

Calcium Renal Stone in Relation to Salivary and Urinary Constituents

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ABSTRACT

Background: Renal stone which is actually renal calculi commonly termed as kidney stone may contribute to the development of chronic kidney disease and its incidence is rising rapidly; this study conducted to investigate the ability of using saliva as an indicator of susceptibility to calcium renal stone formation by investigating some salivary and urinary constituents among patients with idiopathic calcium renal stone then comparing the results with healthy looking subjects.

Materials and Methods: The study group selected of thirty patients with idiopathic calcium renal stone with an age range (25-30) year's old and 30 gender and age matched healthy looking subjects selected as control. Stimulated salivary samples in addition to fasting second morning urinary samples collected then chemically analyzed to determine the concentrations of salivary and urinary calcium, phosphate, magnesium in addition to urinary creatinine.

RESULTS High significant elevation in the concentration of salivary phosphate with high significant reduction in salivary magnesium concentration recorded within study group compared control one while for salivary calcium concentration, found higher in study group but with no significant difference between them. The calcium stone formers found to have no hypercalciuria but they had higher level of urinary phosphate/creatinine ratio with high significant difference compared with the non stone formers while they had a high significant reduction for urinary magnesium/creatinine ratio compared with the control group.

Conclusion: Saliva may provide an investigative tool for calcium renal stone disease by using the salivary magnesium concentration as an indicator of susceptibility to calcium renal stone formation for both males and females.

Key Words: Saliva, salivary composition, idiopathic calcium renal stone, kidney stones, urolithiasis.

INTRODUCTION

Renal stone is a major cause of morbidity due to its association with renal colic, urinary tract obstruction, urinary tract infection, and increase the risk of: Renal parenchymal damage ⁽¹⁾, bone disease, and hypertension. Although many inherited and systemic diseases are associated with calcium renal stones, most of such stones are idiopathic ⁽²⁾. The process of stone formation is complex. It begins with urine that becomes supersaturated with stone-forming salts, such that dissolved ions or molecules precipitate and form crystals or nuclei. Once formed, crystals may flow out with the urine or become retained in the renal system at anchoring sites that promote growth and aggregation leading to stone formation ⁽³⁾ that the type of stone formed correlates with the supersaturations found in the urine ⁽⁴⁾. Calcium-containing renal stones make up 90% of all stones and are generally composed of a mixture of calcium oxalate and calcium phosphate. In mixed stones, calcium oxalate usually predominates; pure calcium oxalate stones are more common than pure calcium phosphate stones ^(1, 5).

Saliva is the glandular secretion which constantly bathes the teeth and the oral mucosa. It is consti-

tuted by the secretions of the three paired major salivary glands (parotid, submandibular and sublingual) and minor salivary glands; the complex physical and chemical composition of salivary secretion performs a considerable number of protective functions which are part of the total body's ability to maintain homeostasis ⁽⁶⁾. Saliva through its flow rate and constituents may play an essential role in maintaining the integrity of soft and hard tissues in the oral cavity ^(7, 8). Many oral and systemic conditions manifest themselves as changes in the flow and composition of saliva ⁽⁹⁾. Saliva represents a useful auxiliary means of diagnosis that used to diagnose systemic illnesses, monitoring general health, and as an indicator of risk for diseases creating a close relation between oral and systemic health ^(9, 10).

MATERIALS AND METHODS

In the present study, the study group composed of thirty patients (15 females and 15 males) with an age range (25- 30) years according to the last birthday ¹¹. They were diagnosed as having calcium renal stone (in renal pelvis, the ureter, or the bladder) based on new X-ray with general urine examinations; they were attending the Specialized Surgeries Hospital in

Baghdad city for their treatment; the design of the study was illustrated in Figure 1. The study group was fulfilled the following criteria:

- No presence of another medical problem and the cases of pregnancy, bone fractures, immobilization, previous bowel resection and cases under calcium or vitamin D supplements were also excluded. By that the study group was with idiopathic type renal stone.
- The size of renal stone is equal or less than 2 Cm (20 mm).
- The study group presents in fasting condition.

