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Allopurinol Improves Endothelial Function and Oxidative Stress in Patients with Chronic Kidney Disease

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ABSTRACT

In uremia, mechanistic alterations of vessel wall properties, which have a profound impact causing decreased arterial compliance or its inverse; increased arterial stiffness, early wave reflections and decreased endothelial function. Allopurinol an xanthine oxidase inhibitor, has been found to improve endothelial function in patients with hypercholesterolemia, diabetes mellitus and heart failure. Twenty five Patients with chronic kidney disease in the age group between 20 to 60 years were enrolled into allopurinol study. Reflection index (RI), Serum Malondialdehyde (MDA) and Total antioxidant activity (TAA) were estimated before and after treatment with allopurinol. Allopurinol significantly lowered serum uric acid concentrations from 487 ± 119 to 384 ± 101 µmol/L, P<0.01 and serum MDA from 108 ± 27 to 87 ± 32 nmol/L, P<0.01. Allopurinol independently of renal functions; improves endothelial function, probably through either uric acid lowering effect or improving oxidative stress, which are preventable risk factors of cardiovascular morbidity in renal failure patients.

Keywords:- Allopurinol, Endothelial dysfunction, Reflection index, Malondialdehyde, Antioxidant activity, Uric acid

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Introduction

Cardiovascular disease is the prime cause of death among the patients with chronic renal failure [1]. It is possible that, in renal failure, an increased uric acid level contributes to endothelial dysfunction, possibly via proinflammatory mechanism [2]. In addition, the oxidants generated via xanthine oxidase (XO) can potentially reduce endothelial dependent vasodilatory capacity through effects on nitric oxide (NO) inactivation and bioavailability, suggesting increased oxidative stress as another possible mechanism of endothelial dysfunction [3]. Thus, hyperuricemia, oxidant stress and endothelial dysfunction all linked. Therefore, XO inhibition with allopurinol may improve endothelial function and reduce arterial stiffness. Allopurinol has been found to improve endothelial function in patients with hypercholesterolemia, diabetes mellitus and heart failure [4-6]. We quantitatively analyzed RI a marker of digital artery tone from pulse volume record. We also determined endothelial dependent vasodilatory function expressed as percent change in RI to

salbutamol challenge, MDA, TAA and SUA levels before and after treatment with allopurinol.

Materials and methods

The study was conducted as an open label study. The Institutional Ethical Committee approved the study protocol. The control population was recruited from the general population and the patients were from the outpatient Nephrology Department. Participants declared their willingness, once the details of the study and the treatment had been explained and provided a written informed consent for study participation. All subjects underwent screening by clinical history and physical examination. The values of laboratory parameters as requested by the nephrology consultant were noted. Twenty five Patients with chronic renal failure in the age group between 20 to 60 years, either with radiological evidence of renal impairment or with serum creatinine levels above 1.5mg/dL for a minimum duration of renal failure of more than 3 months, serum albumin above 3.5 gm/dL were enrolled into allopurinol study. Patients were excluded from the study, if they were current smokers or alcoholics, pregnant or lactating, having hepatic, and peripheral vascular disorders and those who were currently on drugs that influence endothelial function like vitamins E, C, statins, anti-oxidants, nitrates, ACE inhibitors and if they were hypersensitive to allopurinol. Enrolled patients received 100 mg of Allopurinol for four weeks once daily orally. Six mL blood sample was collected for estimation of MDA, SUA and TAA before and after the treatment.

Measurement of Endothelial Function

The reflection index (RI) was calculated manually from the contour of the arterial wave forms obtained from pulse plethysmography apparatus. The first peak is formed mainly by the pressure transmitted along a direct path from left ventricle and the second peak is formed by reflected wave from lower part of the body along the aorta. The RI is the height of the second wave of the arterial pressure waveform expressed as a percentage of the waveform peak. RI=b/a*100 where b and a represents heights of second and first waves respectively and the final reflection index is expressed as the mean of three reflection indices. The RI was measured at base line. Then the subject was asked to inhale 400 micrograms of salbutamol from a spacer and after 15 minutes; once again, RI was measured. The percentage decrease in RI at the end of the test compared to baseline was used to assess the endothelial dependent vasodilator function [7]. All recordings were performed while the patients were on their regular medications.

