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STUDY OF THE URINE AND BLOOD BIOCHEMICAL PARAMETERS OF CONTROLLED DIABETIC NON CALCULOGENIC AND CONTROLLED DIABETIC CALCULOGENIC RATS

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ABSTRACT

Experimental studies are important as prerequisite to clinical trials. The objective of the present study is to understand the changes produced in the urine and blood biochemical values in the controlled diabetic non-calculogenic and controlled diabetic calculogenic rats. The experimental work was conducted in male rats of wistar species. Diabetes was induced by injecting 3% aqueous solution of alloxan monohydrate in a dose of 150mg/kg body weight and calculogenesis by giving 0.5% of ethylene glycol orally in drinking water. Diabetes was controlled by giving lente insulin. The 24-hour urine samples intermittently and blood at the end of the study was analyzed to assess the urine parameters calcium, phosphorous, uric acid, oxalate, magnesium and citrate and serum parameters calcium, phosphorus, uric acid and magnesium. The mean values of the promoters urine uric acid and serum calcium ($p<0.05$, $p<0.05$) and inhibitors urine citrate ($p<0.01$) and magnesium ($p<0.001$) and serum magnesium ($p<0.01$) were significantly lower in controlled diabetic non-calculogenic rats. Controlled diabetic calculogenic rats had significantly low urine calcium ($p<0.05$), uric acid ($p<0.0001$) and serum uric acid ($p<0.04$) and magnesium ($p<0.01$). Serum phosphorus ($p<0.01$) was significantly higher while the serum magnesium ($p<0.01$) was significantly lower in the controlled diabetic calculogenic rats. Comparative study of the non-calculogenic and calculogenic controlled diabetic rats showed serum uric acid to be significantly higher ($p<0.05$) and urine citrate significantly lower ($p<0.05$) in the controlled diabetic non-calculogenic rats. Serum magnesium values of the controlled diabetic non-calculogenic and controlled diabetic calculogenic rats were significantly lower. Controlling diabetes of rats

showed lower value of urine and serum promoters but did not elevate the levels of inhibitors which indicate that the tendency for stone formation in the diabetic rats is not completely reversed on controlling the diabetic state.

KEYWORDS: Controlled diabetic non-calculogenic (CD NC), Diabetic non-calculogenic (D NC), Diabetic ethylene glycol induced calculogenic (D EG), Calculogenesis.

INTRODUCTION

Urinary Stone Disease which affects both children and adults and cause pain, suffering and financial burden is seen increasing in number considerably in the recent decades. Life-time risk of urolithiasis varies from 1-5% in Asia, 5-9% Europe, 10-15% USA and 20-25% middle-east; lowest prevalence is reported from Greenland and Japan.[1,2] This increase in the incidence of the disease can be mainly due to the modifications made in the dietary habits and physical activity.

Diet is one of several factors that can promote or inhibit kidney stone formation. Stones form when calcium, oxalate, and phosphorus in the urine become highly concentrated. High concentrations of calcium in the urine can combine with oxalate and phosphorus to form stones. Meats and other animal protein such as eggs and fish contain purines, which can break down into uric acid in the urine. Pathogenesis of calcium oxalate stone formation is a multi-step process and includes nucleation, crystal growth, crystal aggregation and crystal retention. Deficiency of inhibitors or an abundance of promoters in the urine can modify the physiochemical conditions and can predispose to stone disease. Citrate and magnesium are known to be the most common inhibitors of urinary stones. They are known to complex oxalate and calcium, respectively and reduce the driving force for calcium oxalate monohydrate crystallization.[3] The four major types of kidney stones that can form are the calcium stones, uric acid stones, struvite stones, and cystine stones. Majority of kidney stones are composed of calcium oxalate and phosphate crystals (80%). Most of the rest are composed of uric acid (5-10%) and struvite (5-15%).[4]

Compositions vary in different geographical conditions and ethnicities. It is understood that high blood pressure, diabetes, and obesity can increase the risk of stone disease. Diabetes mellitus, the most common non-communicable disease is now growing to an alarming level in the present century. It is understood that type 2 diabetes can increase the risk for developing kidney stone since insulin resistance can cause a deficit in ammonium production in the kidney, which lowers urinary pH, thus generating a favorable milieu for stone

formation.[5] The current epidemiologic evidence suggests that persons with diabetes mellitus are at increased risk of stone formation.[6] The objective of the present study is to understand the changes produced in the urine and blood biochemical values in the controlled diabetic non-calculogenic and controlled diabetic calculogenic rats.

