Evaluation of role of Doxycycline (A Matrix Metalloproteinase Inhibitor) on renal functions in patients of Diabetic Nephropathy

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INTRODUCTION

- Diabetic Nephropathy (DN), an important long term complication of Diabetes Mellitus (DM) is the most common cause of End Stage Renal Disease (ESRD) and secondary nephrotic syndrome.
- Increased extracellular matrix (ECM) is a characteristic feature of diabetic nephropathy that is directly linked to declining renal functions.
- The matrix metalloproteinases (MMPs), play an important role in the remodelling of connective tissue and are involved in the degradation of all components of the extracellular matrix.
- Changes in the balance between ECM synthesis and degradation may lead to expansion of the glomerular ECM i.e. glomerulosclerosis and decline in renal function.
- Tetracycline and their analogues inhibit protein synthesis by binding to 30S ribosomes in susceptible organisms.
- They are potent inhibitors of MMPs, both collagenases and gelatinases.
- They have been investigated in the treatment of various disorders in which MMP activity is amplified such as pyoderma gangrenosum, sarcoidosis, rheumatoid arthritis, scleroderma, aortic aneurysms and amyloidosis.(1)
- Doxycycline is a potent non-selective MMP inhibitor. It can inhibit both mammalian collagenases and gelatinases as well as inhibit the synthesis of MMPs in human endothelial cells.
- The remodeling and excess deposition of ECM could be attenuated by doxycycline due to its property of MMP inhibition.(2)

AIMS AND OBJECTIVES

- The aim of the present study was to evaluate the effect of doxycycline-a matrix metalloproteinase inhibitor on renal functions especially proteinuria in patients having Diabetic nephropathy.

MATERIAL AND METHODS

- Forty clinically proven adult cases of diabetic nephropathy (DN), fulfilling the inclusion criteria were taken up for the study.
- It was an open randomized trial conducted over a period of 6 months.
- The patients were enrolled in the study after obtaining a pre-informed consent.

Inclusion criteria

- All adult patients (>18years) with type II DM
- Patients with overt proteinuria (>300 mg/24 hours)
- All patients had to be on stable doses of ACEs and/or ARBs for at least 2 months before enrolment in the study.

Exclusion criteria

- Any history of systemic long term anti microbial therapy over the past 6 months
- History of hypersensitivity to tetracycline derivatives
- Chronic renal insufficiency (serum creatinine levels >1.4mg/dl)
- Hepatic dysfunction (transaminase levels greater than twice the upper limit of normal)
- Incompatibility of doxycycline with the patient's concurrent medications.
- The patients were divided into two groups of 20 patients each.
  Group A (Control) (n=20) – Subjects with DN on ACE inhibitors and/or ARBs.
  Group B (Study) (n=20) – Subjects with DN on ACE inhibitors and/or ARBs and Doxycycline.
- The control group received only ACE inhibitors and/or ARBs while the study group was given doxycycline in a dose of 100mg per day in addition to ACE inhibitors and/or ARBs for a period of 3 months.
- Investigations to assess the renal parameters including proteinuria were carried out in the beginning of the study, after one month and 3 months and after 6 months (washout period of 3 months).
- Adequate glycemic control was achieved with insulin and/ or oral hypoglycemic agents.
- All the patients were observed for the side effects of doxycycline like gastrointestinal intolerance and photo toxicity.