Estimation of Serum MDA and TAA

MDA is a Lipid peroxidation product generated in the tissues by free radical injury. The lipid peroxidation products react with thiobarbituric acid forming a pink coloured adduct on boiling, which was measured at 532nm [8]. The concentration of MDA was read from standard calibration curve plotted using TEPP (1, 1, 3, 3-tetra ethoxy propane). The results were presented in nano-moles per mL. TAA in plasma was measured by decolorization assay [9]. The pre-formed radical mono-cation of 2,2-azinobis-(3-ethylenbenzothiozoline-6-sulfonicacid) (ABTS) is generated by oxidation of ABTS (Sigma Chemical Co., USA) with potassium persulfate and is reduced in the presence of hydrogen-donating antioxidants. The inhibition percentage of the ABTS radical cation formation by the added serum sample at a fixed time point is quantified as the result. Trolox (6-hydroxy-2, 5, 7, 8-tetramethychroman-2-carboixylic acid, Aldrich Chemical Co, UK) a water-soluble vitamin-E analogue was used as a standard. The antioxidant capacity of the serum then was expressed in molar Trolax equivalents per L.

Statistical Evaluation

The statistical analysis was carried out with Sigma graph pad software, USA Version-4. All the data was presented as mean and standard deviation. Paired "t" test was used for comparing post treatment with baseline. All the efficacy parameters were presented as percentage change from base line. The negative sign indicates decrease in the value from baseline. For statistical significance, the two-tailed probability value of less than 0.05 was considered.

Results

The age range of patients was 42 ± 14 years. There were 13 males and 12 females. The various laboratory parameters and hemodynamic parameters before and after treatment with allopurinol are shown in Table-1. Allopurinol significantly lowered serum uric acid concentrations from 487 ± 119 to 384 ± 101 µmol/L, $P<0.01$ and serum MDA from 108 ± 27 to 87 ± 32 nmol/L, $P<0.05$ as shown in Figure 1. Other laboratory parameters did not change significantly.

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Percentage change in RI to salbutamol inhalation before and after treatment with allopurinol was -12±12 to -20±11, p<0.001 as shown in Figure-2. Administration of allopurinol perse did not significantly alter all hemodynamic parameter. However, there was a significant fall in mean arterial pressure, diastolic blood pressure during the second visit. None of the patients developed adverse effects.

Parameter	Before Treatment (n-25)	After Treatment (n-25)
Serum creatinine (µmol/L)	283 ± 115	301 ± 133
Creatinine clearance (mL/s)	0.47 ± 0.23	0.43 ± 0.22
Serum uric acid (µmol/L)	487 ± 119	384 ± 101 **
Serum albumin (gm/L)	37.6 ± 1.0	37.2 ± 0.9
Malandialdehyde (nmoles/L)	108 ± 27	87 ± 32 *
Total antioxidant activity (mmoles/ L)	1.12 ± 0.18	1.10 ± 0.15
Heart rate (bpm)	82 ± 17	83 ± 15
Systolic blood pressure (mmHg)	137 ± 31	129 ± 28
Mean arterial pressure (mmHg)	104 ± 23	94 ± 21
Diastolic blood pressure (mmHg)	83 ± 14	77 ± 16
Pulse pressure (mmHg)	54 ± 19	53 ± 14
Reflection index (%)	75 ± 15	74 ± 15
Percentage change in parameters to salbutamol inhalation		
Reflection index	-12±12	-20±11***
Data expressed as Mean ± SD. *P<0.05 **P<0.01, ***P<0.001.		

Table-1-Comparison of clinical parameters of patients treated with Allopurinol.

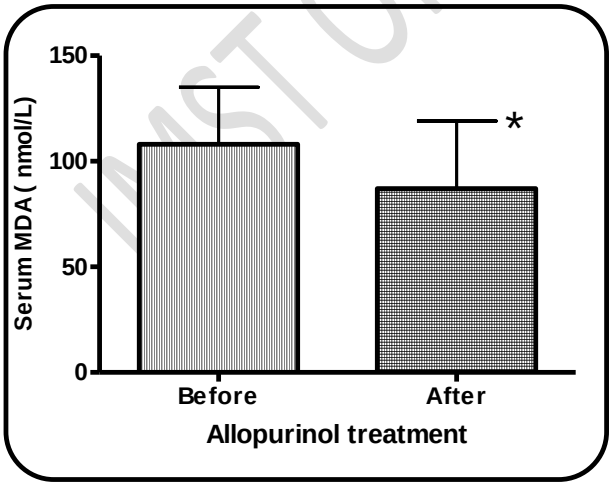

Figure 1: MDA levels in patients with CRF before and after treatment with Allopurinol

