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Research Paper

Diabetes-induced oxidative stress in the vitreous humor

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ABSTRACT

Purpose: Diabetes is accompanied by fundamental rearrangements in redox homeostasis. Hyperglycemia triggers the production of reactive oxygen and nitrogen species which contributes to tissue damage in various target organs. Proliferative diabetic retinopathy (PDR) is a common manifestation of diabetic complications but information on the possible role of reactive intermediates in this condition with special regard to the involvement of the vitreous in PDR-associated redox alterations is scarce.

The aim of the study was to determine key parameters of redox homeostasis [advanced glycation endproducts (AGE); protein carbonyl and glutathione (GSH)] content in the vitreous in PDR patients.
Methods: The study population involved 10 diabetic patients undergoing surgery for complications of proliferative diabetic retinopathy and 8 control (non-diabetic) patients who were undergoing surgery for epiretinal membranes. Vitreal fluids were assayed for the above biochemical parameters.
Results: We found elevated levels of AGE in the vitreous of PDR patients (812.10 vs 491.69 ng AGE/mg protein). Extent of protein carbonylation was also higher in the samples of diabetic patients (2.08 vs 0.67 A/100 μg protein). The GSH content also increased in the vitreous of PDR patients as compared to the control group (4.54 vs 2.35 μmol/μg protein), respectively.

we determined protein oxidation, whereas the antioxidant status has been assessed by measuring glutathione levels.

2. Materials and methods

2.1. Patient groups and tissue samples

All procedures performed as part of this study were in accordance with the ethical standards of Semmelweis University and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. The study has been approved by ETT-TUKEB (Scientific and Research Committee of the Medical Research Council, Hungary) under protocol number: 9683-1/2012/EKU. Informed consent was obtained from all individual participants included in the study.

The study population involved diabetic patients (n=10; 6 males and 4 females) with an average age of 54 (range 34–69) and the control group (n=8; 2 males and 6 females) operated with epiretinal membranes. Average age of control patients was 72 (range 67–80). In the diabetes group (7 type 2 diabetic patients and 3 type 1 diabetic patients with all but one patient under insulin treatment) the disease has persisted for an average of 17.7 years (2–34) and surgery was performed due to complications of proliferative diabetic retinopathy (bleeding, retinal ablation, proliferation bundles). With regard to the higher average age of control patients it is important to note that according to our current understanding, the age of the diabetic patients does not significantly affect the phenotype of diabetic retinopathy [15]. Moreover, it has also been reported that total protein carbonyl levels don't correlate with age [16] and the ophthalmological features of diabetic retinopathy are independent from the type of diabetes [17].

Undiluted vitreal samples were obtained by pars plana vitrectomy and samples were stored in eppendorf tubes at −80 °C for further analysis. The glutathione (GSH) assay was performed in 96-well plates. Potassium phosphate buffer (1 M) and o-phthalaldehyde (0.5%) were added to the samples and after 30 min incubation at room temperature fluorescence was measured at 390/460 nm. The mixture of supernatant and N-ethylmaleimide was used as a blank for each sample. Standard curve was prepared with GSH. Protein concentration was determined with the BCA (bicinchoninic assay) method.

