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Biochemical markers of hypertensive retinopathy

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SUMMARY

THE RESEARCH CONCEPTUAL FRAMEWORK

The relevance and importance of the researched problem. High blood pressure (HBP), the most common cardiovascular disease, is a major public health problem, its prevalence ranging from 5-10% in underdeveloped countries till 20-30% in industrialized countries, with a worldwide impairment of approx. 40% of people over the age of 25 [1]. Extensive population-based epidemiological studies have indicated that 3-14% of non-diabetic adults > 40 years of age have characteristic hypertensive retinopathy (HR) changes, with a higher prevalence in the 40-60 age group, with men being more predisposed to develop retinopathy, according to studies in India, while in Europe there is a greater affectation of the female [2].

Called the "silent killer", hypertension is a risk factor for a number of eye conditions, but HR, in which the retinal vessels undergo a series of changes, is the most common. The danger of the condition is due to the unawareness of the presence of the disease, because of non-existence of premonitory signs, the symptoms usually developing in the late stages [3]. In the latest articles, researchers use and consider HR a marker for a number of vascular diseases, as well as a harbinger of the risk of stroke, cardiovascular disease, microalbuminuria, chronic kidney disease and even death. However, for the moment, the evaluation of hypertensive signs of retinopathy appears only in the clinical guidelines for the management of patients with hypertension [4].

HR has a complex vascular phenotype, including three separate pathophysiological stages, which creates a broad spectrum of retinal vascular changes, reflecting the severity and duration of BP amplification. These changes are explained by the specific ability of self-regulation of the retinal vessels in response to changes in BP that allows a stable retinal vascular flow to be maintained by active arterial vasoconstriction. Self-regulatory mechanisms explain arterial vasoconstriction, as well as the specificity of "soft" exudates and deep haemorrhages associated with arteriolar occlusions [5]. In addition to self-regulation, the second characteristic of retinal circulation is the presence of a blood-retinal barrier (BRB), the lesion of which is responsible for superficial retinal bleeding, retinal oedema and hard exudates ("dry exudates") [2].

There is an interrelation between the development of microvascular and macrovascular hypertensive complications and the lesions generated by the presence of free radicals. Several biochemical pathways have been associated with the amplification of free radical formation in patients with HTN, which could be considered one of the pathogenic mechanisms of HTN itself and, probably, of the mechanisms of HR development [6]. Oxidative and nitrosative stress have been directly involved in the mechanisms of development of vascular rigidity and endothelial dysfunction in correlation with HTN [7].

The role of oxidative (OS) and nitrosative stress (NS) in the development of HTN and HR is unanimously recognized. At the same time, the number of studies on the mechanisms by which this process is involved in retinal damage remain to be incompletely elucidated. The role of OS and NS in the development of HR has been indirectly demonstrated by identifying the amplification of gamma-glutamyl transferase activity and ferritin levels in the blood of patients with HR, the correlation of changes in GSH/GSSG and LDL-Col with retinal vessel dynamics in patients with HTN [8]. However, it is still controversial whether OS and NS have a causative effect on the development of HR or are a consequence of tissue damage [9]. The existing data are insufficient to ascertain with certainty the role of various factors (OS, NS, antioxidant system, inflammation, etc.) in the chain of pathogenic links of HR, as well as the use of specific markers in diagnosis, prognosis and monitoring.

Previous studies of HR-associated metabolic changes and assessment of the usefulness of

markers in the diagnosis of pathological condition have been performed in patients' blood serum. At the same time, the eye is an organ with a certain degree of structural, functional and metabolic autonomy, the blood modifications not being a faithful reflection of the ocular changes. The tear film is a polycomponent biological fluid, the composition of which is directly correlated with the ocular structures (their integrity and functionality) and primarily reflecting local physiological and pathological changes. Being conventionally accessible, the tear is a valuable biological material for visual apparatus research [10]. The investigation of different representative tear markers in various ocular pathologies could be a useful tool in their diagnosis due to the significant variety and the presence in different compartments of the eye of multiple chemical compounds [10].

However, so far, the diagnosis of HR is mainly based on ophthalmological evaluation of patients. The uniqueness of the retinal vessels is related to the easy accessibility for a physical examination, but the physical consult depends on the dexterity and experience of the ophthalmologist and is largely subjective. In this context, biochemical markers could be useful not only in understanding of the HR pathogenic mechanisms and fully estimation of pathological metabolic changes in the retina caused by HTN, but also in objectifying and quantifying these changes at the level of HR diagnosis and in establishing an accurate, personalized treatment tactic, as well as in monitoring the effectiveness of treatment and the evolution of retinopathy.

Following the above, it is imperative to identify objective markers of ocular damage in hypertension, which should be outlined as promising explanatory indices and laboratory markers of metabolic disorders.

The aim of the research was to study the role of oxidative stress, antioxidant system (AOS) and renin-angiotensin system in the pathogenesis of hypertensive retinopathy and the identification of laboratory markers for the diagnosis and monitoring of the pathology.

Research objectives:

1. The study of changes in OS and antioxidant system indices in serum and tear of patients with primary hypertension with varying degrees of HR.
2. The evaluation of serum and tear RAS markers in patients with primary hypertension with varying degrees of HR.
3. The correlations identification of biochemical changes with the severity of HR.
4. The establishment of biochemical markers for early diagnosis and prognosis of HR evolution.

Scientific research methodology. An analytical, observational study was performed on a representative sample of patients with primary HTN with HR without specific antihypertensive treatment, divided into study subgroups according to the severity of HR, respecting all scientific requirements and ethical principles of institutional, national and international research. In order to achieve the objectives of the thesis, tears and blood serum were investigated to assess the metabolic indices of OS, antioxidant and RAS systems and ischemia. The Research Ethics Committee positive decision was obtained on 08.02.2018 (no. 35/34).

The novelty and scientific originality of the obtained results. The research complemented the current knowledge on the pathogenesis of HR, analyzing biochemical changes and their reflection in blood and tears. Markers of OS, antioxidant protection, ischemic damage and RAS were measured in both fluids, with the appreciation of the presence of tear–blood serum correlation of the evaluated indices. It has been shown that the eye is structurally autonomous, with specific defensive systems, which later allows it to withstand the effects of OS. The idea of the lack of a single reliable index, relevant for all HR stages was suggested, the appreciation of clinical

manifestations tending to remain a priority.

Approval of scientific results. The research results were presented, discussed and approved at several national and international scientific forums: Annual Scientific Conference of Institute of Emergency Medicine Specialists "News and controversies in medical-surgical emergency management", Chisinau, Moldova, November 10, 2017; 7th International Medical Congress for Students and Young Doctors MedEspera, "Nicolae Testemitanu" SUMPh, Chisinau, Moldova, May 3-5, 2018; Annual scientific conference of young specialists within IMSP IMU "Performances and perspectives in medical and surgical emergencies", Chisinau, Moldova, May 18, 2018; Annual Scientific Conference of Institute of Emergency Medicine "News and controversies in the management of medical and surgical emergencies", Chisinau, Moldova, December 7, 2018; VII Bukovinian International Medical Congress, BIMCO 2020, Bukovinian State Medical University, Chernivtsi, Ukraine, April 7-10, 2020. Congress dedicated to the 75th anniversary of the founding of "Nicolae Testemitanu" SUMPh, Chisinau, Moldova, October 20-23, 2020; XXIst International Scientific and Practical Conference International Trends in Science and Technology, Warsaw, Poland, January 31, 2020; 7th Lublin International Medical Congress, LIMC 2020, Medical University of Lublin, Lublin, Poland, 26-28 November 2020; VIIIth Bukovinian International Medical Congress, BIMCO 2021, Bukovinian State Medical University, Chernivtsi, Ukraine, April 6-9, 2021; International Scientific Conference on Medicine 2021, University of Latvia, Riga, Latvia, 23-24 April 2021; EURETINA 2021 Virtual, 9-12 September 2021.

Keywords: hypertensive retinopathy, oxidative stress, reactive oxygen species, antioxidant system, hypoxia, renin-angiotensin system.

1. THE MECHANISMS INCRIMINATED IN THE DEVELOPMENT OF HYPERTENSIVE RETINOPATHY

1st chapter presents a synthesis of the literature, designed to highlight the actuality of the study. The implications of OS in the pathogenesis of visual system impairment, the mechanisms generating ROS and the biochemical changes induced by them, as well as the mechanisms of antioxidant protection, with representative biochemical indices were described in the 1st subchapter. 2nd subchapter demonstrates the relevance of endothelial dysfunction and endothelial and inflammatory factors as an effect of OS in the development of HR, exploring the role and consequences of the involvement of the RAS in HTN and HR, as well as of inflammation in the generation of reactive oxygen intermediates, with a crucial role in the pathogenesis and development of HTN and HR, respectively. In 3rd subchapter are exposed particularities of biochemical mechanisms in the development of clinical manifestations in HR.

2. MATERIALS AND METHODS OF STUDY OF BIOCHEMICAL MARKERS IN HYPERTENSIVE RETINOPATHY

2.1. Research methodology. The study is analytical, observational, performed on a representative sample of patients with primary HTN with HR, divided into study subgroups according to the severity of HR. The research was conducted during the years 2018-2020, in strict accordance with the Principles of the Helsinki Declaration on the Study of Human Subjects, which were included in the research only after the signing of the informed agreement. The research protocol was approved by the Research Ethics Committee of "Nicolae Testemitanu" SUMPh, with the issuance of the favorable opinion under no. 34 of 12.02.2018.

The study included 90 hypertensive patients: 38 (42.2%) men and 52 (57.8%) women. The respondents from the research group were stratified according to the degrees of severity of HR, using the Keith-Wagner-Barker classification system, based on the examination of the fundus of the eye. Group 1 (GI) included 36 patients with grade I of HR - with mild generalized retinal arteriolar narrowing. The age of the patients varied from 27 to 73 years, the average age being 52.56 $\pm$ 12.20 years. The gender division included: 15 men (42%) and 21 women (58%). Group 2 (GII): 35 patients with grade II of HR with defined focal narrowing and arteriovenous nipping. The age of the patients varied from 37 to 88 years, the average age being 63.49 $\pm$ 11.23 years. The gender partition included: 15 men (43%) and 20 women (57%). Group 3 (GIII): 19 patients with grade III of HR with signs of grade II as well as retinal haemorrhages and soft exudates. The age of the patients varied from 45 to 84 years, the average age being 64.63 $\pm$ 13.01 years. The gender division included: 8 men (42%) and 11 women (58%). Patients with grade IV of HR - with severe grade III HR and papillary oedema, were not included in the study, due to the association with other pathologies, which was considered as an exclusion criterion.