MATERIALS AND METHODS

Male rats of wistar species each weighing 200-250 gm was used for the experimental work. Animals were acclimatized to laboratory conditions before the experiment procedure used in this study were reviewed. The control and experimental rats were maintained in cages and were fed on standard laboratory feed pellets and water / drugs ad libitum. Six rats each were included in the control and experimental group and the experiment was conducted for a period of three months. Diabetes was induced in the experimental group of rats by injecting 3% aqueous solution of alloxan monohydrate in a dose of 150mg/kg body weight prepared by weighing the requisite dose of the drug and dissolving it immediately in distilled water and administering to the rats. Diabetes was controlled in the experimental group by giving 2 units of Lente insulin subcutaneously. The urine and blood samples of these rats were collected and the percentage of sugar was noted. Calculogenesis was induced in rats by giving 0.5% of ethylene glycol orally in drinking water. The rats were placed individually in metabolic cages and 24-hour urine samples were collected to assess the urine parameters like calcium, phosphorous, uric acid, oxalate, magnesium and citrate and at the end of the experiment, rats were anaesthetized and blood was collected to assess the serum parameters namely calcium, phosphorous, uric acid and magnesium. Student ‘t’ test, Analysis of Variance and Duncan’s Multiple Range Test were performed to study the changes produced in the experimental rats.

RESULTS

On controlling diabetes with lente insulin in the non-calculogenic rats, the mean urine and blood sugar values decreased from 1.63 ± 0.08 mg% and 241.06 ± 12.21 mg% to 0.5 ± 0.06 mg% and 135.50 ± 1.99 mg% respectively. The mean values of urinary promoters namely calcium (NS), phosphorus (NS), uric acid ($p<0.05$) and oxalate (NS) and serum calcium ($p<0.05$) and phosphorus (NS) were lower in the controlled diabetic compared to the diabetic rats. However the serum uric acid was higher (NS) in the controlled diabetic rats (Table.1). The inhibitors namely urine citrate ($p<0.01$) and magnesium ($p<0.001$) and serum magnesium ($p<0.01$) were also significantly lower in the controlled diabetic rats.

Table.1: Comparative study of the urine and serum biochemical parameters of diabetic and controlled diabetic non-calculogenic rats

Parameters	D NC Mean ± SE	CD NC Mean ± SE	t	p
Urine Calcium (mg/day)	0.400 ± 0.052	0.348 ± 0.040	0.79	NS
Urine Phosphorus (mg/day)	2.714 ± 0.336	2.667 ± 0.491	0.07	NS
Urine Uric acid (mg/day)	4.729 ± 0.533	3.394 ± 0.381	2.03	<0.05
Urine Oxalate (mg/day)	0.585 ± 0.125	0.460 ± 0.201	0.52	NS
Urine Magnesium (mg/day)	29.106 ± 4.610	6.319 ± 0.692	4.88	<0.001
Urine Citrate (mg/day)	0.836 ± 0.242	0.173 ± 0.030	2.71	<0.01
Serum Calcium (mg%)	14.244 ± 0.407	12.579 ± 0.665	2.13	<0.05
Serum Phosphorus (mg%)	5.5 ± 0.500	3.730 ± 0.710	2.03	NS
Serum Uric acid (mg%)	7.780 ± 1.110	9.308 ± 1.436	0.84	NS
Serum Magnesium (mg%)	8.890 ± 1.110	2.886 ± 0.266	5.25	<0.01

D NC – Diabetic non-calculogenic

CD NC – Controlled diabetic non-calculogenic

In controlled diabetic ethylene glycol induced calculogenic rats, the mean urine and blood sugar values were 1.00 ± 0.05 mg% and 184.20 ± 4.24 mg% respectively. The mean urinary promoters namely calcium ($p<0.05$), phosphorus (NS) and uric acid ($p<0.0001$) and serum uric acid ($p<0.04$) were lower in the controlled diabetic calculogenic rats compared to the diabetic calculogenic rats (Table.2). However, the serum phosphorus was significantly ($p<0.01$) higher in the controlled diabetic calculogenic rats. The inhibitor namely urine magnesium value was slightly lower in the controlled diabetic calculogenic rats, but the difference was not statistically significant. However, the mean serum magnesium was significantly lower ($p<0.01$) in the controlled diabetic calculogenic rats.

