

Cyclosporin Induced Glomerulosclerosis in Developing Kidney

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ABSTRACT

Aim: To study glomerular changes in developing kidney by administering therapeutic doses of CsA to pregnant Albino mice throughout gestation.

Methods: Twelve pregnant mice were divided into two groups, A and B, having six animals each. Cyclosporin (Sandimmun, Novartis, Switzerland) was freshly prepared in normal saline daily having a concentration of 1mg/ml and administered subcutaneously by a single dose of 50 mg/kg in the morning to experimental group B during pregnancy from day 0 to day 18. The control group A was treated with the comparable volume of normal saline given subcutaneously for 18 days during gestation. The pregnant mice were sacrificed at the end of experimental period. The fetal kidneys were dissected, fixed in 10% formalin and the sections were stained with Hematoxylin and eosin (H & E) for general histological study and Periodic acid Schiff (PAS) for the demonstration of basement membranes. These sections were evaluated for glomerular changes using Calcineurin Inhibitor Toxicity Score.

Results: Our work clearly illustrated statistically significant glomerular changes including thickening of the glomerular basement membrane, retraction of tuft of glomerular capillaries, increased urinary space, mesangial matrix expansion and capillary collapse. These glomerular lesions were more indicative of glomerulosclerosis suggestive of possible damage to the glomeruli. The score for glomerulosclerosis was high (1.68 ± 0.47) in experimental group as compared to (0.43 ± 0.50 ; $p < 0.000$) in control group.

Conclusion: The current study investigated the effects of CsA given to mice during intrauterine life in therapeutic doses. The work clearly illustrated harmful effects of the drug leading to statistically significant glomerular changes suggestive of fetal nephrotoxicity and may eventually lead to renal failure. It might produce comparable effects in human conceptuses after intake of CsA by pregnant mothers

Keywords: Cyclosporin A, nephrotoxicity, glomerulosclerosis

INTRODUCTION

Cyclosporin A (CsA) has been proven to be a powerful immunosuppressive agent commonly used in organ transplantation, grafts and for the treatment of various autoimmune diseases.¹ The introduction of CsA has improved graft and patient survival rate.² Its usage in higher doses is however restricted due to its nephrotoxicity producing a spectrum of clinical manifestations from precipitous decrease in glomerular filtration rate (GFR) to chronically progressive scarring.³

Among patients undergoing CsA therapy, pregnant women undergoing CsA therapy are increasing in number.^{4,5,6} However, maternal CsA treatment during pregnancy is not without fetal side effects. This has presented new challenges regarding the long-term effects of CsA used by pregnant recipients.⁷ The nephrotoxicity of CsA among adults is well documented^{8,9,10} however its effects on developing kidney remain to be established.

Previous studies have shown that CsA and its active metabolites cross the placental barrier^{11,12} and enter the fetal circulation, interfering with its development.¹³ The development of metanephric kidney depends on mesenchymal-epithelial interactions between the metanephric mesenchyme and the arborizing ureteric bud which will induce the mesenchyme to condense and differentiate into glomerular and tubular structure^{14,15}.

Chronic nephrotoxicity is characterized by reduced glomerular filtration rate, pathological changes, such as glomerulosclerosis, proximal tubular swelling and necrosis, infiltration of macrophages and striped interstitial fibrosis¹⁶.

The mechanisms underlying chronic nephrotoxicity are not completely clear. Cyclosporin A induced nephropathy involves overexpression of IL-6, TGF- β and activation of NAD(P)H oxidase in endothelial cells, which damage the integrity of renal endothelium¹⁷. These changes are facilitated by angiotensin II (Ang II)¹⁸ and transforming growth factor beta (TGF- β) dependent pathways¹⁹. Cyclosporine leads to activation of the renin-angiotensin system (RAS), by both direct effects of cyclosporine on juxtaglomerular cells²⁰ and indirect

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effects from the renal vasculature hemodynamic changes (arteriolar vasoconstriction) secondary to decreased vasodilator factors and increased endothelin²¹. At the cellular level, morphologic observations suggest that tubular epithelial cells, vascular, endothelial cells, arteriolar myocytes, and interstitial fibroblasts are all targets for Cyclosporin nephrotoxicity²². At the functional level, Cyclosporin causes vasoconstriction and reduces the renal blood flow and glomerular filtration, damage endothelial cells produce morphologic alterations in glomeruli²³.

Previous studies showed that administration of CsA to pregnant Albino mice throughout gestation was associated with interstitial fibrosis and tubular atrophy; both were recognized as an indicator of renal disease severity and its progression²⁴. Glomerular injury was only occasionally documented and it is not clear whether glomerular changes may have an impact on CsA nephrotoxicity observed in experimental animals and humans. Therefore in current study glomerular changes were studied by administering CsA (15mg/kg sc) to pregnant Albino mice throughout gestation.

