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Major Constituents, Free Amino Acids and Metal Levels in Renal Calculi from Multan Region

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Abstract: Renal calculi collected from adult male/female patients of Multan, Pakistan were analyzed for major constituents, free amino acids and metallic contents. Composition of calculi (whole powder) was determined by chemical method using stone analysis kit. Thirteen distinct types of renal calculi were identified. Most of the calculi were compound in nature, however, some simple calculi containing calcium oxalate only were also found. Two dimensional descending paper chromatography was used to identify free amino acids in some prominent classes of renal calculi. Arginine, aspartinine, glycine, leucine, isoleucine and tryptophan were found in nearly all the calculi whereas glutamic acid, histidine, hydroxyproline, phenylalanine, threonine and tryosine occurred randomly. Analysis of major (Na, K, Ca and Mg) and trace (Fe, Zn, Cu, Ni, Pb and Mn) elements in the calculi was carried out by flame atomic absorption spectrometry. Elements such as Na, K, Ca, Mg, Fe and Zn have shown their presence in nearly all the calculi however Pb, Ni, Mn and Cu levels were below the limit of detection. This study has revealed useful information about the chemical nature of renal calculi from Multan region. It will be helpful in adopting preventive strategies to minimize stone formation and their reoccurrence.

Key words: Major constituents, free amino acids, metals, renal calculi, Multan region

Introduction

Renal calculi are the solid masses of sediments formed in some parts of the urinary tract such as kidney and urinary bladder. This condition is known as lithiasis (Oser, 1965). It is one of the known major health problems in human history all over the world which can be traced back many centuries ago. Renal calculi are generally divided into two categories, i.e., simple and compound calculi. Simple calculi are made up of single constituent (i.e., calcium oxalate or uric acid etc.) whereas compound calculi are those which are made up of two or more individual constituents (i.e., calcium oxalate, uric acid, cystine, tricalcium phosphate, magnesium ammonium phosphate etc.). Several causes have been suggested for renal stone formation including dietary deficiency, altered concentration of solutes and solids in urinary solution, decreased urinary output of citrate, renal infection and inadequate urinary drainage.

Urinary tract stones are very common in Pakistan. Their observed prevalence is high in the Southern Punjab (Khan *et al.*, 1990; Sial *et al.*, 1995; Rafique *et al.*, 2000). Most of the stone formers belong to low socio-economic groups (Zafar *et al.*,1992). Multan is situated in the southern part of the Punjab province of Pakistan. It has very hot and dry climate. It has a population of around two million people. Majority of the inhabitants live in rural areas. Every year a significant number of patients are admitted in the Urology department of the Nishtar Hospital, Multan for treatment.

This study was aimed to find out the chemical composition of renal calculi of residents of Multan region of Pakistan with particular reference to major constituents, free amino acids and metal contents in order to draw useful conclusions about stone formation processes and to develop preventive measures for stone formation and its recurrence.

Materials and Methods

Background of the renal calculi: Thirty six samples of renal calculi were collected from the adult patients of different sex and age admitted to the Urology Ward of Nishtar Hospital, Multan, Pakistan during December 1993 to November 1994. Prior to chemical analysis, calculi were washed thoroughly with chloroform and then rinsed with distilled water to remove blood stains from their surfaces. Samples were dried in a pre-heated oven (Memmert, Germany). The dried samples were crushed and powdered in agate pestle and mortar and then stored in a desicator. Major constituents of the renal calculi were determined semi-quantitatively by chemical method using Merckogonost kit prepared by E. Merck.

Extraction and analysis of free amino acids: Sample solution was prepared following the method reported earlier (Khaskhali *et al.*,1993) as: " 0.5 g of the stone powder was taken in 50 ml Erlenmyer flask and 5 ml of ethyl alcohol (75%) was added to it. The contents of the reaction mixture were washed with 2 ml of hot ethanol. The filtrate was then used as a sample solution for qualitative analysis of amino acids. Two dimensional descending paper chromatography was performed using two different solvent systems I) n-Butanol: Acetic acid-water (4:1:5 v/v) and ii) Phenol-water (4:1 w/v). The paper chromatograms were sprayed with ninhydrin reagent to locate amino acids. Unknown amino acids were identified by comparing their Rf values with those of standards applied.

