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Original Research

Association of Diabetic Autonomic Neuropathy with Red Blood Cell Aldose Reductase Activity

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ABSTRACT

Objective: Activation of polyol pathway based on increased activity of aldose reductase (AR) has been implicated in the development of diabetic complications including diabetic autonomic neuropathy (DAN). The relationship between DAN and hyperglycemia-induced activation of polyol pathway is still uncertain. In the present study, we investigate the association between aldose reductase activity and diabetic autonomic neuropathy by measuring AR level in red blood cells (RBC).
Method: In this study, 145 subjects with diabetes with or without DAN and 32 subjects without diabetes have been included. All subjects have been investigated for autonomic function tests and RBC aldose reductase activity. DAN was defined if results of any 2 of the tests of parasympathetic function were abnormal. RBC aldose reductase level was determined spectrophotometrically and expressed as unit/g of hemoglobin. The values were expressed as mean ± standard deviation, and ANOVA test has been applied for comparison between groups.
Results: RBC aldose reductase activity was found to be significantly higher in people with diabetes with autonomic neuropathy in comparison to people with diabetes without autonomic neuropathy and healthy individuals without diabetes. Aldose reductase (AR) level ranges from 0.8 units/g Hb to 14.2 units/g Hb. The mean AR level was 8.6±2.95 units in subjects of DM with autonomic neuropathy, while mean AR level was 4.1±1.78 units and 2.0±0.89 units in people with diabetes without neuropathy and normal healthy individuals, respectively ($p<0.001$).
Conclusions: High aldose reductase activity is associated with the presence of autonomic neuropathy in subjects of type 2 DM.

Mots clés :
voie des polyols
aldose réductase
neuropathie diabétique autonome
explorations fonctionnelles du système nerveux autonome

RÉSUMÉ

Objectif : L'activation de la voie des polyols du fait de l'augmentation de l'activité de l'aldose réductase (AR) a été impliquée dans le développement des complications liées au diabète, y compris la neuropathie diabétique autonome (NDA). Le lien entre la NDA et l'activation de la voie des polyols induite par l'hyperglycémie est encore incertaine. Dans l'étude actuelle, nous examinons le lien entre l'activité de l'aldose réductase et la neuropathie diabétique autonome en mesurant la concentration d'AR dans les globules rouges (GR).
Méthode : Dans cette étude, 145 sujets diabétiques ayant ou non une NDA, et 32 sujets non diabétiques ont été inclus. Tous les sujets ont subi des explorations du fonctionnement du système nerveux autonome et de l'activité de l'aldose réductase dans les GR. La NDA était définie si les résultats de l'une des deux explorations du fonctionnement du système nerveux parasympathique étaient anormaux. La concentration de l'aldose réductase dans les GR était déterminée par spectrophotomètre et exprimée en U/g d'hémoglobine. Les valeurs étaient exprimées par la moyenne ± l'écart type, et l'analyse de la variance a été appliquée pour comparer les groupes.
Résultats : L'activité de l'aldose réductase dans les GR s'était avérée significativement plus élevée chez les diabétiques ayant une neuropathie autonome que chez les diabétiques n'ayant pas de neuropathie

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autonome et les individus non diabétiques en santé. La concentration de l'aldose réductase (AR) variait de 0,8 U/g d'Hb à 14,2 U/g d'Hb. La concentration moyenne d'AR était de 8,6 ± 2,95 unités chez les sujets ayant un DS et une neuropathie autonome, tandis que la concentration moyenne d'AR était de 4,1 ± 1,78 unités et de 2,0 ± 0,89 unités respectivement ($p<0.001$) chez les diabétiques n'ayant pas de neuropathie et chez les individus ayant une bonne santé.

Conclusions : Une forte activité de l'aldose réductase est associée à la présence d'une neuropathie autonome chez les sujets ayant le DS de type 2.

