Original Article

HEALTH SCIENCES
MEDICINE

DOI: 10.32322/jhsm.941655
J Health Sci Med 2021; 4(5): 589-597

The association between urine pH and abnormal glucose tolerance in adults

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Cite this article as: Sözel H, Yılmaz F. The association between urine pH and abnormal glucose tolerance in adults. J Health Sci Med 2021; 4(5): 589-597.

ABSTRACT

Aim: Urine Ph (U-pH) is a clinical indicator of acid excretion in the urine and acid load in the diet. The association between low U-pH and net acid secretion with obesity, metabolic syndrome, diabetes, chronic kidney disease, and uric acid nephrolithiasis was showed. The aim of this study is to evaluate the U-pH in patients with different glucose tolerance statuses.

Material and Method: This study was designed as single-center, retrospective, and cross-sectional. A total of 1666 subjects (male/female: 531/1135) were divided into three groups according to their oral glucose tolerance test (OGTT) results: group 1=normal glucose tolerance (NGT), group 2=prediabetes, group 3=T2DM. Then subjects were divided into five groups according to their OGTT results: group 1=NGT, group 2=impaired fasting glucose (IFG), group 3=isolated impaired glucose tolerance (IGT), group 4=both IFG and IGT, and group 5=T2DM. Additionally, patients were divided into three groups according to their glycated hemoglobin (HbA1c) results: group 1=NGT, group 2=prediabetes, and group 3=T2DM. U-pH values and other outcomes were compared between groups.

Results: Age, male gender, hemoglobin, creatinine, triglycerides, and OGTT groups showed significant association with low U-pH through univariate logistic regression analyses. In model 1 (with OGTT 3 groups), it was found that creatinine (OR: 3.471; 95% CI: 1.377-8.749; p=0.008) and triglycerides (OR: 1.001; 95% CI: 1-1.003; p=0.013) were positively associated with low U-pH. Patients with T2DM (OR:1.437; 95% CI: 1.015-2.035; p=0.041) had higher risk for low U-pH compared to patients with NGT. In Model 2 (with OGTT 5 groups), creatinine (OR:3.423; 95% CI: 1.354-8.654; p=0.009) and triglycerides (OR:1.001; 95% CI: 1-1.003; p=0.014) were identified as independent predictive factors associated with low U-pH. Patients with IFG+IGT (OR:1.522; 95% CI: 1.083-2.138; p=0.015) and T2DM (OR:1.447; 95% CI: 1.022-2.049; p=0.037) had higher risk for low U-pH compared to patients with NGT.

Conclusion: In this study, the frequency of diabetes was found to be increased in patients with low U-pH. More detailed clinical studies are needed to evaluate whether different glucose tolerance statuses such as NGT, IFG, IGT, and T2DM are associated with U-pH.

Keywords: Urine pH, glucose, prediabetes, diabetes mellitus

INTRODUCTION

Type 2 diabetes mellitus (T2DM) is a frequent chronic disease and has reached an epidemic proportion worldwide. Diabetes, which is expected to reach 4.4% in 2030, its prevalence continues to increase, and the total number of patients with diabetes mellitus will reach 366 million by 2030 (1). Diabetes is often associated with protein and fat metabolic disorders, and electrolyte and acid-base imbalance. The frequency of eye, heart or renal vascular disease in diabetic patients is higher than in healthy subjects (2,3). Impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are the two main components of prediabetes. Prediabetes is a transition process from normal glucose tolerance (NGT) to diabetes, which represents a state that often progresses to overt diabetes within a few years, and may be associated with an increased risk of micro and macrovascular complications (4,5). IFG is defined as the 0-hour plasma glucose level in the oral glucose tolerance test (OGTT) from 100 mg/dL to 125 mg/dL. IGT is a condition defined as 2.hour plasma glucose level ranging from 140 mg/dL to 199 mg/dL in OGTT (6). The level of glycated hemoglobin (HbA1C) ranging from 5.7% to 6.4% is another prediabetic condition, ≥6.5 diabetes ≤5.7 is considered as normal glucose homeostasis (7).

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Received: 24.05.2021 **Accepted:** 09.08.2021

In the human body, acidic substances consisting of intracellular metabolic events and dietary nutritional sources are excreted through the lungs and kidneys. Volatile acids are excreted from the lungs via the respiratory tract as such CO_2 (15000 meq per day), while nonvolatile acids are excreted from the kidneys in the urine (1meq/kg/day per day) (8). Urine Ph is a clinical indicator of acid excretion in the urine and acid load in the diet. Also, several studies revealed the association between low urine Ph (U-pH) and net acid secretion with obesity, metabolic syndrome, T2DM, obesity, insulin resistance, chronic kidney disease, and uric acid nephrolithiasis (9–13). The role of acid-base imbalance in patients with diabetes is mediated by insulin resistance (14,15). Previous studies reported the association between diet acid load with metabolic syndrome, hypertension, and T2DM (16–19). In a study with a large number of patients, it was shown that the risk of developing diabetes in male patients with U-pH≤ 5 over a 5-year period significantly increased compared to those with U-pH≥5 (20). However, in Turkish patients, no study has previously been published that has investigated the relationship between U-pH, which is a useful marker for acid load, and OGTT findings.

