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SERUM DEPLETION AND TEAR INCREASE OF TOTAL ANTIOXIDANT CAPACITY IN HYPERTENSIVE RETINOPATHY

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Abstract

Introduction. Arterial hypertension (HTN) is one of the leading public health problems, causing a challenging and complex visual impairment known as hypertensive retinopathy (HR). Its pathogenesis is caused not only by changes in retinal homeostasis because of high blood pressure (BP) but, undoubtedly, other pathological mechanisms are involved, including oxidative stress (OS).

The aim of the study was to evaluate the serum and tears total antioxidant capacity (TAC), as a single parameter of oxidative stress associated with the development of HR and to establish whether there is a correlation between its level and the grade of HR.

Materials and methods. 90 hypertensive patients divided according to the Keith-Wagner-Barker grading system of HR into three groups: GI – 36 patients; GII – 35 patients; GIII – 19 patients. TAC in tears and serum was measured and the obtained results were

Résumé

Déplétion dans le sérum et elevation en larmes de la capacité antioxydante totale dans la rétinopathie hypertensive

Introduction. L'hypertension (HTN) est l'un des principaux problèmes de santé publique, provoquant l'une des déficiences visuelles difficiles et complexes connues sous le nom de rétinopathie hypertensive (RH). Sa pathogenèse est causée non seulement par des modifications de l'homéostasie rétinienne dues à l'hypertension artérielle, mais sans aucun doute, d'autres mécanismes pathologiques sont impliqués, notamment le stress oxydatif (SO).

L'objectif de l'étude était d'évaluer la capacité antioxydante totale (CAT) du sérum et des larmes, comme paramètre unique du stress oxydatif associé au développement de la RH et d'établir s'il existe une corrélation entre son niveau et le grade de RH.

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analysed by Kruskal-Wallis and Mann-Whitney tests in SPSS Statistics. The Spearman correlation coefficient was determined. A p≤0.05 was considered statistically significant.

Results. TAC values in the tears samples of hypertensive patients increased statistically significant as HR advanced in grade (p=0.003). Serum level of TAC showed only a tendency of decrease (p=0.182). Only tears TAC levels correlate significantly with low power with HR degree (r=0.357**, p=0.001). Serum and tears levels of TAC have a statistically significant negative low power correlated with each other (r= – 0.226*, p=0.032).

Conclusions. The continuous rise in TAC levels in tears along with HR progression is a possible mechanism by which the eye copes with the damaging reactive oxygen species produced by OS. The increase in TAC may partially explain the gradual and delayed development of retinopathy stages in HTN.

Keywords: hypertensive retinopathy, total antioxidant capacity, oxidative stress.

List of abbreviations:
HTN – hypertension
BP – blood pressure
HR – hypertensive retinopathy
TAC – total antioxidant capacity
OS – oxidative stress

Matériel et méthodes. 90 patients hypertendus divisés selon le système de classement Keith-Wagner-Barker de RH en trois groupes: GI – 36 patients; GII – 35 patients; GIII – 19 patients. Le CAT dans les larmes et le sérum a été mesuré et les résultats obtenus ont été analysés par les tests de Kruskal-Wallis et de Mann-Whitney dans SPSS Statistics. Le coefficient de corrélation de Spearman a été déterminé. p≤0,05 a été considéré comme statistiquement significatif.

Résultats. Les valeurs de CAT dans les échantillons de larmes des patients avec RH s'élèvent de manière statistiquement significative à mesure que RH progresse en grade (p=0.003). Le taux sérique de CAT n'a montré qu'une tendance à la baisse (p=0.182). Seuls les niveaux de CAT des larmes sont corrélés de manière significative avec une faible puissance avec le degré de RH (r=0.357**, p=0.001). Les niveaux de CAT dans le sérum et les larmes étaient statistiquement significativement négatifs corrélés les uns aux autres (r= – 0.226*, p=0.032).