Metal analysis: Analytical reagent grade chemicals were used without further purification. Deionized water was used to prepare standard and sample solutions throughout this work. Atomic absorption measurements were made with a atomic absorption spectrometer (Hitachi A-1800, Japan).

Standard stock solutions (1000 mg l^{-1}) of each element were prepared by dissolving respective salts supplied by Merck in deionized water. Dilutions of the stock solutions were made to prepare working acidic standards (0.1M HNO_3).

Sample solution was prepared as " 0.1 g of dried powder sample, 2 mL of $HClO_4$: HNO_3 (1:1) mixture was added. Sample mixture was left overnight for digestion. Sample was then heated slowly to near dryness. Then 5 mL of HNO_3 was added to each sample and filtered the solution (if necessary) and diluted upto 50 mL with deionized water (further dilutions were also made in some cases)". Sample solutions were aspirated into atomic absorption spectrophotometer and absorbance measurements were made for each element. Elemental analysis was carried using specific instrumental conditions (Table 1). Calibration of the instrument was repeated periodically during operation. Metal concentrations were computed using computer.

Table 1: Instrument conditions for metal analysis by flame atomic absorption spectrometry.

Element	Wavelength (nm)	Lamp current (mA)	Flam composition	Detection limit mg l^{-1}
Ca	422.7	7.5	air : C_2H_2	0.200
Mg	285.7	7.5	air : C_2H_2	0.004
Na	589.0	10	air : C_2H_2	0.003
K	766.5	10	air : C_2H_2	0.010
Fe	248.3	10	air : C_2H_2	0.400
Ni	232.0	10	air : C_2H_2	0.300
Mn	279.6	7.5	air : C_2H_2	0.100
Cu	324.8	7.5	air : C_2H_2	0.040
Zn	213.8	10	air : C_2H_2	0.010
Pb	283.3	7.5	air : C_2H_2	0.100

Results and Discussion

Renal stone formation processes can be best understood after studying chemistry of the calculi. In this study, renal calculi obtained from the patients of Multan region have been analyzed for their major constituents, free amino acids and trace metal contents. Based on major constituents, thirteen distinct types of renal calculi have been identified in the patients of Multan region. Table 2 presents details about the major constituents (with % wt of each component) in different types of renal calculi. Most of the calculi were compound calculi with varying concentrations of major constituents such as calcium oxalate, uric acid, cystine, tricalcium phosphate, magnesium ammonium phosphate, ammonium urate etc. however, some simple calculi containing calcium oxalate only were also found. Similar types (classes A, B, C, D, F & G reported in this work) of calculi obtained from other areas of Pakistan have also been reported by other workers (Afzal *et al.*, 1992; Rafique *et al.*, 2000). Reported data on chemical composition of upper renal tract calculi of Multan showed that majority of stones were uric acid calculi (28.1%), followed in frequency by calcium oxalate calculi (26.1%), mixed calculi containing calcium oxalate and uric acid (21.8%) and calculi containing calcium oxalate and calcium phosphate (10.4%). Other varieties of calculi were reported to be less common. Presence of calcium oxalate in almost all the stones may be attributed to the dietary habits of inhabitants of Multan region. Vegetables (spinach, cabbage & tomato etc.), milk and milk products are the main components of food menu of the local people. Such vegetables are rich in contents of oxalate and its precursors (Contarow & Schepartz, 1967). Qualitative analysis of free amino acids in the prominent classes of renal calculi was carried out for 19 amino acids by two dimensional descending chromatography. Presence of 9 to 13 different free amino acids was observed in these samples (Table 3). Arginine, aspartinine, glycine, leucine, isoleucine and tryptophan were found in nearly all the calculi whereas glutamic acid, histidine, hydroxyproline, phenylalanine, threonine and tryosine occurred randomly.