We planned to examine U-pH values in participants with different glucose tolerance statuses to evaluate whether OGTT findings such as NGT, IFG, IGT, and T2DM are associated with U-pH.

MATERIAL AND METHOD

The study was carried out with the permission of Akdeniz University Faculty of Medicine Clinical Researchs Ethics Committee (Date: 08.07.2020, Decision No: 490). All procedures were carried out in accordance with the ethical rules and the principles of the Declaration of Helsinki.

Participants

This study was designed as single-center, retrospective, and cross-sectional. The study consisted of outpatients who presented to Akdeniz University Medical Faculty, Department of Internal Medicine outpatient clinic and were administered OGTT. Laboratory data were obtained from electronic patient files. The study included patients who were aged >40, had fasting blood glucose between 100-126 mg/dL, had a family history of diabetes mellitus, and symptoms of reactive hypoglycemia. Exclusion criteria for the study were lack of data for U-pH, patients using metformin and other oral antidiabetic agents due to insulin resistance, lack of data for serum creatinine, chronic liver disease, and chronic kidney disease at baseline. Furthermore, individuals who had gestational diabetes, acute or chronic inflammation, urinary infection, cardiovascular disease with corticosteroid treatment, malign disease, or other known chronic diseases were excluded from the study. After applying the inclusion and exclusion criteria, a total of 1666 subjects (male/female: 531/1135) who received between January 2015 and June 2020 were included in the study.

All individuals were divided into three groups according to the OGTT results, group 1=NGT, group 2=prediabetic, group 3=T2DM. Then, all individuals were divided into five groups based on the OGTT results, according to the World Health Organization diagnostic criteria for diabetes (6): group 1=NGT, group 2=IFG, group 3=IGT, group 4=both IFG and IGT, group 5=T2DM. Furthermore, individuals were divided into three groups based on the results of HbA1c: group 1=normal range (HbA1c <5.7%), group 2=prediabetic status (HbA1c: 5.7-6.4%), and group 3=T2DM (HbA1c ≥6.5%). Finally, participants were divided into three groups as normal, prediabetes, and T2DM by evaluating both OGTT and HbA1c results together.

The estimated glomerular filtration rate (eGFR) was calculated as follows based on CKD-EPI 2009 (Chronic Kidney Disease Epidemiologic Collaboration):eGFR=141 x min (Scr/k, 1) a x max (Scr/k, 1) -1,209 x0,993 Age x 1,018 [Women] x 1,159 [Black race]. -Scr=serum creatinine, k=0.7 for women and 0.9 for men, a=-0.329 for women and -0.411 for men. min=Scr/k minimum.

Statistics

Statistical analyzes were conducted by using IBM SPSS Statistics for Windows, Version 23.0 (IBM Corp., Armonk, NY). Normality assumptions were controlled by the Shapiro–Wilk test. Descriptive analyzes were presented using mean±SD, median (min-max) or n (%), where appropriate. Categorical data was analyzed using Pearson's chi-square. Kruskal Wallis test was used for comparison of nonparametric variables between groups and Bonferroni-Dunn test was used as a post hoc test for significant cases, while One-Way ANOVA with post hoc Tukey HSD test was used for parametric variables. Univariate and multivariate logistic regression analyzes were used to determine independent risk factors associated with low U-pH (pH=5.0). Odds ratio (OR) was reported with the corresponding 95% confidence intervals (95% CI). A p-value of less than 0.05 was considered statistically significant.

RESULTS

Baseline Characteristics

The mean age of the 1666 patients included in the study was 50.77±13.23 years, and 68.1% were women. When participants were divided into three groups according to their OGTT results as NGT, prediabetes, and T2DM; the mean age of the diabetic group and the percentage of male participants were higher than in the other

groups. Additionally, the creatinine, HbA1c, and 1st hour glucose values were higher in the diabetic group. eGFR was highest in the NGT group and lowest in the diabetic group (Table 1). The U-pH values of the diabetes and prediabetes groups were lower than those of the NGT group, while the percentage of patients with pH=5.0 was lower in the NGT group than in the other two groups. The CRP and triglyceride values of the prediabetes and diabetic groups were higher and the HDL values were lower (**Table 1**).

Relationship Between U-pH and OGTT

When the prediabetic group was divided into three groups, IGF, IGT, and IFG+IGT; while the eGFR values were highest in NGT group and lowest in the T2DM group (p<0.001). The percentage of male patients and serum creatinine in the IFG+IGT and diabetic group were higher than in the other groups (p<0.001). The HbA1c values of the IFG and IGT groups were similar. As the prevalence of diabetes increased, the glucose levels at the first hour also increased (p<0.001). The U-pH values of the IFG+IGT and T2DM groups were lower than those of the NGT group (p=0.007). The percentage of patients with pH 5.0 in the NGT group was lower than in the IFG+IGT and T2DM groups (p=0.026) (**Table 2**). Other biochemical parameters compared for the patients are shown in **Table 2**.

