal controls.

**As regard IOP POAG patients were grouped into:**
- Normotensive patients (NTG) (IOP < 21mmhg).
- High-tension patients (IOP > 21mmhg).

**As regard age of onset high tension patients were divided into:**
- Juvenile Onset POAG (JPOAG) (age of onset > 3 years & < 40 years).
- Adult Onset High Tension POAG (AO HTPOAG) (age of onset > 40 years).
Normal control individuals were divided into 2 age matched groups:

. Normal 1: control group for NTG and AO HTPOAG groups (20 eyes).
. Normal 2: control group for JPOAG group (10 eyes).

So, the study had 5 groups:

- Normal 1: control group for NTG and AO HTPOAG groups (20 eyes).
- Normal 2: control group for JPOAG group (10 eyes).
- NTG group (15 eyes).
- JPOAG group (15 eyes).
- AO HTPOAG group (15 eyes).

# Inclusion criteria:

Patients suffering from POAG with the following criteria:

- Presence of GON.
- RNFL defect that is visible in red free slit lamp biomicroscopy or red free fundus photography.
- Glaucomatous VF changes.
- Open angle in gonioscopic examination.
# Exclusion criteria: (for all)

- Any retinal disease affecting retinal vascularity as BRAO and BRVO.
- Any media opacity interfering with clinical examination or investigations (corneal opacity, dense cataract, VH and RD).
- Any disease that may cause VF loss or OD abnormalities.
- Any physical and or mental handicapping preventing investigation.
- Patients with previous ocular laser and or intraocular surgery.

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Methods

1. **Complete history taking.**

   - Past history
   - **Family history:** glaucoma in 1st degree relatives.
   - **History of the disease (glaucoma):** onset, duration, topical medication.
   - **History of other ocular diseases or surgical intervention.**

2. **Systemic blood pressure and pulse rate measurement.**

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Patients and methods

3. Complete ophthalmologic examination:

a) visual acuity measurement:
   (UDVA, CDVA) using Snellen chart in decimal notation.

b) Anterior segment examination using slit lamp: for any abnormality.

c) Gonioscopic examination:
   The anterior chamber angle was examined using Goldmann 3 mirror contact lens. Four quadrants (upper, lower, nasal and temporal) are examined carefully to exclude closed angle glaucoma.

3. Complete ophthalmologic examination:

d) Intraocular pressure measurement:
   • using Goldmann applanation tonometer.
   • by the same examiner.
   • in different times in the morning, afternoon and at the evening to avoid diurnal variation and the mean was taken.

e) Posterior segment examination:
   • using slit lamp fundus bio microscopy.
   • OD examination as regard; its color, C/D ratio, RA, edge, CV and cup asymmetry. RNFL loss and exposed LC can be seen.
Patients and methods

3. Complete ophthalmologic examination:

f) Central corneal thickness (CCT) measurement: using Pachymetry.

g) Axial length measurement:
by IOL Master to detect cases with axial myopia (axial length > 24 mm) to be excluded as it may affect vascular density of the retina and OD.


5. Colored fundus photography.

6. Optical coherence tomography of optic disc.

7. Optical coherence tomography angiography of optic disc.

OCT and OCTA were performed using swept source TOPCON 3D OPTICAL COHERENCE TOMOGRAPHY DRI OCT Triton.

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Patients and methods

**Technique of OCT and OCTA Image Acquisition and Processing:**

1. All subjects underwent pupillary dilatation using tropicamide 1%.
2. The peripapillary RNFLT and macular GCLT were measured.
3. OCTA was performed using 4.5x 4.5 mm scan centered on ONH in all cases. Segmentation was done manually with different slabs to determine vascularity at different levels.

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**The ONH was assessed in 4 levels:**

- **The superficial Papillary level (VDI-1):** extends from a point at level of internal limiting membrane to a point at level of outer boundary of inner plexiform layer.

- **The deep papillary level (VDI-2):** extends from a point at level of outer boundary of inner plexiform layer to a point at the outer boundary of outer plexiform layer.

