Level of vegf in chronic nephropathy models

The number of patients with chronic kidney disease (CKD) progressing to end-stage renal disease (ESRD) and requiring renal replacement therapy are increasing worldwide. In India, the age-adjusted incidence rate of ESRD is estimated to be 229 per million population (pmp), and > 100, 000 new patients enter renal replacement programs annually (1). Diabetic nephropathy (DN) is the most common cause of ESRD development among other disorders predisposing to ESRD. The costs of DN are significantly higher than those from other diabetic complications because the patients are subjected to haemodialysis programs and renal transplant when failure occurs. Thus, the burden of DN on public health is enormous (2). The current therapy for patients with renal injury includes glycemic control by antidiabetic medications. Blockage of renin angiotensin system (RAS) is the most commonly practiced way of controlling blood pressure in DN. However, angiotensin-converting enzyme inhibitors and angiotensin receptor blockers moderately slow the rate of progression but do not arrest or reverse the progression of disease. Moreover, RAS blockade is usually initiated only after DN manifests itself clinically with persistent proteinuria in both type 1 and type 2 diabetes. But despite knowledge of the devastating effects of these complications and the involved costs to patients, to date, there is still no method that is sufficiently sensitive and accurate for subclinical diagnoses of diabetic nephropathy. The pathomechanisms leading to these changes are not yet clearly understood and therefore, therapeutic approaches for relief of this disease are scarce or do not permit a favorable pharmacological intervention.

Angiogenesis – the development of new blood vessels from pre-existing ones – is involved in physiological events and in pathological disorders including cancer, proliferative retinopathy, rheumatoid arthritis, psoriasis, and neointimal formation. Angiogenesis is controlled by the balance between proangiogenic and anti-angiogenic factors. Experimental studies have demonstrated the involvement of an imbalance of angiogenesis-related factors in the progression of CKD and the potential therapeutic effects of modulating these factors have been identified (3, 4). Vascular endothelial growth factor (VEGF)-A, a potent pro-angiogenic factor, is involved in the development of the kidney, and also plays an important role in maintaining the glomerular capillary structure and in the repair process following injuries of glomerular endothelial cells and peritubular capillaries (5-7). It is constitutively expressed in podocytes, proximal tubular cells and medullary thick ascending limb cells in the juxtamedullary region of the normal kidney. Evidence is emerging that VEGF plays a critical role in maintaining renal homeostasis (8, 9). Altered (increased or decreased) expression of VEGF leads to glomerular dysfunction and proteinuria (3, 10-15). It has been demonstrated that VEGF administration has a beneficial effect in both acute and chronic nondiabetic renal disease. In the remnant kidney model and cyclosporine nephropathy, decreased VEGF expression was observed. These observations were correlated with renal dysfunction and capillary loss. VEGF administration was found to reverse the renal dysfunction in these models (4, 16, 17). In contrast, both circulating and local VEGF levels are high in diabetes. In diabetic nephropathy , the increases in the number of glomerular capillaries and in the glomerular levels of VEGF-A and its receptor VEGFR-2 are observed (3, 18). The role of abnormal angiogenesis induced by

VEGF has been implicated in diabetic retinopathy and diabetic nephropathy associated with progression of disease and the excessive VEGF has been shown to have a role in mediating glomerular hypertrophy (3). The precise mechanism is unclear for contradictory status of VEGF-A in diabetic and non diabetic kidney disease. So, in the present study we decided to investigate the level of VEGF in two different chronic nephropathy models; one was diabetes induced chronic nephropathy and the other was non diabetic nephropathy.

VEGF and NO interaction has been explained as one of the regulating mechanism in causing paradoxical effects of VEGF by Takahiko Nakagawa *et al.; 2007.* The author explained the dark side and the bright side of VEGF effects. VEGF normally stimulates endothelial nitric oxide (NO) release and acts in coordination with elevated NO levels as a trophic factor for vascular endothelium. The increased NO derived from the endothelial cell acts as an inhibitory factor that prevents excess endothelial cell proliferation, vascular smooth muscle cell proliferation, and macrophage infiltration. Normally, an elevation in VEGF expression should result in elevated endothelial NO levels, since VEGF increases both endothelial NOS (eNOS) expression and NO release from endothelial cells. However, in diabetes, despite high levels of VEGF, endothelial NO levels are low. The authors have summarized several mechanisms to explain the low endothelial NO bioavailability. First, glucose can scavenge NO. Second, there is an impairment of eNOS activation. A third mechanism could be oxidative stress, which quenches NO to form peroxynitrite. Fourth, the formation of advanced glycation products in diabetes may also result in the consumption of endothelial NO. Fifth, both

asymmetric dimethyl arginine and uric acid are commonly elevated in diabetes and can reduce endothelial NO bioavailability. Finally, NO may bind to glycosylated deoxyhemoglobin. Thus in diabetic settings high levels of VEGF, in absence of NO; lead to excessive endothelial cell proliferation, stimulation of macrophage chemotaxis, and vascular smooth muscle cell activation resulting in vascular injury (19, 20). Based on these observations we decided to investigate level of NO, in addition to VEGF; in two different chronic nephropathy models.