Therefore, the final study included 90 patients who applied for a routine consult at the "Ovisus" Medical Center, from which blood and tear samples were collected for biochemical analysis (simultaneously with routine clinical manipulations).

Selection/inclusion criteria 1. Patients with high values of BP and primary HTN and HR, confirmed after a detailed specific ophthalmological consult (determination of visual acuity, autorefracto-keratometry, perimetry, anterior segment and fundus biomicroscopy, ultrasonography, tonometry, pachymetry, gonioscopy, OCT of the macular area and papilla of the optic nerve at necessity); 2. The age between 27-88 years; 3. Patients who have signed the informed consent; 4. Patients who are able to understand and answer at the questions.

Exclusion criteria 1. The request not to participate/leave the study; 2. Age under 27 years and over 88 years; 3. Patients with chronic diseases that influence the metabolic picture (endocrine diseases with prevalence of diabetes, metabolic diseases, renal pathologies, neurological pathologies and other severe somatic comorbidities); 4. People with visual system diseases (history of ocular and craniocerebral trauma, optic nerve atrophies of different genesis, glaucoma, diabetic retinopathy, acute and chronic inflammatory processes, uveitis); 5. Treatment that can influence marker changes.

2.2. Preparation of biological material and biochemical methods of investigations

2.2.1. Sample collection. For the analysis of markers of interest, venous blood samples (5 ml) were collected, which after coagulation were centrifuged for 7 minutes at 1500 rotations/minute. The serum was separated and transferred to Eppendorf test tubes and stored at -45°C. The tear sample was collected from the outer corner of the eyelid using a disposable insulin syringe, after a slight preventive irritation of the outer corner of the eyelid with "Golden Star" Vietnamese aromatic balm. Serum and tear were distributed in Eppendorf microtubes and frozen (-40°C) until biochemical testing. All samples were coded.

2.2.2. Biochemical investigations were performed according to the methods adapted by the researchers of Biochemistry Laboratory of the SUMPh “Nicolae Testemitanu” for the Synergy H1 microplate spectrofluorometer (Hydrid Reader) (BioTek Instruments, USA) and the Power Wave HT spectrophotometer (BioTek Instruments, USA). By spectrophotometric and immunoenzymatic methods, the indices of **oxidative stress and antioxidant system** were evaluated in serum and tear: *nitric oxide (NO)* [11], *S-nitrosothiols* [12], *malonic dialdehyde* (MDA) [13], *advanced oxidation protein products* (AOPP) [14], *total antioxidant activity* (TAA)

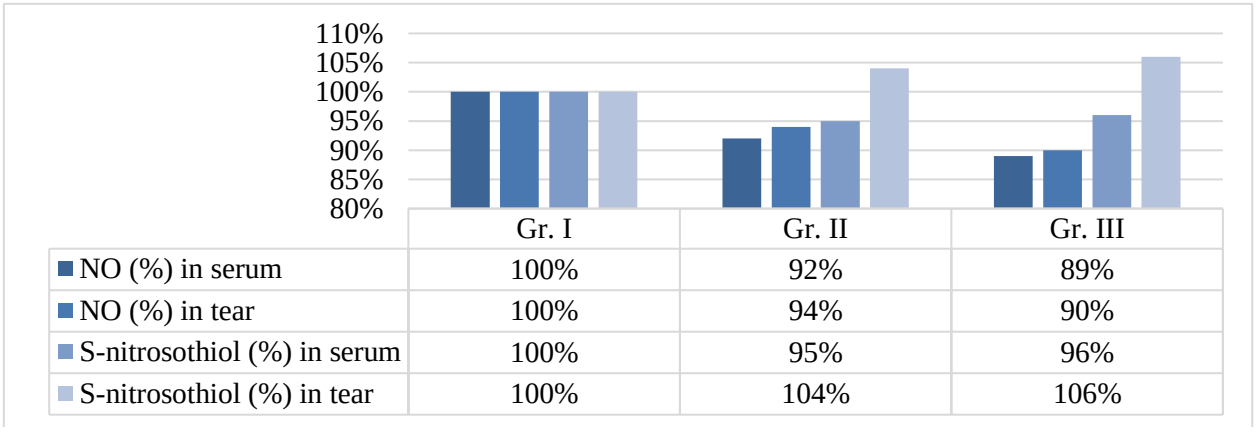
[15], *catalase* (CAT) [16], *superoxide dismutase* (SOD) [17], *reduced glutathione* (GSH) [18], *glutathione peroxidase* (GPx) [19], *glutathione reductase* (GR) [20], *SH-thiol groups* [21]; -ischemia: *ischemic-modified albumin* (IMA) [22]; **-renin-angiotensin system:** *angiotensin II* (Ang II), *angiotensin converting enzyme* (ACE) activity - according to the technical instructions of the standard Human angiotensin II, ANG-II Elisa Kit and ACE Elisa Kit of MyBioSource, Inc. (USA); **-lipid metabolism:** *triglycerides* (TAG), *total cholesterol* (total COL), *LDL-cholesterol*, *HDL-cholesterol* - following the technical instructions of the standard kits of the company DAC-Spectromed (Moldova); ***-total proteins*** - according to the technical instructions of the standard kits of the company DAC-Spectromed (Moldova).

2.3. Information technologies and procedures for statistical analysis of the results. Statistical processing of the results was performed using the SPSS software package (Statistical Package for the Social Sciences), version 23.0, using the Kolmogorov-Smirnov and Shapiro-Wilk normality tests, the Lavene test, the Kruskal-Wallis non-parametric test, the post-hoc test for comparisons PostHocDunn, the correlation coefficient of the ranks (Spearman).

3. BIOCHEMICAL CHANGES IN THE EVOLUTION OF HYPERTENSIVE RETINOPATHY

The study aimed to elucidate the impact of OS, ischemia and modifications in RAS on the evolution of HR, performing the analysis and interpretation of changes in selected markers.

3.1. Changes in oxidative stress indices in HR. In the study, statistically significant differences were recorded between the amount of NO determined in the blood serum, between groups, along with the amplification of HR (p=0.039), but not in the tear of patients (p=0.158) (figure 1).



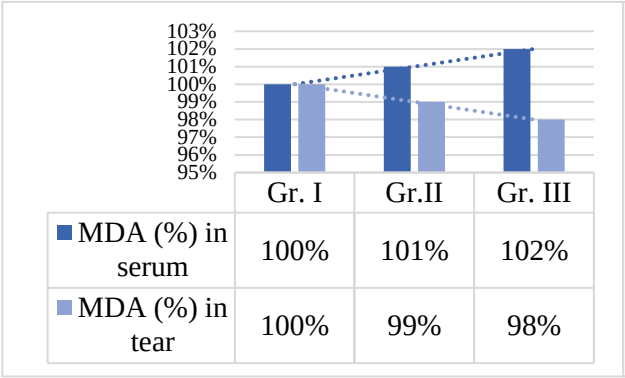
	Gr. I	Gr. II	Gr. III
NO (%) in serum	100%	92%	89%
NO (%) in tear	100%	94%	90%
S-nitrosothiol (%) in serum	100%	95%	96%
S-nitrosothiol (%) in tear	100%	104%	106%

Figure 1. **The change in the amount of NO and S-nitrosothiol values in the serum and tear of patients with different degrees of HR.**

It was attested a weak correlation between the degree of retinopathy and the level of NO in serum (r_s=-0.265, p=0.012), but not in tears (r_s=-0.203, p=0.056), nor between NO values in the both studied fluids (r_s=0.070, p=0.513). The level of S-nitrosothiols in the researched groups did not register statistically true changes neither in serum (p=0.694) nor in tear (p=0.706) (figure 1). No correlation was determined between the degree of retinopathy and the level of S-nitrosothiols in serum (r_s=-0.049, p=0.647) and in tears (r_s=-0.076, p=0.475) nor between the values of S-nitrosothiols in those 2 studied probes (r_s=0.142, p=0.181).

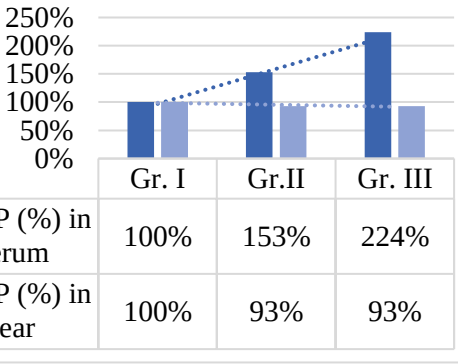
In order to analyze the harmful effects of OS on biomolecules, the level of MDA was determined - the final product of lipid peroxidation, as well as of AOPP - the product of oxidative changes of proteins [23]. MDA values revealed only statistically inconclusive trends of increase

in serum (p=0.628) and decrease in tear (p=0.527) (figure 2). Serum amplification of MDA values was noted in the serum, unlike the tear decreased values. No correlation was detected between the studied biochemical index and the degree of retinopathy in serum (r_s=-0.102, p=0.338) nor in tears (r_s=-0.008, p=0.408), but there was a weak positive correlation (r_s=0.277, p=0.008) between the MDA values from the both samples of interest.



