

# Effects of Alpha Tocopherol on Renal Glomeruli in Diabetic Mice

Saadia Rashid, Shadab Ahmed Butt, Samina Anjum

Army Medical College Rawalpindi

## Abstract

**Background:** To evaluate the effects of alphatocopherol supplement on renal glomeruli of diabetic mice.

**Methods:** In this randomized control trial, thirty adult male mice were randomly divided into three groups. Group A served as control group. Group B was made diabetic by the intraperitoneal injection of streptozotacin. Group C received injection streptozotocin and fed with alphatocopherol supplemented diet. After 12 weeks animals were sacrificed and their kidneys were removed for histomorphological study.

**Results:** Diabetes caused significant changes in glomeruli of Experimental Group B as compared to Control Group A but these changes were prevented in alphatocopherol treated group. Majority (80%) of animals in Group B showed glomerulosclerosis of Grade 4 and 20% showed Grade 3 glomerulosclerosis. In Group C 70% animals showed no glomerulosclerosis where as 30% showed glomerulosclerosis of Grade 1.

**Conclusion:** Alphatocopherol, as an antioxidant, has a protective role in improving streptozotocin induced glomerulosclerosis.

## Introduction

Diabetes mellitus, a chronic metabolic disorder characterized by hyperglycaemia is increasing world wide with its complications being a very important health issue. Increased free radical generation and oxidative stress are hypothesized to play an important role in pathogenesis of diabetes and its late complications. There is widespread acceptance of possible role of reactive oxygen species (ROS) generated as a result of hyperglycaemia in causing many of the secondary complications of diabetes such as retinopathy, neuropathy, cardiomyopathy and nephropathy.<sup>1</sup> Diabetic nephropathy is a leading cause of end-stage renal failure, accounting for 35 to 40% of all new cases that require dialysis therapy worldwide. Diabetic subjects are shown to have increased oxidative stress and decreased antioxidant levels. Antioxidants are claimed to work as anti-stress

agents by decreasing oxidative stress. Antioxidants, in particular vitamin E, have been reported to protect against diabetic renal injury. Vitamin E is a fat soluble vitamin which prevents lipid peroxidation. Out of 8 different forms, alphatocopherol is most active form.<sup>2,3</sup>

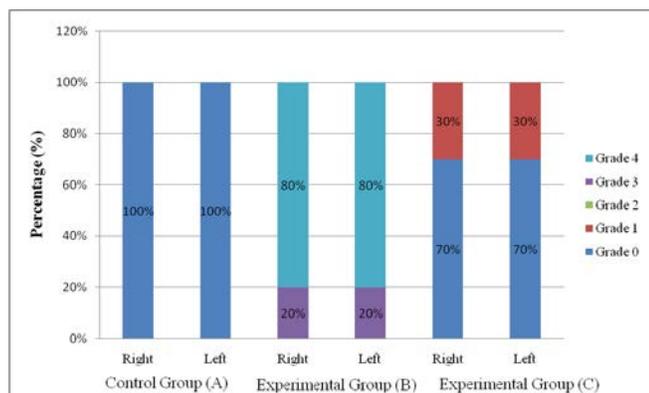
## Materials and Methods

This study was conducted in NIH, Islamabad and Army Medical College Rawalpindi. Thirty adult female mice BALB/C (Weight 25-40 g) were obtained from animal house of NIH, Islamabad. Mice were kept under standard laboratory conditions and were maintained on pelleted form of laboratory which was prepared at animal house and water ad libitum. Animals were divided into 3 groups each containing 10 mice. Group A received standard daily diet for 12 weeks. Group B was made diabetic by giving injection streptozotocin 120 mg/kg body wt. per animal intraperitoneally. Injection was given by holding the mice from back and then drug was injected by insulin syringe in its abdomen. They served as disease control. They were given standard diet. Group C received injection streptozotocin and fed with alphatocopherol (500 mg/kg of diet) enriched diet. Animals were sacrificed after 12 weeks and their kidneys were removed for histomorphological analysis. Glomerulosclerosis was assessed by semi quantitative score (sclerosis index), in which grade 1 represented sclerosis of 25% of glomerulus, grade 2 sclerosis of 50% of glomerulus, grade 3, sclerosis of 75% of glomerulus and grade 4 represented sclerosis of 100% of glomerulus. The whole kidney sclerosis was obtained by averaging scores for all glomeruli in ten fields at 40X power.<sup>4</sup>

## Results

In diabetic group (group B) the mice were sluggish throughout experiment, while in group C, the mice were sluggish initially but later on they become apparently healthy and active.

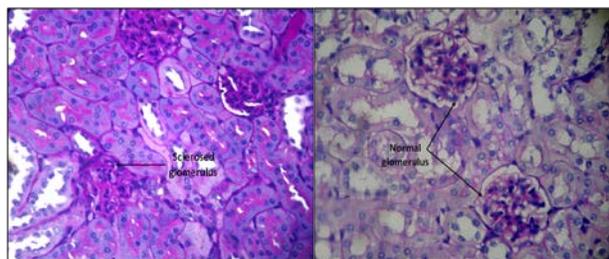
Assessment of glomerulosclerosis was done according to criteria by Schwarz.<sup>5</sup>In control group A there was no glomerulosclerosis. In group B there was glomerulosclerosis of grade 3 in 20% of animals while 80% of animals showed glomerulosclerosis of grade 4. In group C, 70% of animals revealed no glomerulosclerosis (grade 0) while 30% of animals showed glomerulosclerosis of grade 1 (Fig 1). Histological features regarding glomerulosclerosis were hyaline deposition in the renal glomeruli with capillary basement membrane thickening and increased mesangial matrix. Mesangial expansion and glomerular basement membrane thickening are a consequence of extracellular matrix (ECM) accumulation, with increased deposition of the normal ECM local components of types IV and VI collagen, laminin and fibronectin due to their increased production, decreased degradation or both (Fig 2).



**Fig1: Scoring of Glomerulosclerosis in kidneys of different groups**

## Discussion

Glomerulosclerosis in diabetic nephropathy is the end point of multifactorial mechanisms that lead to excessive accumulation of extracellular matrix proteins as collagen type I, III and IV and fibronectin in mesangial space which through stages of mesangial expansion finally result in glomerulosclerosis. Oxidative stress is thought to be increased in system where free radical production is increased or antioxidant system is impaired. In recent years, the oxidative stress induced free radicals have been implicated in the pathology of insulin dependent diabetes mellitus. Drug therapy, and change in life style remains best preventive and therapeutic approach, but many antioxidants are being developed side by side to oppose the spread of diabetes.<sup>5</sup> A multitude of in vivo studies have been performed



**Fig 2: Comparison between the normal and sclerosed glomeruli in group A and B**

utilizing antioxidants in experimental diabetic models. The effects of antioxidants on oxidative stress are measured through certain biomarkers. Treatment with Vit C and vitamin E is known to decrease the glomerular basement membrane thickness and kidney weight in streptozotocin diabetic rats.<sup>6</sup> Recently it was reported that antioxidant treatment with vitamin E, lipoic acid or taurine normalized not only diabetes induced renal dysfunction such as albuminuria but also glomerular pathologies. It was also demonstrated that astaxanthine, a strong natural antioxidant reduced glomerular histological changes in DN mice with little effect on blood glucose levels.<sup>8</sup>

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