Table.2: Comparative study of the urine and serum biochemical parameters of diabetic and controlled diabetic ethylene glycol induced calculogenic rats

Parameters	D EG Mean ± SE	CD EG Mean ± SE	t	p
Urine Calcium (mg/day)	0.390 ± 0.039	0.297 ± 0.021	2.09	<0.05
Urine Phosphorus (mg/day)	3.628 ± 0.597	2.499 ± 0.181	1.81	NS
Urine Uric acid (mg/day)	4.626 ± 0.334	2.737 ± 0.403	3.61	<0.0001
Urine Oxalate (mg/day)	0.651 ± 0.163	0.800 ± 0.416	0.33	NS
Urine Magnesium (mg/day)	10.426 ± 2.285	7.109 ± 0.238	1.44	NS
Urine Citrate (mg/day)	0.254 ± 0.043	0.377 ± 0.085	1.29	NS
Serum Calcium (mg%)	14.150 ± 0.353	12.580 ± 1.550	0.98	NS
Serum Phosphorus (mg%)	1.363 ± 0.501	5.395 ± 0.536	5.49	<0.01
Serum Uric acid (mg%)	9.367 ± 1.687	4.770 ± 0.324	2.67	<0.04
Serum Magnesium (mg%)	8.375 ± 0.125	2.710 ± 0.210	23.17	<0.01

D EG – Diabetic ethylene glycol induced calculogenic

CD EG – Controlled diabetic ethylene glycol induced calculogenic

On comparing the non-calculogenic and calculogenic controlled diabetic rats, the mean values of promoters of stone formation namely urine and serum calcium and phosphorus and urine oxalate and uric acid were not significantly higher in the controlled diabetic non-calculogenic rats. However, the serum uric acid was significantly ($p<0.05$) higher in the controlled diabetic non-calculogenic rats (Table.3). The inhibitor namely urine citrate was significantly lower ($p<0.05$) in the controlled diabetic non-calculogenic rats.

Table.3: Comparative study of the urine and serum biochemical parameters of controlled diabetic non-calculogenic and controlled diabetic ethylene glycol induced calculogenic rats

Parameters	CD NC Mean ± SE	CD EG Mean ± SE	t	p
Urine Calcium (mg/day)	0.348 ± 0.040	0.297 ± 0.021	1.13	NS
Urine Phosphorus (mg/day)	2.667 ± 0.491	2.499 ± 0.181	0.32	NS
Urine Uric acid (mg/day)	3.394 ± 0.381	2.737 ± 0.403	1.18	NS
Urine Oxalate (mg/day)	0.460 ± 0.201	0.800 ± 0.416	0.73	NS
Urine Magnesium (mg/day)	6.319 ± 0.692	7.109 ± 0.238	1.07	NS
Urine Citrate (mg/day)	0.173 ± 0.030	0.377 ± 0.085	2.27	<0.05
Serum Calcium (mg%)	12.579 ± 0.665	12.580 ± 1.550	0.005	NS
Serum Phosphorus (mg%)	3.730 ± 0.710	5.395 ± 0.536	1.87	NS
Serum Uric acid (mg%)	9.308 ± 1.436	4.770 ± 0.324	3.08	<0.05
Serum Magnesium (mg%)	2.886 ± 0.266	2.710 ± 0.210	0.51	NS

