ERYTHROCYTE TRANSMEMBRANE FLUX AND MEMBRANE TRANSPORT ABNORMALITY IN PATHOGENESIS OF HYPERTENSION LEADING TO NEPHROLITHIASIS

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Abstract: The epidemiological relationship between nephrolithiasis and hypertension is well-known. Patients with hypertension are at increased risk for nephrolithiasis and those with nephrolithiasis are at risk for hypertension. An anomaly in RBC Oxalate transport and reduced activities of adenosine triphosphatases has been reported in patients with hypertension when compare to control subjects. This study presents an abnormal increase in transmembrane flux of oxalate in RBC of hypertensive subjects and it might be due to membrane degradation caused by oxalate-induced free radicals depleting erythrocyte thiol contents and impaired adenosine triphosphatases activity resulting in tissue injury and defective membrane transport. Thus relative risk of hypertension was significantly associated with increased oxalate flux rate and impaired adenosine triphosphatases activity in stone formers. This association is important when treating patients with nephrolithiasis since those with hypertension may require unique dietary and medical therapy.

Keywords: Nephrolithiasis, Hypertension, Transmembrane flux, Adenosine triphosphatases, Erythrocytes.

1. INTRODUCTION

Essential hypertension is characterized by significant and persistent elevations in arterial pressure. Hypertension is a multifactorial disorder that may involve abnormalities in the functions of the heart pump, the blood vessels, and the kidneys. Short-term and long-term regulation of arterial pressure is influenced by changes in cardiac function, the peripheral vascular resistance, and the renal control mechanisms of plasma electrolytes and volume. Increases in the heart rate and stroke volume lead to increases in the cardiac output and could contribute to increases in arterial pressure particularly in relatively young individuals
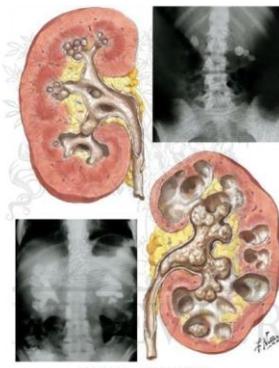
ASSOCIATION BETWEEN HYPERTENSION AND KIDNEY STONE DISEASE

The prevalence of nephrolithiasis has been reported to be 30% to 79% in hypertensive than normotensive subjects. Alterations in calcium metabolism may play an important role in the pathgenesis of both hypertension and nephrolithiasis and have been suggested as plausible mechanism linking this to disorder (Starzzullo and Mancini 1994). A higher prevalence of hypercalciuria has been repoted in patients with essential hypertension and alterations of calcium metabolism such as primary hyperparathyroidism that lead to hypercalciuria have been associated with a increased prevalence of hypertension. Several other mechanisms that may link hypertension and nephrolithiasis have been suggested, which include high dietary intake of sodium, low intake of potassium and renal damage.

PERTURBATION IN THE OXALATE METABOLISM AND ITS ROLE IN NEPHROLITHIASIS

The most common types of kidney stones are composed predominantly of calcium oxalate. A smaller proportion is composed mainly of calcium phosphate. Many stones can be mixtures of calcium oxalate and phosphate, but usually oxalate will predominate.

Oxalate is a major component of kidney stones and represents a relevant risk factor in calcium oxalate disease. Several studies have evaluated the importance of urinary oxalate as a determinant of calcium oxalate precipitation. Changes in urinary oxalate concentration are more relevant than calcium variations determining urine over saturation with calcium oxalate. The hyperoxaluria is usually secondary to high dietary oxalate intake caused by ingestion of foods or liquids containing large quantities of oxalate. Some of these foods and liquids include baked beans, collard greens, green beans, rhubarb, tea, cocoa, peanut butter, and vegetable soup. In other cases, the hyperoxaluria occurs in the setting of gastrointestinal malabsorption, seen in patients with inflammatory bowel disease. When patients malabsorb fat, dietary calcium binds to the fat rather than to dietary oxalate, which is the norm. This results in a larger amount of unbound intestinal oxalate that passes into the colon, from which it is absorbed. In patients with ileostomies (colon excluded), this enhanced oxalate absorption does not occur. A rare cause of hyperoxaluria is the inherited condition known as primary hyperoxaluria. 9 In this condition, the hepatic enzyme that converts glyoxylate to glycine is deficient. As a result, there is increased production of oxalate from glyoxylate calcium oxalate.