- **Outer retina level (VDI-3):** extends from a point at level of outer boundary of outer plexiform layer to a point at the level of Bruch’s membrane.

- **Choriocapillaries level (VDI-4):** extends from the level of Bruch’s membrane to 353 micrometer below it.
The ONH was assessed in 4 levels:

VDI-1

VDI-2

VDI-3

VDI-4

1. Patients and methods

2. Density map images gave;

- **qualitative data**, the more flow the more hot colors will be present and vice versa.
4. **Density map images allowed**;

- **quantitative assessment**, these images were processed using Image J program. The program gave us quantitative data by determining **vascular density index (VDI)** as percentage by taking 350 x 350 pixel images and converting it to binary images in which any flow has threshold could be detected as white color and those of very low or no flow could be detected as dark color.

Results
The study had 5 groups

GROUPS INCLUDED IN THE STUDY

Demographic Data:

General: No significant differences regarding the age and gender distribution.

<table>
<thead>
<tr>
<th>Type of Participants</th>
<th>Normal control 1</th>
<th>NTG</th>
<th>AO HTPOAG</th>
<th>Test of sig.</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>20 eyes</td>
<td>15</td>
<td>15</td>
<td>t = -0.047</td>
<td>0.962</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mean ± SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>9 (45%)</td>
<td>8 (53.3%)</td>
<td>9 (60%)</td>
<td>x^2 = 0.654</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>11 (55%)</td>
<td>7 (46.7%)</td>
<td>6 (40%)</td>
<td></td>
</tr>
</tbody>
</table>
Results

<table>
<thead>
<tr>
<th>Type of Participants</th>
<th>Normal control 2</th>
<th>JPOAG</th>
<th>Test of sig.</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>10 eyes</td>
<td>15 eyes</td>
<td>t=</td>
<td>0.971</td>
</tr>
<tr>
<td>Age (years)</td>
<td>Mean ± SD</td>
<td>26.80±7.8</td>
<td>26.67±9.492</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Median</td>
<td>27.00</td>
<td>28.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Range</td>
<td>14-40</td>
<td>13-38</td>
<td></td>
</tr>
<tr>
<td>Sex</td>
<td>Male</td>
<td>6 (60%)</td>
<td>8 (53.3%)</td>
<td>c²=</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>4 (40%)</td>
<td>7 (46.7%)</td>
<td>1.326</td>
</tr>
</tbody>
</table>

After processing of density map images for the 4 groups we could measure VDI of the 4 levels of segmentation and the average was calculated then all are tabulated and compared.

Measurements of vascular density in normal control (1) subjects.

<table>
<thead>
<tr>
<th>VDI-1</th>
<th>VDI-2</th>
<th>VDI-3</th>
<th>VDI-4</th>
<th>Average VDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S. D.</td>
<td>58.57±2.676</td>
<td>64.18±2.922</td>
<td>47.16±4.037</td>
<td>26.12±4.612</td>
</tr>
<tr>
<td>Median</td>
<td>58.285</td>
<td>63.466</td>
<td>47.076</td>
<td>26.007</td>
</tr>
<tr>
<td>Range</td>
<td>55.13-67.44</td>
<td>60.36-69.81</td>
<td>48.26-58.79</td>
<td>19.2-37.69</td>
</tr>
</tbody>
</table>
In NTG group there was statistically significant difference at all levels.

Measurements of VDI in NTG patients and comparison with normal subjects.