	Gr. I	Gr.II	Gr. III
MDA (%) in serum	100%	101%	102%
MDA (%) in tear	100%	99%	98%

Figure 2. **The evolution of MDA levels in patients' serum and tears simultaneously with the progression of HR**



	Gr. I	Gr.II	Gr. III
AOPP (%) in serum	100%	153%	224%
AOPP (%) in tear	100%	93%	93%

Figure 3. **AOPP changes in the serum and tears of patients in varying degrees of HR**

Among the laboratory indices that have been analyzed in the context of oxidative changes of OS-induced proteins are advanced oxidation protein products (AOPP). In our study, no statistically significant differences were reported between AOPP in the blood serum (p=0.071) and in the tear (p=0.655) of patients along with the progression of HR (figure 3). A weak positive correlation was noted between the degree of retinopathy and the level of serum AOPP (r_s=0.243, p=0.021), suggesting an enhancement in OS and oxidative protein damage simultaneously with the evolution of retinopathy, as opposed to tear AOPP (r_s=-0.037, p=0.731), which would suggest a possible local protection capable of counteracting the effects of OS, attested by the increase of TAA in tears. No correlation was established (r_s=0.035, p=0.745) between the AOPP values between the 2 samples of interest.

3.2 Changes in antioxidant system indices in HR. The tear values of the TAA gave promising results, being determined an indisputable, statistically significant increase of the TAA values as HR advanced in degree (p=0.003) (figure 4).

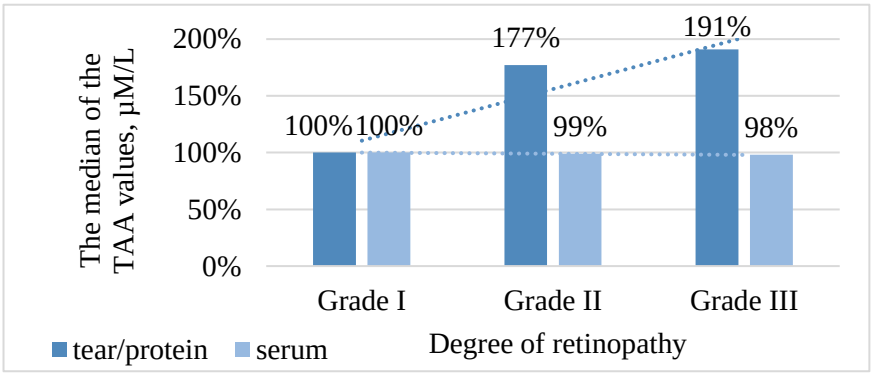


Figure 4. **Evolution of TAA values in the serum and tear of patients with HR**

There was no statistically significant change between groups in serum TAA values, unlike those in tears, but there was a trend of gradual decrease as HR progressed (p=0.182), by about 3% (p>0.05) (figure 5). A statistically significant weak negative correlation was established between

tear and serum AAT levels (r_s=-0.226, p=0.032). No significant correlation was recorded between serum TAA with HR grade (r_s=-0.164; p=0.123), while tear TAA showed a significant weak positive correlation with HR grade (r_s=0.357, p=0.001). It is worth mentioning the statistically significant, moderate correlation of tear TAA with individual antioxidants such as SOD (r_s=-0.446, p=0.000) and tear catalase (r_s=0.365, p=0.000), which possibly explains the increase in tear TAA in patients with HR.

The tear activity of SOD was statistically significantly lower than in serum in all groups studied by 25%. A significant weak positive correlation was attested between tears and serum SOD levels (r_s=0.336, p=0.001). A statistically significant difference in serum (p=0.035) and tears (p=0.027) SOD was established between groups, with values decreasing in both cases as HR progressed (Table 1). In both researched fluids, SOD activity showed a significant, weak, negative correlation with the degree of HR (r_s=-0.246, p=0.019 in serum; r_s=-0.284, p=0.007 in tears).

Tear catalase activity was statistically significantly lower than serum by 30% in all studied groups (p=0.033). There were no differences in serum catalase levels (p>0.05) between groups. There was a tendency for patients' serum catalase activity to increase as HR progressed. Serum catalase activity did not show a correlation with the degree of HR (r_s=0.143; p=0.177). No correlations were identified between serum and tears levels of catalase (r_s=0.125, p=0.239), while tears catalase activity showed a significant weak, positive correlation with the degree of HR (rs=0.261, p=0.013). The results obtained showed a decreased antioxidant activity of SOD and an amplified catalase, which could be interpreted as a mechanism of cellular resistance against an exacerbated OS. Eventually, the decrease in SOD activity, established in the research, conditioned the increase in the production of $O_2^{\bullet -}$ and subsequently of hydrogen peroxide. As a result, the need for H_2O_2 detoxification induced an enhancement in catalase activity.

Table 1. **SOD and catalase levels in serum and tear related to the evolution of HR**

Kruskal-Wallis	**SOD**/Me (LQ, UQ)		**Catalase**/Me (LQ, UQ)	
	serum (u/mL)	tear (u/mL)	serum (μM/L)	tear (μM/L)
	p=0.035	p=0.027	p=0.362	p=0.033
GI	1467.37 (1343.54, 1530.62) 100%	1123.89 (974.55, 1193.58) 100%	32.20 (27.25, 38.55) 100%	22.07 (15.35, 25.75) 100%
GII	1451.40 (1315.58, 1515.31) 99%	1057.52 (933.63, 1163.72) 94%	33.03 (29.58, 40.39) 103%	24.77 (21.47, 28.08) 112%
GIII	1352.86 (1212.92, 1431.29) 92%	942.48 (898.23, 1121.24) 84%	34.23 (29.43, 44.59) 106%	26.13 (20.57, 27.63) 118%

Note: Me – median; LQ – lower quartile; UP – upper quartile;

To examine one of the cardinal links of the antioxidant defence system - the glutathione system, in patients with HR, the concentration of reduced glutathione (GSH) and the activities of the main enzymes involved in glutathione metabolism - glutathione peroxidase (GPx) and glutathione reductase (GR) were evaluated. The values in tears of the three researched markers showed increases correlated with the advancement of the HR degree.

A trend of statistically insignificant fluctuation of reduced glutathione levels (GSH) between groups (p=0.357) was observed in tears. The values identified for GI - 153.36 μM /L, IQR 92.97, GII - 153.36 μM/L, IQR 62.15 and respectively GIII - 153.36 μM/L, IQR 123.62 [24]. In tears, GPx values were 289.75 nM/s·L, IQR 93.38 in group I, 308.55 nM/s·L, IQR 146.16 (+6%,

p=0.182) in group II and 357.27 nM/s·L, IQR 129.92 (+17%, p=0.077) in the third group of patients with HR. A statistically significant difference was observed between group I - 289.75 nM/s·L, IQR 93.38 and III - 357.27 nM/s·L, IQR 129.92 (p=0.004). Moreover, a true fluctuation

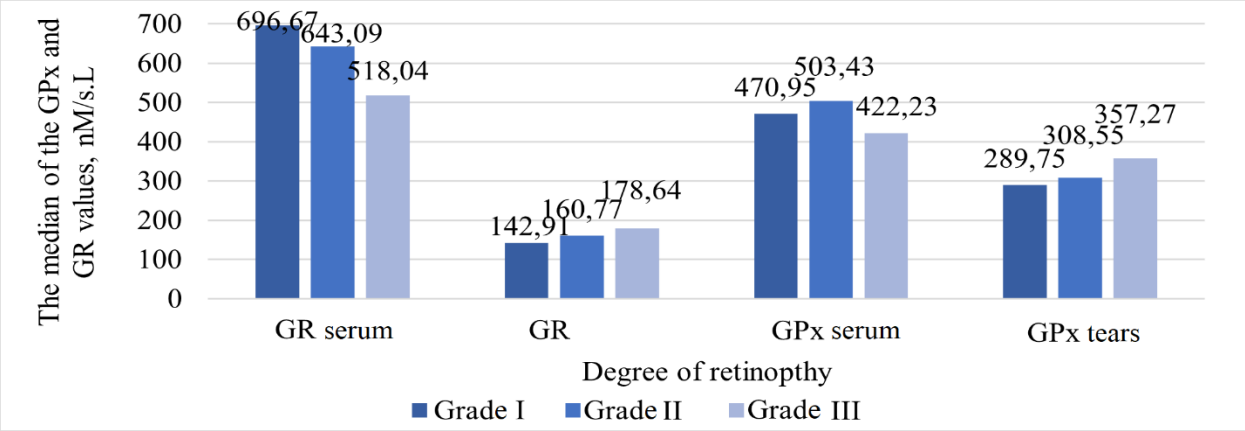


Figure 5. **Dynamics of GPx and GR activity in serum and tears reported at the HR stage**

of GPx levels between groups in tears (p=0.015) was attested as HR progressed (figure 5).

GR activity showed a statistically significant differences between groups, similar to GPx changes, with an amplification of values in the lacrimal sample (p=0.032) with the advancement of HR, in GII compared to GI +12%, p=0.492) and in GIII compared to GII (+ 25%, p=0.009). In addition, a conclusive change was observed between GIII and GI, p=0.045 (figure 5). In serum, the values of GSH content and GPx activity did not change cardinally between groups, unlike GR activity whose value was substantially decreased (p=0.010).

Statistically insignificant changes in GPx activity in serum (p=0.223) were attested during the evolution of the degree of retinopathy. The serum level of GPx in GII increased compared to GI + 7%, p>0.05), decreased in GIII compared to GII (-18%, p>0.05) (figure 5). Unlike GSH and GPx, serum GR activity showed a different, decreasing, statistically significant dynamics between groups (p=0.010). In group comparisons, the serum level of GR in GII decreased compared to GI - 8%, p=0.345 and continues to decrease in GIII -18%, p=0.027. A statistically significant change was observed in GIII compared to GI - p=0.003.