The control group also composed of thirty subjects and they were in healthy condition (normally looking) according to their medical history matching with age and gender the study group, they were subjects who working near and at the same hospital where the study done; the control group was fulfilled the following criteria:

- No history of previous renal stone and without familial history from the first relative degree.
- No presence of serious medical problems.
- Presence of a new ultrasound examination to ensure that there is no renal stone.
- The control group presents in fasting condition.

Collection of Salivary Samples

Stimulated salivary samples ⁽¹²⁾ were collected in this study at (9-12 AM). Each salivary sample was centrifuged at 3000 r.p.m for 10 minutes then the clear supernatants was separated by micropipette and then stored at (-20°C) in a deep freeze till the time of biochemical analysis.

Collection of Urinary Samples

Fasting second morning specimens were collected in which all individuals that participated within this study were in fasting condition from 9 PM on the evening preceding the study. At morning, the subject emptied his bladder (this specimen being discarded) and fasting was continued until second morning specimen taken at (9-12) AM. By using this technique, it can be assumed that the influence of recently ingested food on the excretion is minimal ⁽¹³⁾. Also each urinary sample was centrifuged at 3000 r.p.m for 10 minutes then the clear supernatant was separated by micropipette and then stored at (-20°C) in a deep freeze till the time of biochemical analysis.

Biochemical Analysis

Frozen saliva and urine samples were allowed to thaw and come to room temperature. There after, they were subjected to biochemical analysis for the

common calculi promoters and inhibitor. This was done by using colorimetric method for determination of salivary and urinary (Calcium, phosphate) and urinary Creatinine concentrations while for salivary and urinary Magnesium ions concentration, it was determined by Flame Atomic Absorption Spectrophotometer using standardized procedure by air-acetylene gas. The concentration level of each salivary and urinary constituent was expressed as (mg/dL) unit except for urinary creatinine that was expressed as (g/L) unit and the final expression of urinary constituents' concentration was expressed as a ratio (mg/gm creatinine); in this ratio creatinine serves as a reference standard by virtue of its relatively constant excretion rate throughout the 24 hours ⁽¹³⁾.

Statistical Analysis

Data processing and analysis were carried out using SPSS version 18 (Statistical Package for Social Sciences) which provided calculation and presentation of statistical parameters, means and standard deviation of the means for the biochemical variables examined in the study. The statistical test that used in this study was student's t-test.

* The level of significance was accepted at $P < 0.05$, highly significance at $P < 0.01$ and very highly significance at $P < 0.001$.

RESULTS

Values of inorganic salivary constituents (means and standard deviation) among study and control groups are presented in Table 1. For both study and control groups including males (M) and females (F), phosphate was found to be the highest value followed by calcium and then magnesium. The concentration of phosphate was found higher in study group with statistically very highly significant difference in comparing to control group ($t = 4.87$, $P < 0.001$, $df = 58$) while for concentration of magnesium ions, it was found higher in control group in comparing to study group with also very high significant difference between them ($t = -13.71$, $P < 0.001$, $df = 58$). For calcium ions concentration, it was found higher in study group compared to control group but with no significant difference between them ($P > 0.05$).

Concerning gender differences, salivary phosphate concentration was recorded higher within males than females among study group with a significant difference between them ($t = 2.35$, $P < 0.05$, $df = 28$) while among the control group the difference was statistical-

ly not significant ($P>0.05$) with higher concentration of salivary phosphate within males also. Statistical results revealed that females among study group had higher salivary magnesium concentration than males but with not significant difference ($P>0.05$) while among control group, salivary magnesium concentration within females found to be equal to that within males, so there is no significant difference between them ($P>0.05$). For salivary calcium concentration, it was found higher within males than females among study group in contrast to that found among control group that this concentration found to be higher within females than males with statistically not significant difference among both groups ($P>0.05$).

Mean and standard deviation of urinary constituents which was expressed as a ratio (Calcium, Phosphate and Magnesium)/ Creatinine illustrated in Table 2. The highest mean value of urinary constituents was recorded for phosphate/creatinine ratio which was recorded in the study group with statistically high significant difference compared with control group ($t= 3.59$, $P<0.01$, $df= 58$), in urine this value was followed by magnesium/creatinine ratio which was found higher in control group with very high significant difference compared to study group ($t= -9.26$, $P<0.001$, $df=58$). For calcium/creatinine ratio, it was found higher in study group compared to control group but with not significant difference between them ($P>0.05$).