Presence of relative pattern of free amino acids in different types of stones using high speed amino acid analyzer has been reported. However, the individual amino acid patterns varied

Table 2: Types of renal calculi found in patients of Multan region

Class	No. of Calculi	Major constituents * (% wt)
A	6	Ca-Ox (83-100 %)
B	4	Ca-Ox (17-66%) + Urate (5 %) + UA (40-60 %)
C	6	Ca-Ox (17-83 %) + Struvite (10-30 %)
D	2	Ca-Ox (17 %) + Struvite (50 %) + Cystine (10-20 %)
E	1	Ca-Ox (17 %) + UA (35 %) + Urate (15 %) + Cystine (20 %)
F	4	Ca-Ox (83 %) + Cystine (10 %)
G	5	UA (40 %) + Cystine (10-20 %)
H	3	Ca-Ox (17-50 %) + UA (10-40 %) + Cystine (10-20 %)
I	1	Weddellite (83 %) + Whitlokite (12 %)
J	1	UA (13 %) + Urate (31 %) + Cystine (10 %)
K	1	Weddellite (66 %) + Struvite (10 %) + Whitlokite (18 %)
L	1	Weddellite (17 %) + Whitlokite (2 %) + Brushite (4 %) + UA (31 %) + Urate (10 %) + Cystine (20 %)
M	1	Weddellite (17 %) + Struvite (30 %) + Whitlokite (16 %) + Brushite (28 %)

*Presence of more than one constituents is indicated by + sign

Ca-Ox = Calcium oxalate monohydrate

UA = Uric Acid

Urate = Ammonium acid urate

Struvite = Magnesium ammonium phosphate hexahydrate

Weddellite = Calcium oxalate dihydrate

Whitlokite = Tricalcium phosphate

Brushite = Calcium hydrogen phosphate dihydrate

Table 3: Occurrence of free amino acids in some prominent classes of renal calculi from Multan region.

Amino acid	Classes of renal calculi					
	B	C	D	F	G	K
Leucine	+	+	+	+	-	+
Isoleucine	+	+	+	+	-	+
Tryosine	-	+	-	-	-	+
Glutamic acid	+	+	-	-	-	-
Glycine	+	+	+	-	+	+
Histidine	+	+	-	+	+	-
Proline	-	-	-	-	-	-
Theronine	+	-	+	-	+	+
Aspartic acid	+	+	-	+	+	+
Lysine	+	+	-	+	-	+
Alanine	-	-	+	-	-	+
Valine	+	-	-	+	+	+
Cystine	-	-	+	+	+	-
Methionine	-	+	-	+	-	-
Phenylalanine	+	+	-	-	+	-
Hydroxyproline	-	+	+	+	-	-
Arginine	+	+	-	+	+	+
Aspartinine	+	+	+	+	+	-
Tryptophane	+	-	+	+	+	+
Total	13	13	9	12	10	11

+ Present; − Absent.

Table 4: Metal concentrations in different classes of renal calculi from Multan region.

