USE OF BLOOD MARKERS IN EARLY DIAGNOSIS OF OXIDATIVE STRESS IN AGE RELATED MACULAR DEGENERATION

Raluca Dănulescu¹, D. Costin²
University of Medicine and Pharmacy “Grigore T. Popa” - Iasi
1. Ph.D. student
    Faculty of Medicine
2. Discipline of Ophthalmology

USE OF BLOOD MARKERS IN EARLY DIAGNOSIS OF OXIDATIVE STRESS IN AGE RELATED MACULAR DEGENERATION (Abstract): Age related macular degeneration (AMD) is the most frequent cause of irreversible blindness in the elderly population, affecting approximately 30-50 million people around the world. **Aim:** to establish the role of oxidative stress in retinal structural lesions. **Materials and methods:** It is a case-control study that included 19 patients diagnosed with AMD. Depending on the severity of the diagnosis, patients were divided into 3 groups: group 1 - mild AMD, group 2 – moderate, atrophic AMD, group 3 – severe, neovascular AMD. They were followed by assessment of visual acuity, optical coherence tomography (OCT) and oxidative stress markers like superoxide dismutase (SOD), thiobarbituric acid reactive substances (TBARS) and inflammatory marker – C-reactive protein (CRP). **Results:** Risk factors involved in patients with AMD are arterial hypertension, smoking, hyperlipidemia and diet poor in antioxidants, as revealed in the questionnaire. Retinal thickness assessed by optical coherence tomography showed that values in patients with severity level 2 is within normal limits, while in patients with severity level 3, these values are significantly increased due to macular edema. The mean values of SOD, TBARS and CRP in the studied group were significantly higher compared to controls, being higher in group severity level 3. **Conclusions:** This study shows the role of oxidative stress and inflammation in retinal structural lesions in AMD and the importance of blood markers in early detection of oxidative stress and thus of retinal lesions in this disease. **Keywords:** AGE RELATED MACULAR DEGENERATION, OXIDATIVE STRESS, RETINAL THICKNESS.

Age-related macular degeneration (AMD) is the most common cause of irreversible blindness in the elderly population, affecting approximately 30-50 million people (1). AMD consists of degenerative and inflammatory lesions of the macular region, occurring in a previously normal eye and resulting in deterioration of central vision and macular function (2). The disease begins as a non-neovascular or dry stage, which can progress to geographic atrophy or macular neovascularisation. Recently, the research has focused on the risk factors that could be involved in the development of AMD, such as oxidative stress, inflammation and phototoxic stress (3, 4).

The aim of this study is to establish the role of oxidative stress in retinal structural
and functional changes in AMD. Also, it is meant to highlight the role of blood markers in early diagnosis of oxidative stress and consequently in early identification of retinal lesions.

**MATERIAL AND METHODS**

This is a case-control study that included a group of 19 patients diagnosed with AMD, selected from the "N. Oblu Hospital” in Iaşi, and two control groups, one with younger controls and another with controls with similar ages as the cases. Patients with systemic and ocular diseases that could increase oxidative stress, such as diabetes, inflammatory and infectious diseases, and vascular occlusions were excluded.

According to the law of patients' rights, they signed an informed consent and completed a questionnaire concerning risk factors, associated diseases, living conditions, subjective complaints and an Amsler grid. A score was calculated for each patient.

During a follow-up period for a median of 8 months, we recorded the visual acuity, anterior pole biomicroscopy, posterior pole appearance (Volk 78 D lens), Optical Coherence Tomography (OCT) and laboratory tests. As regards the laboratory tests, we followed the oxidative stress markers such as thiobarbituric acid reactive substances (TBARS), antioxidant enzymes - superoxide dismutase (SOD), and markers of inflammation - C-reactive protein (CRP).

Depending on the severity of the diagnosis, patients were divided into 3 groups according to the classification of "Age-Related Eye Disease Study" (AREDS) of the U.S. National Eye Institute: group 1 - mild AMD, group 2 – moderate, atrophic AMD, group 3 - severe neovascular AMD. According to the appearance of the posterior pole and the OCT results, we maintained the same classes. In the study group there were included only patients with severity level 2 and 3. The retinal thickness and retinal nerve fiber layer were measured with Spectral OCT OPTOPOL in 9 quadrants.