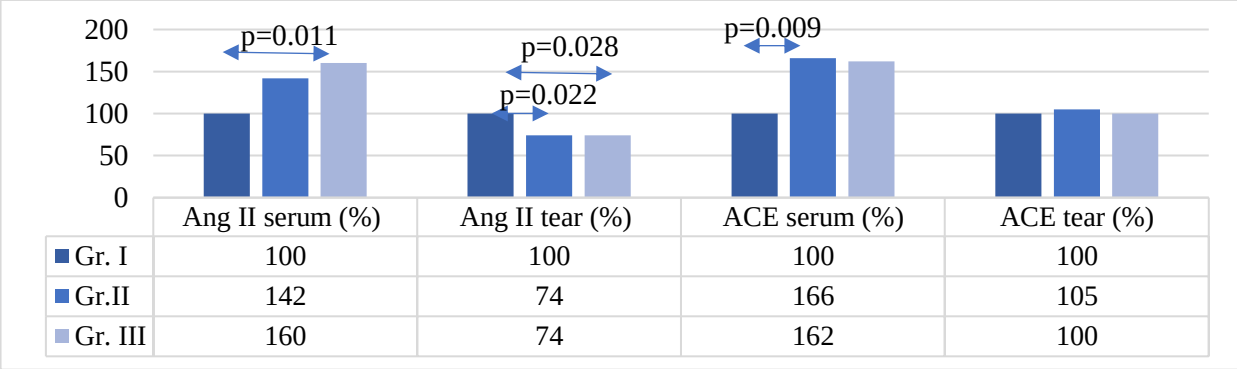
A weak significant negative correlation was established between tear and serum GSH levels (r_s=-0.361, p=0.000), while no correlation exists between GPx activities (r_s=-0.170, p=0.109) and GR (r_s=-0.039, p=0.714) in tear and serum. Analysis of feasible associations of GSH level changes with enzyme activity, which consumes GSH as a cofactor for detoxification and antioxidant activity (Table 3) identified significant mean positive correlations between the following markers: GPx and GR in tear (r_s=0.417, p=0.000) and GSH and serum GPx (r_s=0.409, p=0.000). In both fluids investigated, the GSH level did not correlate with the HR degree (r_s=-0.008, p=0.941 in serum; r_s=0.039, p=0.716 in tears). Serum GPx activity did not show a correlation with HR grade (r_s=-0.053; p=0.621), while tear GPx had a significant weak positive correlation with HR grade (r_s=0.299, p=0.004). In both investigated fluids, GR activity showed a significant weak and positive correlation with the degree of HR (r_s=0.297, p=0.004 in serum/r_s=0.252, p=0.017 in tears).

The content of thiol groups of proteins was also noted by a statistically insignificant distribution both in tears (p=0.877) and in serum (p=0.640). It was found that the amount of thiol groups of proteins in serum in GII increased compared to GI +12%, p>0.05, followed by a diminution in values in patients in GIII compared to GII -8%, p>0.05. In the tear sample, the thiol group content of proteins was 157.72 µM/g·prot (IQR 45.83) in group I, 150.98 µM/g·prot (IQR

32.35) in group II and 150.98 μM/g·prot (IQR 32.35) in the third group of patients with HR. No sero-lacrimal correlations were identified for the content of thiol groups of proteins (r_s=0.162, p=0.128) nor for each of the samples with HR degree (r_s=-0.040, p=0.709 for tear, r_s=0.039, p=0.718 for serum).

3.3 The changes in ischemia indices in HR. HR is characterized by a progressive damage of the retinal vessels, which may be associated with tissue ischemia. IMA is a specific marker of ischemic impairment. IMA was noted by different values, statistically significant in the blood serum (p=0.006), of patients during the evolution of HR. In group comparisons, the level of serum IMA in GII did not differ compared to GI (+3%, p=0.378), but increased in GIII patients compared to GII (+17%, p=0.016). There were no differences in IMA content in tear samples (p=0.160) between groups. No sero-lacrimal correlations were identified for IMA (r_s=-0.159, p=0.134), while serum IMA showed a significant moderate positive correlation, with the degree of HR (r_s=0.307, p=0.003). Amplified serum IMA levels in HR and the correlation with the severity of retinopathy suggests that the subsequent ischemia of microcirculatory disorders on the background of OS and dyslipidemia may be incriminated in the mechanism of its development.

3.4 The changes in SRA indices in the evolution of HR. There was a statistically significant increase in serum Ang II levels at the same time as the degree of HR increased (p=0.039). An inverse picture, expressed by the statistically significant decrease of Ang II values in groups, was observed in tears (p=0.035). In group comparisons, the serum level of Ang II in GII increases compared to GI (+ 42%, p = 0.264) as well as in GIII compared to GII (+18%, p=0.153) (figure 6). A statistically significant difference was observed between GI and GIII with p=0.011. The tear shows a gradual decrease in values, with a statistically significant decrease of -26% between GI and GII, with p=0.022 and between GI and GIII of - 26% with p=0.028 (figure 6).



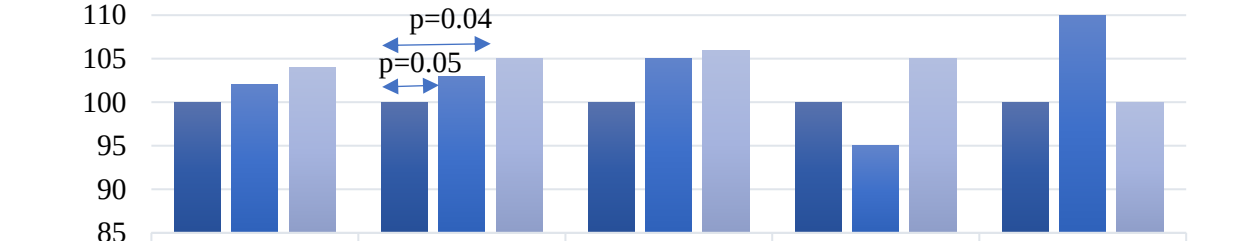
	Ang II serum (%)	Ang II tear (%)	ACE serum (%)	ACE tear (%)
Gr. I	100	100	100	100
Gr.II	142	74	166	105
Gr. III	160	74	162	100

Figure 6. **Ang II and ACE values in serum and tears in patients with varying degrees of HR**

Negative, low-strength, statistically significant sero-lacrimal correlations of Ang II were identified (r_s=-0.323, p=0.045). Moreover, Ang II levels in both serum and tear showed a statistically significant mean correlation with the degree of HR, in serum being positive - r_s=0.413, p=0.009, while negative in tear - r_s=-0.357, p=0.026. At the same time, there was a statistically significant increase in serum ACE levels (p=0.032) and insignificant in tears (p=0.536). In group comparisons, the serum level of ACE in GII increases compared to GI (+66%, p=0.009), remaining at the same level in GIII, p=0.242) (figure 6). The tear shows a statistically untrue fluctuation of the values starting with an amplification of +5% in GII compared to GI, p>0.05 and subsequent return to the initial values in GIII (figure 6). The ACE level in both serum and tear did not demonstrate a statistically significant correlation with HR grade. There was also a statistically significant increase in serum Ang II levels at the same time as the degree of HR progressed (p=0.039). The serum level of Ang II in GII increased compared to GI by +42%, and in GIII

compared to GII by +18%. Moreover, Ang II levels in both serum and tear showed a statistically significant mean correlation with the degree of HR, in serum being positive - r_s=0.413, p=0.009, while negative in tear - r_s=-0.357, p=0.026. Therefore, the statistically significant increase in serum Ang II levels in patients with HR and its correlation with the severity of retinopathy, confirms the active participation of RAS in the development of HR.

3.5 The changes in lipid metabolism indices in HR progression. A complete picture of changes in lipid status in serum samples of hypertensive patients with varying degrees of retinopathy are shown in Figure 7.



	TAG (%)	Total-Chol (%)	LDL-Chol (%)	HDL-Chol (%)	AI (%)
Gr. I	100	100	100	100	100
Gr.II	102	103	105	95	110
Gr. III	104	105	106	105	100

Figure 7. **Serum lipid levels and plasma atherogenic index in different degrees of HR**

Note: TAG - triglyceride; LDL-Chol - LDL-cholesterol; HDL-Chol - HDL - cholesterol; IA - atherogenic index;

No changes in serum TAG levels were observed between groups over time along with HR progression (p=0.061). In pair comparisons, the TAG level in GII tends to increase compared to GI (2.03 (IQR 0.18) mM/L vs. 2.07 (IQR 0.15) mM/L) and in GIII compared to GII (2.10 (IQR 0.25) mM/L) vs. 2.07 (IQR 0.15) mM/L). TAG levels demonstrated a statistically significant weak positive correlation with the degree of HR (r_s=0.249, p=0.018). There was a statistically significant increase in serum total cholesterol levels between groups as HR advanced (p=0.017). In group comparisons, the total cholesterol levels in GII and GIII were significantly higher compared to GI: 5.63 (IQR 0.69) mM/L compared to 5.49 (IQR 0.51) mM/L, p=0.05 and respectively 5.76 (IQR 0.82) mM/L compared to 5.49 (IQR 0.51), p=0.04 (figure 7).

The total cholesterol level demonstrated a positive correlation, statistically significant (p=0.005), weak (r_s=0.292) with the degree of HR. Serum LDL-Chol values showed an insignificant correlation with the degree of HR (r_s=0.129, p=0.225), while those of HDL-Chol were not correlated with the degree of pathology (r_s=0.084, p=0.429). None of these markers of lipoprotein metabolism changed statistically conclusively between groups (LDL-Chol - p=0.302, HDL-Chol - p=0.153). The LDL-Chol content showed a negligible upward trend of about 6% (GI=2.94 (IQR 0.55) mM/L; GII = 3.09 (IQR 0.37) mM/L; GIII = 3.13 (IQR 1.16) mM/L), and that of HDL-Chol fluctuated insignificantly with the advancement of HR (GI = 1.24 (IQR 0.28) mM/L; GII = 1.18 (IQR 0.20) mM/L; GIII = 1.30 (IQR 0.36) mM/L) (figure 7). The results of the studies suggest the involvement of lipid disorders in the development of HR, through endothelial dysfunction and damage to the retinal blood barrier, which will promote serum lipids and exudation of lipoproteins.

3.6 Correlation analysis. Analyzing the serum and tear marker changes during the evolution of HR, few sero-lacrimal correlations were attested, all being of weak power (table 2).