The therapeutic effects of anti-VEGF-A strategies and anti-angiogenic factors in diabetic nephropathy have been reported (21). The beneficial effects of administration of VEGF in non diabetic CKD have been reported (17). The contrasting effects of VEGF in non-diabetic and diabetic kidney disease prompted us to review factors modulating VEGF expression in CKD. Hypoxia and certain cytokines are major regulators of VEGF expression (22-29). Physiological adaptation to hypoxia is an area of intense investigation. Adenosine is a critical mediator during ischemia and hypoxia and contributes to diseases as diverse as inflammation and carcinogenesis (30). Inhibition of adenosine kinase and the dephosphorylation of ATP and AMP by surface apyrases (e. g., CD39) and ecto-5’ nucleotidase (CD73), respectively, represent the major pathways of extracellular adenosine liberation during oxygen supply imbalances. Once liberated in the extracellular space, adenosine is either recycled (e. g., through dipyridamole-sensitive carriers) or interacts with cell surface Adenosine Receptors (ARs). Presently, four subtypes of G protein-coupled ARs exist, designated A_1 , A_{2A} , A_{2B} , and A_3

. They are classified according to utilization of pertussis toxin sensitive pathways (A_1 and A_3) or adenylate cyclase (A_{2A} and A_{2B}).

The A_{2B} AR have been recently much investigated for their role on renal functions. A_{2B} AR have been reported to inhibit PDGF induced growth of mesangial cells , they also protect the kidney from ischemia (31, 32). A_{2B} AR have also been reported to inhibit inflammation, so it is remained to be determined which type of A_2 AR are involved in inflammation associated with diabetic nephropathy. A_{2B} receptors have a lower affinity compared with other subtypes and require higher concentrations of adenosine for their stimulation and such high levels can be reached during hypoxia, ischemia, inflammation, and injury. A_{2B} AR regulate various pathological processes, including mast cell activation, vasodilatation, inhibition of cardiac fibroblast and vascular smooth muscle growth, stimulation of endothelial cell (EC) growth, and angiogenesis (8, 9, 10, 11, 12). The functional aspects of ARs responses may be determined by surface expression profiles. Microarray analyses of cDNA derived from endothelial cells subjected to various periods of hypoxia revealed significant changes in the ARs profile, wherein the prominent phenotypic change favored A_{2B} AR expression, with concomitant down regulation of A_1 AR and A_3 AR(2). As chronic state of nephropathy also involve hypoxic intra renal environment(33), we decided to find the expression of A_{2B} AR in two different models of chronic nephropathy.

The most potent stimuli for VEGF production is hypoxia as stated above. The evidence of line also suggests the hyperglycemic state of diabetes to be hypoxic. Particularly, it has been demonstrated that in a mouse podocytes cell line the expression of VEGF increases under exposition to high D-glucose

concentrations. At present however, it is not clear how glomerular VEGF production is unregulated in response to diabetes or high glucose concentration (13). Ex vivo exposure of rat kidney glomeruli to adenosine leads to an increase in VEGF content. Activation of A_{2B} AR subtypes augments expression and releases VEGF beyond basal levels in rat glomeruli. Additionally, the status of VEGF and NO axis in non diabetic nephropathy is not well investigated. Based on these observations we decided to investigate the effects of A_{2B} AR modulators on VEGF and NO in chronic diabetic nephropathy. Reconstitution of endothelial NO synthesis and/or its availability in glomeruli of diabetic nephropathy animal models via the A_{2B} AR modulation, remains an interesting matter. We thus hypothesize that differential expression of VEGF in diabetic and non-diabetic kidney diseases is mediated by A_{2B} AR. The expression of A_{2B} receptor is disease specific.

Cyclosporine A (CsA) is a potent immunosuppressive agent with definite efficacy to prevent organ allograft rejection. However, CsA causes significant nephrotoxicity that might contribute to long-term kidney graft loss (34). Acute CsA nephrotoxicity is characterized by renal vasoconstriction, which is dose-related and reversible with dose reduction. In contrast, chronic CsA nephrotoxicity may be progressive and irreversible, the histological lesion of which includes tubular atrophy, afferent arteriolar hyalinosis. We resolved to investigate the mechanisms of cyclosporine induced nephropathy as non diabetic chronic nephropathy model in present study.

A line of evidence has demonstrated reduction in vascular endothelial growth factor (VEGF) and nitric oxide (NO) in CsA nephropathy(35, 36). VEGF is an

endothelial cell mitogen that increases angiogenesis and vascular permeability. Endogenous VEGF has a relevant role in the renal tubular defense against CsA toxicity. Blockade of the VEGF by α-VEGF results in intensification of the tubular injury the CsA nephropathy(37). The occurrence of both in-vivo and in-vitro effects of VEGF blockade provides evidence of a direct protective effect of VEGF on the tubular cell.

Numerous studies have reported a important role of NO in regulation of the effects of VEGF on angiogenesis, vascular permeability, and blood pressure regulation (38, 39). A $_{2B}$ AR have been known to mediate NO release in various pathological settings (40, 41). In the late phase of CsA nephropathy, nitric oxide synthase activation is reduced (42). However, it is necessary to determine whether or not A $_{2B}$ AR agonist induces VEGF in chronic CsA nephropathy. Previous in vitro studies using vascular smooth muscle cells as well as macrophages suggest administration of A $_{2B}$ AR agonists results in increased VEGF expression, potentially stimulating angiogenesis. Accordingly, it was hypothesized that A $_{2B}$ AR agonists induce expression of key angiogenic factors such as VEGF in CsA induced chronic nephropathy. Such an increase in renal VEGF expression by A $_{2B}$ AR activators may initiate the angiogenic response at the site of renal injury. Hencepresent study was designed to investigate the effects of A $_{2B}$ AR modulators on VEGF expression and NO levels in kidneys of chronic CsA induced nephropathy.