CD NC – Controlled diabetic non-calculogenic

CD EG – Controlled diabetic ethylene glycol induced calculogenic

Analysis of variance was done to study the difference in mean values of the urine and serum parameters of different groups of experimental rats namely diabetic non-calculogenic, diabetic ethylene glycol induced calculogenic, controlled diabetic non-calculogenic and controlled diabetic ethylene glycol induced calculogenic rats. The mean urine and serum magnesium and phosphorus values showed significant difference in the groups (Table.4). The urine magnesium showed significant difference in mean ($p=0.001$) with the highest mean value of 29.10 ± 4.61 mg/day in the diabetic non-calculogenic group and lowest mean value of 6.31 ± 0.69 mg/day in the controlled diabetic non-calulogenic rats. The serum phosphorus showed significant difference in mean ($p=0.01$) with the highest mean value of 5.5 ± 0.50 mg% in the diabetic non-calculogenic group and lowest mean value of 1.36 ± 0.50 mg% in the controlled diabetic calculogenic rats. The serum magnesium also showed significant difference in mean ($p=0.0001$) with the highest mean value of 8.89 ± 1.11 mg% in the

diabetic non-calculogenic group and lowest mean value of 2.71 ± 0.21mg% in the controlled diabetic ethylene glycol induced calculogenic rats.

Table.4: Mean values, F ratio and Duncan's Multiple Range Test of the urine and serum parameters of diabetic non-calculogenic, diabetic ethylene glycol induced calculogenic, controlled diabetic non-calculogenic and controlled diabetic ethylene glycol induced calculogenic rats

Parameter	D NC	D EG	CD NC	CD EG	F	p
U.Calcium (mg/day)	0.40 *a*	0.39 *a*	0.35 *a*	0.30 *a*	0.38	NS
U.Phosphorus (mg/day)	2.71 *a*	3.63 *a*	2.67 *a*	2.50 *a*	1.02	NS
U.Uric acid (mg/day)	4.73 *a*	4.63 *a*	3.39 *a*	2.74 *a*	1.60	NS
U.Oxalate (mg/day)	0.59 *a*	0.65 *a*	0.46 *a*	0.80 *a*	0.26	NS
U.Magnesium (mg/day)	29.11 *b*	10.43 *a*	6.32 *a*	7.11 *a*	6.08	<0.001
U.Citrate (mg/day)	0.84 *a*	0.254 *a*	0.17 *a*	0.38 *a*	1.57	NS
S.Calcium (mg%)	14.24 *a*	14.15 *a*	12.58 *a*	12.58 *a*	2.13	NS
S.Phosphorus (mg%)	5.50 *b*	1.36 *a*	3.73 *a,b*	5.40 *b*	5.97	<0.01
S.Uric acid (mg%)	7.78 *a*	9.37 *a*	9.31 *a*	4.77 *a*	1.98	NS
S.Magnesium (mg%)	8.89 *b*	8.38 *b*	2.89 *a*	2.71 *a*	50.14	<0.0001

D NC – Diabetic non-calculogenic CD NC – Controlled diabetic non-calculogenic

D EG – Diabetic ethylene glycol induced calculogenic

CD EG – Controlled diabetic ethylene glycol induced calculogenic

Duncan's Multiple Range Test with significance level at 0.05

***a*: Homogeneous Subset 1 *b*: Homogeneous Subset 2**

On applying Duncan's multiple range test, the urine magnesium value of the diabetic non-calculogenic rats was found to be significantly higher than that of the values of the diabetic ethylene glycol induced calculogenic, controlled diabetic ethylene glycol induced calculogenic and controlled diabetic non-calculogenic rats. The values of the other parameters namely calcium, phosphorus, uric acid, oxalate and citrate did not show significant difference. The serum calcium and uric acid values also did not show any significant difference. The serum phosphorus values of the diabetic non-calculogenic and controlled diabetic ethylene glycol induced calculogenic rats were significantly higher than the values of the diabetic ethylene glycol induced calculogenic rats. The serum magnesium values of the controlled diabetic ethylene glycol induced calculogenic and controlled diabetic non-calculogenic rats were significantly lower from the diabetic non-calculogenic and diabetic ethylene glycol induced calculogenic rats.