Conclusions. Il y a une correlation directe entre l'augmentation continue des niveaux de CAT dans les larmes et la progression de la rétinopathie hypertensive. L'augmentation de CAT peut constituer un mécanisme possible par lequel l'œil fait face aux espèces réactives de l'oxygène nuisibles produites par le SO, et expliquer en partie le développement progressif et retardé des stades de la rétinopathie dans l'HTN.

Mots-clés: rétinopathie hypertensive, capacité antioxydante totale, stress oxydatif.

Introduction

Hypertension (HTN) ranks first among the leading causes of morbidity and mortality worldwide. The advances in the study of its pathogenesis make it possible to clarify the complex origins of the disease[1].

A series of previous studies have shown a decrease in the concentration of numerous antioxidant substances, which suggests the presence of oxidative stress (OS) in hypertension. There is a relationship between the development of microvascular and macrovascular hypertensive complications and free oxygen radicals damage. Several biochemical pathways have been associated with increased generation of free radicals in patients with HTN, that can be considered one of the likely mechanisms in the development of the most serious ocular complication, hypertensive retinopathy (HR)[2].

Retina is a very complex tissue of the eye. With a high metabolic rate and fully working on aerobic respiration, it has a complex circulatory network, able to provide oxygen and nutrients for metabolic needs, normal functioning of neurotransmission, phototransduction and, at the same time, interactions of metabolites, growth factors and vasoactive agents[3].

The eye is constantly exposed to a number of exogenous factors (ionizing radiation, toxins, ultraviolet light, etc.) and endogenous stress, with potentially harmful effects, and an imbalance between oxidants and antioxidants, that will cause retinal alterations[4]. An overcome of the protective factors is supposed to lead to retinal changes visualized at fundoscopy.

OS causes changes in various biological structures and molecules; therefore, it is extensively investigated as one of the most probable causes of HR. In time, proofs of OS involvement in HR development have been demonstrated by the increased of gamma-glutamyl transferase (GGT) activity and ferritin levels[5]. However, it is still controversial whether OS has a causal effect in the development of HR or whether it may be a consequence of tissue damage[6].

In eye diseases, the investigation of the local antioxidant defense state can be useful, due to significant variety of enzymatic and non-enzymatic antioxidants

present in different compartments/structures of the eye[7]. Moreover, the tears film is a significant constituent of the defence mechanism and a valuable biological material for eye research[8]. Our previous studies identified a statistically significant increase in tears' glutathione peroxidase and glutathione reductase activity in HR and a direct correlation of their activity with the HR degree[9]. Nevertheless, studies of the antioxidant defence mechanisms in eye tissues and liquids are limited.

Undoubtedly, extra markers with an established mechanism of implication in the pathogenesis of HR will be beneficial for a reliable assessment of quantitative and qualitative changes in the retina.

Determination of total antioxidant capacity (TAC) might be considered for a fast quantification of antioxidant efficacy[10]. TAC is known as the "cumulative action of all antioxidants present in plasma and body fluids, providing an integrated parameter rather than the simple sum of measurable antioxidant values"[11]. The antioxidants of a particular system can act through different mechanisms and have different effectiveness; therefore, the assessment of TAC is expected to be much more informative than the evaluation of the individual antioxidant's concentration[11]. Moreover, biological antioxidants imply both enzymatic antioxidants – as superoxide dismutase, catalase and glutathione peroxidase – and non-enzymatic antioxidants, such as vitamin C, vitamin E, plant polyphenol, carotenoids, and glutathione[12].

From all the methods of TAC assessment, 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonate) (ABTS) assay is simple, applicable to both lipophilic and hydrophilic systems and determines the antioxidant capacity of the enzymes, as well[13].

In the literature there are no published studies about the correlation between changes in the TAC level in tears and serum, which would allow to be used in the diagnosis and monitoring of HR.

The objective of the study was to define the change of TAC in tears and serum of patients with different stages of HR and to examine their relationship with HR, analysing a possible connection between their levels and the degree of HR.

