Variations of Biochemical and Cellular Urine Parameters among a Healthy Lebanese Group

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Abstract Objectives: Urine analysis, a simple and inexpensive test, is the cornerstone in the evaluation of the kidney function and may reflect some pathophysiologies. Materials: A cross sectional study was carried out in Lebanese University of Lebanon. One hundred asymptomatic students and staff were enrolled in this study. Urine samples were obtained from students and staff and were tested by dipstick method and trypan blue for urine cell count. Results: Among all the samples, leukocyturia and hematuria were the most common abnormalities found with a prevalence of 39% and 24% respectively, followed by proteinuria (13%), nitrituria (8%) and glycosuria (8%). Urinary abnormalities were more common in females than in males. Number of living urine cells was associated to the presence of glucose, proteins and leucocytes in urine ($p=0.001$, $p=0.049$ and $p=0.027$ respectively). Urine viable cells were also significantly correlated with the gender ($p=0.000$), alcohol and theine consumption ($p=0.023$, $p=0.034$ respectively) and smoking status ($p=0.003$). Conclusion: Asymptomatic urinary abnormalities might be detected by urine screening. Our results confirm that gender and environment factors might affect urine composition.

Keywords Dipstick Urine Analysis, Kidney Function, Environmental Factors, Urine Analysis Screening, Urine Cell Count

patient-specific kidney cells that can be harvested noninvasively. The greatest advantages of urine as a source of viable cells are the easy collection and less complicated ethical issues [3]. General urine examination includes macroscopic, chemical and microscopic urinalysis. The macroscopic analysis includes examination of urine appearance and color. The chemical examination includes specific gravity, qualitative presence of proteins, blood, nitrites, glucose, ketones, bilirubin, urobilinogens, etc. The microscopic examination refers to the presence of erythrocytes, leukocytes, epithelial cells, cylinders, bacteria, crystals, etc.

In recent years, urine has emerged as a source of urine cells [4]. Various cells may be encountered in the urine sediment. Cells of Hematologic Origin like Erythrocytes (RBCs), Leukocytes (WBCs), Neutrophils, Glitter Cells (a type of neutrophil seen in hypotonic urine of specific gravity 1.010 or less), Eosinophils (Although difficult to recognize in the usual wet preparation of the urine sediment) and Lymphocytes (although they are rarely recognized, a few small lymphocytes are normally present in urine). Other type of cells, like Epithelial Origin example Renal Epithelial Cells (or renal tubular epithelium), Renal Tubular Fat Bodies (renal epithelial cells or macrophages that have filled with fat or lipid droplets), transitional Epithelial Cells (Urothelial Cells) and Squamous Epithelial Cells. Finally, some Microorganisms Encountered in the Sediment example bacteria and yeast [5].

Urine analysis, a simple and inexpensive test, is the cornerstone in the evaluation of the kidney function. Serious renal diseases may be present without any symptoms. Proteinuria as well as hematuria may be the only early signs of renal disease [6]. Also, the presence of detectable nitrites in urine has been used to diagnose urinary tract infection. Urinary tract infection is very common with severe consequences on the kidney function leading to chronic kidney disease and hypertension if left untreated [7,8].

It is well known that various biological factors affect the metabolic composition of biological fluids. They are intrinsic

1. Introduction

Traditionally, clinicians and researchers have relied on a skin biopsy or blood extraction as relatively accessible supplies for *in vitro* cell expansion and biological studies. Perhaps surprisingly, limited attention has been given to a totally noninvasive source, urine, which eliminates the discomfort associated with other procedures [1]. Urine may be a waste product, but it contains an enormous amount of information [2]. It represents an unlimited source of

(e.g. age, gender) and extrinsic (e.g. diet, smoking, stress, exercise). The urine composition is typical among all individuals but anatomical difference in the urinary tract between men and women can generate a difference in the number of urine cells.

Epidemiological data on the incidence and prevalence of chronic kidney disease in the pediatric population is currently limited, imprecise, and flawed by methodological differences between various data resources. It is estimated to be approximately 18 per million [6].