<table>
<thead>
<tr>
<th>NTG</th>
<th>VDI-1</th>
<th>VDI-2</th>
<th>VDI-3</th>
<th>VDI-4</th>
<th>Average VDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S. D</td>
<td>50.00±9.715</td>
<td>53.79±13.99</td>
<td>37.34±12.19</td>
<td>15.295±2.695</td>
<td>39.11±8.225</td>
</tr>
<tr>
<td>Median</td>
<td>52.234</td>
<td>53.784</td>
<td>38.072</td>
<td>14.178</td>
<td>41.635</td>
</tr>
<tr>
<td>Range</td>
<td>26.06-60.20</td>
<td>9.07-66.69</td>
<td>10.27-55.64</td>
<td>12.05-19.49</td>
<td>14.4-47.11</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>8.573</td>
<td>10.392</td>
<td>9.819</td>
<td>10.82</td>
<td>9.9</td>
</tr>
<tr>
<td>T test</td>
<td>3.325</td>
<td>2.830</td>
<td>2.999</td>
<td>8.091</td>
<td>4.585</td>
</tr>
<tr>
<td>P value</td>
<td>0.004*</td>
<td>0.013*</td>
<td>0.008*</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

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In AO HTPOAG there was only significant difference in VDI-1 and VDI-4.

Measurements of VDI in AO HTPOAG patients comparison with normal subjects.

<table>
<thead>
<tr>
<th>AO HTPOAG</th>
<th>VDI-1</th>
<th>VDI-2</th>
<th>VDI-3</th>
<th>VDI-4</th>
<th>Average VDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S. D</td>
<td>53.01±5.919</td>
<td>62.19±6.84</td>
<td>46.79±7.0</td>
<td>21.58±4.972</td>
<td>45.892±3.954</td>
</tr>
<tr>
<td>Median</td>
<td>53.305</td>
<td>62.664</td>
<td>49.45</td>
<td>20.7</td>
<td>45.4</td>
</tr>
<tr>
<td>Range</td>
<td>44.41-59.57</td>
<td>49.99-69.7</td>
<td>32.23-53.62</td>
<td>12.45-28.21</td>
<td>38.95-52.18</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>5.567</td>
<td>1.986</td>
<td>0.365</td>
<td>4.538</td>
<td>3.114</td>
</tr>
<tr>
<td>T test</td>
<td>3.394</td>
<td>1.055</td>
<td>0.179</td>
<td>2.786</td>
<td>2.849</td>
</tr>
<tr>
<td>P value</td>
<td>0.003*</td>
<td>0.305</td>
<td>0.859</td>
<td>0.009*</td>
<td>0.011*</td>
</tr>
</tbody>
</table>


Vascular density in normal subjects and AO HTPOAG patients.
In JPOAG group there was statistically significant difference in all levels except in outer retina level.

Measurements of VDI in JPOAG patients and comparison with age matched normals.

<table>
<thead>
<tr>
<th>JPOAG</th>
<th>VDI-1</th>
<th>VDI-2</th>
<th>VDI-3</th>
<th>VDI-4</th>
<th>Average VDI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean ± S. D</td>
<td>48.92±8.09</td>
<td>58.33±4.5</td>
<td>43.41±10.77</td>
<td>18.26±3.851</td>
<td>42.23±4.71</td>
</tr>
<tr>
<td>Median</td>
<td>52.234</td>
<td>60.120</td>
<td>46.82</td>
<td>17.874</td>
<td>42.518</td>
</tr>
<tr>
<td>Range</td>
<td>30.82-59.78</td>
<td>49.5-63.9</td>
<td>24.22-55.92</td>
<td>12.92-27.69</td>
<td>30.96-47.89</td>
</tr>
<tr>
<td>Mean Difference</td>
<td>9.389</td>
<td>4.747</td>
<td>2.618</td>
<td>7.547</td>
<td>6.076</td>
</tr>
<tr>
<td>T test</td>
<td>4.317</td>
<td>3.427</td>
<td>0.867</td>
<td>5.836</td>
<td>4.673</td>
</tr>
<tr>
<td>P value</td>
<td>0.001*</td>
<td>0.002*</td>
<td>0.397</td>
<td>&lt;0.001*</td>
<td>&lt;0.001*</td>
</tr>
</tbody>
</table>

VDI of Normal vs JPOAG

Vascular density in normal subjects and JPOAG patients

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Comparison between vascular density in the 3 types of glaucoma.