RESULTS

- The mean age of the patients in group A was 52.8±8.74 years and in group B 56.7±10.3 years.
- Majority of the patients were above 50 years of age.
- There were 9 males and 11 females in group A while in group B there were 13 males and 7 females.
- All patients were having type II diabetes mellitus.
- The basal values for the various renal parameters were largely within normal limits and did not vary significantly throughout the study.
- The mean basal levels of proteinuria were 1.74±1.70g/day for group A and 2.17±2.95g/day for group B. It reduced to 1.56±1.52 for group A and 1.89±2.72 for group B, at the end of one month.
- At the end of 3 months, a significant decline of proteinuria was observed in both the groups.
- A statistically significant difference existed between the two groups ($p<0.05$),at 3 months.
- The levels of proteinuria increased nearing the pre-treatment levels (1.98±2.65) after a washout period of 3 months i.e., at 6 months in group B. (Table 1)
- The mean change in the level of proteinuria was 0.956 g/day in the study group (Group B) after 3 months of Doxycycline therapy while it was 0.253 g/day in the control group (group A) indicating beneficial effect of doxycycline in decreasing proteinuria.
- In Group B, there was no significant difference in the mean change of proteinuria at the baseline to that at 6 months, indicating that there was no carry forward effect of doxycycline and the increase of proteinuria seen was due to withdrawal of the additive effect of the drug. (Table 2)

Table 1:Level of proteinuria (g/day)

Level of proteinuria	Group A (mean ±SD)	Group B (mean ±SD)	P value
Basal	1.74±1.70	2.17±2.95	>0.05
1 month	1.56±1.52	1.89±2.72	>0.05
3 months	1.50±1.50	1.22±2.01	<0.05
6 months (after a washout period of 3 months)	1.24±1.31	1.98±2.65	>0.05

Table 2: Mean change in the level of proteinuria (g/day)

	Group A	Group B
Baseline vs. 1 month	0.189	0.289
Baseline vs. 3 months	0.253	0.956
Baseline vs. 6 months (end of washout period)	0.502	0.193

DISCUSSION

- Increased extracellular matrix (ECM) is a characteristic feature of diabetic nephropathy that is directly linked to declining renal functions.
- The matrix metalloproteinases (MMPs) are zinc dependent endopeptidases that play an important role in the remodelling of connective tissue and are involved in the degradation of all components of the extracellular matrix.
- Changes in the balance between ECM synthesis and degradation may lead to expansion of the glomerular ECM i.e. glomerulosclerosis and decline in renal function.(3)
- The tetracyclines are potent inhibitors of the MMP enzyme family.
- Their role has been investigated in several disorders in which MMP activity is amplified.
- Doxycycline is a potent, broad spectrum, non selective MMP inhibitor, acting on both mammalian collagenases and gelatinases and inhibiting the synthesis of MMPs invivo.
- This is achieved by binding of the drug to zinc or calcium associated with the enzyme and blocking the active site, resulting in decreased enzyme activity leading to decreased expression of TGF-β, therefore attenuating mesangial cell proliferation, ECM deposition, glomerular hypertrophy and proteinuria.
- In the present study,diabetic proteinuria was seen to decrease significantly after 3 months of doxycycline therapy, though the other renal parameters which were normal at baseline remained unaltered.
- When interpreted in terms of mean change in the levels of proteinuria, the mean change in the levels of proteinuria in group B at 3 months was high as compared to the basal values and this mean change decreased significantly on withdrawal of doxycycline.
- Naini demonstrated the favourable effects on diabetic proteinuria by using low dose doxycycline in a similar study performed over a period of 2 months on 35 patients.(2)
- A similar dose was used by Ahuja who reported a 70% decline in proteinuria in a patient with glomerulonephritis.(4)
- The present study showed that the level of proteinuria in patients with overt diabetic nephropathy can be decreased with a low dose doxycycline therapy.
- The delayed response seen after 3 months and not immediately after 1 month may be due to altered expression of MMPs and degradation of ECM proteins in the presence of the drug.
- This was achieved with a high degree of compliance and no apparent serious adverse effects.

CONCLUSIONS

- Although promising, the studies published so far including present study have been too short term to clarify whether doxycycline treatment in diabetic patients is capable of curing DN, instead of simply influencing one of its surrogate end points, albuminuria.
- Further long term multicentric trials are required to determine the benefits of doxycycline in patients of diabetic nephropathy.

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