Table 2. **Sero-lacrimal correlations of biochemical indices in patients with HR**

Index	**Correlation coefficient r_s**	**p veracity**

MDA	r_s= 0.277	p = 0.008
TAA	r_s = - 0.226	p = 0.032
SOD	r_s = 0.336	p = 0.001
GSH	r_s = - 0.361	p = 0.000
Ang II	r_s = - 0.323	p = 0.045

The eye is a highly specialized organ with a relatively autonomous structure that is characterized by the presence of blood-retinal barrier and blood-aqueous humor barrier, peculiarities of fluid dynamics and metabolic processes, as well as specific operating conditions caused by direct contact with the environment. Thus, it is possible that the structural autonomy determines the lack of sero-lacrimal correlations, and the presence of the aforementioned barriers ensures the maintenance of different biochemical environments, in order to ensure the optimal functioning of the individual sections of the eye. A likely contributing factor would be vascular disorders specific to retinopathy, which can be explained by the specific ability of self-regulation of retinal vessels in response to changes in BP, ultimately ensuring the maintenance of a stable retinal vascular flow by active arterial vasoconstriction [7]. In addition to vasoconstriction, damage to local barriers may be responsible not only for the development of superficial retinal bleeding, retinal oedema and deep exudates ("dry exudates"), but also for disorders of the exchange of substances between the ocular structures and blood. A consequence of this phenomenon may be the lack of correlations between changes in local indices (tear) and systemic changes (blood) [2].

Another hypothesis would be that local changes have a lower share of changes at general level, both due to the structural-metabolic features previously revealed, and due to the relatively small size of the visual organ. It should also be mentioned that the level of parameters in the serum could be conditioned by the presence of other systemic factors.

The analyzed sero-lacrimal correlations could be questionable. However, it is not possible to compare the results obtained with the results of other researchers, due to the insufficiency of other studies referring to HR, and the lack of research to analyze predictive or explanatory markers or possible sero-lacrimal correlations. A larger study with the use of a larger group of patients could elucidate the questions.

The analysis of the obtained data showed that the levels of markers investigated in the serum and tear of patients would not always be an independent predictor of HR severity, supported by weak correlations between marker values and HR degree (table 3).

Although less researched in retinopathy, there is compelling evidence that a local SRA exists in the retina, and Ang II influences vascular dysfunction and inflammation. In HR, an increase in serum Ang II levels and a decrease in tear values were identified at the same time with HR progression, the changes being statistically significant in both serum (p=0.039) and tear (p=0.035). The tear and serum levels of Ang II showed a negative correlative connection, of average power, statistically significant (r_s=- 0.323, p=0.045). Also, Ang II values were truthfully correlated with the degree of HR both in tears (r_s=-0.357, p=0.026) and in serum (r_s=0.413, p=0.009), the strength of the association being average, but more important compared with the other correlations identified.

Table 3. **Correlations of serum and tear biochemical indices with RH degree**

Serum			**Tear**		
Index	**Correlation coefficient**	**Veracity**	**Index**	**Correlation coefficient**	**Veracity**
NO	r_s=- 0.265	p = 0.012	AAT	r_s = 0.357	p = 0.001

PPOA	$r_s = 0.243$	$p = 0.021$	SOD	$r_s = - 0.284$	$p = 0.007$
SOD	$r_s = - 0.246$	$p = 0.019$	CAT	$r_s = 0.261$	$p = 0.013$
GR	$r_s = 0.297$	$p = 0.004$	GPx	$r_s = 0.299$	$p = 0.004$
AIM	$r_s = 0.307$	$p = 0.003$	GR	$r_s = 0.252$	$p = 0.017$
Ang II	$r_s = 0.413$	$p = 0.009$	Ang II	$r_s = - 0.357$	$p = 0.026$
TAG	$r_s = 0.249$	$p = 0.018$			
Col	$r_s = 0.292$	$p = 0.005$			

Positive correlations of the serum ACE activity with the serum level of Ang II (r_s=0.356, p=0.026) and the negative correlations with lacrimal Ang II (r_s=-0.509, p=0.001) were identified. The detection of correlational connections confirms the involvement of RAS in the pathogenesis of HR. ACE plays a key role in the functioning of the system, possessing the ability to transform Ang I into Ang II - a powerful vasoconstrictor, which amplifies BP and at the same time disintegrating bradykinin with opposite properties. Due to the fact that Ang II receptors have been located in retinal blood vessels, a role of SRA in regulating retinal vascular tone is plausible, with arterioles responding to Ang II with vasoconstriction. Additional studies are needed to thoroughly assess the particularities of ocular RAS changes and their role in HR.

Almost all markers of the antioxidant system in tears (TAA, SOD, CAT, GPx, GR) correlate with the degree of HR. The correlations of the antioxidant system indices are all positive (AAT - r_s=0.357, p=0.001, CAT - r_s=0.261, p=0.013; GPx - r_s=0.299, p=0.004 and GR - r_s = 0.252, p = 0.017), with exception of SOD (r_s=- 0.284, p=0.007). Therefore, in HR the initial protection against ROS (neutralization of O_2- by SOD) is diminished, and subsequently compensatory increase the activities of enzymes (CAT, GPx, GR) that attenuate the effects of subsequently formed compounds - H_2O_2, lipid peroxides, etc. Overall, the correlated changes in antioxidant enzyme activity ensure a continuous, statistically significant increase in TAA values as HR advanced in degree (p=0.003), which resulted in a higher degree of local antioxidant protection compared to the systemic level.

A correlated change in SOD and CAT activity was attested in serum (r_s=0.390, p=0.000), which provides primary $O_2^{-\cdot}$ and H_2O_2 protection in OS, as well as SOD and GPx activity (r_s=0.438, p=0.000) and SOD activity with GSH content (r_s=0.395, p=0.000) which ensures the elimination of organic peroxides - by-products of OS. Thus, the body provides a balanced protection in unison by the enzymatic component of the body's AOS from the effects of HTN.

At the lacrimal level, fewer correlations were established between the AOS elements, TAA being negatively moderately correlated with SOD activity (r_s=-0.446, p=0.000) and moderately positively correlated with GSH content (r_s=0.405, p=0.000). The low number of correlations at the tear level does not allow conclusions to be drawn regarding the coordination of the AO response at the tear level as opposed to the systemic response reflected by the serum correlations.

At the same time, there was a link between the functionality of the AO and RA systems, reflected by the average negative correlations of Ang II with TAA (r_s=- 0.611, p=0.000) and the GSH content (r_s=-0.447, p=0.004) and positive with SOD activity (r_s=0.493, p=0.001). Additionally, were established correlations between GPx and GR in tear (r_s=0.417, p=0.000) and serum GSH and serum GPx (r_s=0.409, p=0.000). Considering all the experimental evidence, it is assumed that OS and disturbances in glutathione metabolism are imperatively related to the pathogenesis of HR.

The correlation of OS markers with lipid status indices and of the last ones in serum was also identified. The MDA content was directly correlated, with average strength with the TAG level (r_s=0.464, p=0.000), the values of which increased progressively with increasing HR

severity. We consider that the attested phenomena could be an additional proof of the impact of dyslipidemia in the spread of OS in HTN and the development of its complication - HR.

Additionally, the lack of a higher number of correlations could be explained by the relatively limited number of patients, the extension of the study may elucidate some uncertainties. Another option would require increasing and diversifying the number of representative markers, such as isoprostanes, lipid hydroxyperoxides, thioredoxins, glutaredoxins, VEGF, etc., which could be much more reliable in explaining the pathobiochemistry of HR.

4. GENERALISATION OF THE STUDY RESULTS

The serum and tear changes identified in the research allow us to deduce some hypotheses regarding the impact of OS and RAS, the defensive role of the antioxidant system, as well as their evolution within the 3 degrees of HR, as well as outlining the most sensitive laboratory indices of the researched pathology.

In serum, it was initially found in grade II of HR, the attempt to maintain the relatively clinically compensated status by logical fluctuation of markers of interest, either by amplifying or decreasing them (Table 4).

Table 4. **The evolution serum markers (%) related to the advancement of the HR degree**

	The degree of retinopathy			**p veracity**
	GI	**GII**	**GII**	
	Indices of oxidative stress			
1. NO	100%	92%	89%	**p=0.039**
2. S-nitrosothiols	100%	95%	96%	p=0.694
3. DAM	100%	101%	102%	p=0.628
4. AOPP	100%	153%	224%	p=0.071
	Changes in ischemia indices			
5. IMA	100%	103%	120%	**p=0.006**
	AOS activity			
6. TAA	100%	99%	97%	p=0.182
7. SOD	100%	99%	92%	**p=0.035**
8. CAT	100%	103%	106%	p>0.05
9. GSH	100%	102%	97%	**p=0.010**
10. GPx	100%	107%	90%	p=0.223
11. GR	100%	92%	74%	**p=0.010**
12. gr.-SH	100%	112%	104%	p=0.640
	RAS changes			
13. Ang II	100%	142%	160%	**p=0.039**
14. ACE	100%	166%	162%	**p=0.032**
	Changes in lipid metabolism			
15. TAG	100%	102%	104%	p=0.061
16. Chol	100%	103%	105%	p=0.017
17. LDL-Chol	100%	105%	106%	p=0.302
18. HDL-Chol	100%	95%	105%	p=0.153

In our study, the decrease of NO by 8% in gr. II of HR, most probably explained by the fact that NO•, as a second messenger, interacts with the superoxide anion forming peroxynitrite (ONOO−), being a factor determined in the induction of OS. Endothelial dysfunction, which is characterized by impaired NO bioavailability, is an important risk factor for both the development of HTN and HR and may be a major link between diseases. Understanding the role of NO in BP

regulation may have implications for improving treatment and reducing the risk of developing morbidity [25]. Subsequently, we hypothesized that a decrease by 5% in the circulating concentration of S-nitrosothiols in HTN in grade II of the disease, results in endothelial lesions and affects NO biosynthesis. These changes may contribute to the development of specific vascular lesions in the retina in HR. The slight increase in the concentration of MDA in the serum indicates an intensification of lipid peroxidation and therefore the fact that the tissues were exposed to OS, and the pathological structural transformations of proteins, with the formation of AOPP, attest to the action of ROS on them. Thus, the amplification of oxidative damage of proteins in grade II by ↑53%, which is stimulated under OS conditions, comes in tandem with the slight increase of the values of the classic products of lipid peroxide oxidation (MDA +1%), these results confirming the fact that proteins are more susceptible compared to lipids in conditions of HTN and retinal damage.