Materials and methods

The study was approved by the Research Ethics Committee of the "Nicolae Testemitanu" State University of Medicine and Pharmacy, Chisinau, Republic of Moldova (12.02.2018). Participants signed a written informed consent prior to enrolment in the study. All procedures performed were in accordance with the national law and ethical standards of the Research Ethics Committee, the 1975 Declaration of Helsinki, as revised in 2008(5).

Patient selection

A total number of 90 hypertensive patients, 38 (42.2%) males and 52 (57.8%) females, with a mean age of 59.79±12.29 years (range 38 – 88 years), were enrolled in the study (Figure 1A). The patients were divided into three groups according to the Keith-Wagner-Barker HR grading system, based on fundus examination (Figure 1B). Group 1 (GI) included 36 patients with first grade HR, mild generalized retinal arteriolar narrowing; group 2 (GII) included 35 patients with second grade HR, with pronounced focal narrowing and arteriovenous nipping and group 3 (GIII) included 19 patients with third grade HR, with retinal haemorrhages, exudates and cotton wool spots.

Patients with fourth grade HR (with additional papilledema) were excluded from the study, due to

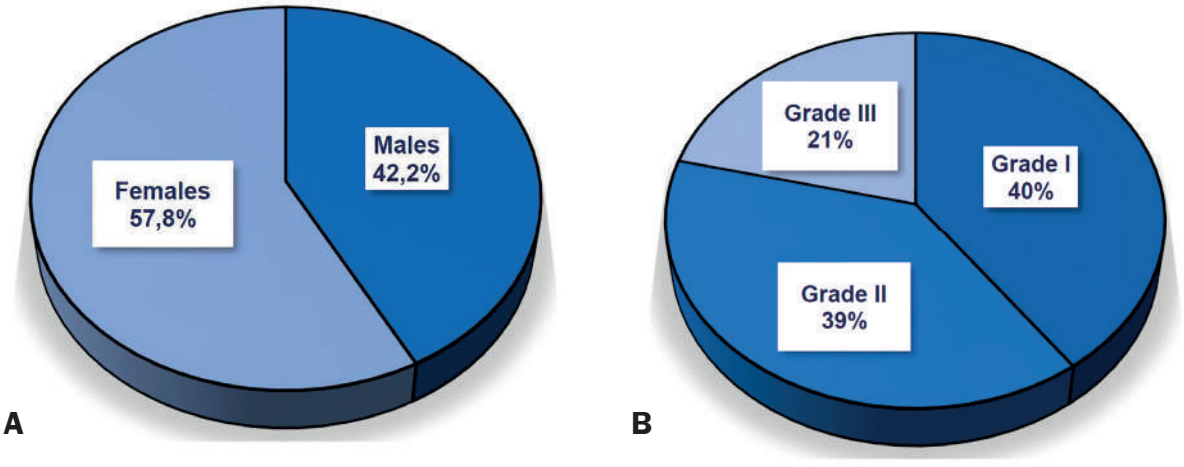

Figure 1. Repartition per gender (A) and per grade of HR (B) of the patients with HTN enrolled in the study.

