Can J Diabetes 40 (2016) 115–119

Contents lists available at ScienceDirect

Canadian Journal of Diabetes

journal homepage: www.canadianjournalofdiabetes.com

Original Research

Aqueous Humour and Serum Levels of Nitric Oxide, Malondialdehyde and Total Antioxidant Status in Patients with Type 2 Diabetes with Proliferative Diabetic Retinopathy and Nondiabetic Senile Cataracts

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ARTICLE INFO

Article history:
Received 24 February 2015
Received in revised form
21 May 2015
Accepted 13 July 2015

Keywords:
cataract
diabetic retinopathy
malondialdehyde
nitric oxide
total antioxidant status

ABSTRACT

Objectives: Diabetic retinopathy is associated with enhanced oxidative stress and/or reduction of antioxidant status. Several studies have examined the oxidative agents, such as nitric oxide (NO) and malondialdehyde (MDA), in the body fluids of patients with diabetes. However, total antioxidant status (TAS) in the aqueous humour of patients with diabetes has not been determined to date. To address this issue, we analyzed the aqueous humour and serum levels of NO, MDA and TAS in patients with type 2 diabetes and nondiabetic senile cataracts.
Methods: This prospective study included 35 patients with type 2 diabetes and 35 age- and sex-matched healthy subjects in whom cataract surgery was indicated. Aqueous humour and serum MDA, NO and TAS levels were determined by spectrophotometric methods, respectively.
Results: The analysis of MDA levels in the serum and aqueous humour revealed no significant differences in any of the groups ($p>0.05$). At the level of aqueous humour, patients with type 2 diabetes had significantly increased NO levels, compared to the controls ($p=0.003$). The control group also presented significantly higher TAS levels than the subgroup with type 2 diabetes in serum ($p=0.001$). However, there were no significant differences in the TAS levels of aqueous humour and serum NO levels in the groups ($p>0.05$).
Conclusions: Our results seem to demonstrate that the development of diabetic retinopathy is associated with high levels of aqueous humour NO and reduced serum antioxidant defenses. Therefore, inhibition of reactive oxygen species production and substitution of serum antioxidant status may be a therapeutic target for eye diseases associated with oxidative stress.

Mots clés:
cataracte
rétinopathie diabétique
malondialdéhyde
oxyde nitrique
statut antioxydant total

RÉSUMÉ

Objectifs: La rétinopathie diabétique est associée à l'augmentation du stress oxydatif et/ou à la réduction du statut antioxydant. De nombreuses études ont examiné les agents oxydants comme l'oxyde nitrique (NO) et le malondialdéhyde (MDA) des liquides organiques des patients diabétiques. Cependant, le statut antioxydant total (SAT) dans l'humeur aqueuse des patients diabétiques n'a pas encore été déterminé à ce jour. Pour répondre à cette question, nous avons analysé les concentrations du NO, du MDA et du SAT dans l'humeur aqueuse et le sérum des patients souffrant du diabète de type 2 et de cataractes séniles non associées au diabète.
Méthodes: Cette étude prospective comptait 35 patients souffrant du diabète de type 2 et 35 sujets sains appariés selon l'âge et le sexe chez qui la chirurgie de la cataracte était indiquée. Les concentrations du MDA, du NO et du SAT dans l'humeur aqueuse et le sérum ont été déterminées par les méthodes spectrophotométriques, et ce, de manière respective.
Résultats: L'analyse des concentrations du MDA dans le sérum et l'humeur aqueuse n'a révélé aucune différence significative dans aucun des groupes ($p>0,05$). Pour ce qui est de l'humeur aqueuse, les

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1499-2671
http://dx.doi.org/10.1016/j.jcjd.2015.07.002

patients souffrant du diabète de type 2 ont montré une augmentation significative des concentrations de NO comparativement aux témoins (p=0,003). Le groupe témoin a également présenté des concentrations sériques du SAT significativement plus élevées que celles du sous-groupe souffrant du diabète de type 2 (p=0,001). Cependant, les groupes n'ont montré aucune différence significative dans les concentrations du SAT de l'humeur aqueuse et des concentrations sériques du NO (p>0,05).
Conclusions: Nos résultats semblent démontrer que le développement de la rétinopathie diabétique est associé à des concentrations élevées du NO dans l'humeur aqueuse et à une réduction des défenses antioxydantes dans le sérum. Par conséquent, l'inhibition de la production des espèces réactives de l'oxygène et la substitution du statut antioxydant dans le sérum peuvent être des cibles thérapeutiques des maladies de l'œil associées au stress oxydatif.

Introduction

Cataract is the most common cause of blindness in the world (1). The incidence of cataract has increased dramatically over the past 2 decades (2). The causes of cataract formation are multifactorial. The exact mechanism of cataract formation has not been clearly defined. Cataract is often related to diabetes mellitus, hereditary syndromes, chronic renal failure and aging (3).