MATERIAL & METHODS

Sixteen mice (6-8week old) weighing 25-30 gm were used; comprising twelve females and four males. They were kept under standard condition of temperature (24±1°C) and humidity (55±5%) with regular 12 hour light/ dark cycle; the animals were fed with pellet food and tap water ad libitum. Three females and one male mouse were housed in a single cage for mating²⁵. When pregnancy was confirmed by vaginal plug, twelve pregnant mice were divided into two groups, having six animals each. The experimental group B was subjected to single daily subcutaneous injections of 50mg/kg CsA (Sandimmun, Novartis, Switzerland) prepared in normal saline, for 18 days. The control group A received daily subcutaneous injections of comparable volume of normal saline for 18 days during gestation. The pregnant mice were sacrificed on 18th day of gestation. The fetuses were removed, examined macroscopically and weighed; their kidneys were removed, dissected and fixed in 10% formalin for histological examination. Kidney pieces were processed in a usual way to prepare paraffin blocks; 5 µm thick sections were obtained, using rotary microtome and, those were mounted on albuminised glass slide before staining with Haematoxylin and Eosin (H&E) for histology and Periodic acid Schiff (PAS) stain for the demonstration of basement membranes. The sections were evaluated for glomerulosclerosis using Calcineurin inhibitor toxicity (CNIT) score²⁶.

Glomerulosclerosis was defined as thickening of glomerular basement, adhesion formation and capillary obliteration^{27,28}. The glomerulus is a specialised structure which is composed of cells (epithelial, mesangial, endothelial) and matrix (mesangium, basement membrane) in a complex network of capillaries.

Glomerulosclerosis was examined in six randomly selected fields in juxtamedullary region in PAS stained sections. The extent of glomerular sclerosis was analyzed by the presence of increased amount of PAS positive material within the glomeruli²⁹. First each glomerulus was graded (0 to 4) according to the percentage of sclerosis/glomerular area, grade 0 being given to glomeruli with no sclerosis and grade 4 to obsolescent glomeruli³⁰. Subsequently, a final score was attributed to each animal according to the percentage of sclerotic glomeruli previously graded, score 0: none; score 1: 1 to 25%; score 2: 26 to 50%; score 3: >50%

Statistical Analyses: Mean±SD were given for normally distributed quantitative variables, frequencies and percentages were given for qualitative variables. Independent sample "t" test was applied to group differences. A "p" value of < 0.05 was considered statistically significant.

RESULTS

Histological section showed that all glomeruli were not affected equally; the pathological changes varied from a slight mesangial matrix expansion to glomerular capillaries filling most of Bowman's space in severely affected areas. The characteristic histologic feature was sclerosis of segments of the glomerular tuft in some glomeruli, with mesangial expansion and capillary lumen loss in these segments. Many glomeruli displayed normal cyto-architecture with normal cellularity and well defined Bowman's capsule. Some glomeruli showed very mild lesions, without mesangial matrix expansion or mesangial cell proliferation. Hypercellular glomeruli were also observed, exhibiting slight endothelial or mesangial proliferation (Fig.1).

In the treated group the retraction of tuft of glomerular capillaries, increased urinary space, mesangial matrix expansion, capillary collapse and thickening of the glomerular basement membrane were observed, these lesions seen were more indicative of focal segmental glomerular sclerosis. An eosinophilic amorphous material, which was strongly PAS positive, filled the capsular space; this was suggestive of possible damage to the glomeruli (Fig.2). Increased urinary space and retraction of tuft of glomerular capillaries toward the vascular pole was also observed in the treated groups (Fig.3).

The number of normal glomeruli was 13.00 ± 2.90 in experimental group as compared to 39.00 ± 3.70 in the control group. The number of hypercellular glomeruli was 11.10 ± 1.59 in experimental group as compared to 1.02 ± 1.05 in the control group. The number of sclerosed glomeruli was 2.60 ± 1.00 in experimental group compared to 0.43 ± 0.50 in the control group. The atrophic glomeruli were negligible in both control and experimental group (Tab. 7). All changes were significant ($P < 0.000$) except those in the number of atrophic glomeruli (Fig.5).

Fig.1. Photomicrograph of fetal kidney from group B showing different profile of glomeruli A hypercellular glomerulus (red arrow) was seen with increased cellularity, prominent urinary space (red arrow head) and retraction of tuft of glomerular capillaries (green arrow). An atrophic glomerulus (yellow arrow) was also visible with decreased cellularity, increased urinary space (yellow arrow head) and a small area of sclerosis (green arrow head). A normal glomerulus (blue arrow) was also visible with normal cellularity and prominent urinary space (blue arrow head). H&E. X400.

