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Serum nitric oxide levels in subjects with high creatinine value in comparison of healthy control subjects

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Abstract: Creatinine is a chemical waste molecule that is generated from muscle metabolism and is produced from creatine. The kidneys maintain blood creatinine levels in normal range. Creatinine has been found to be a fairly reliable indicator of kidney function. Elevated creatinine level signifies impaired kidney function or kidney disease. Since nitric oxide (NO) is produced by three types of Nitric Oxide Synthases (NOSs), rapid changes in stable oxidized metabolites (nitrite and nitrate) in the tissues and blood should be represented by the amount of stable forms in the serum and may reflect changes in the body. The serum samples were collected from the individuals with high levels of creatinine and normal range. Nitrite was measured by a Griess reaction while nitrate was measured using the enzymatic one step assay with nitrate reductase. The total 36 samples (18 normal range (N) and 18 high creatinine values (H)) were evaluated for the NO levels. The age group varies from 6-74 and 20-80 for normal and high level of creatinine respectively. The levels of creatinine in the normal range and high values varies from 0.71-0.93 (mean=0.86±0.01) and 1.59-11.59 (mean=4.34±0.77), respectively. When the nitrite (15.19±1.73 µM versus 12.84±1.19 µM, P>0.05) and nitrate (24.94±2.60 µM versus 27.76±2.42 µM, P>0.05) levels were compared between these groups no significant differences were observed. Results of this study reveal that there is no correlation between nitric oxide production and the serum creatinine levels. However, those results are preliminary and have to be confirmed in sample of larger size.

Key words: Nitric oxide; iNOS; Creatinine; Kidney function

Introduction

Nitric oxide (NO) is heat-labile, unstable compound and is one of the few gaseous signaling molecules known (1). It is involved in many physiological and pathological processes within the body, both beneficial and detrimental (2,3). Appropriate levels of NO production are important in protecting organs from ischemic damage (4), whereas chronic expression of NO is associated with various malignancies and inflammatory conditions including juvenile diabetes, multiple sclerosis, arthritis and ulcerative colitis (5,6). Genetic factors including endothelial nitric oxide synthase (eNOS) were implicated in pathogenesis of rheumatoid arthritis, and extra-articular manifestations of rheumatoid arthritis were significantly greater among the carriers (7).

Since NO is involved in various pathological states and is produced by three types of Nitric Oxide Synthases (NOSs), rapid changes in stable oxidized metabolites (nitrite and nitrate) in the tissues and blood should be represented by the amount of stable forms in serum and may reflect vascular activities and circulatory or inflammatory changes in the body (8). NO is produced in all tissues and organs by constitutive NOS (cNOS), which includes endothelial NOS (eNOS; isoform III) and neuronal NOS (nNOS; isoform I) and inducible NOS (iNOS; isoform II) (9). Therefore, pathophysiological changes such as atherosclerosis with coronary artery diseases (10, 11), endothelial dysfunction (12), pro-inflammation and inflammation seen in various diseases (13-16) may be to some extent studied by measuring NO metabolites in the peripheral blood (17- 20).

Recent evidence suggests that NO deficiency is both a cause and consequence of chronic kidney disease (CKD) (21). Clinical data show decreased total NO production in patients with CKD and end-stage renal disease (ESRD) (22-26). In animal models of CKD, renal NO deficiency is evident irrespective of the initial insult (27–33) and enhanced progression is seen with superimposed NO synthase (NOS) inhibition and protection with L-arginine supplementation (34, 35). Furthermore, chronic NOS inhibition alone leads to hypertension, proteinuria, and renal injury (36). Within the kidney, loss of the neuronal isoform of NOS always associates with injury in multiple models of CKD (28-31, 33, 37) and correlates with level of damage and declining renal function (38).