Relationship Between U-pH and HbA1c

The patients were divided into three groups according to their HbA1c values as <5.7 (n=531), 5.7-6.49 (n=1019), and ≥ 6.5 (n=116). There were no significant differences between the gender distributions (p=0.115) and the U-pH values (p=0.534) of the groups. It was observed that as the HbA1c level increased, the glucose levels in the first hour also increased (p<0.001). The hemoglobin (p=0.006) and eGFR (p<0.001) values of the group with HbA1c<5.7 were higher than the other two groups, and CRP (p<0.001), triglyceride (p=0.015), and uric acid (p=0.031) were lower. HDL was lower in the group with HbA1c≥6.5 (p=0.004) (**Table 3**).

Relationship Between U-pH and OGTT/HbA1c

The patients were divided into three groups according to their OGTT and HbA1c values such as NGT (n=240), prediabetes (n=1071), and T2DM (n=355). There was no significant difference between the groups in terms of albumin (p=0.206), and U-pH values (p=0.132). The hemoglobin value of the diabetic group was higher than that of the prediabetes group (p=0.017) and its creatinine was higher than that of the other two groups (p<0.001). It was observed that as the frequency of diabetes increased, the eGFR values decreased and the triglyceride values increased (p<0.001). The HDL of the diabetic group was lower than that of the NGT group (p=0.009) (**Table 4**).

Table 1. Comparison of patients' characteristics according to OGTT groups

Variables	Overall	NGT	Prediabetes	T2DM	p values
Number (%)	1666	532 (31.9)	826 (49.6)	308 (18.5)	-
Age (years)	50.77±13.23	45.72±14.34[a]	52.23±12.14[b]	55.57±11.16[c]	<0.001
Gender					
Male	531 (31.9)	149 (28)[a]	258 (31.2)[a]	124 (40.3)[b]	0.001
Female	1135 (68.1)	383 (72)	568 (68.8)	184 (59.7)	
Hemoglobin (g/L)	13.51±1.54	13.27±1.62[a]	13.55±1.45[b]	13.86±1.54[c]	<0.001
Creatinine (mg/dL)	0.75±0.16	0.72±0.15[a]	0.75±0.16[b]	0.78±0.16[c]	<0.001
eGFR (mL/min/1.73m²)	110.48 (65.9-188.76)	116.84 (70.72-162.11)[a]	109.03 (65.9-188.76)[b]	105.99 (67.51-144.95)[c]	<0.001
CRP (mg/dL)	0.27 (0-30.39)	0.18 (0-2.86)[a]	0.32 (0.01-30.39)[b]	0.35 (0.01-4.79)[b]	<0.001
Uric acid (mg/dL)	5.45±1.48	5.13±1.42	5.57±1.54	5.59±1.41	0.114
Triglycerides (mg/dL)	138 (30.62-1265.54)	124 (30.62-1020)[a]	141 (35-1265.54)[b]	150.68 (40-1201)[b]	<0.001
LDL (mg/dL)	134.9±37.23	132.82±38.59	135.55±36.54	136.46±36.86	0.430
HDL (mg/dL)	46.75 (18-109.7)	49.4 (23-109.7)[a]	46.05 (18-109.5)[b]	44.4 (21-90.2)[b]	0.001
Albumin (g/dL)	4.45 (2.9-5.65)	4.4 (3.64-5.18)	4.47 (2.9-5.65)	4.46 (3.78-5.14)	0.082
HbA1c (%)	5.9 (4-11.6)	5.7 (4-7)[a]	5.9 (4.2-7.1)[b]	6.2 (4.4-11.6)[c]	<0.001
<5.7	531 (31.9)	240 (45.1)[a]	253 (30.6)[b]	38 (12.3)[c]	<0.001
5.7-6.49	1019 (61.2)	285 (53.6)[a]	533 (64.5)[b]	201 (65.3)[b]	
≥6.5	116 (7)	7 (1.3)[a]	40 (4.8)[b]	69 (22.4)[c]	
1st hour glucose (mg/dL)	177.94±49	139.96 ±35.98[a]	188.42±37.19[b]	242.93±36.06[c]	<0.001
U-pH	5 (5-9)	5.5 (5-8.5)[a]	5 (5-8.5)[b]	5 (5-9)[b]	0.004
pH 5.0	864 (51.9)	247 (46.4)[a]	442 (53.5)[b]	175 (56.8)[b]	0.003
pH 5.5	237 (14.2)	82 (15.4)[a]	119 (14.4)[a]	36 (11.7)[a]	
pH 6.0	178 (10.7)	58 (10.9)[a]	90 (10.9)[a]	30 (9.7)[a]	
pH 6.5	178 (10.7)	62 (11.7)[a]	81 (9.8)[a]	35 (11.4)[a]	
pH ≥7	209 (12.5)	83 (15.6)[a]	94 (11.4)[a]	32 (10.4)[a]	

OGTT, oral glucose tolerance test; NGT, normal glucose tolerance; T2DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HbA1c: glycated hemoglobin; U-pH, Urine pH
Data are presented as mean±SD, median (min-max), or n (%). ANOVA, Kruskal-Wallis test, Pearson chi-square test. Different lower case letters in a row indicate statistically significant difference between groups.