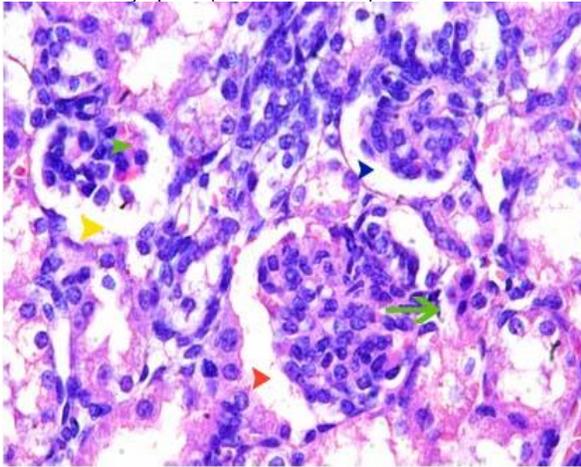


Fig. 2: Photomicrograph of fetal kidney from group B showing a glomerulus showing thickening of glomerular basement membrane (yellow arrow); an eosinophilic material (A) was also visible within the mesangium. The proximal convoluted tubules (yellow arrow head) and distal convoluted tubules (blue arrow head) were also demonstrating thickening of basement membrane. PAS stain. X630.

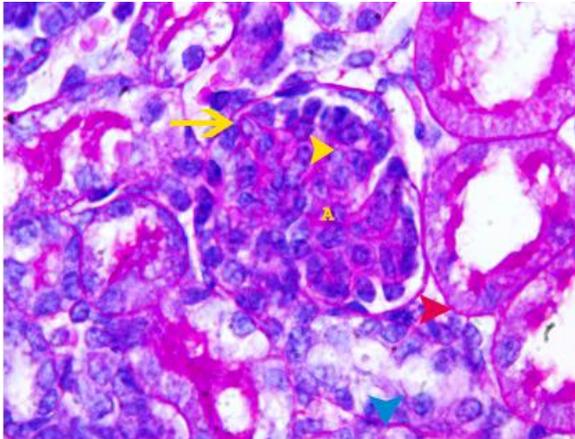
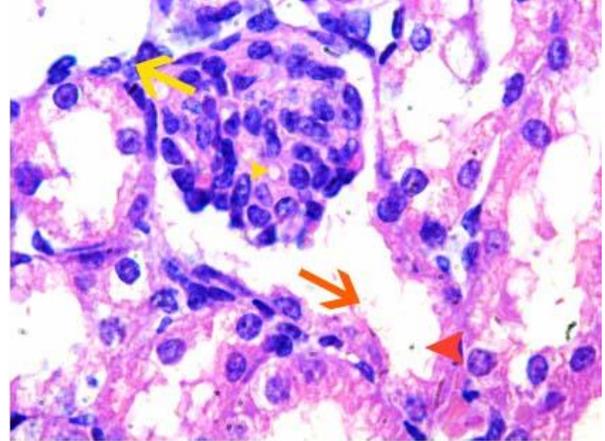
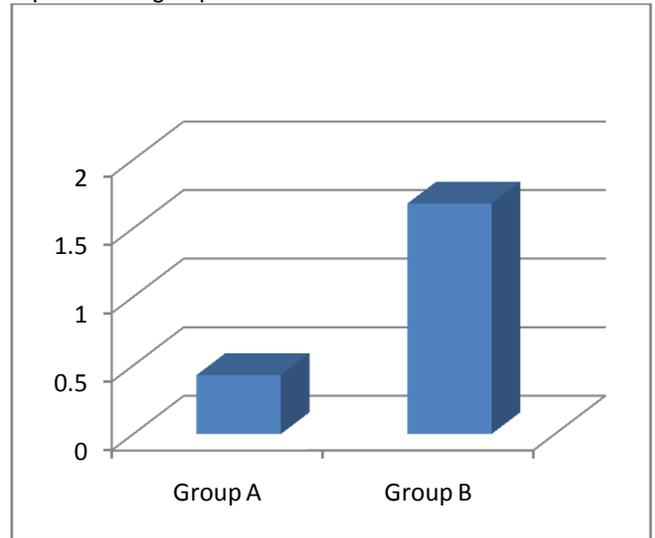


Fig.3: Photomicrograph of fetal kidney group B showing a glomerulus with retraction of the capillary tuft (yellow arrow) and increased urinary space (red arrow head) continuous with the lumen of proximal convoluted tubule (red arrow head) at the urinary pole. The glomerular capillaries (yellow arrow head) could be outlined in the expanded mesangium. H&E stain. X400.