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Kidney failure is considered one of the most serious chronic disorders. Though the incidence of Chronic Renal Failure (CRF) or kidney disease is not as common as Coronary Heart Disease, because of the population density and lack of adequate healthcare to match there is a constant clamor for Dialysis and treatment facilities in India. Long term care for kidney disease continues to be expensive. Usually the functioning of the kidney is determined by the biochemical estimation of creatinine. The normal range of creatinine in healthy individual is 0.5-1.2 mg/dL. Creatinine is a breakdown product of creatine which is primarily synthesized in the liver from the methylation of glycocyamine by S-Adenosyl-L-Methionine. It is then transported through blood to the other organs, muscle, and brain where, through phosphorylation, it becomes the high energy compound phosphocreatine. During the reaction creatine: phosphocreatine, catalyzed by Creatine Kinase, spontaneous conversion to creatinine may occur. Creatinine is chiefly filtered out of the blood by the kidneys (glomerular filtration and proximal tubular secretion). There is little or no tubular reabsorption of creatinine. If the filtering of the kidney is deficient, creatinine blood levels rise. Therefore, creatinine levels in blood and urine may be used to calculate the creatinine clearance (CrCl), which reflects the glomerular filtration rate (GFR). Elevated creatinine level signifies impaired kidney function or kidney disease. Therefore, we have done a study to evaluate the association between serum creatinine and NO levels.

Materials and Methods

Study group

Serum samples from eighteen subjects (10 females and 8 males) having high creatinine values and 18 individuals (11 females and 7 males) who have normal creatinine values were collected from Maha Rani Laxmi Bai Medical College, Jhansi, Uttar Pradesh and stored at -80^0 C. Approval for the study was obtained from the institutional research ethical committee (IEC/IRB No: BU/Pharma/032).

Nitrite determination

Nitrite was measured by using a Griess reaction as described elsewhere (39). The results were given as µM.

Nitrate determination

Nitrate was measured using the enzymatic one-step assay with nitrate reductase. This method is based on the reduction of nitrate to nitrite by nitrate reductase in the presence of β-NADPH. Tubes containing 250 μl of 100 mmol/l potassium phosphate buffer (pH 7.5), 50 μl of 12 mmol/l β-NADPH, and 100 μl sample were equilibrated at 25°C. To start the enzymatic reaction, 40 μl of 500 U/l nitrate reductase was added. The samples were incubated for 45min in the dark. The oxidation of β-NADPH was monitored in terms of the decrease in absorbency at 340 nm. The method of standard addition was used to minimize the effect of interfering substances from the serum. The results are given as µM. Samples with internal standard, and serum and reagent blanks were also analyzed.

Statistical analysis

To compare differences in nitrite-nitrate levels in different groups (normal creatinine vs high creatinine values), all values were expressed as mean ± standard of means (SEM) unless stated otherwise. Statistical significance level was set to 0.05 for all calculations.

Results

The total 36 samples (18 normal range (N) and 18 high Creatinine values (H)) were evaluated for the NO levels. The age group varies from 6-74 years and 20-80 years for normal and high level of creatinine respectively. The levels of Creatinine in the normal range and high values varies from 0.71-0.93 %mg (mean=0.86±0.01) and 1.59-11.59 %mg (mean=4.34±0.77), respectively. When the nitrite (15.19±1.73 µM vs 12.84±1.19 µM, P>0.05) and nitrate (24.94±2.60 µM vs 27.76±2.42 µM, P>0.05) levels were compared between these groups no significant differences were observed (Fig. 1 and 2).

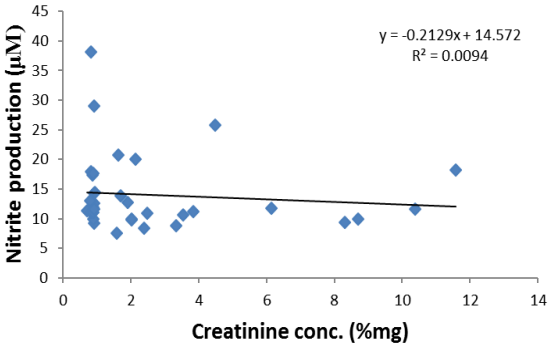

Figure 1: Serum levels of Nitrite in subjects with normal and high values of creatinine