Table 2. Comparison of patients' characteristics according to OGTT subgroups

Variables	NGT	Prediabetes IFG	Prediabetes IGT	Prediabetes IFG+IGT	T2DM	p values
Number (%)	532 (31.9)	371 (22.3)	148 (8.9)	307 (18.4)	308 (18.5)	-
Age (years)	45.72±14.34[a]	51.39±12.24[b]	51.14±13.06[b]	53.77±11.41[b,c]	55.57±11.16[c]	<0.001
Gender						
Male	149 (28)[a]	109 (29.4)[a]	35 (23.6)[a]	114 (37.1)[b]	124 (40.3)[b]	<0.001
Female	383 (72)	262 (70.6)	113 (76.4)	193 (62.9)	184 (59.7)	
Hemoglobin (g/L)	13.27±1.62[a]	13.52±1.45[a,b]	13.3±1.33[a,b]	13.7±1.48[b]	13.86±1.54[c]	<0.001
Creatinine (mg/dL)	0.72±0.15[a]	0.74±0.15[a]	0.74±0.17[a]	0.77±0.17[b]	0.78±0.16[b]	<0.001
eGFR (mL/min/1.73m²)	116.84 (70.72-162.11)[a]	109.49 (67.51-160.8)[b]	109.81 (72.66-154.67)[b]	107.9 (65.9-188.76)[b,c]	105.99 (67.51-144.95)[c]	<0.001
CRP (mg/dL)	0.18 (0-2.86)[a]	0.33 (0.01-30.39)[b]	0.35 (0.01-5.07)[b]	0.31 (0.01-6.39)[b]	0.35 (0.01-4.79)[b]	<0.001
Uric acid (mg/dL)	5.13±1.42	5.31±1.47	5.68±1.71	5.75±1.48	5.59±1.41	0.167
Triglycerides (mg/dL)	124 (30.62-1020)[a]	133 (35-1265.54)[a]	143.83 (47-1066)[b]	148.31 (38.94-743)[b]	150.68 (40-1201)[b]	<0.001
LDL (mg/dL)	132.82±38.59	135.4±37.41	138.28±36.24	134.4±35.63	136.46±36.86	0.654
HDL (mg/dL)	49.4 (23-109.7)[a]	48 (18-94.2)[a,b]	47.9 (21.9-102.3)[a,b]	44.1 (22-109.5)[b]	44.4 (21-90.2)[b]	0.002
Albumin (g/dL)	4.4 (3.64-5.18)	4.47 (3.37-5.65)	4.47 (3.79-5.17)	4.46 (2.9-5.16)	4.46 (3.78-5.14)	0.285
HbA1c (%)	5.7 (4-7)[a]	5.8 (4.2-7.1)[b]	5.86 (4.6-7.04)[b]	6 (4.7-6.88)[c]	6.2 (4.4-11.6)[d]	<0.001
<5.7	240 (45.1)[a]	128 (34.5)[b]	52 (35.1)[b]	73 (23.8)[c]	38 (12.3)[d]	<0.001
5.7-6.49	285 (53.6)[a]	226 (60.9)[a,b]	92 (62.2)[a,b]	215 (70)[b]	201 (65.3)[b]	
≥6.5	7 (1.3)[a]	17 (4.6)[b]	4 (2.7)[a,b]	19 (6.2)[b]	69 (22.4)[c]	
1st hour glucose (mg/dL)	139.96±35.98[a]	171.71±36.84[b]	184.57±28.99[c]	209.67±29.97[d]	242.93±36.06[e]	<0.001
U-pH	5.5 (5-8.5)[a]	5.5 (5-8.5)[a,b]	5 (5-8.5)[a,b]	5 (5-8)[b]	5 (5-9)[b]	0.007
pH 5.0	247 (46.4)[a]	183 (49.3)[a,b]	79 (53.4)[a,b]	180 (58.6)[b]	175 (56.8)[b]	0.026
pH 5.5	82 (15.4)[a]	67 (18.1)[a]	16 (10.8)[a]	36 (11.7)[a]	36 (11.7)[a]	
pH 6.0	58 (10.9)[a]	47 (12.7)[a]	17 (11.5)[a]	26 (8.5)[a]	30 (9.7)[a]	
pH 6.5	62 (11.7)[a]	32 (8.6)[a]	15 (10.1)[a]	34 (11.1)[a]	35 (11.4)[a]	
pH ≥7	83 (15.6)[a]	42 (11.3)[a]	21 (14.2)[a]	31 (10.1)[a]	32 (10.4)[a]	

OGTT, oral glucose tolerance test; NGT, normal glucose tolerance; ; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HbA1c: glycated hemoglobin; U-pH, Urine pH
Data are presented as mean±SD, median (min-max), or n (%). ANOVA, Kruskal-Wallis test, Pearson chi-square test. Different lowercase letters in a row indicate statistically significant difference between groups.