The scope of this study is to diagnose oxalate flux rate and ATPhase activity of RBC in hypertensive subjects before the condition become worse which progress to Nephrolithiasis.

2. MATERIAL AND METHODS

Hypertensive patients were those from the OPD of hypertensive clinic, department of general medicine, Royapettah Medical College, Chennai.

Patients who exhibited a systolic pressure of >=160mmHg and / or a diastolic pressure of >=95mmHg were included in the present study and the hypertensive patients selected for the study were receiving regular drug treatment for high blood pressure. These patients were established to be essential hypertensives.

The exclusion criteria which established the patients to be essential hypertension excluded patients who presented hypertension as a secondary cause: - Diabetes Mellitus, Acute Respiratory Distress Syndrome, Acute Myocardial infarction, Chronic renal failure, Bronchial asthma, Cerebrovascular accidents, Inflammatory bowel disease, Rheumatoid arthritis, Smokers, Alcoholics, Aged people who were above 60 years and cases who had Cirrhosis, Cholelithiasis, Insecticides exposure, Drugs- thyroid replacements, Antidepressants, Nonsteroidal anti-inflammatory drugs[NSAIDS], Allopurinol.

10ml of bloods was collected by vein puncture from the antecubital vein from normal and hypertensive person and immediately transferred to stoppered tube containing 0.1 ml heparin as anticoagulant and transported to lab using a cool container.

OXALATE EXCHANGE STUDIES IN THE ERYTHROCYTE MEMBRANE.
Oxalate exchange studies were determined by the method of Baggio.
REAGENTS
1.   Tris HCl 20mM
2.   Potassium chloride 10mM
3.   Sodium Chloride 150mM
4.   Reagents 1,2 and 3 were mixed and the pH was adjusted to 7.4
5.   Sodium oxalate 10mM
PROCEDURE
10 ml blood samples were washed 3 times in a solution of sodium chloride, potassium chloride and Tris-HCl. They were resuspended to a haematrocrit of 50% in the same solution. The intact RBC was then supplemented with sodium oxalate and incubated at room temperature for 2hrs, after centrifugation, the RBC was resupended to a volume to yield an equivalent of 20% haematocrit in the same solution and subdivided into many fractions to which a tracer amount of 14C-oxalate(8000) was added. After 10, 20,40,60,90 and 120 min, a fraction was centrifuged and the 14C-oxalate activity of the supernatant was counted.
The flux rate was calculated according to the formula:
ln(at-aa)= ln(at-aa)-K

PHOSPHATASES

Na+ K+ - Adenosine triphosphatase

$Na^+ K^+$ - Adenosine triphosphatase activity was assayed by the method of Bonting(1970)

REAGENTS

1. Tris-Hydrochloric acid buffer : 184mM, pH7.5.
2. Magnesium sulphate   :50mM
3. Potassium Chloride   :50mM
4. Sodium Chloride   :600mM
5. EDTA: :1mM
6. Adenosine triphosphate   :40mM
7. Trichloroacetic acid   :10%
8. Ammonium molybdate   :2%
9. Amino napthol sulphonic acid:0.5g of ANSA was dissolved in 5ml of 20% Na_2SO_3 and then 195ml of 15% sodium metabisulphite was added, stirred well, filtered and stored in brown bottle.
10. Standard Phosphate:35.1mg Potassium dihydrogen phosphate was dissolved in 100ml of distilled water, which had a concentration of 80µg phosphorus/ml.