Furthermore, many factors could be the reason for changes in the urine composition or even in the number of urine cells. In this study, we searched for a correlation between the number of urine cells and different parameters involving gender, lifestyle, family history, environmental factors, medications and urine biochemical composition. The aim of this study was to investigate variability in the biochemical urinary profile and urine cell count between male and female in a Lebanese population. The dipstick urinalysis screening was also conducted to detect abnormal urinary findings in asymptomatic persons.

2. Materials and Methods

2.1. Study Group

The study population consisted of 100 Lebanese University personals (73 females and 27 males) whose ages ranged between 17 and 60. All participants signed a written informed consent and completed a questionnaire about their nutritional habits, age, current health status, smoking habits, physical exercise, daily water intake, medicine intake and other factors that affect the quality of urine and urination, the presence of any pathology or disease family history, or any drug taking history.

2.2. Urine Dipstick Chemical Analysis

Appropriate containers (up to 100ml) were sterilized before urine collection. The first micturition of the day was routinely avoided. Besides, only the mid-stream of urine was later on collected into sterile containers. Urine samples were collected from all the participants and urine were analyzed using a Dipstick. The usual volume of specimens was 50-100ml. Dipstick urinalysis was done using Multistix 10 SG (Siemens) and Clinitek Advantus Analyzer. The reagent strip contains tests pads for protein, blood, leukocyte esterase, nitrite, glucose, ketone, pH, specific gravity, bilirubin and urobilinogen.

Urinalysis was considered abnormal, if the following findings were detected: >5-10 RBC/ μ l; hematuria; 1+ or greater proteinuria (trace, 1+, 2+, 3+, 4+ correspond to 10mg/dl, 30mg/dl, 100mg/dl, 300mg/dl, 1000mg/dl respectively); 1+ or greater glycosuria (1+, 2+, 3+

corresponds to 100mg/dl, 300mg/dl, 1000mg/dl respectively); Positive nitrites; and >25 WBC/ μ l; leukocyturia.

2.3. Urine Viable Cell Count

The urine cell count was determined by the trypan blue exclusion test. Urine samples were then transferred into 15 ml tubes inside a tissue culture hood and these tubes were centrifuged at 900rpm for 5 minutes at room temperature. The supernatant were discarded and cells re-suspended in 500 μ l of PBS (Dulbecco's Phosphate Buffered Saline, Sigma-Aldrich, USA), centrifuged again and re-suspended in 100 μ l DMEM- F12 (Dulbecco's Modified Eagle's Medium F-12 Ham, Sigma-Aldrich, USA) containing 10% Fetal Bovine Serum FBS (Sigma-Aldrich, St. Louis, USA), 1% penicillin/streptomycin and 1% amphotericin B (PSA) (Lonza, Basel, Switzerland). The cell suspension was diluted (1:1, v/v) with trypan blue to reach 0.4 %. The number of viable cells was determined.

2.4. Statistical Analysis

Statistical analysis was performed using the ANOVA test followed by post hoc tests of Duncan or Turkey for more precision. The study of the evolution between 2 time points was performed using the paired samples Student t-test. Differences were considered significant for p values less than 0.05. All analyses were done using the GraphPad Prism software (version 7.0).

3. Results

3.1. Description of the Sample

Table 1 presents the main characteristics of each of the two groups: Male and females. In total, 100 samples were evaluated; they were aged between 17 and 60 years, 27% males and 73% females. Moreover, we describe in Table 1 some variables, such as dwelling region, the blood group, time of urination per day, the presence of certain diseases, the consumption of caffeine, theine and alcohol, and the smoking status; there were no statistically significant differences between males and females regarding the listed characteristics. A significant difference in smoking status was observed, which is lower in female group compared to male group with a value of $P < 0.05$. (Table 1)

3.2. Urine Test Analysis and Cell Count

Among all the parameters studied in the urine test, there was a significant difference in the number of leucocytes between females and males; 35 females and only 4 males had a considerable number of leucocytes (p value=0.002).

Concerning the results of the cell count, there was a very significant difference between males and females; females showed a higher total cell count (P value=0.036) and living cell count (p value=0.000) compared with males (Table 2).