Table 3. Comparison of patients' characteristics according to HbA1c groups

Variables	<5.7	5.7-6.49	≥6.5	p values
Number (%)	531 (31.9)	1019 (61.2)	116 (7)	-
Age (years)	45.52±13.73[a]	53.12±12.45[b]	54.2±10.28[b]	<0.001
Gender				
Male	164 (30.9)	320 (31.4)	47 (40.5)	0.115
Female	367 (69.1)	699 (68.6)	69 (59.5)	
Hemoglobin (g/L)	13.7±1.51[a]	13.42±1.51[b]	13.41±1.79[b]	0.006
Creatinine (mg/dL)	0.74±0.15	0.75±0.17	0.77±0.16	0.084
eGFR (mL/min/1.73 m²)	116.39 (72.66-162.11)[a]	108.92 (65.9-188.76)[b]	106.94 (76.86-154.81)[b]	<0.001
CRP (mg/dL)	0.2 (0-30.39)[a]	0.32 (0-6.39)[b]	0.55 (0.08-2.13)[b]	<0.001
Uric acid (mg/dL)	5.12±1.55[a]	5.59±1.39[b]	5.93±1.65[b]	0.031
Triglycerides (mg/dL)	131.95 (30.62-1066)[a]	141.27 (35-1265.54)[b]	137 (40-442)[b]	0.015
LDL (mg/dL)	131.74±36.5	136.19±38.09	136.64±31.9	0.168
HDL (mg/dL)	47.48 (20.9-109.7)[a]	47.1 (18-109.5)[a]	41.2 (27-90.2)[b]	0.004
Albumin (g/dL)	4.42 (3.77-5.18)	4.45 (2.9-5.65)	4.51 (3.81-4.98)	0.863
HbA1c (%)	5.37 (4-5.69)[a]	6 (5.7-6.49)[b]	6.7 (6.5-11.6)[c]	<0.001
1st hour glucose (mg/dL)	159.25±45.65[a]	185.31±47.03[b]	217.79±50.16[c]	<0.001
U-pH	5 (5-9)	5 (5-8.5)	5 (5-8)	0.534
pH 5.0	270 (50.8)	533 (52.3)	61 (52.6)	0.735
pH 5.5	69 (13)	146 (14.3)	22 (19)	
pH 6.0	60 (11.3)	107 (10.5)	11 (9.5)	
pH 6.5	57 (10.7)	110 (10.8)	11 (9.5)	
pH ≥7	75 (14.1)	123 (12.1)	11 (9.5)	

eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HbA1c: glycated hemoglobin; U-pH, Urine pH
Data are presented as mean±SD, median (min-max), or n (%). ANOVA, Kruskal-Wallis test, Pearson chi-square test. Different lowercase letters in a row indicate statistically significant difference between groups.

Table 4. Comparison of patients' characteristics according to OGTT-HbA1c combined groups

Variables	NGT	Prediabetes	T2DM	p values
Number (%)	240 (14.4)	1071 (64.3)	355 (21.3)	-
Age (years)	40.28±13.39[a]	51.61±12.66[b]	55.34±10.97[c]	<0.001
Gender				
Male	69 (28.7)[a]	323 (30.2)[a]	139 (39.2)[b]	0.004
Female	171 (71.3)	748 (69.8)	216 (60.8)	
Hemoglobin (g/L)	13.58±1.58[a,b]	13.43±1.49[a]	13.72±1.63[b]	0.017
Creatinine (mg/dL)	0.72±0.14[a]	0.74±0.16[a]	0.78±0.16[b]	<0.001
eGFR (mL/min/1.73 m²)	123.07 (84.54-162.11)[a]	110.2 (65.9-188.76)[b]	106.2 (67.51-154.81)[c]	<0.001
CRP (mg/dL)	0.16 (0-2.09)[a]	0.28 (0-30.39)[b]	0.36 (0.01-4.79)[b]	<0.001
Uric acid (mg/dL)	4.86±1.52[a]	5.55±1.43[b]	5.57±1.53[b]	0.035
Triglyserides (mg/dL)	115 (30.62-484.88)[a]	138.45 (35-1265.54)[b]	149.45 (40-1201)[c]	<0.001
LDL (mg/dL)	127.13±36.08[a]	136.04±37.85[b]	135.87±35.63[b]	0.027
HDL (mg/dL)	49.3 (26.1-109.7)[a]	47.1 (18-109.5)[a,b]	44.4 (21-90.2)[b]	0.009
Albumin (g/dL)	4.4 (3.77-5.18)	4.45 (2.9-5.65)	4.49 (3.78-5.14)	0.206
HbA1c (%)	5.3 (4-5.69)[a]	5.9 (4.2-6.49)[b]	6.3 (4.4-11.6)[c]	<0.001
1st hour glucose (mg/dL)	131.46±35.98[a]	176.68±40.59[b]	233.21±41.72[c]	<0.001
U-pH	5.5 (5-8.5)	5 (5-8.5)	5 (5-9)	0.132
pH 5.0	118 (49.2)	545 (50.9)	201 (56.6)	0.597
pH 5.5	36 (15)	157 (14.7)	44 (12.4)	
pH 6.0	24 (10)	120 (11.2)	34 (9.6)	
pH 6.5	26 (10.8)	113 (10.6)	39 (11)	
pH ≥7	36 (15)	136 (12.7)	37 (10.4)	

OGTT, oral glucose tolerance test; NGT, normal glucose tolerance; T2DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; CRP, C-reactive protein; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HbA1c: glycated hemoglobin; U-pH, Urine pH
Data are presented as mean±SD, median (min-max), or n (%). ANOVA, Kruskal-Wallis test, Pearson chi-square test. Different lowercase letters in a row indicate statistically significant difference between groups.