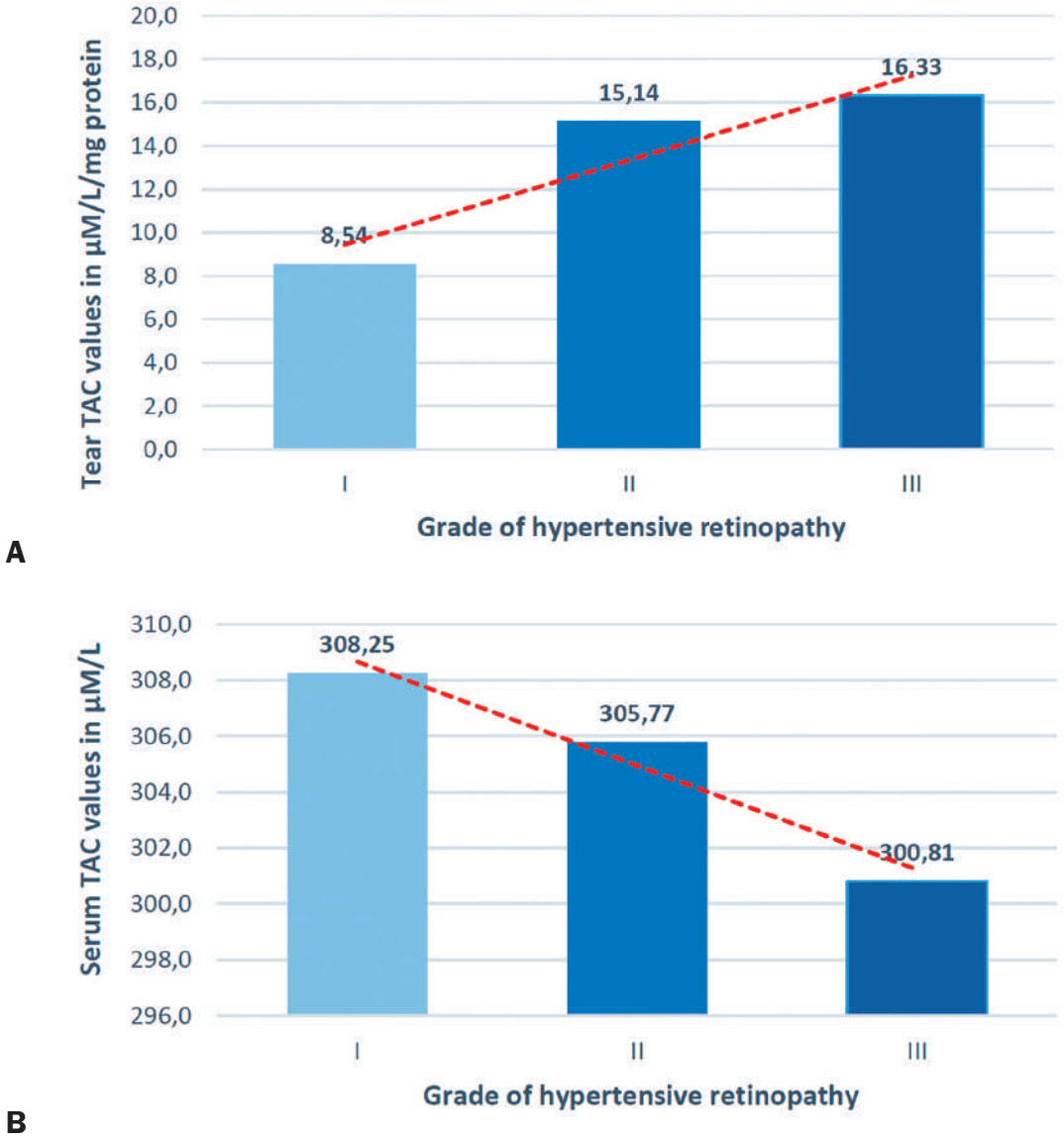

Figure 2. Changes of the total antioxidant capacity in tears (A) and serum (B) of patients with different grades of HR.

their insufficient number and the association of additional pathologies that could compromise the study results[14].

The study enrolled hypertensive patients, who presented for a random check-up to the Ovisus Medical Center, Chisinau, between 2018-2019, and who were first diagnosed with HR, after a complex specialized ophthalmological investigation. When selecting patients, the following exclusion criteria were applied: use of antihypertensive drugs or other medication that can interfere with the results, presence of metabolic disorders like diabetes and severe obesity, serious somatic comorbidities, neurological pathologies, ocular trauma, optic nerve atrophies of various causes and ocular diseases such as glaucoma, diabetic retinopathy, acute and chronic inflammatory processes, uveitis, etc.

