ORIGINAL ARTICLE

Role of vitamin E in preventing arteriohyalinization in kidneys of streptozotocin induced diabetic mice

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Abstract

Objective: To evaluate the role of vitamin E on the arteriolar hyalinisation in kidneys of diabetic mice.

Methods: The laboratory-based randomised control trial was conducted at the Department of Anatomy, Army Medical College, Rawalpindi, in collaboration with National Institute of Health, Islamabad, from November 2009 to November 2010. Adult female BALB/C mice were randomly divided into three groups. Group A served as control group. Group B was made diabetic by the intraperitoneal injection of streptozotocin. Group C received streptozotocin injection and was fed with vitamin E (alphatocopherol) supplemented diet. After 12 weeks,the animals were sacrificed and their kidneys were removed for histomorphological study. SPSS 16 was used for statistical analysis.

Results: Diabetes caused significant histomorphological changes in arteriole of kidneys of Experimental Group B compared to Control Group A ($p>0.05$), but these changes were prevented in Group C. In experimental group B, 2(20%) animals had arteriolar hyalinisation of score 1, while score 2 was revealed in 8(80%) animals. Experimental group C showed no hyalinisation in any arteriole.

Conclusion: Vitamin E prevents the arteriohyalinization in kidneys of mice with STZ induced diabetes.

Keywords: STZ, Alphatocopherol, Diabetic nephropathy. (JPMA 65: 1085; 2015)

Introduction

Diabetes mellitus is a chronic metabolic disorder characterised by a high blood glucose concentration (fasting plasma glucose >125mg/dl or plasma glucose >180mg/dl, 2 hours after a meal) due to insulin deficiency and/or insulin resistance.[1] It is generally considered that hyperglycaemia is a major factor in the pathogenesis of diabetic complications.[2] Oxidative stress is thought to be increased in body system where free radical production is increased or antioxidant system is impaired. In recent years, the oxidative stress (OS)-induced free radicals have been implicated in the pathology of insulin-dependent diabetes mellitus (IDDM).[3] Although drug therapy and change in lifestyle remains the best preventive and therapeutic approach for diabetic complications, but many antioxidants are being developed side by side to offset the spread of diabetes.[4]

Several morphological changes are related to many complications of diabetes. In individuals with strict control of diabetes, the onset may be delayed. These morphological changes may be found in arteries (microvascular disease), basement membrane of small vessels (microangiopathy), kidneys (diabetic nephropathy), retina (retinopathy) and other tissues.[5] Diabetic nephropathy, one of the causes of end-stage renal disease (ESRD) and frequently found in subjects with diabetes, is a condition characterised pathologically by hyalinisation of arterioles.

Vitamin E is said to have a protective role against renal arteriolar hyalinisation (AH) which may be an indicator of diabetic nephropathy.[6] To prevent damages induced by OS various strategies are adopted. Role of antioxidants are increasingly investigated nowadays and are first line of defence against free radical damage.

Vitamin E has been reported to protect against diabetic renal injury. For this reason, there has been an increased interest in the use of dietary antioxidant supplementation as an intervention to attenuate diabetic complications. Vitamin E, a membrane bound lipid soluble and naturally occurring antioxidant has been shown to protect animal tissue against oxidative damage such as lipid peroxidation both in vitro and in vivo.[1] This generic term is used for tocopherols. Naturally occurring forms of vitamin E are known as α, β, γ, and δ and the most common and biologically active form is alpha-tocopherol.[7]

The current study was performed to evaluate the protective role of vitamin E on renal AH.

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Material and Methods

The laboratory-based randomised control trial was conducted at the Department of Anatomy, Army Medical College (AMC), Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad, from November 2009 to November 2010. Adult female BALB/C mice weighing 25-40g each were obtained from the animal house of NIH. The mice were kept under standard laboratory conditions and were maintained on water ad libitum and palleted form of laboratory diet which was prepared at the animal house. The animals were divided into 3 equal groups of 10 each.