The patients were divided into five groups according to U-pH values of 5.0 (n=864), 5.5 (n=237), 6.0 (n=178), 6.5 (n=178) and ≥7 (n=209). There was no difference between the groups in terms of the combined group distribution of LDL-c, glucose in the first hour, HbA1c, and OGTT-HbA1c combined group distribution (**Table 5**). The mean age of the pH 5.5 group was lower than that of the pH 5.0 and 6.5 groups (p=0.007). In the pH 5.0 group, the percentage of male patients and normal OGTT was higher than in the pH≥7 group (p=0.032, p=0.026, respectively). Hemoglobin value was higher in the pH 5.5 group than the ≥7 group (p=0.042). Creatinine in the pH 5.0 group was higher (p<0.001). The lowest eGFR values were observed in the pH 5.0 and 6.5 groups (p=0.001). The uric acid value of the pH 5.5 group was higher than that of the pH 6.5 and grub ≥7 group (p=0.005). Triglyceride values were higher in the pH 5.0 and 6.5 group compared to the pH 6.0 and ≥7 group (p=0.003). HDL-c was lower in the pH 5.0 group than in the pH 6.0 and ≥7 group (p=0.004).

Logistic Regression Analysis

Age, male gender, hemoglobin, creatinine, triglycerides, and OGTT groups showed a significant association with low U-pH through univariate logistic regression analyzes. In model 1 (with OGTT 3 groups), it was found that creatinine (OR:3.471; 95% CI:1.377-8.749; p=0.008) and triglycerides (OR:1.001; 95% CI:1-1.003; p=0.013) were positively associated with low U-pH. Patients with T2DM (OR:1.437; 95% CI:1.015-2.035; p=0.041) had a higher risk of low U-pH compared to patients with NGT. In Model 2 (with OGTT 5 groups), creatinine (OR:3.423; 95% CI:1.354-8.654; p=0.009) and triglycerides (OR:1.001; 95% CI:1-1.003; p=0.014) were identified as independent predictive factors associated with low U-pH. Patients with IFG+IGT (OR:1.522; 95% CI:1.083-2.138; p=0.015) and T2DM (OR:1.447; 95% CI: 1.022-2.049; p=0.037) had a higher risk of low U-pH compared to patients with NGT (**Table 6**).

DISCUSSION

We present a study with a large number of subjects who had no chronic disease at baseline, its results showed that the level of U-pH was significantly associated with various glucose tolerance statuses.

Providing a fixed intracellular and extracellular pH level is essential for the body to perform its normal physiological function, and it is regulated by complex biological processes. Goel and Calvert have demonstrated that the systems that provide the balance of acid and base are made up of the intracellular and extracellular buffering systems, the respiratory system, and the urinary system (21). Additionally, all fats, carbohydrates, and proteins affect the pH of the body. As our body produces approximately 2-3 mEq/kg H^+ ions daily, changes in acid-base balance regulated by cellular metabolism are highly associated with diet components.

Table 5. Comparison of patients' characteristics according to U-pH groups

Variables	U-pH groups					p values
	pH 5.0	pH 5.5	pH 6.0	pH 6.5	pH ≥7.0	
Number (%)	864 (51.9)	237 (14.2)	178 (10.7)	178 (10.7)	209 (12.5)	
Age (years)	51.55±12.58[a]	48.35±14.72[b]	50.16±13.28[a,b]	52.03±13.04[a]	49.73±13.84[a,b]	0.007
Gender						
Male	296 (34.3)[a]	79 (33.3)[a,b]	53 (29.8)[a,b]	55 (30.9)[a,b]	48 (23)[b]	0.032
Female	568 (65.7)	158 (66.7)	125 (70.2)	123 (69.1)	161 (77)	
Hemoglobin (g/L)	13.55±1.51[a,b]	13.71±1.56[a]	13.39±1.53[a,b]	13.47±1.55[a,b]	13.26±1.57[b]	0.042
Creatinine (mg/dL)	0.76±0.16[a]	0.74±0.15[a,b]	0.74±0.16[a,b]	0.74±0.17[a,b]	0.7±0.15[b]	<0.001
eGFR (mL/min/1.73 m²)	109.49 (65.9-188.76)[a]	112.27 (71.56-162.11)[b]	112.39 (78.14-150.28)[b]	107.78 (70.81-157.68)[a]	113.34 (70.72-154.81)[b]	0.001
Uric acid (mg/dL)	5.49±1.56[a,b]	6.26±1.61[a]	5.62±1.38[a,b]	5.11±1.09[b]	4.9±1.21[b]	0.005
Triglycerides (mg/dL)	142.11 (33-1066)[a]	133.08 (35-743)[a,b]	131 (30.62-442)[b]	144.28 (31.08-1265.54)[a]	127 (35.78-1201)[b]	0.003
LDL (mg/dL)	133.77±37.3	136.18±40.05	133.47±36.07	138.21±36.48	136.49±35.47	0.699
HDL (mg/dL)	44.9 (18-109.7)[a]	45.9 (27-89.8)[a,b]	51 (20.9-109.5)[b]	47.75 (25.2-80)[a,b]	49.8 (21.9-102.1)[b]	0.004
HbA1c (%)	5.9 (4-11.2)	5.9 (4.2-10.6)	5.89 (4.4-11.6)	5.89 (4.3-7.71)	5.8 (4.2-7.75)	0.428
<5.7	270 (31.3)	69 (29.1)	60 (33.7)	57 (32)	75 (35.9)	0.735
5.7-6.5	533 (61.7)	146 (61.6)	107 (60.1)	110 (61.8)	123 (58.9)	
≥6.5	61 (7.1)	22 (9.3)	11 (6.2)	11 (6.2)	11 (5.3)	
1st hour glucose (mg/dL)	181.34±48.41	172.66±50.17	175.34±48.29	173.86±49.53	175.89±49.61	0.084
OGTT						
NGT	247 (28.6)[a]	82 (34.6)[a,b]	58 (32.6)[a,b]	62 (34.8)[a,b]	83 (39.7)[b]	0.026
IFG	183 (21.2)[a]	67 (28.3)[a]	47 (26.4)[a]	32 (18)[a]	42 (20.1)[a]	
IGT	79 (9.1)[a]	16 (6.8)[a]	17 (9.6)[a]	15 (8.4)[a]	21 (10)[a]	
IFG+IGT	180 (20.8)[a]	36 (15.2)[a]	26 (14.6)[a]	34 (19.1)[a]	31 (14.8)[a]	
T2DM	175 (20.3)[a]	36 (15.2)[a]	30 (16.9)[a]	35 (19.7)[a]	32 (15.3)[a]	
Combined groups						
NGT	118 (13.7)	36 (15.2)	24 (13.5)	26 (14.6)	36 (17.2)	0.597
Prediabetes	545 (63.1)	157 (66.2)	120 (67.4)	113 (63.5)	136 (65.1)	
T2DM	201 (23.3)	44 (18.6)	34 (19.1)	39 (21.9)	37 (17.7)	