Concerning gender differences in each group, among study group, within males, statistical analysis found that the mean value of urinary phosphate/creatinine ratio is higher than that of females with statistically highly significant difference ($t= 3.15$, $P<0.01$, $df=28$) in contrast to the control group in which that this ratio recorded higher within female than males but with very high significant difference ($t= -6.21$, $P<0.001$, $df= 28$). For the mean value of urinary magnesium/creatinine ratio, it was recorded to be higher within females than males among study group with statistically high significant difference ($t= -3.10$, $P<0.01$, $df= 28$), among control group this ratio also recorded higher within females than males but with statistically not significant difference ($P>0.05$). Among males within study and control group, it was found that the mean values of urinary calcium/creatinine ratio are higher than females within the two groups with very high significant difference and high significant difference respectively between them ($t= 4.33$, $P<0.001$, $df= 28$; $t= 3.88$, $P<0.01$, $df=28$, re-

spectively).

DISCUSSION

Renal stone has been studied extensively but unfortunately, there was no Iraqi study able to be found about its influence on salivary and urinary composition and to examine the ability of using saliva as an indicator of susceptibility to calcium renal stone formation, for that this study was designed.

The concentration of salivary phosphate was investigated in this study and the result is higher level of salivary phosphate concentration was recorded among calcium stone formers with highly significant differences than non stone formers. The high level of salivary phosphate among calcium stone formers may be interpreted by that phosphate regulation is primarily achieved by the kidneys⁽¹⁴⁾ which they expected to be affected by presence of stone, so more phosphate secreted in saliva which may be a reflect to their increase in serum. Since saliva is act as a mirror of serum^(15, 16) when there is increased in its secretion⁽¹⁶⁾. Other explanation for this result will discussed later on. In addition to salivary phosphate, other salivary elements including calcium and magnesium were evaluated in the present study and found that there was no significant difference between calcium stone formers and non stone formers in regarding to salivary calcium but with higher level among the stone formers. However, highly significant difference was recorded among males within study group in compared to males within the control one while among females; there was no significant difference between two groups. For salivary magnesium, present study revealed highly significant reduction in salivary magnesium concentration among calcium stone formers in compared to non stone formers. Regarding gender differences, it was found that there were no significant differences among males and females in concerning to salivary calcium and magnesium while the males in this study had higher concentration of salivary phosphate than females with significant difference. One can notice from the result of salivary magnesium among control group that the numerical value of salivary magnesium concentration is similar among males and females which it is (1.24) mg/dL. Since saliva offers an alternative to serum as a biologic fluid^(15, 16) when there is increased in its secretion⁽¹⁶⁾, so these differences in the inorganic salivary constituents (Calcium, phosphate and magnesium) among calcium stone formers as compared to non stone formers may

be related to differences in their level in serum as a result in which that the majority of patients with idiopathic stones have metabolic abnormalities ⁽²⁾.

Similar to that found in saliva about phosphate concentration, present study revealed present of higher level of urinary phosphate/creatinine ratio among study group with high significant difference in compared to control one this may be related to dietary factors that the excretion of urinary phosphate in normal adults is related to the amount of dietary phosphate ⁽¹⁷⁾ and may be related also to metabolic abnormalities which present among the majority of idiopathic stone formers ⁽²⁾. About gender differences, similar to that revealed in saliva in regarding salivary phosphate, higher level of urinary phosphate/creatinine ratio was recorded among males within study group with high significant difference compared to females. This difference among males and females may expect to be due to differences in dietary habits, since it must be considered that the urine composition is directly related to diet ⁽¹⁸⁾. For urinary magnesium/creatinine ratio, present study revealed a result also similar to that found about the concentration of magnesium in saliva. It was revealed a reduction in this ratio among study group with high significant difference compared to control one. The reduction in magnesium/creatinine ratio is an indicator for insufficient magnesium intake ⁽¹³⁾ and also may relate to intestinal malabsorption, since the majority of idiopathic stone formers have metabolic abnormalities ⁽²⁾. Regarding gender differences and among the study group, females had higher level of urinary magnesium/creatinine ratio than males with high significant difference. This may consider one of explanations for why males have higher renal stone incidence than females, since the magnesium is an inhibitor for calcium stone formation ^(1, 19). This gender differences may also related to differences in dietary habits ⁽¹⁸⁾. Similar to that revealed for salivary calcium in present study, the concentration of urinary calcium/creatinine ratio is in higher level among study group in compared to control one but with no significant difference. Regarding gender differences, males found to have higher level of urinary calcium/creatinine ratio than females in both groups with high significant difference. This result may be due to differences in dietary habits among both genders ⁽¹⁸⁾.