Group A was the Negative Control group and was maintained on routine NIH diet for 12 weeks. Group B was the Positive Control group and was made diabetic by giving single injection of streptozotocin (STZ) 120mg/kg body weight per animal intraperitoneally.[8] They served as disease controls and were maintained on routine NIH diet for 12 weeks. Group C received single injection of STZ and were fed with routine NIH diet enriched with alphatocopherol (500 mg/kg of diet) for 12 weeks.[9] Animals were sacrificed after 12 weeks. Kidneys were dissected and fixed in 10% formalin. Whole kidney tissue was processed for paraffin embedding. Five micron-thick consecutive sections were cut and stained with haemotoxylin and eosin (H&E) for histomorphological analysis.

Hyalinization of renal arterioles was assessed by counting the number of hyalinised vessels that were present within the whole section of the kidneys. Hyaline, an amorphous, eosinophilic, glassy substance, is found in the small arteries and arterioles. It is composed of several constituents, including the third component of complement, hyaluronic acid, cholesterol and fibrin in smaller amounts.[6]

Only the transverse sections of arterioles were considered. The AH scoring was done at 40X by using the criteria mentioned in literature:[10] Score 0: Absent; Score 1: AH in one arteriole; Score 2: AH in more than one arteriole.

Olympics digital professional camera DP2I-2 mega pixel, 4X optical and electronic zoom was used for photomicrography. The images were edited using Microsoft Image Analyser.

Data was analysed using SPSS 16. Comparison of scoring of AH between different groups was determined by using chi square test. The difference was regarded statistically significant at $p<0.05$.

Results

In Group B the mice were sluggish throughout the experiment while in Group C the mice were sluggish initially but later on they became apparently active.

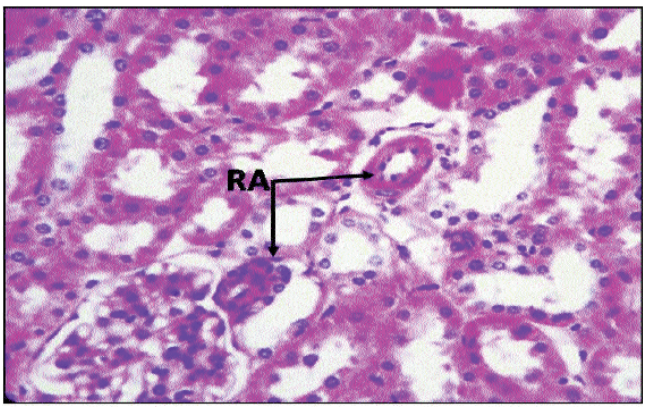

Figure-1: Photomicrograph of specimen of Control Group A showing renal arteriole (RA). Haemotoxylin and eosin (H&E) Stain X 400 (40 objective ×10 occular).

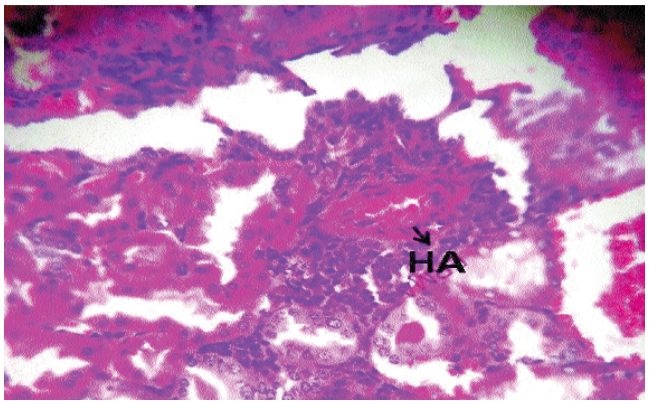

Figure-2: Photomicrograph of specimen of Experimental Group B showing hyalinised arteriole (HA). Haemotoxylin and eosin (H&E) Stain X 400 (40 objective ×10 occular).