To counteract the effects of OS, the human body uses a variety of antioxidant mechanisms. In our study, the presence of a momentarily compensated imbalance in the antioxidant system is supported by relatively unchanged serum values of markers such as TAA and GSH. An enzymatic imbalance was observed in gr. II of HR, expressed by decreasing SOD activity (8%) and increasing CAT activity (6%), as well as by increasing serum GPx values by 7% and reducing GR values by 8% (table 4).

Possible maintenance of the TAA at normal level is determined by the coordinated changes of the key compounds of the AO system. Thus, the decrease of SOD activity is counterbalanced by the increase of CAT activity. Therefore, the evolution of enzymatic diversity: SOD, catalase and peroxidase in order to eliminate reactive intermediates, suggests that a significant proportion of O_2 is reduced in this way. Research in the last decade has shown that HTN is associated with a number of disorders in glutathione metabolism, the phenomenon being established by the current study.

It is also worth noting the increase of -SH groups by 12% in gr II of HR. Increasing the level of thiol groupings in the study patients could be a compensatory mechanism for counteracting OS and its harmful effects, as well as maintaining the structural and functional integrity of cellular elements and the whole cell.

We know that the involvement of RAS in HR argues in favour of most hypotheses to elucidate the pathogenesis of HR, especially ischemic and OS participation. Although initially adaptive, the changes that accompany HTN and possibly HR, namely endothelial dysfunction, vasoconstriction, can eventually become maladaptive and lead to the progression of HR. We observe in grade II an increase in Ang II level by 42% and ACE activity by 66%, a change that is associated with a decrease of -8% in NO content (table 4). At the same time, Ang II decreases the bioavailability of NO by promoting SO.

Grade III HR lesions become more evident. Thus, in grade III HR, the decrease of SOD (-8%) is more obvious in the patients in the current study. Therefore, this decline suggests a deficiency in antioxidant defence mechanisms, which would subsequently affect the ability of hypertensive patients to remove the circulating superoxide anion and cause an increase in ROS-induced vascular damage.

On the other hand, increased catalase expression was determined in HTN, a polymorphism in the catalase promoter region being associated with high BP levels. In our study, a +6% amplification in catalase is observed in GIII, which possibly compensates for the reduced capacity of SOD to neutralize $O_2^{-\cdot}$ and the subsequent increase in the amount of H_2O_2 [26].

Moreover, as far as it is known, our study highlighted for the first time the decline in serum and tear SOD levels and the growth in serum and tear catalase levels in HR. It was also pointed out that in hypertensive patients with RH, in both investigated fluids, SOD activity showed a significant weak, negative correlation with the degree of HR (r_s=- 0.246, p=0.019 in serum/r_s=-0.284, p=0.007 in tears). There was also a significant weak positive correlation between serum and tear SOD levels (r_s=0.336, p=0.001). However, even if no correlation with serum catalase was identified, tear catalase showed a significant weak, positive association with HR grade (r_s=0.261, p=0.013).

Our research highlights the fact that the progression of HTN and HR, respectively, induces in addition to vessel damage and metabolic changes characterized by increased amount of biochemical marker indicator of tissue ischemia, such as IMA, in serum. This is especially noticeable in the third degree of HR. In OS, the formation of IMA is determined by exposure to free radicals, as a result of changes that occur in the microvascular network, including the retina. Thus, statistically significant results in serum were determined with the increase in the third degree of HR of the value by 20%, p=0.006. In gr. III of HR deepens and vascular dysfunction, visible being the decrease of Ang II by another 18%.

Thus, the OS amplification is further supported by the decrease by another 3% of NO in gr.III. At the same time, the increase of AOPP levels by 124% compared to gr.I of HR indicates that proteins are exposed to extreme aggression with a possible deterioration of enzymes, including those studied. This explains the decrease in enzymatic activity.

Subsequently, the imbalance in the antioxidant system deepens, which to a large extent remains compensated, a fact demonstrated by the relatively unchanged TAA values. SOD decreases by -8%, followed by GPx by 10% and more GR by -26%, which favours the substantial generation of $O_2^{\bullet-}$, so H_2O_2, as well as the peroxidation process. By the decline of GPx, it undergoes the process of neutralizing peroxides, as well as by decreasing GR - reconversion of GSSG into reduced glutathione (GSH), in order to maintain the antioxidant efficacy of GSH.

Should also be mentioned and the changes in lipid metabolism (table 4). Increased lipid levels, visualized as HR progressed, induce vascular endothelial dysfunction and subsequently a reduced bioavailability of NO, also noted in our research. Peroxidation of lipids from lipoproteins of vascular wall generates local production of reactive carbonyl species, which interferes with macrophage involvement, cell activation and proliferation, and ultimately leads to chemical changes in vascular proteins through advanced lipoxidation end products. As a result, all these processes will affect the structure and function of the vascular wall [27]. We have noticed that disorders of lipid metabolism can certainly influence the evolution of HR. HTN and hyperlipidemia not only stimulate the development of atherogenesis, but also lead to a series of degenerative changes in the arterial walls of medium and large size. The results of the performed studies demonstrated disorders of lipid status biomarkers in hypertension-induced retinopathy. TAG and total cholesterol levels were correlated with the degree of HR, changes noticed over time by other scientists. These disturbances can be explained by the fact that lipids play a key role in the development of HTN and its most common complications. Subsequently, we can suggest the involvement of lipid metabolism disorders in the development of HR, through endothelial dysfunction and damage to the retinal blood barrier, which will promote serum lipids and exudation of lipoproteins. The discrepancies highlighted attest to the need for further research.

In tears, the picture of OS manifestations differs from that seen in serum. Initially, a similar decrease of NO is observed, with -6% in grade II and with another -4% in grade III, which supports

the hypothesis of OS amplification. Additionally, however, the results obtained in grade II of HR showed a decreased antioxidant activity of SOD of -6% and an amplified catalase +12%, which could be interpreted as a cellular defence mechanism against an exacerbated OS. Eventually, the further decrease of SOD activity of -10% in grade III, established in the research, as well as the persistent correlations conditioned the increase of ROS production and subsequently of hydrogen peroxide. As a result, the need for H_2O_2 detoxification induced an increase in catalase activity by another 6% in grade III (table 5).

Table 5. **The evolution of the indices investigated in tears (%) related to the degree of HR**

	The degree of retinopathy			p veracity
	GI	GII	GII	
	Indices of oxidative stress			
1. NO	100%	94%	90%	p=0.158
2. S-nitrosothiols	100%	104%	106%	p=0.706
3. MDA	100%	99%	98%	p=0.527
4. AOPP	100%	93%	93%	p=0.655
	AOS activity			
5. TAA	100%	177%	191%	p=0.003
6. SOD	100%	94%	84%	p=0.027
7. CAT	100%	112%	118%	p=0.033
8. GSH	100%	100%	100%	p=0.357
9. GPX	100%	106%	123%	p=0.015
10. GR	100%	112%	137%	p=0.032
	Changes in ischemia indices			
11. IMA	100%	89%	94%	p=0.160
	RAS changes			
12. Ang II	100%	74%	74%	p=0.035
13. ECA	100%	105%	100%	p=0.536

OS amplification is indirectly reflected by significant activation of the antioxidant system, which attempts to compensate SOD. We notice a significant increase in grade II of GPx by 6%, GR by 12%. In gr. III, the antioxidant system is activated even more compared to HR II, amplifying CAT by +6%, GPx by +17%, GR by +25%, with preserved GSH. At the same time, the SOD decreases by 10%. These enhancements determined the increase of TAA by 77% in gr.II and by 91% in gr.III of HR compared to gr.I. The tear changes show a gradual, statistically significant increase in TAA levels, explained by the amplification of local mechanisms of protection against OS in patients with HR, while elucidating the potential of retinal resistance to HTN, with superior antioxidant defence due to increased ability of tear antioxidant. Thus, we suggest that patients with HR manage fluctuating oxygen levels more efficiently, due to enhanced local antioxidant protection. Therefore, the eyes of the patients in the study have an enhanced ability to cope with toxic oxygen intermediates, which potentially explains the gradual and delayed evolution of the stages of retinopathy.

The results obtained showed a decreased antioxidant activity of SOD and an amplified catalase, which could be interpreted as a cellular defence mechanism against an exacerbated OS. Eventually, the decrease of SOD activity, established in the research, as well as the persistent correlations conditioned the increase of ROS production and subsequently of hydrogen peroxide. As a result, the need for H_2O_2 detoxification induced an increase in catalase activity. At the same time in the tear there is an inverse result to the serum, marked by amplificated values of GPx, GR,

GSH. Additionally, it was noted that not all laboratory indices investigated persist the sero-lacrimal correlation, which allows us to conclude that we are not talking about the presence of an interdependence of phenomena that occurs in the retina with biochemical changes in serum.

We assume that functional imbalance between Ang II, which decreases significantly in the second degree of HR by -26% and NO plays an important pathogenetic role in the hypertensive lesion of the visual apparatus. Over time, it has been suggested that Ang I, Ang II and angiotensinogen are not able to cross the barriers between the eyes and the circulating blood.

We observe that in grade III of HR, starting from a certain level, Ang II is produced in the same amount of available substrate and remains diminished. The given results would support the presence of a functional RAS located in the vascular walls. The results of our study suggest that the eye's resistance to HR triggers may be due to the presence of a local regulation and protection system, but this hypothesis requires further research.

In OS, the generation of IMA is determined by exposure to free radicals, as a consequence of changes in the microvascular network, including the retina. But unlike serum where the level of IMA is significantly amplified, in tear in grade II there is an atypical picture by decreasing IMA by 11%, but followed by a slight increase by 5% in grade III of HR. The phenomenon requires further studies to elucidate the veracity, magnitude and role in HR.

Being a multifactorial disease, over time a number of mechanisms have been proposed to explain the ways of developing ocular microvascular complications in patients with HR.

The results of the study and the identified correlations allowed us to conclude that there is a potentiation of systemic production, as well as potential and local ROS, which will subsequently induce oxidative changes of various molecules investigated and reduce the antioxidant protection activity of some enzymes. These conclusions can be supported by the decrease of even statistically insignificant NO in serum by -11%/in tears -10%, the amount of S-nitrosothiols in serum - 4%, the increase in the level of MDA in serum by +2% and serum AOPP by + 124%. It is worth mentioning that such markers as S-nitrosothiols, MDA and AOPP in tears showed antagonistic values to those in serum, MDA -2%, S-nitrosothiols + 6%, AOPP - 7%.

