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Original Article

Biochemistry Section

Is Urolithiasis Associated with Increased Levels of High Sensitivity C-Reactive Protein and Interleukin-6 in Diabetic Patients?

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ABSTRACT

Background: The prevalence and incidence of urolithiasis is increasing worldwide. Type 2 diabetes mellitus is well known to be associated with insulin resistance which increases the risk of urolithiasis by altering the composition of urine. Both urolithiasis and diabetes mellitus are associated with inflammation. The aim of the study was to assess the serum levels of inflammatory markers i.e. high sensitivity C-reactive protein (hsCRP) and Interleukin-6 (IL-6) in diabetes mellitus patients with urolithiasis in comparison to those without urolithiasis.

Materials and Methods: The study involved two groups. Group A consisted of diabetic mellitus patients with urolithiasis (n=30) and Group B consisted of diabetic mellitus patients without urolithiasis (n=30). Blood samples were obtained and analysed for HbA_{1C}, lipid profile, calcium, phosphate and uric acid, and inflammatory markers (C-reactive protein and Interleukin-6) were also measured.

Results: We found a significant increase in CRP and IL-6 levels in diabetic urolithiasis cases as compared to diabetes mellitus cases without urolithiasis. However, no significant difference was observed in calcium, phosphorus and uric acid in diabetic patients with and without urolithiasis. We also found that total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol levels were significantly increased, and HDL-cholesterol was significantly decreased in diabetic urolithiasis cases. IL-6 was significantly correlated with total cholesterol in diabetic urolithiasis cases.

Conclusion: The data from the present study shows that lipid profile is altered, and Interleukin-6 and C-reactive protein levels are significantly increased in patients with diabetes mellitus and urolithiasis when compared to diabetes mellitus alone.

Keywords: Diabetes mellitus, Inflammation, Inflammatory markers, Renal stones

INTRODUCTION

Diabetes is a common endocrine disorder affecting all age groups, with a worldwide prevalence ranging from 2.8% in 2000 to a predictive value of 4.4% in 2030 [1]. Type 2 diabetes mellitus is characterized by insulin resistance, a metabolic derangement that may increase the risk of renal calculi by changing the composition of urine [1,2].

Urolithiasis is a common disorder among the general population [3]. The prevalence of kidney stones is approximately 6–9% in men and 3–4% in women. The etiology of renal stones is multifactorial including climate, occupation, diet and obesity [4,5]. Components of metabolic syndrome have been established as risk factors for kidney stones [6,7].

Even though few studies have established the association between diabetes mellitus and urolithiasis, the pathophysiology of urolithiasis remains unclear [1,2]. Subclinical inflammation and inflammatory pathways are known to contribute to the development of diabetes mellitus and its complications [8]. It has been demonstrated that hyperglycemia stimulates the release of interleukin-6 (IL-6) from various cell types and results in the release of acute-phase reactants by adipocytes [9]. Previous studies have shown that the levels of interleukin-6 and C-reactive protein are elevated in patients with diabetic nephropathy [10,11].Interleukin-6 plays a key role in inflammation and also induces the synthesis of C-reactive protein [12]. The levels of interleukin-6 (IL-6) and high sensitivity C-reactive protein (hsCRP) were not explored till date in diabetic patients with urolithiasis. The aim of the present study is to determine blood levels of IL-6 and hsCRP in diabetes mellitus patients with renal stones. The secondary objectives of the study are to estimate the lipid parameters in serum and correlate them with these inflammatory markers in diabetic urolithiasis cases.

MATERIALS AND METHODS

This was a cross-sectional study conducted for a period of one year from January 2013 to December 2013 in Jawaharlal Institute of Postgraduate Medical Education and Research (JIPMER), Puducherry, India with the approval of Institute ethical review committee for human studies. A total of 60 patients were included in the study.

The subjects recruited for the study were categorized into two groups–

- Group A include all cases of urolithiasis diagnosed on the basis of ultrasonography/computed tomography/X-RAY who presented with Type II diabetes mellitus (n=30).
- Group B include Type II diabetes mellitus patients without urolithiasis who were matched for duration of diabetes with above mentioned cases (n=30).

The patients were on treatment with oral hypoglycemic drugs for a period of 5-10 y. Patients with obstructive uropathy, ureteric colic, renal failure, inflammatory and infectious diseases, malignancy and ischemic heart disease were excluded from the study. Diabetic patients taking insulin and those with glycated hemoglobin levels more than 8g % were also excluded from the study as these patients are likely to have more severe disease with renal complications which may have influence on renal stone formation.