SOD activity was determined by xanthine oxidase method, TBARS were quantified by colorimetric method and CRP measurement was made by immun-turbidometry with calibrator. The statistical analysis (performed with the Statistical Programme 7.0), was intended to highlight significant correlations between the severity of the diagnosis and results of the OCT, visual acuity and results of the blood tests. The statistical comparison was made with "Difference tests" from the sub-set of descriptive statistic programs, being significant those differences which stood at a safe threshold of 95% (p <0.05).

**RESULTS**

We investigated a group of 19 subjects, 8 males (42.11%) and 11 women (57.89%) with a mean age of 72.47 ± 7.74 years (59 to 88). The first control group (older group) included 11 subjects (3 men and 8 women), with ages ranging between 60 and 85 years. We used the second control group (young group), which comprised of 40 subjects (11 men and 29 women), aged between 28 and 60 years, in order to compare the laboratory results.

We evaluated the risk factors in the study group and in the control group and we showed that there was a higher frequency of arterial hypertension (HTA), smoking, lack of antioxidants and UV exposure in the study group (fig. 1).

There were enrolled 16 patients with ne-
Ocular AMD, severity level 3, and 3 patients with dry form, severity level 2. We present the average retinal thickness, depending on the severity of diagnosis (tab. I).

**TABLE I**

**Indicators of the average retinal thickness in the two severity groups**

<table>
<thead>
<tr>
<th>Quadrant</th>
<th>Severity level 3 – retinal thickness</th>
<th>Severity level 2 – retinal thickness</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average (μ)</td>
<td>Minimum (μ)</td>
</tr>
<tr>
<td>Quadrant 1</td>
<td>381.62</td>
<td>164.00</td>
</tr>
<tr>
<td>Quadrant 2</td>
<td>372.68</td>
<td>224.00</td>
</tr>
<tr>
<td>Quadrant 3</td>
<td>357.00</td>
<td>180.00</td>
</tr>
<tr>
<td>Quadrant 4</td>
<td>377.81</td>
<td>257.00</td>
</tr>
<tr>
<td>Quadrant 5</td>
<td>358.62</td>
<td>187.00</td>
</tr>
<tr>
<td>Quadrant 6</td>
<td>307.06</td>
<td>185.00</td>
</tr>
<tr>
<td>Quadrant 7</td>
<td>317.37</td>
<td>156.00</td>
</tr>
<tr>
<td>Quadrant 8</td>
<td>318.87</td>
<td>210.00</td>
</tr>
<tr>
<td>Quadrant 9</td>
<td>340.75</td>
<td>266.00</td>
</tr>
<tr>
<td>Average</td>
<td>347.27</td>
<td>228.88</td>
</tr>
</tbody>
</table>

In patients with the neovascular form, mean retinal thickness was 347.27 μ, signifying the presence of macular edema (over 340μ), with a maximum of 495μ.
In patients with dry form, mean retinal thickness was 257.47μ, falling within the normal range. Next we evaluated the retinal nerve fiber layer thickness, depending on the severity of diagnosis. The average thickness was much lower than normal both in severity level 2 group and severity level 3 group.

The average visual acuity is significantly higher in subjects with a diagnosis of severity level 2 (0.67) compared with 0.25 in those with level 3 severity (p = 0.005). The score was significantly higher in group severity 3 (39) compared to group severity 2 (28).

Depending on the aspect of the posterior pole and the OCT results, the average thickness of the retina is significantly higher in cases with severity level 3 compared to those with severity level 2 (fig. 2).

![Fig. 2. The average thickness depending of the OCT results](image)

We determined the levels of SOD, TBARS and CRP in the study group, older control group, and in the younger control group. To highlight the differences between groups it was used the t Student test, appreciating as significant those with p <0.05.

**TABLE II**

<table>
<thead>
<tr>
<th>Groups</th>
<th>Number</th>
<th>SOD (U/ml)</th>
<th>TBARS (nmols/ml)</th>
<th>CRP (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Study group</td>
<td>19</td>
<td>331.8</td>
<td>4.6</td>
<td>3.2</td>
</tr>
<tr>
<td>Older control group</td>
<td>11</td>
<td>269.5</td>
<td>3.9</td>
<td>1.5</td>
</tr>
<tr>
<td>Younger control group</td>
<td>40</td>
<td>196.1</td>
<td>3.1</td>
<td>0.9</td>
</tr>
</tbody>
</table>

Comparing the mean values in the three groups, we found that the mean values of SOD, TBARS and CRP were significantly higher in the study group compared to con-
trols; the mean values of SOD and TBARS were significantly higher in the older control group compared to younger control group (tab. II). We also found that the values were higher in the group with severity 3 diagnosis, compared to severity 2 (fig. 3, 4).