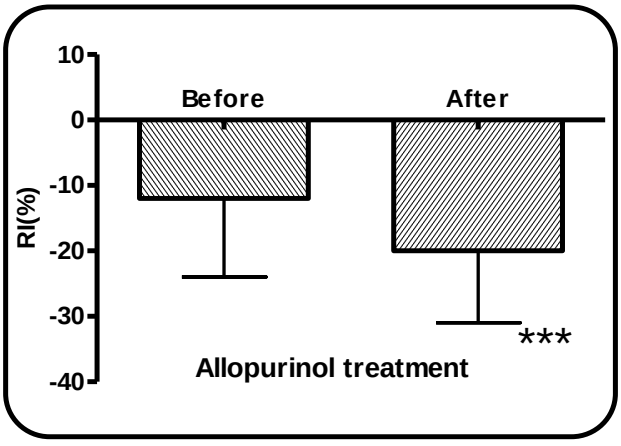

Figure 2: Percentage change in RI before and after treatment with Allopurinol

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Discussion

All portions of arterial wall undergo structural changes, such as proliferation of intima, lipid core formation, calcification of medial and fibrosis of adventitia in renal failure. Thus for these structural reasons, increase in inflammatory markers,, increase in biochemical parameters oxidative stress and uric acid, endothelial function is grossly abnormal in chronic renal failure. [10]. Moreover, both endothelial dysfunction and increased arterial stiffness commonly coexist in risk factor groups of cardiovascular disease including renal failure. Endothelial dysfunction has been reported in both resistance and conduit arteries in patients receiving haemodialysis [11], and peritoneal dialysis [12]. Evidence shows that, endothelial dysfunction is an early event in the pathogenesis of arterial disease, and is not restricted to the vessels that show clinical manifestations of atherosclerosis. In the context of cardiovascular diseases, endothelial dysfunction is characterized as loss of its vasodilatory predominance, in particular to endogenous nitric oxide deficiency.

In the present study, we observed that RI was higher in patients with renal failure than healthy controls taken from our data base, suggesting the early return of arterial wave reflections. Further inhalation of salbutamol, the test applied to study endothelial dependent vasodilatory function, revealed blunting of response, suggesting possible endothelial dysfunction in patient group. It is possible that any of these coexisting risk factors in our study could also be responsible for increased endothelial dysfunction. We observed elevated levels of Serum MDA in uremic patients, in addition to blunting of vasodilatory response to salbutamol challenge. Therefore, a possible mechanism for endothelial dysfunction in our study group could be increased oxidative stress. Oxidative stress can promote the production of free radicals. These free radicals attack cell membrane bound lipids and result in formation of lipid peroxidation products. The lipid peroxidation products such as DC (diene-conjugate), LOOH (lipid hydropreoxidases) and MDA thus formed directly injure endothelial cells and diminish its capacity to synthesise NO [13].

Xanthine oxidase is a key free radical producing enzyme system in the vascular endothelium [14] and orally active allopurinol probably reduces oxidative stress and prevents inactivation of endogenous NO. When our patients received allopurinol, we found improvement in endothelial dysfunction. This improvement in endothelial function was associated with reduction in the level of serum uric acid and serum MDA. Other probable cause for altered endothelium-dependent vasodilatation in uremic patients may be malnutrition. However, we did not find any significant change in serum albumin (a marker on nutrition) between treatment visits. In addition, markers of renal disease like creatinine clearance and blood urea nitrogen were unchanged between two visits suggesting the observed change was not due to improvement in renal function.

The likely mechanism for the improvement in endothelial function with allopurinol may be due to in vivo bioavailability of endothelium-derived nitric oxide, presumably by blocking the production of reactive oxygen species mediated by XO [3]. This is further substantiated by finding of a significant reduction in plasma MDA with allopurinol. Our results were consistent, with the studies with allopurinol showing improvement of endothelial function in- patients with hypercholesterolemia [4], diabetes mellitus [5] and heart failure [6]. Another possible benefit of allopurinol may be due to decreasing serum uric acid levels [15]. Hyperuricemia is common in patients with renal disease, and has been linked both to the progression of renal disease and development of cardiovascular disease [16-17]. Recent studies showed that uric acid level is an independent risk factor for cardiovascular events and mortality in-patients with hypertension and end stage renal disease, as well as in general population [18-19]. High serum uric acid level was a significant and independent predictor of cardiovascular mortality in 146 hemodialysis patients (Hsu *et al*). Kang et al observed a correlation between elevated uric acid levels and endothelial dysfunction in predialysis patients [2]. Hyperurecemia has been shown to induce preglomerular arterial disease and hypertension via with activation of renin-angiotensin and cyclo-oxygenase-2 systems. Earlier studies have demonstrated beneficial effects of allopurinol in reducing cardiovascular complications after coronary artery bypass [20], and in- patients with dilated cardiomyopathy [21]. In addition, allopurinol speeds up the repletion of high-energy phosphates by blocking dephosphorylation of ATP to AMP to hypoxanthine during ischemia [22].

Conclusion

The results from our present study, demonstrates increased abnormalities of wave reflection in patients with chronic kidney disease. Inhalation of salbutamol, revealed the presence of

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endothelial dysfunction of conduit artery. In addition, our study also demonstrates that allopurinol independently of renal function; improves endothelial function, probably through uric acid lowering effect or improvement in oxidative stress, which are preventable risk factor of cardiovascular morbidity in renal failure patients. Further studies in more number of patients are required to confirm our observation.

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