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Original Article

Correlation of blood glucose with urinary titrable acidity, urinary ammonia and net acid excretion among type 2diabetes mellitus patients in south India.

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ABSTRACT

Objective: In this paper, an attempt is made to correlate blood glucose with urine titrable acidity, ammonia and net acid excretion among type 2 diabetes mellitus patients in south india . Materials and methods: one hundred individuals are included in this study, 70 cases (36 males&34 females) and 30 controls (16 males &14 females). Estimation of blood glucose and estimation of serum creatinine were done by commercial kit methods. Urine titratable acidity is done by titrating urine with 0.1m NaOH using phenolphthalein indicator and the same titrated urine is used for estimating ammonia by adding formaldehyde and again titrating with 0.1m NaOH. Results: blood glucose was statistically correlated, significantly with creatinine (r=0.292, p=0.007) and blood glucose was statistically not correlated with urinary titratable acidity, urinary ammonia and net acid excretion. There is statistically significant intercorrelation is seen between Urinary titratable acidity, urinary ammonia and net acid excretion. Comparision between cases and controls showed, there is increase in urinary titrable acidity, urinary ammonia and net acid excretion in cases compared to controls. Conclusion: If blood glucose is increased there is increase in creatinine levels and increased levels of urinary ammonia, urinary titratable acidity and net acid excretion is noticed.

Introduction

Diabetes has emerged as major health problem worldwide with serious health related and socioeconomic impacts on individual and population alike. Cross-sectional and metabolic studies have demonstrated a link between insulin resistance, a hallmark of type 2 diabetes, and low urine pH [1] . Diabetes is associated with increase prevalence of cardiovascular diseases and micro vascular complications affecting nerve, eye and kidney.

Blood Ph in a narrow range of 7.35-7.4, fluid volume and extra cellular osmolality is regulated in mammals by kidneys [2], Carbon dioxide and water are involved in production of protons and these are transported via H+ -ATPase pump to distal lumen, presence of phosphate buffer in high concentration buffers H+ ions, which represent titratable acidity. At minimum urinary PH, creatinine will account for some of the titratable acidity. If ketoacids are present, they also contribute to titratable acidity. In severe diabetic ketoacidosis, beta-hydroxybutyrate (pKa 4.8) is the major component of titrable acidity. The titrable acidity can be measured in the urine from the amount of sodium hydroxide needed to titrate the urine pH back to 7.4, hence the term titratable acidity.

Ammonia is a low-molecular-weight molecule that plays an important role in renal physiology. It is produced in the proximal tubule [3] concentrated in the renal interstitium by the loop of Henle, and secreted into the luminal fluid in the collecting duct [4].

During the synthesis of ammonia excess of protons are buffered by bicarbonate buffer system. There is an excretion of ammonia more than 80% in urinary net acidity in human during metabolic acidosis [5, 6].

Ammonia (NH_4^+) is the primary component of net acid excretion under basal conditions, and is the primary mechanism by which the kidney increases net acid excretion in response to metabolic acidosis. Ammonia secretion by the collecting duct epithelia accounts for the urinary ammonium. It is driven by interstitum to lumen ammonia gradient, due to accumulation of ammonia in the medullary and papillary interstitium. Several transport proteins have shown to mediate medullary thick ascending limb of Henle's loop NH_4^+ reabsroption [7]. However, the mechanisms that underlie the maintenance of high interstitial NH_4^+ concentrations in the medulla and papilla, thereby avoiding back flux into the systemic circulation, have remained unexplored.

Materials and methods:

The study was conducted in known type 2 diabetes mellitus subjects who came to department of biochemistry, central laboratory, GSL Medical college, Rajahmundry, for routine blood investigations. This as case-control study. Hundred individuals are included in the study, 70 cases (36 males&34 females) and 30 controls (16 males &14 females). This study was conducted with approval of institutional ethical committee, informed consent was obtained from all 100 subjects after explaining the nature of the study. 2ml of random venous blood was collected in EDTA 5mg% and without EDTA vials, for blood glucose and creatinine estimation. Blood samples are centrifuged at 1000 rpm for 5

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minutes, plasma and serum are seperated. 100 ml of urine was also collected in a sterile container and assay was done immediately after collection of urine. The following parameters were applied to the samples for assays, estimation of blood sugar by GOD-POD method, estimation of creatinine by alkaline picrate method, commercial kits were supplied by transasia biomedicals. Urine titratable acidity is done by titrating urine with 0.1m NaOH using phenolphthalein indicator and the same titrated urine is used for estimating ammonia by adding formaldehyde and again titrating with 0.1m NaOH. Urine titratable acidity was done by titrating with0.1m NaOH.