After obtaining written informed consent, five ml of fasting blood sample was collected from all subjects. Two ml blood was collected

with EDTA and used for HbA_{1C} estimation, and the remaining three ml was collected in tubes free of anticoagulant. The serum was separated by centrifuging the blood at 5000rpm for ten minutes at room temperature. The serum thus separated was used to analyse the other study parameters i.e., hs-CRP, IL-6, lipid profile, blood levels of calcium, phosphate and uric acid.

Serum lipid profile was estimated using Olympus AU400 fully automated clinical chemistry analyser by enzymatic method [13-16]. Serum calcium was assayed by OCP method using reagent kit from AMS Diagnostics, Italy [17]. Serum phosphorus was assayed by the phosphomolybdate method using reagent kit from Genuine Biosystem, India [18]. Serum uric acid was assayed by uricase/POD method using BEACON reagent kit [19]. Serum hs-CRP levels were estimated using commercially available ELISA kits (Diagnostics Biochem Canada In, Ontario, Canada) [20]. Serum levels of IL-6 were estimated using commercially available ELISA kits (Ani Biotech, Orgenium Laboratories Business Unit, FINLAND) [21].

STATISTICAL ANALYSIS

The statistical analysis was performed using SPSS version16. The results were expressed as mean ± standard deviation and median with range. The comparison of the biochemical parameters between the study groups (diabetic patients with and without urolithiasis) was carried out by using Independent Student t-test and Mann-Whitney U-test for normally and non-normally distributed data respectively. The association between various parameters was assessed by Pearson's and Spearman's correlation analysis. p-value <0.05 was considered as statistically significant.

RESULTS

In the present study, two study groups were included. Group A consisted of diabetic mellitus patients with urolithiasis (n=30), and group B consisted of diabetic mellitus patients without urolithiasis (n=30). [Table/Fig-1] shows the lipid profile in diabetes mellitus patients with and without urolithiasis. The total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol levels in diabetic urolithiasis cases (Group A) were significantly increased as compared to control cases (Group B) and there was a concomitant decrease in the level of HDL-cholesterol in diabetic urolithiasis cases. However, no significant difference was observed in serum calcium, phosphorus and uric acid in diabetic patients with and without urolithiasis.

[Table/Fig-2] compares the inflammatory markers between diabetes mellitus patients with and without urolithiasis. The CRP and IL-6 levels in diabetic urolithiasis cases were significantly increased when compared with the other group. On correlating inflammatory markers with the routine parameters [Table/Fig-3], it was seen that IL-6 was significantly correlated with total cholesterol and CRP was significantly correlated with uric acid in diabetic cases with urolithiasis. However, there was no significant correlation between IL-6 and CRP as shown in [Table/Fig-3].

DISCUSSION

Type 2 diabetes mellitus is characterized by insulin resistance, a metabolic derangement that may increase the risk of kidney stones by altering the urine composition [22]. Insulin resistance results in high levels of plasma free fatty acids, which enters the proximal tubule cells and interferes with the utilization of glutamine in the production of ammonia. Insulin resistance results in defective generation of ammonia in the kidneys and thus decreases the urinary pH [7,23]. This predisposes to uric acid stones. Further, a decrease in renal citrate excretion increases the risk of calcium stones too [22,24].

Subclinical inflammation and inflammatory pathways are known to contribute to the development of diabetes mellitus and its complications. Insulin resistance causes an increase in macrophage infiltration into adipose tissue and the kidney. The infiltration of

Parameter	Diabetes mellitus (n=30)	Diabetes mellitus with urolithiasis (n=30)	p-value
Total cholesterol(mg/dl)	137.17 ± 27.99	163.63 ± 46.68	0.01*
Triglycerides(mg/dl)	91.73±24.5	143.53±61.1	0.0001*
HDL-cholesterol(mg/dl)	37.8±12.49	29.13±5.44	0.0012*
LDL-cholesterol(mg/dl)	81.17 ± 24.59	105.60 ± 44.40	0.011*
VLDL-cholesterol(mg/dl)	18.2±4.94	28.9±12.27	<0.0001*
Calcium(mg/dl)	8.65 ± 0.60	8.87 ± 0.65	0.18
Phosphorus(mg/dl)	3.46 ± 0.54	3.74±0.62	0.064
Uric acid(mg/dl)	4.8±1.23	4.88±1.96	0.863