PROCEDURE

The incubation mixture containing 1ml of Tris hydrochloric acid buffer, 0.2ml each of magnesium sulphate, potassium chloride, sodium chloride, EDTA, adenosine triphosphate and erythrocyte membrane. The mixture was incubated at 37°C for an hour and the reaction was arrested by the addition of 1ml of 10% Trichloroacetic acid and mixed well, centrifuged. The control tubes received enzyme after incubation. The phosphate content of the supernatant was estimated by the method of Fiske and Subbarow.

0.5ml of ammonium molybdate and 0.2ml of ANSA was added and mixed well. The readings were taken after 20 minutes at 650nm.

The enzyme activity was expressed as micromoles of phosphate liberated/mg protein/hour.

Ca^{2+} Adenosine triphosphatases
Ca^{2+} Adenosine triphosphatases acitivity was assayed by the method of Hjerten and Pan,

REAGENTS

1. Tri Hydrochloric acid   : 125mM, pH 8.
2. Calcium Chloride   : 50mM
3. Adenosine triphosphate   : 10mM
4. Trichloro acetic acid   : 10%

PROCEDURE
The incubation mixture containing 0.1ml each of Tris Hydrochloric acid buffer, calcium chloride, adenosine triphosphate and erythrocyte acid buffer, calcium chloride, adenosine triphosphate and erythrocyte membrane and was incubated at 37°C for an hour and the reaction was arrested by the addition of 1ml of 10% Trichloroacetic acid and mixed well, centrifuged.

The phosphate content of supernatant was estimated by the method of Fiske and Subbarow as described.

$Mg^{2=}$ Adenosine triphosphatases

$Mg^{2=}$ Adenosine triphosphatases activity was assayed by the method of Ohnishi.

REAGENTS

1. Tris hydrochloric acid buffer   : 37mM, pH 7.6
2. Magnesium Chloride   : 25mM
3. Adenosine triphosphate   : 10mM
4. Trichloroacetic acide   : 10%

PROCEDURE

The assay was initiated by the addition of 0.1ml of enzyme to an incubation mixture containing 0.1ml each of Tris hydrochloric acid buffer, magnesium chloride and adenosine triphosphate. The mixture was incubated at 37°C for an hour and the reaction was arrested by the addition of 1ml of 10% Trichloroacetic acid and mixed well, centrifuged. The phosphate content of supernatant was estimated by the method of Fiske and Subbarrow as described earlier.

The enzyme activity was expressed as micromoles of phosphate liberated/mg protein/hour.

Total Adenosine Triphosphatase

Total Adenosine triphosphatase activity was assayed by the method of Evans.

REAGENTS

1. Tris Hydrochloric acid   : 0.1M, pH7.4
2. Magnesium chloride   : 25mM
3. Adenosine triphosphate   : 0.01M
4. Trichloro acetic acid   : 10%
5. Potassium Chloride   : 50mM
6. Sodium Chloride   : 600mM

PROCEDURE

The assay was initiated by the addition of 0.1ml enzyme to an incubation mixture containing 1.5ml of Tris-Hydrochloric acid buffer, 0.1ml each of magnesium chloride, adenosine triphosphate, sodium chloride and potassium chloride. The mixture was incubated at 37°C for an hour and the reaction was arrested by the addition of 1ml of 10% Trichloroacetic acid and mixed well, centrifuged. The phosphate content of supernatant was estimated by the method of Fiske and Subbarrow as described earlier.

The enzyme activity was expressed as micromoles of phosphate liberated/mg protein/hour.