Class	Calculi (n)	Metal concentrations * (% wt)					
		Na	K	Ca	Mg	Zn	Fe
A	6	0.102±0.050	0.062±0.028	37.679±8.615	0.088±0.050	0.037±0.036	0.021±0.007
B	4	0.046±0.024	0.041±0.005	7.837±9.002	0.025±0.024	BDL	0.016±0.005
C	6	0.267±0.346	0.106±0.080	23.578±12.874	0.098±0.075	0.009±0.016	0.020±0.005
D	2	0.342±0.047	0.212±0.032	11.282±1.600	0.248±0.003	0.025±0.006	0.020±0.001
E	1	1.473	0.089	8.200	0.055	0.002	0.027
F	4	0.089±0.016	0.060±0.016	33.472±4.145	0.073±0.028	0.003±0.003	0.017±0.006
G	5	0.062±0.051	0.072±0.058	5.069±9.332	0.033±0.034	0.034±0.038	0.017±0.006
H	3	0.041±0.011	0.052±0.036	10.462±10.726	0.021±0.011	0.001±0.001	0.024± 0.008
I	1	0.126	0.193	32.038	0.183	0.018	0.020
J	1	0.054	0.038	0.362	0.015	BDL	0.016
K	1	0.388	0.124	32.117	0.208	0.079	0.028
L	1	0.054	0.426	0.457	0.065	BDL	0.007
M	1	0.147	0.231	4.375	0.241	0.025	0.010

* Mean ± Standard deviation; BDL Below Detection Limit.

according to the types of the stone analyzed (Khaskhali *et al.*, 1993). The frequent occurrence of amino acids and uric acid in renal calculi may be due to disturbance in purine metabolism and also due to high intake of dietary proteins mainly in the form of meat, liver and kidneys. It is suggested that the residents of this area in general and patients in particular should change their dietary habits and eat less meat.

Hot and dry weather conditions of Multan region, may be considered as a major contributory factor in stone formation in the local people. Usually the Summer prolongs for about 7 to 9 months due to which labourers / poor people, while working in hot weather conditions, loose excessive amount of the body water through perspiration. It may result in over saturation of the urine causing crystal aggregation and eventually stone formation. To avoid over saturation of urine, it is suggested that the required daily water intake, i.e., 2300 ml per day for an adult (Guyton & Hall, 1996) or more should be observed especially in hot weather conditions.

Although most mechanisms concerning the behaviour of trace elements in biological systems are far from being understood, many researchers are convinced that trace elements play a major role in causing disease such as urolithiasis. The quantitative determination of trace elements in calculi is essential for the understanding of their aetiology. It is now accepted that the crystallization processes occurring during the formation of stones are influenced by these elements, even if these are present in minute concentrations only (Meyer & Angino, 1977; Hesse *et al.*, 1978). Analysis of major (Na, K, Ca and Mg) and trace (Fe, Zn, Cu, Ni, Pb and Mn) elements in the calculi was carried out by flame atomic absorption spectrometry. Table 4 shows variable metal concentration levels in different types of renal calculi. Metals such as Na, K, Ca, Mg, Fe and Zn have shown their presence in nearly all the calculi however Pb, Mn, Ni and Cu levels were below the limit of detection. Very low levels of trace metals in these samples suggest the use of a highly sensitive analytical method such as GFAAS or ICP-MS.

Renal stones are usually thought to form by nucleation of the nidus from a supersaturated urine followed by the growth of the nidus through processes of crystal growth (Robertson, 1973; Pak & Ruskin, 1970) epitaxial growth (Neuman & Neuman, 1958) and crystal aggregation (Harding *et al.*, 1984). Some workers studied the formation of low molecular weight complexes of metal ions of Ca, Cu, Fe, Pb, Mg and Zn with carbonate, oxalate, phosphate, citrate, lactate and amino-acids (histidine, cystine etc. in the plasma of healthy human (Fiabance & Williams, 1977). These results also indicate that calculi containing higher amounts of oxalate and cystine (classes D, E & G) show the presence of high concentrations of major and trace metals. This may be attributed to the greater complexing ability of these chemical species towards metal ions. Some workers have analyzed urinary and biliary calculi using several modern physical techniques and concluded that the growth of calculi, i.e., the nucleation is heterogeneous and is initiated by trace elements (Conville, 1981, Schulz *et al.*, 1982). Further research work is needed to establish correlation between trace metals and free amino acids in the renal calculi.

Information obtained from this study permits more accurate diagnosis of kidney stone disease. This information would be quite helpful in suggesting the preventive measures against renal stone formation in the people of Multan.

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