One can notice from urinary results of present study that calcium stone formers didn't have hypercalciuria but they had high urinary level of phosphate which is consider one of calcium stone promoters ³ and highly reduction in urinary magnesium level which is one of calcium stone inhibitors ⁽²⁰⁾, so one can conclude that the calcium renal stone to be formed the hypercalciuria may be not a condition to be formed but the supersaturation with other urinary stone minerals like phosphate and oxalate which found to be correlates better with the activity of stone disease than urinary calcium ⁽²¹⁾, and lack of adequate levels of inhibitors like magnesium and citrate in the presence of calcium, all these factors may play an important role for formation of calcium renal stone rather than hypercalciuria ⁽²⁰⁾.

In contrast to other ions, serum Mg^{2+} concentration is not under tight hormonal regulation. Bone, the major intracellular Mg^{2+} reservoir, does not readily exchange with extracellular Mg^{2+} , and equilibration with bone stores may take several weeks ⁽²²⁾, so for that and for the result that revealed by current study, one can notice for urinary magnesium/creatinine ratio that this value is higher for both males and females among control group compared to males and females among study one, so one may be use the magnesium/creatinine ratio as an indicator of susceptibility to calcium renal stone formation. On other words, the salivary magnesium concentration may be used as an indicator of susceptibility to calcium renal stone formation due to the similarities that revealed by results of current study in regarding magnesium between saliva and urine among calcium stone formers compared with the non stone formers. This conclusion will need further studies to improve and to use saliva as one of tools that used in primary and secondary preventive programs to avoid the risk of calcium renal calculi and also dental calculi. Since magnesium is an inhibitor of the calcification process ^(18, 23).

The measurement of concentration of magnesium in saliva will be easier than that in urine, since that in urine; it needs 24 hours urine collection or at least a fasting second morning urine specimen with measurement of urinary creatinine, since urine composition is directly related to diet ⁽¹⁸⁾.

