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Original article

Interrelationship of elevated serum Advanced Glycation End-product levels and malnutrition (Subjective Global Assessment) scores with the severity of retinopathy in type II diabetes

Yashodhara Sharma [a, *], Sandeep Saxena [a], Anita Saxena [b], Arvind Mishra [c], Shankar Madhav Natu [d]

[a] Department of Ophthalmology, King George Medical University, Lucknow, Uttar Pradesh, India
[b] Department of Nephrology, Sanjay Gandhi Post Graduate Institute of Medical Sciences, Lucknow, Uttar Pradesh, India
[c] Department of Medicine, King George Medical University, Lucknow, Uttar Pradesh, India
[d] Department of Pathology, King George Medical University, Lucknow, Uttar Pradesh, India

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SUMMARY

Background and aims: Hyperglycemia in diabetes causes endogenous formation of Advanced Glycation End-products (AGEs) which accumulate in various body parts including retina causing diabetic retinopathy. AGEs also originate from exogenous dietary sources contributing to the body's AGE pool. Currently, curing of diabetic retinopathy is mainly focused on medication, surgical or laser interventions and not much emphasis is given on preventing or halting its occurrence or advancement to more severe stages, nutritionally. Planning a 'low glycemic index-low AGE' diet therapy for diabetic subjects can reduce endogenous and exogenous origin AGEs in the body and help in controlling retinopathy. Sound and accurate assessment of nutritional status is a crucial step for planning a therapeutic diet for this condition. As this aspect has not gained sufficient attention till now we are assessing the association of serum Advanced Glycation End-product (AGE) levels with the severity of diabetic retinopathy and for the first time estimating the nutritional status of subjects with this eye disorder for long term patient care.
Methods: This was a tertiary care centre-based, case–control study involving sixty three consecutive cases with diabetes divided as 21 cases with diabetes but no retinopathy, 21 cases with non proliferative diabetic retinopathy (NPDR), 21 cases with proliferative diabetic retinopathy (PDR) along with 21 healthy controls. Serum AGE levels of all the cases and controls were evaluated by Enzyme Linked Immuno Sorbent Assay (ELISA) and nutritional status was assessed by anthropometric measurements and SGA scores.
Results: Serum AGE levels were found significantly elevated in PDR group when compared with no retinopathy ($p < 0.05$) and control ($p < 0.001$) group. Control group was also significantly different from ($p < 0.05$) from NPDR group. Increase in SGA scores was statistically significant amongst the four study groups though other indices of nutritional status showed no definite trend with the increasing severity of retinopathy.
Conclusion: Our study shows that serum AGE levels are potential risk markers of diabetic retinopathy and SGA can be used as a regular tool for the assessment of nutritional status of diabetic retinopathy subjects which will help planning a 'low glycemic index-low AGE' therapeutic diet for halting this morbidity.

1. Introduction

Retinopathy is one of the most common diabetic microangiopathy [1] and one of the leading causes of blindness around the world [2]. Hyperglycemia linked pathways have emerged as one of the most important factors in the initiation and progression of diabetic retinopathy [3]. Chronic hyperglycemia leads to endogenous formation of AGEs which accumulate in various parts of the body including retina [4–7]. AGEs have pathogenic implications in retinal functions such as primary retinal damage by inflammatory process and induction of toxic effects on retinal

* Corresponding author. Tel.: +91 9452205997.
E-mail address: yashodhara.sharma@live.com (Y. Sharma).

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pericytes due to oxidative stress resulting in apoptosis culminating into full-blown diabetic retinopathy [8–10]. Several clinical studies have reported correlation of AGEs with the degree of diabetic retinopathy [11–14]. Presently medication and low glycemic index foods in the diet are administered for controlling hyperglycemia and arresting the complications arising from it. But apart from endogenous formation, AGEs also originate from exogenous dietary sources which are significant contributors and directly correlated to circulating AGEs in humans and their restriction reduce markers of oxidative stress and inflammation, in turn halting key steps to tissue destruction and thus controlling retinopathy [15–18]. Sustained reduction in dietary AGEs can be achieved by planning a low- AGE diet therapy for diabetic subjects [19]. Hence a 'low glycemic index-low AGE' therapeutic diet can be a novel nutrition based approach for the reduction of both endogenous and exogenous origin AGEs. For planning and recommending therapeutic diet to diabetic subjects, accurate assessment of their nutritional status is the primary step. As no previous study was undertaken in this direction previously, we for the first time have taken the initiative to assess the nutritional status of diabetic retinopathy subjects. For assessing the nutritional status, along with anthropometric measurements; Subjective Global Assessment (SGA) was also done, as subjects in different stages of diabetic retinopathy were typical in terms of anthropometric measurements as these indices of the nutritional status showed no correlation with the severity of this eye disorder. Subjective Global Assessment (SGA) is a reliable and reproducible clinical assessment method based on medical history and physical examination of the subjects providing sound estimation of the nutritional status [20–22]. Thus, the objectives of our study were to assess the association of serum AGE levels and SGA scores with the severity of diabetic retinopathy.