OGTT, oral glucose tolerance test; NGT, normal glucose tolerance; ; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2DM, diabetes mellitus; eGFR, estimated glomerular filtration rate; LDL, low-density lipoprotein; HDL, high-density lipoprotein; HbA1c: glycated hemoglobin; U-pH, Urine pH
Data are presented as mean±SD, median (min-max), or n (%). ANOVA, Kruskal-Wallis test, Pearson chi-square test. Different lowercase letters in a row indicate statistically significant difference between groups.

Table 6. Univariate and multivariate logistic regression analysis for low U-pH (U-pH ≤5.0)

Variables	Univariate analysis		Model 1 with OGTT 3 groups		Model 2 with OGTT 5 groups	
	OR (95%CI)	P values	OR (95%CI)	p	OR (95%CI)	p values
Age (years)	1.009 (1.002-1.017)	0.012	1.002 (0.993-1.011)	0.644	1.002 (0.992-1.011)	0.724
Male Gender	1.257 (1.022-1.547)	0.030	0.959 (0.682-1.349)	0.811	0.954 (0.678-1.341)	0.785
Hemoglobin (g/L)	1.097 (1.021-1.178)	0.011	0.966 (0.88-1.059)	0.458	0.964 (0.879-1.058)	0.439
Creatinine (mg/dL)	3.27 (1.742-6.138)	<0.001	3.471 (1.377-8.749)	0.008	3.423 (1.354-8.654)	0.009
Triglycerides (mg/dL)	1.002 (1.001-1.003)	0.002	1.001 (1-1.003)	0.013	1.001 (1-1.003)	0.014
OGTT 3 groups						
NGT	Reference	-	Reference	-	-	-
Prediabetes	1.328 (1.067-1.652)	0.011	1.296 (0.994-1.689)	0.055	-	-
T2DM	1.518 (1.144-2.014)	0.004	1.437 (1.015-2.035)	0.041	-	-
OGTT 5 groups						
NGT	Reference	-	-	-	Reference	-
IFG	1.123 (0.861-1.465)	0.391	-	-	1.188 (0.866-1.632)	0.286
IGT	1.321 (0.917-1.903)	0.135	-	-	1.173 (0.763-1.804)	0.468
IFG+IGT	1.635 (1.231-2.172)	0.001	-	-	1.522 (1.083-2.138)	0.015
T2DM	1.518 (1.144-2.014)	0.004	-	-	1.447 (1.022-2.049)	0.037

OGTT, oral glucose tolerance test; NGT, normal glucose tolerance; ; IFG, impaired fasting glucose; IGT, impaired glucose tolerance; T2DM, diabetes mellitus; OR, Odds ratio; CI,confidence intervals

The intracellular buffering system is managed by a system that uses proteins and organic phosphates for the regulation of acid-base balance. Intracellular buffering occurs by binding bicarbonate (HCO_3) and H+ ions and performing extracellular carbon dioxide (CO_2) and water (H_2O) secretion (22). Intracellular and extracellular buffering systems provide short-term solutions to alkalemia and acidemia. For this reason, there are supporting mechanisms such as the respiratory and urinary systems to regulate extracellular pH. When an increase in extracellular H^+ ions is felt, the respiratory system may accelerate respiration to remove CO_2, which is a weak acid, from the body. On the contrary, in the event of a decrease in the amount of H^+ ions, the brain chemosensors are stimulated to slow respiration and keep CO_2 in the body (23). The effect of the respiratory system is observed very quickly and it can change the pH level in hours or even minutes. The urinary system is much more complex and slow, and, for this reason, it takes hours and days for it to change H^+ ion levels during urination, excessive H^+ ions and ammonia are excreted into urine. The ammonia produced in renal tubular cells spreads in the intraluminal space and is bound to H^+ ions and provides excretion from the body in the acidosis period. Furthermore, potassium ions, calcium ions, and urinary phosphate are also excreted from the body by urine in the acute and chronic acidosis periods (8,24). As plasma bicarbonate has a buffering function, it hides, and it is reabsorbed by the urinary system and secreted back to plasma. Cellular metabolism is responsible for the continuous changes in the acid-base balance and is regulated by one or several of the systems that regulate the acid-base balance.