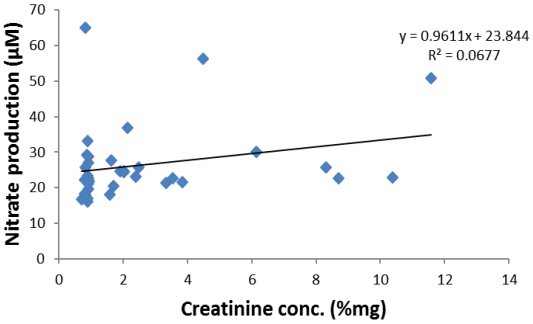

Figure 2: Serum levels of Nitrate in subjects with normal and high values of creatinine

Table 1: Age, Sex, and Serum levels of Nitrite and Nitrate in subjects with normal and high values of creatinine with respect to individuals.

S.No.	Age	Sex	Creatinine value (% mg)	Nitrite (µM)	Nitrate (µM)
Normal values of creatinine					
1	74	M	0.91	12.56	26.93
2	25	F	0.86	17.37	29.18
3	14	M	0.91	14.31	22.75
4	42	F	0.91	29.06	28.68
5	40	F	0.88	11.87	23.37
6	17	F	0.80	11.25	18.18
7	70	F	0.83	38.18	65.00
8	30	F	0.82	17.93	25.62
9	30	F	0.71	11.37	16.75
10	42	F	0.91	9.25	19.62
11	30	F	0.90	11.62	16.18
12	10	F	0.80	13.00	22.31
13	41	M	0.90	9.93	16.93
14	30	M	0.83	11.06	18.00
15	25	F	0.89	11.12	23.37
16	25	F	0.88	17.68	33.12
17	6	F	0.93	14.37	21.81
18	36	F	0.92	11.56	21.06
High values of creatinine					
1	75	F	3.53	10.68	22.62
2	75	F	3.33	8.87	21.43
3	45	F	11.59	18.25	50.81
4	45	F	4.49	25.81	56.25
5	45	M	3.85	11.18	21.56
6	35	F	1.63	20.75	27.75
7	45	F	6.15	11.75	30.00
8	37	F	1.59	7.50	18.06
9	32	M	2.38	8.43	23.12
10	80	M	2.14	20.06	36.75
11	45	M	1.70	13.81	20.56
12	40	F	2.01	9.75	24.68
13	65	F	1.91	12.68	24.68
14	40	M	10.39	11.56	22.87
15	66	M	8.3	9.31	25.62
16	20	F	2.01	10.0	24.50
17	50	M	8.70	9.87	22.62
18	40	M	2.47	10.87	25.75

Discussion

Total NO production can be assessed using the stable oxidation products of NO (NO_2+NO_3 = NO_X), although this is only valid under conditions of dietary NO_X control (41). It has been suggested that the physiologically important vasodilator nitric oxide (NO) is deficient in chronic progressive renal disease (CRD) and in end-stage renal failure (ESRD) (42, 43). This could result from substrate (arginine) deficiency (27) caused by a loss of functional renal mass, increased endogenous NO synthase (NOS) inhibitors that accumulate in renal failure (43), and/or other causes, such as increased oxidative stress (44). In addition to being caused by CRD, low NO production may contribute to and/or exacerbate the progression of CRD by both hemodynamic and renal growth-promoting actions (45). Wever *et al.,* (26) also concluded that NO production was decreased in humans with CKD. Most evidence suggests decreased total NO production in humans with renal disease, although there is one report of increased arginine-to-citrulline conversion in humans with ESRD (46). This might reflect activation of inducible NOS (iNOS) by dialysis in this particular population. In the absence of acute inflammatory events, decreased total NO production (measured by urinary NOx output) has also been reported in different animal models of CKD, including renal mass reduction, chronic glomerulonephritis, chronic puromycin amino nucleoside (PAN) nephritis, and normal aging (27, 29, 30, 33, 34, 37, 39).