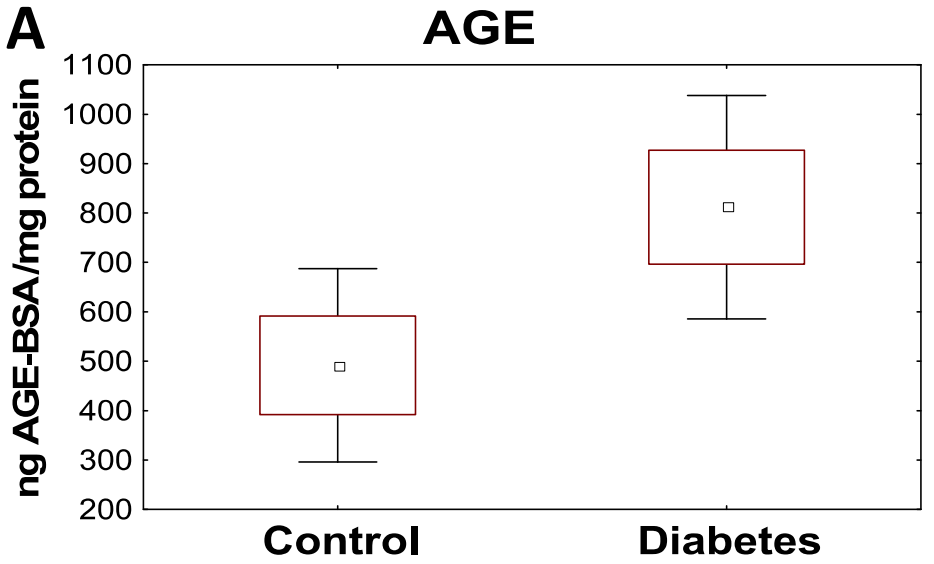
2.5. Statistical analysis

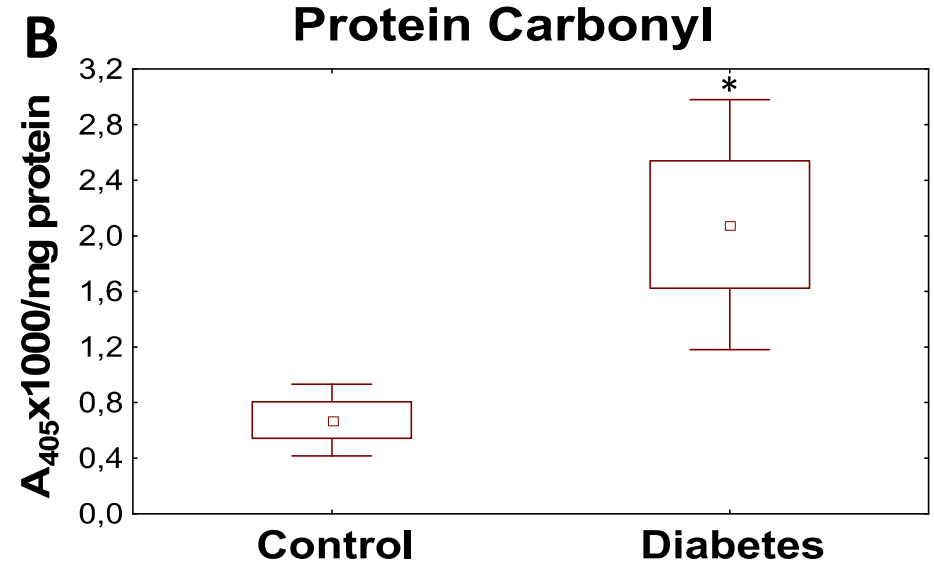
Concentration values of vitreous samples obtained from diabetic and non-diabetic patients were compared by Statistica 11.0 (Statsoft, Tulsa OK, USA) software using the Mann-Whitney *U* Tests. Difference was considered significant if $p < 0.05$.

3. Results

3.1. Glycation products in the vitreous

Efficiency of glycemic control in diabetics is mirrored by serum (and tissue) levels of AGEs [18]. AGEs are stable endproducts formed in a non-enzyme catalyzed reaction between reducing sugars such as glucose and amino groups of proteins (or lipids). The Schiff base formed in this reaction may in turn undergo further chemical rearrangements to form stable Amadori products. Glycated proteins (e.g. glycated hemoglobin A1c) are extensively used in the clinical praxis to provide information about serum glucose levels of the past 6–8 weeks [19]. Since to our best knowledge AGE levels have not yet been measured in the vitreous, we set out to investigate whether increased glycation can also be detected in the vitreous of PDR patients. We found increased AGE levels in the vitreous of PDR patients (Fig. 1A) compared to control samples (obtained from patients undergoing surgery for epiretinal





the blood and in various tissues [28]. Several lines of evidence indicate that in diabetes AGEs form in the eye and contribute to diabetic complications [29]. This is most prominent in the retina where antioxidant enzymes, transcription factors and mitochondrial proteins have been found to undergo glycation which may impair their functions [30,31]. Thus protein glycation may contribute to oxidative stress in the diabetic eye [32]. Our finding that AGE levels increase in the vitreous suggests that glucose levels are increased in this ocular compartment. Since metabolic exchange and equilibration between systemic circulation and vitreous humor is considered to be slow [33], elevated vitreal AGE levels may indicate occurrence of sustained hyperglycemic periods in our study patients. By analogy to what we know about the mechanism of oxidative stress in the retina [34], it may be plausible to hypothesize that glycation of vitreal proteins may also contribute to the development of vitreal oxidative stress.

Tissue oxidative stress is typically accompanied by oxidative protein, DNA and lipid modifications [35]. We measured protein carbonyl content as protein oxidation marker. Elevated protein carbonyl levels in the vitreous of diabetic patients provide further support for the hypothesis that the vitreous humor is no exemption from oxidative diabetic environment in the eye. Vitreous humor abounds in a high variety of proteins with albumin and type II collagen being the most notable ones [36]. Thus ROS/RNS may hit protein targets with high probability.

In oxidative stress situations antioxidants are often depleted by continuous attack of prooxidant stimuli [37]. Therefore we expected lower antioxidant activities in the vitreous of diabetic patients. In the contrary, we found that vitreal level of reduced glutathione was increased in the diabetic patients. This may indicate an adaptive response to increased ROS/RNS production just like it has been reported for the extracellular antioxidant enzyme superoxide dismutase in PDR [38]. Glutamate cysteine ligase, the

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