Until now, possible explanations have been proposed for the association between U-pH and diabetes. In this study incidence of diabetes and prediabetes was observed to increase in patients with urine PH 5. Furthermore, as PH progressed from 5 to 7, it was observed that the eGFR of the patients decreased and their levels of triglycerides and uric acid decreased.

U-pH is also low in people with a low or high body mass index. However, morbid obese patients were excluded in this study and their BMI values were not present.

Acid-base alterations are associated with insulin resistance, through defective renal ammoniagenesis and reduced insulin action in its signaling pathways (10,25,26). One of the possible explanations is the influence of insulin on renal excretion and ammoniac (NH4) production, which is an important urinary buffer (27). Insulin physiologically stimulates NH4 production and secretion. In the case of insulin resistance, this production and secretion of the proximal tubules is impaired (28). Metabolic acidosis increases cortisol secretion by stimulating glucocorticoid synthesis, increased cortisol production causes insulin resistance, and increased visceral obesity. Metabolic acidosis causes insulin resistance by affecting the levels of calcium and magnesium ions that are effective in insulin metabolism. In addition, the acidotic state disrupts insulin-like growth factor secretion, causing insulin ineffectiveness and hyperinsulinemia (14). Insulin level and insulin resistance were not evaluated in our study.

U-pH decreases due to increased excretion of hydrogen ions (H^+) into the urine or decreased elimination of urinary H^+ (29,30). Acceleration of the renin-angiotensin system and increased intrarenal oxidative stress lead to the supply of H^+ by activating the sodium-hydrogen exchanger (31–32). The supply of NH4 to urine controls, elimination of urinary H+, and lower plasma bicarbonate levels have been reported to pose a risk of incident diabetes (9,34). As U-pH correlates positively with body fluid pH, the U-pH level will be a surrogate marker of insulin resistance in the body.

A cohort study with 3119 men showed evidence that the risk of incident diabetes was significantly higher in men with the lowest U-pH (OR: 2.69; subjects with U-pH ≥6.5 as a reference) (20). In a population-based prospective study in 64660 Japanese adults, there was an association between dietary acid load score and type 2 T2DM in men, while in women it was not (19). Univariate and multivariate analysis showed that urine with low creatinine and triglyceride levels are risk factors for PH. In the univariate analysis, age, male gender, and hemoglobin were found to be associated with low U-pH levels. In many studies, low U-pH was found to be associated with obesity, diabetes, and insulin resistance in men, while the same was not found in women (35). U-pH in women has been reported to be higher than in men and this is associated with increased citrate excretion in the urine in women (35). In the same study, the risk of developing diabetes in male patients with low U-pH during a 10-year follow-up was approximately twice as high as in women. In this low rate of diabetes development in women, it is effective that estrogen increases hepatic gluconeogenesis and causes increased glucose entry into skeletal muscle (35,36).

Limitations

Despite the retrospective nature of this study, it also has several strengths, as it contains a large number of subjects. However, there are some acceptable limitations of our study. First, considering the literature showing that fasting U-pH is significantly correlated with 24-hour U-pH, we used spot urine testing to measure U-pH (38) Second, although individuals with a history of obesity in hospital records were excluded from the

study because U-pH was inversely correlated with body weight and body mass index, some individuals could not be separated due to a lack of body composition data (38). Third, although dietary components, the presence of acid-deficiency-related acidification defects, plasma pH, and plasma bicarbonate/lactate concentrations can affect U-pH, we did not have any data on them in our study (34,39,40). Fourth, the lack of electrolyte and ammonia measurements in the urine and, therefore, the urine base deficit could not be evaluated. Another limitation of our study was the lack of evaluation of liver function tests, family history of T2DM, smoking habits, and alcohol consumption. Therefore, more detailed prospective studies are needed.

CONCLUSION

In this study, the frequency of diabetes was found to be increased in patients with low U-pH. More detailed clinical studies are needed to evaluate whether different glucose tolerance statuses such as NGT, IFG, IGT, and T2DM are associated with U-pH.Financial Disclosure: No financial disclosure was declared by the authors.

ETHICAL DECLARATIONS

Ethics Committee Approval: The study was carried out with the permission of Akdeniz University Faculty of Medicine Clinical Research Ethics Committee (Date: 08.07.2020, Decision No: 490).

Informed Consent: Because the study was designed retrospectively, no written informed consent form was obtained from patients.

Referee Evaluation Process: Externally peer-reviewed.

Conflict of Interest Statement: The authors have no conflicts of interest to declare.

Financial Disclosure: The authors declared that this study has received no financial support.

Author Contributions: All of the authors declare that they have all participated in the design, execution, and analysis of the paper, and that they have approved the final version.

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