NO plays a critical role in many vital biological processes, including the control of vascular tone, neurotransmission, ventilation, hormone secretion, inflammation and immunity. Moreover, NO has been shown to influence a host of fundamental cellular functions, such as RNA synthesis, mitochondrial respiration, glycolysis and iron metabolism. Moreover, impaired NO production has been implicated in the pathogenesis of volume-dependent hypertension. This duality of NO's beneficial and detrimental effects has created extraordinary interest in this molecule and the need for a detailed understanding of NO biosynthesis (47).

Although it has been suggested that NO may be important in the pathophysiology of chronic renal diseases, the precise molecular mechanisms involved have not been elucidated. Impaired NO synthetic pathway could have a key role in modulating the complex renal hemodynamic disorders associated with the progression of renal diseases. Data are also available showing, the drugs capable of enhancing renal NO activity may be protective in a variety of renal diseases (48). As a molecule with myriad of activities, NO has many physiological and pathophysiological implications for the renal patient. NO is actively produced in the medulla and the cortex of the kidney. Under conditions, it is synthesized mainly by eNOS and nNOS, and is important for regulating microcirculation and inhibiting platelet adhesion (49, 50).

Localization of NOS activity in the kidney were based on indirect evidence for biosynthesis obtained by measurement of nitrite, nitrate and cGMP levels; specificity for NOS can be assessed by L-arginine dependent activation, cytokine and endotoxin stimulations and or selective pharmacologic inhibition with structural analogoues L-arginine (51, 53). From these it was suggested that renal NO production was not only derived from endothelial cells, but also from smooth muscle cells, mesangial cells, and tubular epithelial cells.

NO is involved in renal physiology and pathophysiology. Endothelium derived NO may relax vascular smooth muscle by activation of soluble guanylatecyclase, which leads to intracellular accumulation of cGMP (54).

Pharmacologically, the enzymatic synthesis of NO can be inhibited by the structural analogues of L-Arginine, leading to an increase in renal vascular resistance and a decrease in the renal blood flow, urine flow and sodium excretion (55). NO has an important role in the local regulation of glomerular arteriolar resistance. Imig and Roman (56) suggested that NO primarily alters afferent vascular tone, thereby modifying the ability of the pre-glomerular vasculature to autoregulate glomerular capillary pressure. Similarly, Deng and Baylis (57) stated that local NO controls afferent arteriolar resistance, whereas efferent resistance is not under tonic control by NO. Endogenous NO may selectively modulate afferent arteriolar angiotensin II actions. It reacts with renin-angiotensin system to control glomerular arteriolar resistance. NO counteracts elevated angiotensin II levels to regulate perfusion of the kidney (58). It has been proposed that NO is involved in mediating the myogenic response of autoregulation (58). The presence of NOS I in the macula densa was thought to be related to the macula densa dependent release of rennin (59).NO may participate in the tubular effects that regulate the pressure natriuresis and diuresis in response to volume changes (60). It appears that NO contributes to renal volume control via a tubular effect, possibly by directly influencing tubular reabsorption (61).

It has been suggested that a deficiency in the synthesis of NO may constitute an important factor in the development of systemic hypertension, as NO interferes with the ability of the kidney to excrete sodium and water and because NO is thought to mediate the renal responses to volume expansion. NO blockade causes reduction of sodium excretion (62). There is evidence indicating that renal NO deficiency occurs in patients with CRF. Significant reduction of thus daily excretion of urinary nitrate/nitrite was significantly lower in patients with moderate and severe renal failure, as compared to those with mild renal failure and controls; the lowest values were found in severe renal failure group has been reported (22). Elevated serum NO could at least in part be due to the declining renal function, due to its increased endogenous production by the kidneys, could be largely be due to in situ production during dialysis.

This study would therefore be useful to determine if there are any changes in the levels of NO in individuals who have high levels of creatinine. We evaluated the levels of nitrite and nitrate in the serum and we could not found any correlation between nitric oxide and creatinine levels. Our study has been supported by the previous study by Ochoa et al and Evans *et al.,* (63, 64). There is not much study has been done to explore the association between serum nitric oxide and creatinine levels. Further, detailed study is required to explore the role of nitric oxide in different clinical stages of renal diseases.

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