Sample collection

Samples of venous blood (5 ml) were collected and centrifuged for 10 minutes with a spin speed of 3500 rpm, with further separation of the serum. Microcapillary tubes were used to collect tears' samples, from the inner tear's lake of the lateral conjunctival sac of the inferior fornix. All the samples were dispensed into Eppendorf microtubes and frozen at minus 40°C prior to biochemical determinations.

Biochemical analysis

The determination of the TAC was based on the electron transfer-based method using ABTS radical as the scavenger molecule. The ABTS radical cation (ABTS•+) chromophore, produced through the reaction between ABTS and potassium persulfate, that has a dark blue colour, is reduced by antioxidants

into colourless ABTS. The colour changes were measured spectrophotometrically at 734 nm[15]. Serum TAC levels were expressed as μM/L and tear TAC levels as μM/L/mg protein.

Statistical analysis

We tested the null hypothesis that the mean values of TAC level are the same for all HR categories. Statistical analysis was performed using SPSS 23.0 Software. Kolmogorov-Smirnov and Shapiro-Wilk normality tests were used to analyse data distribution. As the distribution for TAC was non-parametric, Kruskal-Wallis and Mann-Whitney tests were applied for comparison between groups and within groups and Spearman correlation test to analyse the relationship between HR and TAC. A 0.05 significance threshold was used. The TAC value was presented as median with interquartile range (IQR).

Results

In order to prove our hypothesis, we investigated TAC in the serum and tears of patients with HR of different grades. In the tears it was determined a statistically significant increase in the median with IQR of TAC values as HR advanced in grade (p=0.003). The increase in GII in comparison with GI was of 77% (15.14 μM/L/mg protein, IQR 15.75 vs. 8.53 μM/L/mg protein, IQR 9.09, p=0.006), and in GIII compared to GII of 14% (16.33 μM/L/mg protein, IQR 14.43 vs. 15.14 μM/L/mg protein, IQR 15.75, p=0.478) (Figure 2A). A statistically significant difference was observed between GI (8.53 μM/L/mg protein, IQR 9.09) and GIII (16.33 μM/L/protein, IQR 14.43 (p = 0.002)).

In serum, TAC values were not statistically different between groups as HR progressed (p=0.182). In paired group comparisons, the serum TAC values slightly diminished as HR progressed: in GII compared to GI (- 1%; 305.77 μM/L, IQR 13.46 vs. 308.24 μM/L, IQR 25.84, p>0.05), and in GIII compared to GII (- 2%; 300.81 μM/L, IQR 13.44 vs. 305.77 μM/L, IQR 13.46, p>0.05) (Figure 2B).

A statistically significant weak negative correlation was observed between TAC levels in tears and serum (r = – 0.226, p = 0.032).

Tears TAC had a statistically significant, weak strength, positive correlation with HR grade (r = 0.357, p=0.001), while serum TAC values showed no correlation with HR grade (r = – 0.164; p=0.123).

Discussion

Retina is highly susceptible to OS because of the high oxygen-demand, diminished self-regulation, fluctuating oxygen supply and high content of polyunsaturated fatty acids[16]. Many authors suggested that the development of HR is due to the interaction of multiple mechanisms, among which OS is noted and considered as a factor contributing to its development.

Over time, either increased levels of ROS degradation products or a deficiency of specific antioxidants and a decrease in TAC levels in hypertensive patients provided evidence of OS involvement in HTN pathogenesis[17]. However, relationships between the antioxidant status, blood pressure control, and the risk of complications, including retinopathy, in individuals with HTN are not completely understood[17,18].

The major advantage of TAC testing is that it measures the antioxidant capacity of all antioxidants in a biological sample and not solely the antioxidant capacity of a single compound[19]. A high level of TAC is detected in the serum of patients with chronic kidney damage[20], unchanged TAC levels in Alzheimer's disease, Parkinson's disease, depression and schizophrenia[21] and depleted TAC levels in type 2 diabetes[22], atherosclerosis[23], and ischemic stroke[24].