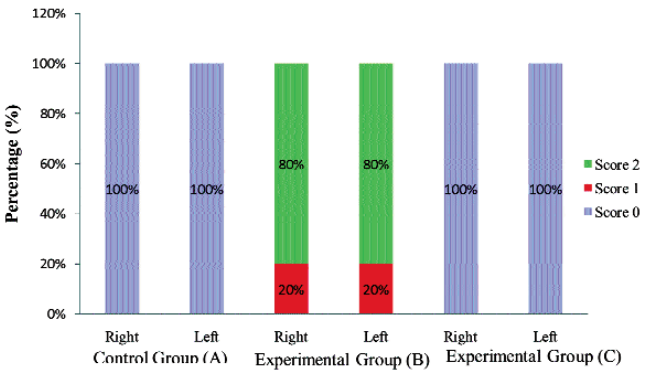

Figure-3: Score of hyalinized arteriole in kidneys of control and experimental groups.

Control Group A revealed no arteriole with hyalinisation (Figure-1). In Experimental Group B, 2(20%) animals had

AH of score 1, while score 2 was revealed in 8(80%) animals (Figure-2). Experimental Group C showed no hyalinisation in any arteriole (Figure-3).

Discussion

The current study was planned to observe the potential protective role of vitamin E in STZ-induced renal damage in mice as evidenced by histomorphological observations with dosage of 500mg/kg of diet for 12 weeks. STZ is widely used to induce experimental diabetes in animals. It generates reactive oxygen species, which contribute to deoxyribonucleic acid (DNA) fragmentation and evoke other deleterious changes in the cells.[11] As a result of the STZ action, beta cells undergo destruction by necrosis. One study investigated the effect of different doses of vitamin E on the biochemical parameters of kidney in normal and STZ-induced diabetic rats. He observed that blood urea nitrogen (BUN) and creatinine were slightly reduced after the oral administration of vitamin E. He did not study the histological parameters of kidney.[12] A study observed that levels of alphatocopherol, chain-breaking antioxidant was significantly decreased in liver and kidney of STZ-induced diabetic rats. This suggests that demand for antioxidant like vitamin E is increased due to activation of free radical-related metabolism in diabetes. Impaired generation of antioxidants results in increase in oxidative injury by failure of protective mechanism.[13] Another study also showed that vitamin C is also an important antioxidant to prevent or alleviate the complications of diabetes mellitus. So any compound with rich antioxidant properties might contribute towards partial or complete alleviation of organ damage.[14] In AH, the most common complication of diabetes, endothelial cell damage, appears to be involved in the initial stages and hyaline masses within the intima might reflect early vascular injury. This early vascular injury may be associated with changes in permeability of the endothelial wall. Increased permeability of arterioles to macromolecules has been demonstrated in subjects with hypertension and diabetes. It is possible that these changes in permeability or necrosis of smooth muscle cells, as others have proposed, may be associated with deposition of hyaline within the vascular wall. One study found that vitamin E has no effect on blood glucose and body weight of diabetic animal whose diet was supplemented with 200 mg of vitamin E/100 g fodder after 6 and 12 weeks of experiment. But it decreases lipid peroxidation and augments the activities of antioxidant enzymes in the kidneys of diabetic rats.[15] The results indicate the potential utility of antioxidant vitamins in the protection against the development of diabetic nephropathy. Regarding AH, scoring was done according to the criteria outlined earlier.[10] Control Group A showed no hyalinisation in any arteriole (Figure-1). Experimental Group B showed AH score 2 in 80% animals, while 20% showed score 1 (Figure-2).

The findings of AH in Experimental Group B compared to Control Group A was in accordance with a study that made the mice diabetic by STZ and then studied the biochemical as well as histological markers of diabetic nephropathy in them.[16] Some researchers proved that glomerulosclerosis and tubulairinterstitial lesions of diabetic mice with vitamin E supplemented diet were much improved and seemed quite normal in appearance compared with diabetic mice.[17]

Our findings suggest that the extent of hyalinisation in renal arterioles may be an important determinant of diabetic nephropathy. Increases in glucose level were associated with significant increases in the likelihood of having an elevated degree of AH, while vitamin E intake showed a strong protective association. These findings suggest that hyalinisation of renal arterioles could be a marker for diabetic nephropathy in particular.

Conclusion

Vitamin E prevented AH in kidneys of diabetic mice. However, exact mechanism of action of this protective effect remains to be discovered.

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