Most likely the increase in the content of S-nitrosothiols in the tear, against the background of decreased NO levels may be a consequence of intensive metabolism of NO, and its involvement in the processes of S-nitrosylation of proteins and other thiol compounds with NO consumption. However, the reduction in MDA in the tear may be due to the fact that its tear level does not reflect the lipid peroxidation activities in the retina.

Currently demonstrated data are not sufficient to propose the independent use of oxidative stress biomarkers evaluated in our research for the diagnosis of hypertensive retinopathy. However, they could be included in the laboratory markers that can be applied in the diagnosis of HR and the monitoring of the progression of the pathological condition, or of the applied treatment, the reference values for the respective laboratory indices needing to be determined by subsequent investigations.

GENERAL CONCLUSIONS

1. The eye is a unique organ with specific defence systems, which allows it to withstand the effects of oxidative stress, as evidenced by insignificant quantitative deviations of products such as nitric oxide, S-nitrosothiols, malonic dialdehyde and protein products of advanced oxidation in tears, compared with significant changes in some of these indices in the blood serum (decrease in nitric oxide and increase in advanced oxidation protein products).

2. In hypertensive retinopathy, the initial protection against reactive oxygen species provided by SOD is diminished, compensating by amplifying the activities of enzymes (catalase, glutathione peroxidase, glutathione reductase) which attenuate the effects of subsequently formed compounds (H_2O_2, lipid peroxides, etc.). The correlated changes in antioxidant enzyme activity induce a persistent, statistically significant increase in AAT values as hypertensive retinopathy progressed (p=0.003), leading to a higher degree of local antioxidant protection compared to the systemic level.

3. In hypertensive retinopathy there was an increase in serum values and a decrease in tear of angiotensin II with the advancement of hypertensive retinopathy, the deviations being statistically true both in serum (p=0.039) and in tears (p=0.035), showing a negative correlative connection, of average power, statistically significant (r_s=-0.323, p=0.045). Angiotensin II values were strongly correlated with the degree of hypertensive retinopathy both in tears (r_s=-0.357, p=0.026) and in serum (r_s=0.413, p=0.009), the strength of the association being stronger compared to the other correlations identified in research.

4. A small number of statistically true, weak correlations were established between the values of the indices studied in tear and serum (malonic dialdehyde, total antioxidant activity, SOD and GSH), between the degree of retinopathy and the level of serum compounds (NO, PPOA, SOD, GR, AIM, TAG and Col) and tear (AAT, SOD, CAT, GPx and GR), only the angiotensin II content showing moderate correlations with the degree of retinopathy, as well as between the values established in the studied fluids.

5. The difficulties of stratifying patients with HR in groups according to the degree of retinopathy according to the results of the clinical examination could be overcome by complementing with the evaluation of serum and / or lacrimal values of angiotensin II and lacrimal AAT, CAT, GPx and GR.

PRACTICAL RECOMMENDATIONS

1. It is recommended to continue the scientific study of hypertensive retinopathy with:

a) increasing the sample of the subjects, including those with grade IV of hypertensive retinopathy, in order to identify the changes specific to the most advanced stage of the pathology;

b) expanding the spectrum of markers of oxidative and nitrosative stress, antioxidant system and vascular endothelial dysfunction to establish with certainty the diagnostic, prognostic and monitoring value of markers that show trends of modification and potential practical utility in the current study;

c) elucidating the possibility of use of natural and synthetic antioxidants in the prevention and / or treatment of hypertensive retinopathy.

2. It is proposed the evaluation in ophthalmological practice the Ang II and the markers of the antioxidant system (TAA, CAT, GPx and GR) levels in order to reliably stage the HR.
3. It is recommended to include in the curriculum at Biochemistry, Pathophysiology and Ophthalmology the topics related to the role of oxidative and nitrosative stress, the antioxidant system and vascular endothelial dysfunction in the development of hypertensive retinopathy.

BIBLIOGRAPHY

1. Fraser-Bell S, Symes R, Vaze A. Hypertensive eye disease: a review. *Clin Exp Ophthalmol*. 2017;45(1):45-53. https://pubmed.ncbi.nlm.nih.gov/27990740/
2. Erden S, Bicakci E. Hypertensive retinopathy: incidence, risk factors, and comorbidities. *Clin Exp Hypertens*. 2012;34(6):397-401. https://pubmed.ncbi.nlm.nih.gov/22468968/

3. Botnaru V, Corlateanu A, Chesov D, Cuzimihih G. *Medicina internă Breviar:Modulul cardiologie*. Chișinău; 2008, pp.150-154. https://www.researchgate.net/publication/341576262_Victor_BOTNARU_Medicina_interna_Breviar_Modulul_CARDIOLOGIE
4. Mancia G, Fagard R, Narkiewicz K, et al. 2013 ESH/ESC Guidelines for the management of arterial hypertension: the Task Force for the management of arterial hypertension of the European Society of Hypertension (ESH) and of the European Society of Cardiology (ESC). *J Hypertens.* 2013;31(7):1281-1357. https://pubmed.ncbi.nlm.nih.gov/23817082/
5. Ambresin A, Borruat FX. Hypertension artérielle et œil [Hypertension and the eye]. *Rev Med Suisse*. 2015;11(499):2366-2372. https://pubmed.ncbi.nlm.nih.gov/26852552/
6. Flaxman SR, Bourne RRA, Resnikoff S, et al. Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. *Lancet Glob Health.* 2017;5(12):1221-1234. https://pubmed.ncbi.nlm.nih.gov/29032195/
7. Mozos I, Luca CT. Crosstalk between Oxidative and Nitrosative Stress and Arterial Stiffness. *Curr Vasc Pharmacol*. 2017;15(5):446-456. https://pubmed.ncbi.nlm.nih.gov/28155616/
8. Karaca M, Coban E, Felek R, Unal M. The association of oxidative stress with hypertensive retinopathy. *Clin Exp Hypertens.* 2013;35(1):16-19. https://pubmed.ncbi.nlm.nih.gov/22571627/
9. Touyz RM. Reactive oxygen species in vascular biology: role in arterial hypertension. *Expert Rev Cardiovasc Ther.* 2003;1(1):91-106 https://pubmed.ncbi.nlm.nih.gov/15030300/
10. McDermott AM. Antimicrobial compounds in tears. *Exp Eye Res.* 2013;117:53-61 https://pubmed.ncbi.nlm.nih.gov/23880529/
11. Gudumac V, Tagadiuc O, Rîvneac V, Sardari V, Pantea V, et al. Investigații biochimice. Volumul II. Micrometode. Elaborare metodică. Tipogr. "Elena-VI", 2010, 97 p. ISBN 978-9975-106-06-1
12. Gudumac V., Rîvneac V., Tagadiuc O., Sardari V., et al. Metode de cercetare a metabolismului hepatic. Elaborare metodică. Sub red. Valentin Gudumac. Univ. de Stat de Medicină şi Farmacie „Nicolae Testemiţanu". Ch.: S.n., 2012. Tipogr. „Tehnica-Info". 162 p.(pag.101)
13. Gudumac V, Tagadiuc O, Andronache L, Ștîrba O, Pantea V. Procedeu de dozare a dialdehidei malonice în materialul biologic. Certificat de inovator nr. 5157 din 14.12.2012
14. Gudumac V, Tagadiuc O, Andronache L, Ștîrba O, Sardari V. Procedeu de dozare a produșilor proteici de oxidare avansată. Certificat de inovator nr. 5164 din 14.12.2012
15. Gudumac V, Rîvneac V, Tagadiuc O. et al. Metode de cercetare a metabolismului hepatic. Elaborare metodică USMF „Nicolae Testemiţanu". Tipografia „Tehnica-Info". Chişinău, 2012; 162 p
16. Baciu E, Nastas I. Procedeu de determinare a activității catalazei. Certificat de inovator nr. 3122 din 09.11.1996.
17. Tagadiuc O, Gudumac V, Pantea V. Procedeu de dozare a activității superoxid dismutazei. Certificat de inovator nr. 4891 din 15.07.2010.
18. Andronache L, Tagadiuc O, Gudumac V, Gulea A. Optimizarea procedeului nitroprusidic de evaluare a glutationului redus în materialul biologic. *Analele Ştiinţifice ale USMF „N. Testemiţanu".* 2009;1(10):184-189. ISSN 1857-1719. https://ibn.idsi.md/ro/vizualizare_articol/2128
19. Tagadiuc O, Andronache L, Ștîrba O, Sardari V, Pantea V. Determinarea glutation peroxidazei. Certificat de inovator nr. 5161 din 26.10.2012.
20. Gudumac V, Tagadiuc O, Andronache L, Ștîrba O, Sardari V. Determinarea glutation reductazei în eritrocite și ser sangvin. Certificat de inovator nr. 5172 din 06.11.2012
21. Gudumac V, Tagadiuc O, Andronache L., Pantea V. Determinarea conținutului de grupe tiolice ale proteinelor. Certificat de inovator nr. 5173 din 07.11.2012.
22. Gudumac V, Tagadiuc O. Metodă de determinare a capacității albuminei ischemic modificate de legare a cobaltului. Brevet de invenție nr. MD 4054 din 11.06.2009. http://www.db.agepi.md/Inventions/details/a%202009%200116/Des~a%202009%200116
23. Nielsen F, Mikkelsen BB, Nielsen JB, Andersen HR, Grandjean P. Plasma malondialdehyde as biomarker for oxidative stress: reference interval and effects of life-style factors. *Clin Chem.* 1997;43(7):1209-1214. https://pubmed.ncbi.nlm.nih.gov/9216458/
24. **Pavlovschi E**, Pantea V, Borovic D, Tagadiuc O. Glutathione-related antioxidant defense system in patients with hypertensive retinopathy. *Rom J Ophthalmol.* 2021 Jan-Mar;65(1):46-53. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC7995505/

25. Perticone F, Ceravolo R, Pujia A, et al. Prognostic significance of endothelial dysfunction in hypertensive patients. *Circulation.* 2001;104(2):191-196. https://pubmed.ncbi.nlm.nih.gov/11447085/
26. **Pavlovschi E**, Pantea V, Borovic D, Tagadiuc O. Tear and serum superoxide dismutase and catalase activities in hypertensive retinopathy. *Russian Open Medical Journal*. 2021; 10: e0305. https://romj.org/2021-0305
27. Fu Z, Chen CT, Cagnone G, et al. Dyslipidemia in retinal metabolic disorders. EMBO Mol Med. 2019;11(10):e10473. https://pubmed.ncbi.nlm.nih.gov/31486227/

LIST OF SCIENTIFIC PUBLICATIONS AND EVENTS

at which the results of the researches for the doctoral thesis in medical sciences with the topic „Biochemical markers of hypertensive retinopathy" were presented

- **Articles in ISI, SCOPUS journals and other international databases:**

1. **Pavlovschi E.**, Pantea V., Borovic D., Tagadiuc O. Glutathione-related antioxidant defense system in patients with hypertensive retinopathy. In: *Rom J Ophthalmol*. 2021; 65(1):46-53. doi: 10.22336/rjo.2021.9 (**SJR: 0.252)**.