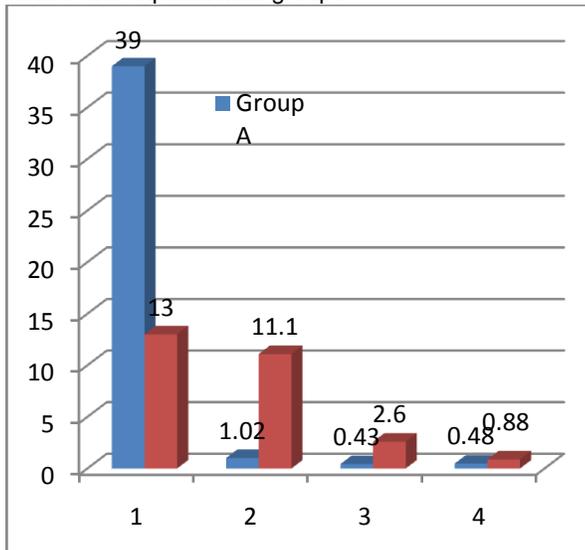
The score for glomerulosclerosis was high (1.68 ± 0.47) in experimental group as compared to (0.43 ± 0.50 ; $P < 0.000$) in control group (Fig.4)

Fig. 4: Comparison of glomerulosclerosis in control and experimental groups



Parameters of glomeruli	Control (n=40)	Experimental (n=40)	Statistical Results (t-test)
	Mean±S.D.	Mean± S.D.	
Normal glomeruli/ mm ²	39.00±3.75	13.00±2.91	p < 0.000
Hypercellular glomeruli/ mm ²	1.02±1.05	11.10±1.59	p < 0.000
Sclerosed glomeruli/mm ²	0.43±0.50	2.60±1.00	p < 0.000
Atrophic glomeruli/mm ²	0.48±0.50	0.88±0.96	p = 0.023

Fig. 5: Comparison of number of different glomeruli in control and experimental groups



1= Normal glomeruli, 2= hypercellular glomeruli, 3= sclerosed glomeruli, 4= Atrophic glomeruli

DISCUSSION

Cyclosporin (CsA) is one of the most potent immunosuppressants used for the management of multiple-organ transplantation. Its clinical use is limited due to the development of chronic nephrotoxicity³¹. The present study stipulates to evaluate the injurious effects of CsA on the glomeruli of developing kidney when given in therapeutic doses to the mice during the whole gestation period of 18 days. Previous description of chronic Cyclosporin renal toxicity commonly included vascular changes, tubular atrophy, and interstitial fibrosis³². Therefore the present study focuses on the glomerular changes. CsA induced nephrotoxicity in our experimental model displays similar glomerular changes as observed earlier in the experimental model showed using adult rat.

Our investigations showed that in the treated groups, retraction of tuft of glomerular capillaries, increased urinary space, mesangial matrix expansion, capillary collapse were observed, these lesions seen were more indicative of focal segmental glomerular sclerosis. An eosinophilic amorphous material, which was strongly PAS positive, filled the capsular space; this was suggestive of possible damage to renal corpuscles (Fig.2). The score for glomerulosclerosis was high (1.68 ± 0.47) in experimental group as compared to (0.43 ± 0.50 ; $p < 0.000$) in control group (Fig.4). The number of sclerosed glomeruli was 2.60 ± 1.00 in experimental group compared to 0.43 ± 0.50 in the control group (Fig.5). These findings indicate that chronic administration of CsA induces in mice glomerular

lesions comparable to the ones reported in human renal transplant. Cyclosporin has renal hemodynamic effects leading to rapid reductions in GFR which, early on, are reversible upon dose reduction or cessation³³. Eventually, GFR loss may become irreversible, reflecting structural changes, including tubular atrophy, interstitial fibrosis and glomerulosclerosis³². Experimental studies show that Cyclosporin can cause juxtaglomerular hyperplasia, by a local activation of the renin-angiotensin system^{34,35,36}. Cyclosporin interact with the renin-angiotensin system, causing vasoconstriction which contribute to glomerulosclerosis through severe compromise of glomerular blood flow and reduces the GFR.³⁷ Eventually GFR loss may become irreversible, reflecting structural changes, including tubular atrophy, interstitial fibrosis, glomerulosclerosis.³⁸ A possible link between glomerulosclerosis and local activation of the renin-angiotensin system is suggested by experiments demonstrating stimulation of extracellular matrix protein synthesis by rat glomerular mesangial cells exposed to angiotensin II³⁹. Mesangial matrix protein synthesis also can be enhanced by TGF- β , a cytokines known to be stimulated by Cyclosporin^{40,41}.

The glomerulosclerosis may result from marked mesangial expansion and capillary occlusion^{42,43}. Cyclosporin A-induced nephrotoxicity model helps to understand the pathological mechanism involved in progressive renal injury and provide important clues for pharmacological manipulations aimed at reducing the long-term consequences of CsA on the kidney.

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