[Table/Fig-1]: Comparison of lipid profile and other routine parameters between diabetes mellitus patients with and without urolithiasis
*p-value less than 0.05

Parameter	Diabetes mellitus (n=30)	Diabetes mellitus with urolithiasis (n=30)	p-value
HbA_{1c} (%)	7.34 ±0.55	7.39 ±0.57	0.713
CRP(µg/l)	79.44±54.64	115.29±50.93	0.011*
IL-6 (pg/ml)	5.8(0.22-28.01)	9.66(0.43-113.41)	0.041*§

[Table/Fig-2]: Comparison of HbA_{1c} and inflammatory markers in serum between diabetes mellitus patients with and without urolithiasis.
§: Mann-Whitney U test used; *: p-value less than 0.05

Parameter	IL6 (pg/ml)§		CRP (µg/l)	
	r	p	r	p
HbA_{1c} (%)	-0.142	0.453	-0.081	0.671
Calcium(mg/dl)	-0.151	0.426	-0.121	0.524
Phosphorus(mg/dl)	0.165	0.383	0.253	0.178
Total cholesterol(mg/dl)	0.420	0.021*	0.330	0.075
Triglycerides(mg/dl)	0.338	0.068	0.223	0.237
LDL-cholesterol(mg/dl)	0.333	0.072	0.243	0.196
HDL-cholesterol(mg/dl)	0.208	0.270	0.280	0.134
VLDL-cholesterol(mg/dl)	0.333	0.072	0.253	0.178
Uric acid(mg/dl)	-0.015	0.937	0.437	0.016*
CRP (µg/l)	0.079	0.677	----	----

[Table/Fig-3]: Correlation between inflammatory markers and routine blood parameters in diabetes mellitus patients with urolithiasis.
§: Spearman's correlation used; *: p-value less than 0.05 (r: correlation coefficient; p:level of significance)

macrophages gives rise to the production of pro-inflammatory cytokines such as interleukin-6. Previous studies have hypothesized that renal cell injury and inflammation are related to the development of renal stones and demonstrated an increase in IL-6 in patients with urolithiasis [25-27]. Boonla et al., also showed increased mRNA expression of IL-6 and monocyte chemoattractant protein -1 in tissue from renal biopsy from kidneys containing calculi [28]. In the present study, we found a significant increase in IL-6 level in diabetes mellitus patient with urolithiasis as compared to those without urolithiasis. Whether IL-6 was released after stone formation due to the mechanical stimulation (irritation) of epithelial cells or it contributes to the formation of renal stones is still unclear [29].

C-reactive protein (CRP), an acute phase protein is used as a marker of low- grade inflammation. Study by Shoag et al., demonstrated the relationship between CRP and kidney stones in younger patients [30]. Urolithiasis is considered as a state of low-grade inflammation and in the present study, we found a significant increase in CRP level in diabetic urolithiasis patients compared to diabetic patients without urolithiasis.

Insulin resistance is associated with an increase in free fatty acid and is directly associated with the pathogenesis of urolithiasis. Abate et al., have shown a significant increase in total cholesterol, triglycerides, LDL-cholesterol and VLDL-cholesterol, and decrease in HDL-cholesterol level in urolithiasis patients [7]. It has been

suggested that IL-6 may cause an increase in circulating lipid levels probably through a decrease in peripheral lipoprotein lipase activity. Cytokines also act on the liver to increase the secretion of very-low density lipoproteins (VLDL), leading to the characteristic diabetic dyslipidemia [31,32]. In our study, we found a significant increase in total cholesterol, triglycerides, LDL-cholesterol, VLDL-cholesterol and decrease in HDL-cholesterol in diabetic urolithiasis patients. Also, IL-6 was significantly correlated with total cholesterol in the urolithiasis group indicating an association between inflammatory markers and dyslipidemia.

The data from the present study shows that IL-6 and CRP are significantly increased in cases of diabetes mellitus with urolithiasis indicating that inflammation may play a role in linking diabetes mellitus with development of urolithiasis in these subjects. Further studies are needed to establish whether anti-inflammatory agents are useful in reducing the incidence of urolithiasis in patients with diabetes mellitus.

LIMITATION

The limitations of our study include smaller sample size and lack of a healthy control group. Also we did not take into account the duration of diabetes and the effect of treatment on the levels of c-reactive protein and interleukin-6.

CONCLUSION

Our study shows that lipid profile is altered, and interleukin-6 and C-reactive protein levels are significantly increased in patients with diabetes mellitus and urolithiasis when compared to diabetes mellitus alone.

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