In this study, many correlations had been done between VDI and

- BCVA
- CCT
- IOP
- OPP
- C/D ratio, RA
- VF (MD, PSD)
- RNFLT
- GCLT
glaucoma patients (45 eyes) are divided into:

- **Mild**: 17 eyes (37.8%).
- **Moderate**: 15 eyes (33.3%).
- **Advanced**: 13 eyes (28.9%).

By comparing means of VDI measurements in different levels in the 3 stages we found that **there was decrease in VDI measurements with increase the stage of severity**.
Case presentation

Case

Normal control 1

50 years old healthy female.

A. Colored photo: normal RA 1.85 mm², CD ratio of 0.31, CV 0.07 mm³.

C. OCT B scan with RNFLT map. RNFLT within the normal range in all four quadrants with average 114 um.

D. MGCLT map showing within normal thickness detected by color coding, with 3mm average thickness 90 um.

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Superficial papillary level: normal dense peripapillary capillary plexus. (VDI-1) 57.85%.

Deep papillary level: normal dense peripapillary capillary plexus with (VDI-2) 67.54%.

Outer retina level:
(VDI-3) 49.36%.

Choriocapilaries level:
(VDI-4) 27.32%.

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Case 2

Moderate NTG

60 years old NTG female patient.

A. Colored photo: RA 1.47mm², CD ratio of 0.62 and CV 0.33mm³.

C. OCT B scan, RNFLT map RNFLT show thinning in inferior quadrant with average 91 um.

D. macular GCL map with 3mm average thickness 97 um.
Superficial papillary level: attenuated PPCP (infero-temporal).

(VDI-1) 50.82%.

Deep papillary level: (VDI-2) 62.76%.

Outer retina level:

(VDI-3) 44.34%.

Choriocapilaries level:

(VDI-4) 15.875%.

Case presentation

Case 3

Mild AO HTPOAG

50 years old AO HTPOAG male patient. Visual field showed mild glaucomatous field changes with MD -3.29 and PSD +2.92.

A. Colored photo shows a RA 1.51 mm\(^2\), with CD ratio of 0.67 and CV 0.46 mm\(^3\)

B. OCT B scan with RNFLT map. RNFLT still normal thickness in all 4 quadrant with average 90 um.

D. macular GCL map hot colors represent thinning with average 3mm thickness 81.75 um.
**Case presentation**

**Case 4**

Severe AO HTPOAG

46 years old AO HTPOAG male patient.

A. Colored photo: RA 0.29mm², CD ratio of 0.99 and CV 1.55mm³.

C. OCT B scan with RNFLT map. RNFLT show diffuse thinning in all quadrants with average 36 um.

D. MGCL map shows hot color (red) representing diffuse thinning with average 3mm thickness 49.25 um.

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Superficial papillary level: large areas with capillary drop out with cold blue color representing decreased vascular density i.e. ischemia. (VDI-1) 44.41%.

Deep papillary level: Diffuse capillary dropout i.e. ischemia. (VDI-2) 49.99%.

Outer retina level: with (VDI-3) 48.95%.

Choriocapilaries level: with (VDI-4) 12.45%.

Case 5

Normal control 2

27 years old healthy female.

A. Colored photo: normal RA 1.65mm², CD ratio of 0.3, and CV 0.06mm³.

C. OCT B scan with RNFLT map. RNFLT is within the normal range in all four quadrants with average 96 um.

D. MGCL map shows within normal thickness with average 3mm thickness 87.50 um.
**Case presentation**

Superficial papillary level: normal dense micro vascular network.

(VDI-1) 59.67%.

Deep papillary level: normal dense micro vascular network.

(VDI-2) 63.55%.

Outer retina level: with (VDI-3) 47.08%.

Choriocapilaries level: with (VDI-4) 26.007%.

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**Case 6**

**Mild JPOAG**

14 years old JPOAG male patient.