Fig. 3. The mean values of SOD in group severity 3 and 2

Fig. 4. The mean values of TBARS in group severity 3 and 2

**DISCUSSION**

We found that AMD correlated with certain risk factors, like arterial hypertension, smoking, hyperlipidemia and low antioxidants diet, which is consistent with the literature (1, 2).

Comparing the mean values of retinal thickness depending on the severity of diagnosis, we found an average value of 347.27 μ in the group of patients with severity level 3, significantly higher (p = 0.02) compared to those with severity level 2 (257.47 μ). The average thickness of the nerve fibers layer is 32.29 μ (ranging between 16.78 and 49.78 μ), being much lower than normal (90 μ). It can be noticed that in severity level 2 group the average thickness of the retina is normal in all quadrants, while in those with severity level 3, it is above the upper limit in 5 of the 9 quadrants. These data are consistent with other studies published in the literature. Kolb (5) studied 75 eyes with neovascular AMD, treated with intravitrean drugs against vascular endothelial growth factor (anti-VEGF). Mean retinal thickness was higher than normal and its reduction was observed after treatment. Garas (6) has shown that AMD affects the macular thickness parameters in eyes without glaucoma.

We showed an increase in antioxidant enzymes (SOD) in the study group (331.8 U/ml) compared with the two control groups (young - 196.1 U/ml and older - 296.5 U/ml). SOD is one of the main antioxidant systems, that increased by a feedback mechanism, trying to keep the balance between antioxidants and free radicals. Dong (7) hypothesized that SOD protects retinal cells from oxidative stress. The results are consistent with the study by Imamura (8) who showed that the retinas of mice deficient in SOD1 had several key elements of human AMD. Kasahara (9) showed that homozygous mice deficient in SOD2 present morphological changes in the RPE and Bruch membrane and accumulation of oxidized proteins. Jia (10) found elevated levels of SOD and malondialdehide (MDA) in patients with AMD as a compensatory response. Shen (11) showed an increase in SOD activity compared to
the control group, along with a decrease in antioxidants. In the POLA study (12) the authors showed that another antioxidant enzyme, glutationperoxidase, increases in patients with AMD.

In this study we evaluated TBARS values, one of the earliest markers of oxidative stress. However, this marker has been less studied in relation to AMD. The mean value of TBARS in the study group was significantly higher (4.6 nmols/ml) than that in the control groups (young control group: 3.0 nmols/ml and older control group: 3.9 nmols/ml), which supports the idea that oxidative stress is involved in the etiopathogenesis of this disease. The mean SOD and TBARS values were significantly higher in elder control group compared with younger control group (SOD: 269.5 vs. 196.05 U/ml, TBARS: 3.9 vs. 3.0 nmols/ml). The mean values of laboratory tests were significantly higher in the group of subjects with severity level 3 compared to severity level 2.

To demonstrate the role of inflammation in AMD, we compared the mean values of CRP. Mean CRP was significantly higher in the study group (3.2 mg/dl) compared with controls (1.5 mg/dl). These data are consistent with other studies, showing an increased value of this marker (13, 14, 15, 16). The Rotterdam Study (15) showed that elevated CRP levels at baseline were associated with progression of AMD. Our study confirmed that elevated CRP levels were associated particularly with advanced form of AMD. Seddon (17) showed that CRP values were higher in subjects with severe maculopathies.

**CONCLUSIONS**

This research highlights the role of oxidative stress and inflammation in retinal structural lesions of age-related macular degeneration and the role of antioxidant enzymes in the early detection of oxidative stress. Risk factors involved in patients with age-related macular degeneration are arterial hypertension, smoking, hyperlipidemia and diet poor in antioxidants, as revealed in the questionnaire. Retinal thickness assessed by optical coherence tomography showed that values in patients with severity level 2 is within normal limits, while in patients with severity level 3, these values are significantly increased due to macular edema. Oxidative stress and inflammatory markers are elevated in the study group compared with control groups. This demonstrates that oxidative stress in relation to inflammatory stress is involved in the pathogenesis of age-related macular degeneration.

**REFERENCES**