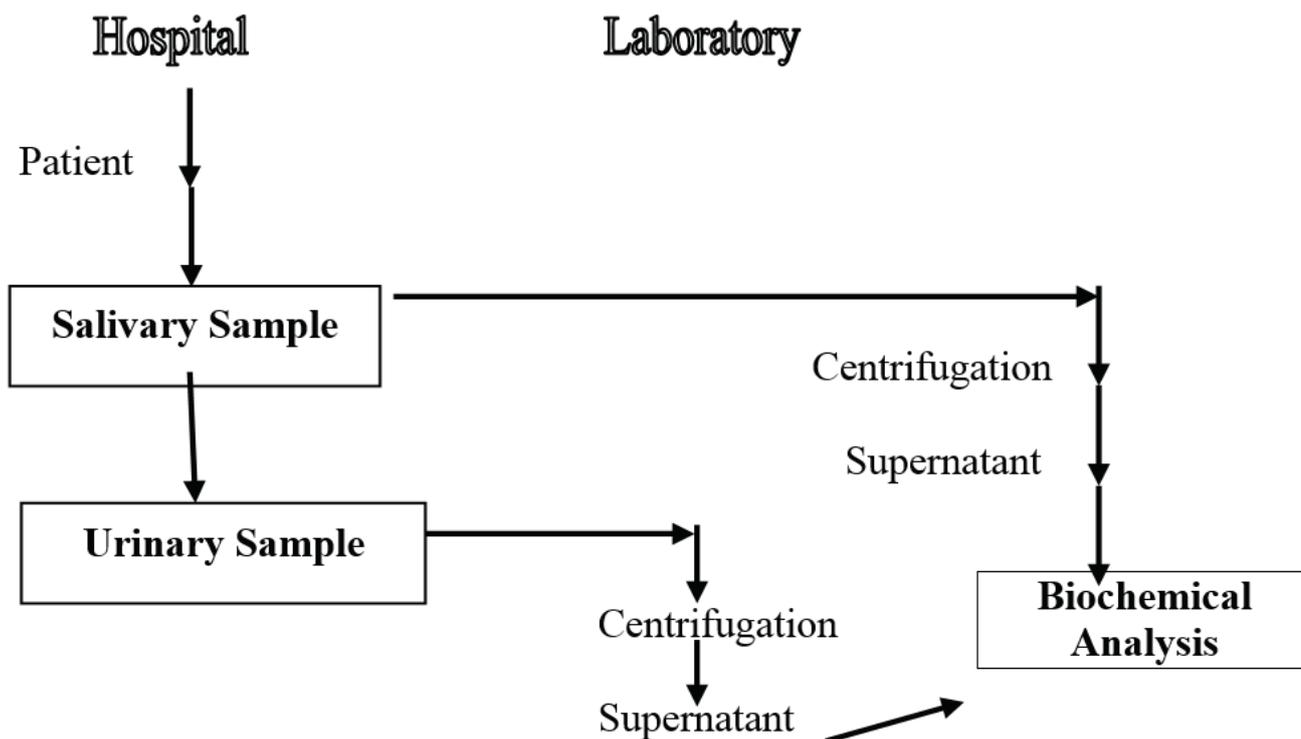


Figure 1: The design of the study.

Table 1: Inorganic Salivary Constituents (Calcium, Phosphate and Magnesium) (Mean and Standard Deviation) among Study and Control Groups.

Elements (mg/dL)	Gender	Study (Mean ± SD)	Control (Mean ± SD)	P-value
Calcium	M	5.48 ± 2.76	2.84 ± 1.44	P<0.01**
	F	4.56 ± 2.32	4.88 ± 4.20	P>0.05
	T	5.00 ± 2.56	3.88 ± 3.24	P>0.05
Phosphate	M	13.21* ± 1.95	9.77 ± 3.63	P<0.01**
	F	11.66 ± 1.64	8.59 ± 2.48	P<0.001***
	T	12.43 ± 1.95	9.18 ± 3.10	P<0.001***
Magnesium	M	0.56 ± 0.12	1.24 ± 0.22	P<0.001***
	F	0.61 ± 0.15	1.24 ± 0.24	P<0.001***
	T	0.58 ± 0.15	1.24 ± 0.22	P<0.001***

* Significant P<0.05 ** Highly significant P < 0.01 *** Very highly significant P < 0.001

Table 2: Urinary (Calcium, Phosphate and Magnesium)/ Creatinine ratios (Mean and Standard Deviation) among Study and Control Groups.

Urinary variables	Gender	Study (Mean ± SD)	Control (Mean ± SD)	P-value
Calcium/ Creatinine (mg/g)	M	69.60*** ± 21.60	57.60** ± 16.80	P>0.05
	F	39.20 ± 16.40	34.80 ± 15.60	P>0.05
	T	54.40 ± 24.40	46.40 ± 19.60	P>0.05
Phosphate/ Creatinine (mg/g)	M	469.03** ± 37.20	310.93 ± 62.00	P<0.001***
	F	409.82 ± 62.31	434.93*** ± 46.19	P>0.05
	T	439.58 ± 58.59	372.93 ± 82.77	P<0.01**
Magnesium/ Creatinine (mg/g)	M	27.46 ± 7.05	63.18 ± 19.93	P<0.001***
	F	35.24** ± 6.80	72.66 ± 19.68	P<0.001***
	T	31.35 ± 8.02	68.04 ± 20.17	P<0.001***

** Highly significant P < 0.01 *** Very highly significant P < 0.001

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