2. **Pavlovschi E.**, Pantea V., Borovic D., Tagadiuc O. Study of ischemia modified albumin (IMA) as a biomarker in hypertensive retinopathy. In: *Med Pharm Rep.* 2021;94(2):185-190. doi: 10.15386/mpr-1815 (**IS: 1.28 / SJR: 0.35)**

3. **Pavlovschi E.**, Borovic D., Pantea V., Lîsîi L., Tagadiuc, O. Biomarkers of Lipid Status and Metabolism in Retinal Hypertensive Disorder. In: *Journal of Biosciences and Medicines*, 2021;9:77-89. https://doi.org/10.4236/jbm.2021.94006 (**IF: 0.87)**

4. **Pavlovschi E**, Pantea V, Borovic D, Tagadiuc O. Tear and serum superoxide dismutase and catalase activities in hypertensive retinopathy. In: *Russian Open Medical Journal* 2021;10:e0305. doi: 10.15275/rusomj.2021.0305 (**SJR: 0.14)**

5. **Pavlovschi E.,** Pantea V., Borovic D., Tagadiuc O. Serum depletion and tear increase of total antioxidant capacity in hypertensive retinopathy. In: *Arch Balk Med Union.* 2021;56(3):291-297. https://doi.org/10.31688/ABMU.2021.56.3.02 **(SJR: 0.15)**

- **Articles in accredited national scientific journals:**

6. **Pavlovschi E**, Stratulat S, Ambros A, Borovic D, Tagadiuc O. Evaluarea modificărilor markerilor OCT în retinopatia hipertensivă. In: *Buletinul Academiei de Ştiinţe a Moldovei. Ştiinţe Medicale*. 2020, nr. 1(65), pp. 296-299. ISSN 1857-011

7. **Pavlovschi E.** Rolul factorilor de creştere endoteliali (VEGF) în retinopatia hipertensivă. In: *Culegeri de lucrări ştiinţifice al Institutului de Medicină Urgentă. Articole originale şi activitatea inovaţională aa. 2015–2018*. Tipogr. „Sirius". 2019; 144 p. ISBN 978-9975-57-2606

- **Articles in international scientific collections:**

8. **Pavlovschi E**. Emerging Concepts of the ocular molecular-metabolic effects of the hypertension, In: *XXI International Scientific and Practical Conference International Trends in Science and Technology*. Warsaw, Poland, 2020, pp. 48-52, ISBN 978-83-956628-1-2

- **Abstracts/theses submitted at national or international scientific conferences:**

✓ **international**

9. **Pavlovschi E**. Nitric Oxide: the synthesis and effects at the level of the retina. In: *7th International medical Congress for Students and Young Doctors MedEspera*. Chișinău, 2018, pp. 197-198.

10.**Pavlovschi E**. Association of hypertensive retinopathy with different laboratory indices in patients with essential hypertension. In: *Abstract book of the congress BIMCO*. Chernivtsi, Ukraine, 2020, p. 75. ISSN 2616-5392.

11.**Pavlovschi E**. Nitric oxide levels in tear and serum of patients with hypertensive retinopathy. In: *7th Lublin International Medical Congress for students and young doctors*. Lublin, Poland, 2020, p. 245.

12.**Pavlovschi E**. Serum and tear malondialdehyde level as a potential stress marker in hypertensive retinopathy. In: *BIMCO Bukovinian International Medical Congress «BIMCO JOURNAL» Abstract book of the congress BIMCO*. Chernivtsi, Ukraine, 2021, p. 157. ISSN 2616-5392.

13.**Pavlovschi E**, Pantea V, Tagadiuc O. Measurement of total antioxidant activity of tear and serum samples of the patients with hypertensive retinopathy, In: *The International Scientific Conference on*

Medicine, organized within the frame of the 79th International Scientific Conference of the University of Latvia. Riga, Latvia, Medicina (Kaunas) 2021;57(Supplement 1), p. 17. ISSN 1648-9233.

14.**Pavlovschi E**, Borovic D, The use of advanced oxidation protein products to monitor oxidative stress levels in patients with hypertensive retinopathy. In: *EURETINA 2021 Virtual,* 9-12 September 2021. https://www.euretina.org/events/euretina-2021-virtual/

- ✓ **national**

15.**Pavlovschi E.** Dereglările activității catalazei în retinopatia hipertensivă. In: *Cartea de abstracte a Congresului consacrat aniversării a 75-a de la fondarea USMF „Nicolae Testemițanu"*. Chișinău, 2020, p.59

- **Invention patents, patents, registration certificates, materials at invention salons**

16.**Pavlovschi E**, Pantea V, Borovic D, Tagadiuc O. Certificat de înregistrare a obiectelor dreptului de autor și drepturilor conexe, "Sistemul antioxidant al glutationului la pacienții cu retinopatie hipertensivă", Seria O Nr.6879 din 25.03.2021 IDNO: 1007600000794

- **Participation with communications at scientific forums:**
- ✓ **international**

17.**Pavlovschi E**. Nitric oxide: the synthesis and effects at the level of retina. *7th International Medical Congress for Students and Young Doctors MedEspera.* Chișinău, 3-5 mai, 2018.

18.**Pavlovschi E.** Emerging Concepts of the ocular molecular-metabolic effects of the hypertension, *XXI International Scientific and Practical Conference International Trends in Science and Technology*. Warsaw, Poland, January 31, 2020.

19.**Pavlovschi E**. Association of hypertensive retinopathy with different laboratory indices in patients with essential hypertension, VII Bukovinian International Medical and Pharmaceutical Congress Of Students And Young Scientists - BIMCO, 7-8 april 2020 **(IInd Place)**

20.**Pavlovschi E.** Nitric oxide levels in tear and serum of patients with hypertensive retinopathy. *Lublin International Medical Congress,* Lublin, Poland, 26th – 28th November 2020

21.**Pavlovschi E.** Serum and tear malondialdehyde level as a potential stress marker in hypertensive retinopathy, *BIMCO Bukovinian International Medical Congress, Chernivtsi, Ukraine, «BIMCO JOURNAL» Abstract book of the congress BIMCO, 6-9 April 2021*, pag.157 ISSN 2616-5392

22.**Pavlovschi E**, Pantea V, Tagadiuc O, Measurement of total antioxidant activity of tear and serum samples of the patients with hypertensive retinopathy, *The International Scientific Conference on Medicine, organized within the frame of the 79th International Scientific Conference of the University of Latvia*. Riga, Latvia, 23-24 April 2021. Medicina (Kaunas) 2021;57(Supplement 1), p.17, ISSN 1648-9233

23.**Pavlovschi E,** Borovic D. The use of advanced oxidation protein products to monitor oxidative stress levels in patients with hypertensive retinopathy, *EURETINA 2021 Virtual*, 9-12 September 2021 https://www.euretina.org/events/euretina-2021-virtual/

- **Participation with posters in scientific forums:**
- ✓ **national**

24.**Pavlovschi E,** Borovic D. Concepte curente a retinopatiei hipertensive. Conferința științifică anuală a specialiștilor din cadrul IMSP IMU "*Actualități și controverse în managementul urgențelor medico - chirurgicale"*. Chișinău, 10 noiembrie 2017

25.**Pavlovschi E,** Tagadiuc O. Posibilii factori de risc biochimici predictivi ai retinopatiei hipertensive. *Conferința științifică anuală a specialiștilor din cadrul IMSP IMU "Actualități și controverse în managementul urgențelor medico - chirurgicale"*. Chișinău, 10 noiembrie 2017

26.**Pavlovschi E.** Rolul factorilor de creştere endoteliali (VEGF) în Retinopatia Hipertensivă. *Conferinţa ştiinţifică anuală a tinerilor specialişti din cadrul IMSP IMU "Performanţe şi perspective în urgenţele medico-chirurgicale"*. Chișinău, 18 mai 2018.

27.**Pavlovschi E,** Borovic D. Angiotensina II în patogenia retinopatiei hipertensive. (Poster) *Conferinţa ştiinţifică anuală a tinerilor specialişti din cadrul IMSP IMU "Performanţe şi perspective în urgenţele medico-chirurgicale".* Chișinău, 18 mai 2018

28.**Pavlovschi E,** Borovic D, Tagadiuc O. *Mecanismele biochimice şi markerii retinopatiei diabetice. Conferinţa ştiinţifică anuală IMSP IMU "Actualităţi şi controverse în managementul urgenţelor medico-chirurgicale."* Chişinău, 7 decembrie, 2018.

29.**Pavlovschi E,** Borovic D. Nivelurile plasmatice ale VEGF ca un marker util pentru detectarea leziunilor microvasculare timpurii în hipertensiune. *Conferinţa ştiinţifică anuală IMSP IMU "Actualităţi şi controverse în managementul urgenţelor medico-chirurgicale*." Chişinău, 7 decembrie, 2018.

30.**Pavlovschi E.** Dereglările activității catalazei în retinopatia hipertensivă. *Congresul consacrat aniversării a 75-a de la fondarea USMF „Nicolae Testemițanu"*. Chișinău, 20 - 23 octombrie 2020