3. RESULT ANDE DISCUSSION

Fig:1 ^{14}C OXALATE FLUX IN ERYTHROCYTE OF CONTROL AND ESSENTIAL HYPERTENSIVE SUBJECTS.
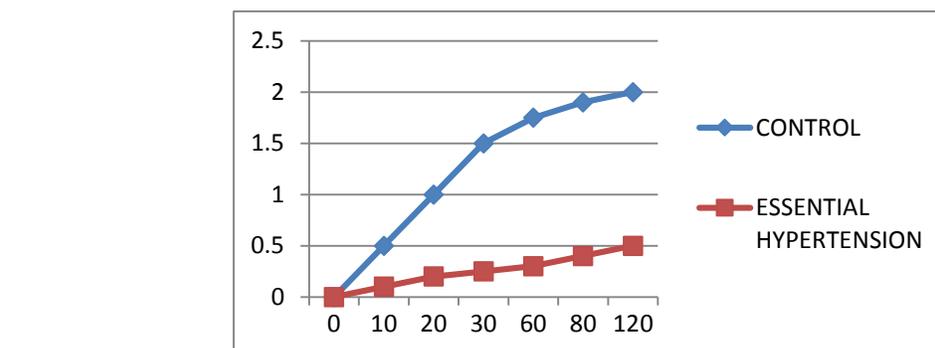

Fig 1 showed the erythrocyte flux rate of control subjects and hyoertensive subiects. Hypertensive subjects show 90% increase in flux rate (0.6Kt/min to 1.2Kt/min)than the normal counterparts at 20 minutes.

TABLE 1: MEMBRANE PHOSPHORYLASES IN CONTROL AND ESSENTIAL HYPERTENSION

PARTICULARS	CONTROL	ESSENTIAL HYPERTENSION
Na$^+$ ATPase	0.52±0.052	0.43±0.01***
Ca^{2+} ATPase	0.26±0.026	0.22±0.02***
Mg^{2+} ATPase	0.89±0.089	0.74±0.03***
Total ATPase	1.6±0.16	1.29±0.04***

Comparisons made between control and essential hypertensive subjects values are statistically significant when *p<0.05, **p<0.001 p<0.001.

Table 1 presents the activity of the ATPases of the respective ions as well as the total ATPases activity all the ATPases were found to be significantly decreased among the hypertensive subjects.

The importance of oxalate in calcium oxalate stone formation can be deduced from studies showing that even slight increase in oxalate can lead to significant changes in oxalate concentration and is 15 times more potent than a similar increase in transmembrane flux of oxalate has been found in red blood cells of stone formers and this process is mediated by band-3-protein which act as an anion exchanger and is adenosine triphosphate dependent. This study presents oxalate flux rates among hypertensive subjects the enhanced oxalate flux rates among the hypertensive subjects might be due to membrane degradation caused by oxalate-induced free radicals depleting erythrocyte thiol contents and impaired adenosine triphosphatases activity resulting in tissue injury and defective membrane transport.

RBC membranes have been shown to have three different adenosine triphosphatases namely Na^+ K^+ adenosine triphosphatase, Ca^{2+} adenosine triphosphatase, Mg^{2+} triphosphatase which are responsible for the transport of respective ions. All these membrane phosphorylase are ion specific adenosine triphosphatases involved in the transport are lipid as well as thiol dependent and membrane bound in mammalian tissues. The functional states of these enzymes are dependent on the presence of specific phospholipids that are attached to proteins of the membrane. Reduced activities of adenosine triphosphatases have been shown in various pathological conditions due to lipid peroxidation.

Hypertension has been proposed as an independent risk factor for nephrolithiasis. Inhypertensive patients, red blood cell (RBC) transmembrane flux is altered and total ATPases activity is decreased. Such a changes might be involved in the physiopathology of hypertension and therefore be the link between hypertension and nephrolithiasis

4. CONCLUSION

Hypertensive patients had higher oxalate flux in erythrocytes and impaired adenosine triphosphatases activity due to membrane degradation caused by oxalate induced free radical depleting erythrocyte thiol contents resulting in tissue injury and defective membrane transport. Hence the deduction of alteration in membrane ATPhases and increased transmembrane flux of RBC in hypertensive patients could be used as early marker for the diagnosis of nephrolithiasis

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