Introduction

Diabetes mellitus (DM) is currently a very common metabolic disorder, and its prevalence is on the rise in developing countries like India, due to changes in lifestyle-related factors, diet and increasing urbanization. The total number of people with diabetes is projected to rise from 171 million in 2000 to 366 million in 2030 (1). According to the Diabetes Atlas (2), the number of people with diabetes in India was around 40.9 million and is expected to rise to 69.9 million by 2025 (3). Type 2 DM accounts for roughly 90% of all diagnosed cases of diabetes (2). Chronic hyperglycemia leads to development of macro- and microvascular complications by various mechanisms like increased polyol pathway flux, increased formation of advanced glycation end products (AGEs), activation of protein kinase C (PKC) and increased hexosamine pathway flux (4,5). Diabetic autonomic neuropathy (DAN) is one of the microvascular complications and a major cause of morbidity and mortality in type 2 DM. DAN frequently coexists with other peripheral neuropathies and other diabetic complications; however, DAN may be isolated, frequently preceding the detection of other complications. Major clinical manifestations of DAN include resting tachycardia, exercise intolerance, orthostatic hypotension, constipation, gastroparesis, erectile dysfunction, sudomotor dysfunction, impaired neurovascular function and hypoglycemic autonomic failure. DAN may affect many organ systems throughout the body (e.g. gastrointestinal [GI], genitourinary and cardiovascular). GI disturbances (e.g. esophageal enteropathy, gastroparesis, constipation, diarrhea and fecal incontinence) are common, and any section of the GI tract may be affected (6). There are various tests available to diagnose DAN; however, tests described by Ewing et al are noninvasive and easy to perform. They described a battery of tests for assessment of cardiovascular autonomic status (7).

As mentioned earlier, activation of polyol pathway based on increased activity of aldose reductase (AR) has been implicated in the development of diabetic complications, including diabetic autonomic neuropathy (DAN) (8,9) and also linked to other cardiovascular risk factors including hyperlipidemia (10). AR is the first rate-limiting enzyme in the polyol pathway, reducing glucose to sorbitol using nicotinamide adenine dinucleotide phosphate (NADPH) as a cofactor. Sorbitol is then metabolized to fructose by sorbitol dehydrogenase (11). AR is an intracellular enzyme present in most mammalian cells including retina, nervous tissue, kidney and erythrocytes. Increased AR activity leads to higher levels of sorbitol and much lower levels of NADPH as well as NAD+, thus causing cell damage by producing osmotic stress to the cells, altered redox potential and generating reactive oxygen species (12).

In this study, we have investigated the association between AR activity and diabetic autonomic neuropathy by measuring AR level in erythrocyte.

Materials and Methods

This was a hospital-based case control study, where subjects were recruited from the patients who visited the Medicine OPD of King George (KG) Medical University, Lucknow. A total of 145 male or female subjects 18 years of age or older with type 2 DM with or without DAN and 32 normal subjects were enrolled. Excluded from the study were: subjects with uncontrolled concurrent disease; subjects having history of stroke or myocardial infarction; females who were pregnant, nursing or planning pregnancy, and subjects with a history or presence of psychiatric disorders. An informed consent was taken from each subject and they were explained about the study in detail. A complete history of each subject regarding their age, gender, clinical symptoms, diabetes duration, medication and socioeconomic background was taken. Assessment of cardiovascular autonomic status: it was carried out in the ANS lab, Department of Physiology, KG Medical University, Lucknow, with the help of device CANWIN (developed by Genesis Medical Systems Pvt. Ltd., Hyderabad, India).

Tests for parasympathetic division

Deep breathing test

Deep breathing at 6 breaths per minute is one of the most convenient and reproducible techniques. The patient is asked to sit quietly and breathe deeply, slowly and smoothly at 6 breaths per minute (5 sec in / 5 sec out) for 1 minute. Proper signal is given by the examiner so that inspiratory and expiratory phase of each respiratory cycle can be maintained for 5 seconds. An electrocardiogram is recorded throughout the period of deep breathing, with a marker used to indicate the onset of each inspiration and expiration. The E:I ratio is the ratio of the longest and shortest R-R intervals averaged over 6 respiratory cycles.

Heart-rate response to Valsalva manoeuvre

The test is performed by the patient blowing into a mouthpiece connected to an aneroid manometer or a modified sphygmomanometer and holding it at a pressure of 40 mm Hg for 15 seconds. The ECG is recorded for 1 minute to get the baseline values and continuously recorded during the manoeuvre and 30 to 45 seconds following release of respiratory strain. The result is expressed as the Valsalva ratio, which is the ratio of the longest R-R interval after the manoeuvre (phase IV reflecting the overshoot bradycardia following release) to the shortest R-R interval during the manoeuvre (phase II reflecting the tachycardia during strain), measured from the electrocardiogram.

Immediate heart-rate response to standing (30:15 ratio)

The test is performed with the patient lying quietly on a couch while continuous electrocardiogram is recorded. The patient is then asked to stand up unaided. The 30:15 ratio is calculated as the ratio between the longest R-R interval at or around the 30th beat and the shortest R-R interval at or around the 15th beat.

Tests for sympathetic division

Blood pressure response to standing

The subject is asked to lie down in the supine position quietly. The test is performed by measuring the patient's blood pressure while he is lying down and again when he stands up. The postural