Statistical analysis: The data has been statistically analyzed by using mean, standard deviation, student t test and p value were used to assess the significance difference of means between the cases and control group. Correlation between parameters in the groups analyzed by Pearson's correlation by using SPSS-16 trail version.

Results:

The parameters were analyzed in 70 type 2 diabetes patients and results are compared with 30 healthy controls. Details representing the total number of males and females in both cases and controls were shown in table 1. Mean values along with standard deviation for various parameters in the diabetic group as well as in controls were shown in table 2 and 3.Table-4 showed that the blood glucose was statistically correlated, significantly with creatinine (r=0.292, p=0.007) and blood glucose was statistically not correlated with urinary titratable acidity, urinary ammonia and net acid excretion. There is statistically significant intercorrelation is seen between Urinary titratable acidity, urinary ammonia and net acid excretion.Table-5 showed that the blood glucose was statistically not correlated with creatinine and blood glucose was statistically not correlated with urinary titratable acidity, urinary ammonia and net acid excretion. There is statistically significant intercorrelation is seen between Urinary titratable acidity, urinary ammonia and net acid excretion.

Table 6 shows, comparison of means between cases and controls, there was significant mean difference between blood glucose of case and control groups ($p<0.001$) and there was as significanct mean difference between creatinine, urinary titratable acidity, urinary ammonia and net acid excretion ($p<0.005$) in case and control groups. Comparision between cases and controls showed, there is increase in urinary titrable acidity, urinary ammonia and net acid excretion in cases compared to controls.

Table-1: Representing the total number of male and female in both cases and controls.

	Males	Females	Total
Cases	36	34	70
Controls	16	14	30

Table -2: Representing mean and standard deviation among cases.

	Age	Blood sugar (mg/dl)	Creatinine (mg/dl)	Titratable acidity (mEq/L)	$NH4^+$ (mEq/l)	net acid excretion (mEq/L)
Mean	51.1	193.7	1.2	44.62	59.2	103.8
SD (±)	9.7	99.4	0.32	22.3	28.9	48.8

Table -3: Representing mean and standard deviation among control.

	Age	Blood sugar (mg/dl)	Creatinine (mg/dl)	Titrable acidity (mEq/L)	$NH4^+$ (mEq/l)	Neta acid excretion (mEq/L)
Mean	45.6	100.77	0.91	33.55	46.66	80.16
SD (±)	13.4	19.2	0.15	17.97	22.35	39.40

Table -4: Representing correlation between parameter in cases

	Blood sugar	Creatinine	Titrable acidity	$NH4^+$	Net acid excretion
RBS	1.0000	r=0.292** p=0.007	r=-0.88 p=0.235	r=-0.107 p=0.189	r=-0.104 p=0.197
Creatinine	r=0.292** p=0.007	1.0000	r=-0.56 p=0.322	r=-0.093 p=0.22	r=-0.81 p=0.252
Titrable acidity	r=-0.88 p=0.235	r=-0.56 p=0.322	1.0000	r=0.805** p=0.000	r=0.936** p=0.00
$NH4^+$	r=-0.107 p=0.189	r=-0.093 p=0.22	r=0.805** p=0.000	1.0000	r=0.962** p=0.00
Net acid excretion	r=-0.104 p=0.197	r=-0.81 p=0.252	r=0.936** p=0.00	r=0.962** p=0.00	1.0000

**Correlation is significant at the 0.01 level (1-tailed).

Table -5: Representing correlation between parameter in controls

	Blood sugar	Creatinine	Titrable acidity	$NH4^+$	Net acid excretion
RBS	1.0000	r=0.14 p=0.417	r=0.307* p=0.49	r =0.220 p=0.121	r=0.265 p=0.79
Creatinine	r=0.14 p=0.417	1.0000	r=-0.084 p=0.329	r=-0.69 p=0.359	r=-0.77 p=0.342
Titrable acidity	r=0.307* p=0.49	r=-0.084 p=0.329	1.0000	r=0.908** p=0.000	r=0.971** p=0.00
$NH4^+$	r =0.220 p=0.121	r=-0.69 p=0.359	r=0.908** p=0.000	1.0000	r=0.982** p=0.000
Net acid excretion	r=0.265 p=0.79	r=-0.77 p=0.342	r=0.971** p=0.00	r=0.982** p=0.000	1.0000