A. Colored photo: RA 1.45mm², C/D ratio of 0.68 and CV 0.58 mm³

C. OCT B scan with RNFLT map with mild thinning at 1 o'clock average 98 um.

D. MGCL map shows scattered localized areas of thinning (yellow color) with average 3mm thickness 88.75um.

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Case presentation

Superficial papillary level: diffuse peripapillary capillary drop out.
(VDI-1) 59.78%.

Deep papillary level: diffuse peripapillary capillary drop out.
(VDI-2) 63.63%.

Outer retina level: with (VDI-3) 46.82%.

Choriocapillaries level: with (VDI-4) 17.52%.

Case 7

Moderate JPOAG

22 years old JPOAG male patient.

A. Colored photo: RA 0.87mm², C/D ratio of 0.80 and CV 0.86 mm³

C. OCT B scan with RNFLT map. RNFLT show thinning in superior quadrant with average 79 um.

D. MGCL map hot colors (red &yellow colors) represent thinning with average 3mm thickness 88um.
Case presentation

Superficial papillary level: absent ONH micro vascular network and attenuated peripapillary capillary plexus.

(VDI-1) 47.49%.

Deep papillary level: areas of capillary drop out.

(VDI-2) 60.16%.

Outer retina level: with (VDI-3) 49.45%.

Choriocapillaries level: with (VDI-4) 19.99%.

Case 8

Severe JPOAG

35 years old JPOAG male patient.

A. Colored photo: RA 0.77mm², C/D ratio of 0.80 and CV 0.97 mm³

C. OCT B scan with RNFLT map. RNFLT show thinning in all quadrants with average 66 um.

D. MGCL map with hot colors (red & yellow colors) represent thinning with average 3mm thickness 67um.
Case presentation

**Superficial papillary level:** absent ONH micro vascular network and diffuse peripapillary capillary drop out.

(VDI-1) 45.02%.

**Deep papillary level:** absent ONH micro vascular network. (VDI-2) 63.63%.

**Outer retina level:** with (VDI-3) 24.22%.

**Choriocapilaries level:** with (VDI-4) 16.99%.

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**Conclusion**
In conclusion

1. glaucoma patients showed markedly reduced ONH vascular density.

- **Quantitative assessment:** VDI reduction was more significant in NTG followed by JPOAG and AO HTPOAG.

- **Qualitative assessment** the normally dense microvascular network of ONH, was attenuated with marked peripapillary capillary drop out.

Conclusion

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In conclusion

2. the most affected level was choriocapilaries level. This level represents the ciliary circulation the main blood supply of ONH.

**So, ischemia in glaucoma may be caused by:**

- **Primary element** as detected by decreased VDI at choriocapilaries level.

- **Secondary effect** to elevated IOP (direct impact) with decrease VDI-1 that is marked in cases with high IOP.
In conclusion

3. The reduction in vascular density has a strong correlation with the functional, structural glaucoma parameters and the disease severity.

3. OCTA may offer insights into the pathophysiology of glaucomatous damage.

Recommendations
Based on the results obtained from the study. we may recommend the following:

- Follow up longitudinal studies on large number of patients.
- Further studies on each glaucoma group separately with patients of different age, sex and race.
- Special studies should be conducted on early glaucoma and glaucoma suspect patients to detect if vascular changes have earlier presentation and its value in detection of glaucoma progression.

Recommendations

Basma Gamal, Ophthalmology Department, Tanta University, Thesis for MSC,August,2018

Based on the results obtained from the study. we may recommend the following:

- Further studies before starting anti-glaucomatous medications to exclude any unclear effect on vascularity.
- Further measurements of the vascularity in hemispheres or quadrants and finding the topographic relationship between it and structural glaucomatous damage might provide more information.

Recommendations

Basma Gamal, Ophthalmology Department, Tanta University, Thesis for MSC,August,2018
• OCTA should not be used alone for glaucoma diagnosis and follow up but can be used as an additional tool beside visual field and conventional OCT.
Thank you!

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August, 2018