DISCUSSION

The mean values of promoters namely urine uric acid and serum calcium were significantly lower ($p<0.05$, $p<0.05$) in the controlled diabetic rats. The inhibitors namely urine citrate ($p<0.01$) and magnesium ($p<0.001$) values were significantly lower in these rats. Thus on controlling diabetes, the promoters as well as the inhibitors of stone formation are lowered. This indicates that even when diabetes is controlled, there are chances for lithiasis in the diabetic rats.

The controlled diabetic ethylene glycol induced calculogenic rats showed that the promoters namely urine calcium ($p<0.05$) and uric acid ($p<0.0001$) and serum phosphorus ($p<0.01$) and uric acid ($p<0.04$) were significantly lower. The inhibitor serum magnesium was significantly lower ($p<0.01$). Thus even though promoters namely calcium and uric acid were lower, significant low serum magnesium seen in the controlled diabetic ethylene glycol induced calculogenic rats are against the concept that controlling diabetes will reduce the risk of stone formation.

On comparing the findings of non-calculogenic and calculogenic rats, it is seen that the promoter oxalate was slightly higher and the inhibitor citrate was significantly higher in the urine of the controlled diabetic ethylene glycol induced calculogenic rats. However the serum uric acid was significantly lower. Thus it is surmised that even though the inhibitors are increased on controlling diabetes, mild high urine oxalate in the controlled diabetic calculogenic rats can still induce calculogenesis.

Analysis of variance showed significant difference in the mean values of promoters namely serum phosphorus and the inhibitor namely magnesium in the urine and serum among the groups. The highest mean value of serum phosphorus was seen in the diabetic calculogenic group and lowest in the controlled diabetic non-calculogenic group. This may indicate the tendency of the diabetic rats with calculogenesis for stone formation, if phosphorus is to be considered as a significant stone promoting factor. The inhibitor urine magnesium and serum magnesium was significantly lower in the controlled diabetic rats. This shows that even on controlling diabetes, chances for urolithiasis are high.

The serum magnesium values of the controlled diabetic non-calculogenic and controlled diabetic ethylene glycol induced calculogenic rats were significantly lower than the diabetic non-calculogenic and the diabetic ethylene glycol induced calculogenic rats. This indicates

that the tendency for stone formation in the diabetic rats is not completely reversed on controlling the diabetic state.

CONCLUSION

The controlled diabetic rats showed significantly low levels of urine uric acid and serum calcium, but had significantly low levels of urine citrate and magnesium. Controlled diabetic ethylene glycol induced calculogenic rats showed significantly high level of serum phosphorus and urine citrate and significantly low levels of urine calcium and uric acid. However, the group had low levels of serum uric acid and magnesium. Controlling diabetes of rats with anti-diabetic drugs produced lowering of urine and serum promoters but not elevation of levels of inhibitors which points that controlled diabetic state also has tendency for stone formation.

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REFERENCES

1. Bartoletti R, Cai T, Mondaini N, Melone F, Travaglini F, Carini M, et al. Epidemiology and risk factors in urolithiasis. Urol Int. 2007; 79(1): 3–7.
2. Mayank Mohan Agarwal, Shwaran K Singh, Ravimohan Mavuduru, Arup K Mandal. Preventive fluid and dietary therapy for urolithiasis: An appraisal of strength, controversies and lacunae of current literature. Indian J Urol. 2011 Jul-Sep; 27(3): 310–319.
3. Nancollas GH, Smeko S A, Campbell AA, Colye Rees M, Ebrshimpour A, BinetenM, Binette J P. Mineralisation inhibitors and promoters. In: Urolithiasis (eds) Valaerie R Walker, Roger AL Sutton, Bert Cameron E C, Charles Y C Pak, William G Robertson, Plenum Press, New York, 1989: 83-91.
4. Moe OW. Kidney stones: Pathophysiology and medical management. Lancet. 2006; 367: 333–44.

5. Abate N, Chandalia M, Cabo-Chan AV, Jr, et al. The metabolic syndrome and uric acid nephrolithiasis: novel features of renal manifestation of insulin resistance. Kidney Int. 2004; 65: 386–392.
6. Daudon M, Jungers P. Diabetes and nephrolithiasis. Curr Diab Rep 2007; 7(6): 443-448.