2. Material and methods

This was a tertiary care centre-based, observational, case–control study involving Type II diabetic patients as cases and healthy individuals as controls, of both the genders. Sixty three consecutive cases with diabetes (all the patients were diagnosed to have Type II diabetes mellitus according to the guidelines proposed by American Diabetes Association) and 21 healthy controls aged 40–65 years were recruited. The subjects were divided into four groups on the basis of classification given by the Early Treatment of Diabetic Retinopathy Study (ETDRS) [23]. Group I included 21 normal healthy controls, Group II included 21 diabetic subjects with no retinopathy. Group III included 21 diabetic subjects with non proliferative diabetic retinopathy (NPDR) and Group IV 21 diabetic subjects with proliferative diabetic retinopathy (PDR). Our study had institutional board clearance and was performed in accordance to the tenets of Helsinki Declaration. Written informed consent was taken from all the subjects after providing them all the study details.

Cases with chronic renal failure, any other ocular or systemic disease like hypertension which can affect the retinal vasculature, who had any previous intravitreal injection(s), ophthalmic, surgical or laser interventions, with current tobacco intake and smoking status or not willing to participate, were excluded.

All the cases underwent detailed fundus evaluation using stereoscopic slit lamp biomicroscopy with 90 diopter lens and indirect ophthalmoscopy. Digital fundus photography and flourescein angiography were done using Zeiss fundus camera FF 450 Plus with pixel width of 0.0054 and image size 2588 × 1958. Subjects with retinal damage were subjected to fluorescein angiography.

A one-time SGA score was calculated as described by Zadeh et al. [24]. In short, the history and physical examination was performed by a trained investigator who was blinded to all clinical and biochemical variables of the patient. The history focused on seven variables namely unintentional weight loss in the past six months, any change in the dietary intake, presence of gastro intestinal (GI) symptoms, loss in functional capacity, presence of any co-morbidity, loss of subcutaneous fat, signs of muscle wasting and edema. These seven variables were given a five point scoring system. The subjects were classified into five groups according to the points scored as follows: normal (score 1–7), mildly malnourished (score 8–14), moderately malnourished (score 15–21), severely malnourished (score 22–28) and very severely malnourished (score 29–35). Total SGA score was obtained after summing up the respective scores of the seven subjective assessments.

Anthropometric measurements included height, weight, Body Mass Index (BMI), Mid Upper Arm Circumference (MUAC), waist circumference, hips circumference and Waist to Hip ratio (WHR).

Fasting plasma glucose level, post prandial plasma glucose, HbA1C, serum cholesterol, triglycerides (TG), high density lipoproteins (HDL), low density lipoproteins (LDL), very low density lipoproteins (VLDL), serum urea, serum creatinine, serum albumin and serum protein levels were assessed within a week of eye examination following the standard protocol. Urine samples were analyzed for detecting albuminuria. Estimated glomerular filtration rate (e-GFR) was calculated using Cockcroft–Gault equation. For the serum analysis of AGEs 3 ml of venous blood sample was drawn from each study subject. The sample was then centrifuged at 1000 × g for 10 min at 4 °C The separated serum was aliquoted into polypropylene tubes, initially stored at −20 °C and then within one week was shifted to dry ice storage at −80 °C.

Assay of AGE in serum was done using the AGE ELISA kit of USCN, Life Science Inc, Wuhan, People's Republic of China. The reagents were prepared following the standard protocol provided with the kit. Briefly, AGE standard provided with the kit was reconstituted with standard diluent. Triple dilution series of the AGE standard (8000, 2666.7, 888.9, 296.3, 98.8, 0 ng/ml) was done following the instructions and run in parallel. Detection reagent A and B were prepared by diluting Stock Detection A and B with Assay Diluent A and B respectively in the ratio 1:100. Standard or sample (50 μl) followed by detection reagent A (50 μl) were added to the appropriate microtiter wells plate precoated with monoclonal antibody, specific for the AGE. Following this, the plate was incubated at 37 °C temperature for one hour. The contents of the plate were removed using multichannel pipettes after the incubation was over. The plate was washed with the wash solution three times to remove unbound antigens, if any. Detection reagent B (100 μl) was added in every well and the plate was incubated at 37 °C temperature for 30 min. After the incubation, the plate was washed with the wash solution five times as previous step. Substrate Solution (90 μl) was added to each well and the well of solution began to turn blue. Following this, the plate was incubated at 37 °C temperature for 20 min in dark. The reaction was ended by adding stop solution (50 μl) to each well. The blue color developed earlier turned yellow. The intensity of the color was read over ELISA plate reader (Bio-Rad, U.S.A) at 450 nm. The calibration curve of the standard AGE was plotted against the AGE optical density on X-axis and the log of concentration on Y-axis. Concentration of AGE in serum sample was calculated based on the standard curve. The values were expressed as ng/ml.

All participants were subjected to clinical examination and interview schedule was used to obtain information regarding age, sex, medical history of any previous or concomitant ocular and systemic disease, duration of diabetes (in years) and current medication for diabetes mellitus.

Data was statistically analyzed by the statistical software package SPSS (version 20, USA). Results are presented as mean ± standard deviation. The significance of differences between