Oxidative stress is 1 of the major factors contributing to cataract formation. Oxidative damage of the lens is involved in the genesis of senile cataract and the development of diabetes-related pathologic changes. It is clearly known that in people with diabetes, oxidative stress plays an important role in the pathogenesis of early and long-term complications of diabetes, such as microangiopathy, neuropathy and retinopathy. Diabetic retinopathy progresses from dysfunction of retinal capillary endothelial cells and pericytes to proliferative retinopathy, characterized by formation of new blood vessels on the retina (4). Although the exact mechanisms underlying the development and progression of diabetic retinopathy still remain unclear, the results of both in vitro and in vivo studies suggest that many processes induced by hyperglycemia may be linked to the causes of diabetic vascular complications. It has been hypothesized that hyperglycemia may damage the vascular endothelium and the retina by inducing the synthesis of oxidative reactive species (5). Other potential mechanisms of oxidative stress include the reduction of antioxidant defense (6).

The most widely used index of lipid peroxidation is malondialdehyde (MDA) formation. MDA is a decomposition product of peroxidized polyunsaturated fatty acids. MDA is the most extensively studied marker in diabetes and is associated with increased formation of free radicals (7).

Nitric oxide (NO) is an important intracellular signalling molecule. NO synthesis may increase during inflammation, re-endothelization and angiogenesis. Vascular damage in the retina may be mediated by NO (8). Direct measurement of NO in vivo has been rare because of experimental difficulties and the short half-life of NO. Therefore, in clinical studies, NO synthesis has been monitored by the detection of nitrite and nitrate levels in biologic fluids.

Reactive oxygen species are produced continuously in cells, as by-products of metabolism or through leakage resulting from mitochondrial respiration. Cells have developed a comprehensive set of antioxidant defense mechanisms to prevent the formation of reactive oxygen species and to limit their damaging effects (7). These mechanisms include enzymes composed mainly of glutathione peroxidase, superoxide dismutase and catalase. Beta-carotene, tocopherols and ascorbic acid are also components of serum antioxidant defense mechanisms (9). All serum antioxidants are usually measured together as total antioxidant status (TAS). Disturbance of balance between oxidative processes and antioxidative defenses causes the oxidative stress that can damage proteins, lipids, polysaccharides and nucleic acids (10). This imbalance has also been proposed in relation to the induction of complications of diabetes mellitus (7). Therefore, it may be proposed that oxidative stress is an important factor in the development of diabetic cataracts, and antioxidants may have a role in decreasing the incidence of cataract.

Several studies have been done of the oxidative agents, such as NO, MDA, hydrogen peroxide and superoxide anion, in the body fluids of patients with diabetes; and antioxidant capacities, such as glutathione peroxidase, superoxide dismutase, catalase and ascorbic acid, have also been analyzed. However, TAS levels in the aqueous humour of patients with diabetes have not been determined to date. Our study is the first to measure TAS levels in aqueous humour of patients with type 2 diabetes. In this study, we analyzed NO, MDA and TAS levels in serum and aqueous humour obtained during cataract surgery from patients with and without type 2 diabetes. We took samples from cataract patients because the patients with cataract were the only ones who had accessible aqueous samples.

Methods

A total of 70 patients were included in the study. Of them, 35 had established diabetes diagnosed according to the World Health Organization diagnostic criteria for type 2 diabetes. The mean age of the subjects with diabetes was 65.74±8.15 (20 males and 15 females), and the mean age of the 35 patients without diabetes was 67.6±11.49 (21 males and 14 females). The duration of diabetes was 13.4±8.1 years. All patients were being treated with dietary measures and insulin. Retinopathy was classified by an independent ophthalmologist using fundus photography and fluorangiographic imaging. On the basis of his findings, all patients with diabetes showed proliferative retinopathy. Patients with active infection, history of current smoking, liver diseases, malignancies, collagen diseases, coronary heart diseases, peripheral vascular diseases, or histories of taking nitrates and antioxidant vitamins were excluded from the study. All patients provided written informed consent, and the local institutional ethics commitee approved the study protocol.

Blood samples were drawn from the antecubital vein after overnight fasting. Serum samples were stored at –80°C until biochemical analysis. Aqueous humour samples (0.1 to 0.3 mL) were collected before and at the beginning of the phacoemulsification surgery through a clear corneal paracentesis. Aqueous humour was aspirated from the central pupillary area using a 27-gauge needle on a tuberculin syringe, with special care taken to avoid vascular contact or damage to the iris and other intraocular structures. Aqueous humour samples were immediately cooled at –80°C until analyses. All samples were protected from light.

Serum and aqueous humour MDA levels were measured according to the method of Yoshioka and were expressed as µmol/mL (11). Serum and aqueous humour TAS levels were measured by the TAS kit (Randox Labs, Crumlin, United Kingdom) and expressed as mmol/L. Serum and aqueous humour NO levels were determined by using a nitrate/nitrite colorimetric assay kit (Cayman Chemical, Ann Arbor, Michigan, USA). Serum glycated hemoglobin (A1C) (%) levels were measured by an Abbott Aeroset autoanalyzer using the Abbott kit (Abbott Diagnostics, Abbott Park, Illinois, USA).