Our study was driven by limited and inconclusive evidence from previous TAC studies in ocular pathologies. So far, TAC level has mainly been assessed in serum and aqueous humour. A low TAC level in aqueous humour and the absence of significant changes in serum in retinitis pigmentosa[25], a decrease in serum TAC level in diabetic retinopathy[26] was identified.

In patients with hypertension, a low anti-oxidant capacity was noted[27,28]. Similar results were observed in experimental studies. In hypertensive rats, TAC values were lower than in healthy ones[29].

We can assume that OS is a contributing factor to HR development, but only a few studies have indicated a link between OS and HR outcomes. Only two OS serum markers have been analysed by now. Karaca et al. highlighted in patients with HR an increased activity of γ-glutamyl transferase, considered to have a pivotal function in glutathione homeostasis and involved in cell's protection against oxidants[6]. Coban et al. demonstrated increased serum ferritin levels in patients with HR, that may be associated with OS due to iron involvement in the Fenton reaction[6].

To the best of our knowledge, our study is the first to investigate the correlation of changes in TAC levels with HR grades. In addition, in our study, the TAC levels were investigated not only in blood serum, but also in tears of the patients with HTN. The analysis of tears in the case of eye diseases is considered more informative and targeted in comparison

with the analysis of blood serum, that reflects the general complications in HTN[30].

TAC encompasses the synergistic effects of the main antioxidants in a given matrix. Biological antioxidants are enzymatic antioxidants such as superoxide dismutase, catalase and glutathione peroxidase, and nonenzymatic ones, such as vitamin C, vitamin E, plant polyphenol, carotenoids, and glutathione[12].

The statistically significant increase of TAC in tears (by 59%, p=0.026 in grade II HR and by 69%, p=0.028 in grade III HR compared with grade I) can demonstrate the augmentation of the antioxidant protective mechanism in response to the OS, that triggers and sustains retina damage in HTN.

At the same time, the determination of TAC in blood serum did not reach statistical significance (p=0.319), as well a significant correlation between TAC in tears and in blood serum was not revealed (r= –0.226, p=0.032). Thus, serum TAC is not representative of HR changes and, for this reason, it should not be used as a marker of HR.

These results are promising and will further deepen our understanding of the mechanisms underlying HR development, that will undoubtedly provide additional information to improve diagnosis, disease control, or predict clinical response to treatment. It would be useful to evaluate changes in individual antioxidants in serum and tear and their correlations in HR of varying degrees, to determine whether individual serum markers would have diagnostic and prognostic value in HR.

Our study has limitations due to the fact that changes in TAC levels cannot be explained solely by HTA, HR, or both. Also, we are not able to confirm if TAC in serum and tears predicts the development of retinopathy or is a consequence. There is a need to expand the research on a higher number of patients.

Conclusions

This study completes the current information on possible local mechanisms of eye protection against OS in patients with HR and potentially explains the apparent resistance of the retina to HTN, demonstrating superior antioxidant protection due to increased antioxidant capacity of tears.

It was found a continuous increase of TAC in tears, along with HR progression, and correlation of TAC values with HG grade. The changes in serum are negligible and there are no relevant serum-lacrimal correlations. The increased TAC level in these patients' eyes is a possible mechanism to cope with toxic oxygen intermediates specific for OS. This fact can partially explain the gradual and delayed evolution of the stages of retinopathy.

The results reinforce the importance of new studies that will assess the changes in TAC and individual antioxidants in all clinical stages of HR and their potential use as diagnostic biomarkers for stratification of patients into groups, as additional criteria for a more reliable grading system.

Author Contributions:

Conceptualization, E.P. and O.T.; methodology, V.P.; software, E.P; validation, E.P., V.P and O.T.; formal analysis, E.P.; investigation, E.P and D.B.; data curation, E.P. and D.B.; writing–original draft preparation, E.P.; writing–review and editing, E.P, V.P., D.B. O.T.; supervision, O.T. All the authors have read and agreed with the final version of the article.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study"

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