*Correlation is significant at the 0.05 level (1-tailed)

** Correlation is significant at the 0.01 level (1-tailed)

Table :6 Representing comparison of means between cases and controls

	Levene's Test for Equality of Variances		t-test for Equality of Means						
								95% Confidence Interval ofthe Difference	
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	lower	upper
SUGAR Equal variances assumed	23.752	.000	5.065	98	.000	92.933333	18.348003	56.522319	129.344348
Equal variances not assumed			7.498	80.188	.000	92.933333	12.394237	68.268903	117.597764
CREATININE Equal variances assumed	5.792	.018	1.804	98	.074	.110952	.061498	-.011089	.232993
Equal variances not assumed			2.331	96.525	.022	.110952	.047604	.016466	.205438
UTA Equal variances assumed	2.608	.110	2.408	98	.018	11.128571	4.621996	1.956370	20.300772
Equal variances not assumed			2.628	67.776	.011	11.128571	4.235185	2.676885	19.580257
NH4+ Equal variances assumed	2.050	.155	2.117	98	.037	12.560000	5.932000	.788139	24.331861
Equal variances not assumed			2.347	70.459	.022	12.560000	5.352150	1.886698	23.233302
TA Equal variances assumed	2.432	.122	2.347	70.459	.021	12.560000	5.352150	1.886698	23.233302
Equal variances not assumed			2.557	67.469	.013	23.688571	9.265409	5.197102	42.180040

Discussion:

In this study, it was observed that high levels of creatinine in diabetic subjects compared with non-diabetics. An 8% of high levels of serum creatinine were observed in diabetic subjects in comparison with non-diabetics. The levels of Titratable acidity were significantly increased in cases compared with controls. A study carried out to determine titrable acidity in urine, collected from diabetic patients and they observed that Titratable acidity was elevated. The observation of present study is in agreement with previous study

H+ is produced from CO2 and H2O (as in the proximal tubular cells) and actively transported into the distal tubular lumen via a H+-ATPase pump. Titratable acidity represents the H+, which is buffered mostly by phosphate, which is present in significant concentration. Creatinine may also contribute to titrable acidity. At the minimum urinary pH, it will account for some of the titratable acidity. Titratable acidity is dependent on the dietary intake of phosphate and cannot be regulated to increase acid excretion. The kidney's main response to an increased acid load is to increase ammonium production and excretion .A very important feature of titrable acidity and ammonium excretion is the regeneration of bicarbonate ions. The kidney must reabsorb all filtered HCO3- in order to maintain acid base balance. Hydrogen ion secretion in the collecting tubule is very important in maximally acidifying the urine. In states of acidosis, maximal acidification of the urine in the collecting tubule must occur for adequate ammonium excretion. In states of acidosis, ammonium excretion is increased by increasing ammonium production and increased hydrogen ion secretion in the collecting duct. Aldosterone stimulates secretion of hydrogen ion in the collecting duct.

In the present study, there is significant difference in ammonia between cases and control. The level of ammonia is a high in cases when compared to controls and it correlated with previous study [8] .The effect of ammonia production on acid-base balance largely depends on the events that determine the distribution of ammonia produced between urine and blood. An additional effect of stimulated ammoniagenesis is kidney hypertrophy. Available data in humans indicate that the response of kidney to metabolic acidosis includes both changes in amino acid uptake and suppression of protein degradation. The latter effect is associated with the increase in ammonia excretion and partition into the urine [9]. In this study, the net acid excretion is more in cases than normal individuals as this study correlates with the previous studies[10]. Recently it has suggested that disturbances in acid base homeostasis may play a vital role in genesis of caridiometaboilic disorders [11].

Conclusion:

Blood glucose is positively correlated with creatinine and negatively correlated with urinary titratable acidity, urinary ammonia and net acid excretion. Creatinine is negatively correlated with urine titratable acidity, urinary ammonia and net acid excretion. Statistically significant intercorrelation is seen between urinary titratable acidity, urinary ammonia and net acid excretion. If blood glucose is increased there is increase in creatinine levels and increased levels of urinary ammonia, urinary titratable acidity and net acid excretion is noticed.

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