Some subjects had more than one abnormality detected. Leukocyturia had the highest prevalence detected in 39% of samples; proteinuria detected in 13%; Hematuria detected in 24% and Glycosuria in only 8%. The other abnormalities found were nitrituria with only 8% among all samples.

3.3. Correlation between Number of Urine Living Cells, Metabolic Urinary Profile and Environmental Factors

There was a correlation between the presence of glucose, proteins and leucocytes in urine (p=0.001, p=0.049 and p=0.027 respectively) and the number of living cells. No significant association was observed between bilirubin, urobilinogen and blood with urine living cell count (p=0.253, p= 0.152 and p= 0.134 respectively) (Figure 1).

Table 1. Comparison of the characteristics of the samples (27 males and 63 females) using t-test for continuous variables and Fisher's exact test for dichotomous variables

Characteristics	Sample		P value
	Male (27)	Female (73)	
Mean Age ($\pm$ SD) year	30.37 $\pm$ 12.50	27.68 $\pm$ 10,43	0.282
Dwelling region, n (%)			
Beirut/Mount Lebanon	14	44	0,981
North	7	20	
South	1	6	
Bekaa	1	3	
Blood group			0,504
A	5	21	
B	3	8	
AB	0	0	
O	15	40	
Urination per day			0,997
< 3 times	5	18	
Between 3 and 5 times	15	39	
>5 times	7	16	
Diabetes			0,083
No	26	73	
Yes	1	0	
Uro-génital Diseases			0,291
No	27	72	
Yes	0	1	
Hypertension Diseases			0.145
No	26	72	
Yes	1	1	
Caffeine consumption (%)			0.077
No	4	20	
Yes	23	53	
Theine consumption (%)			0,903
No	11	46	
Yes	16	27	
Alcohol consumption (%)			0,605
No	21	60	
Yes	6	13	
Smoking status (%)			0,000*
Yes	14	60	
No	13	13	

Table 2. Characteristic of the main urinary factors according to gender using t-test for continuous variables and Fisher's exact test for dichotomous variables

Characteristics	Sample		P value
	Male (27)	Female (73)	
Bilirubine			
No	25	69	0,747
Yes	2	4	
Urobilinogene			
No	21	67	0,057
Yes	6	6	
Ketone bodies			
No	27	71	0,543
Yes	0	1	
Glucose			
No	24	68	0,491
Yes	3	5	
Proteins			
No	12	38	0,545
Yes	4	9	
Trace	11	26	
Blood			
No	23	53	0,195
Yes	4	20	
Nitrites			
No	27	70	0,290
Yes	0	3	
pH			
Normal	27	73	-
Not normal	0	0	
Density			
Normal	27	73	-
Not normal	0	0	
Leucocytes			
No	23	38	0,002*
Yes	4	35	
Total cell count	171031.70±530187.19	2129846.13±5237179.66	0.036*
Viable cell count	21970.88±73535.17	604458.63±841368.65	0,000*
Dead cell count	149060.81±483678.16	1525387.50±4693526.28	0,076

Figure 1. Correlation between viable cell count and many factors (gender, leucocyte, glucose and proteins) using t-test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.0001$

Moreover, urine viable cells was significantly associated with the gender ($p=0.000$), alcohol and their consumption ($p=0.023$, $p=0.034$ respectively) and smoking status ($p=0.003$) (Table 3).

Table 3. Correlation between viable cells count and environmental factor using t-test. * $p < 0.05$, ** $p < 0.001$, *** $p < 0.0001$

Characteristics	Viable cells mean (Mean $\pm$ SD)	P value
Gender		
Male (27)	21970.88 $\pm$ 73535.17	0,000***
Female (73)	604458.63 $\pm$ 841368.65	
Caffeine consumption (%)		
No (27)	647685.18 $\pm$ 963741.94	0,067
Yes (73)	373030.05 $\pm$ 668345.08	
Consumption of Thein/day		
Non (43)	423647.09 $\pm$ 702013.91	0,034*
Oui (33)	150511.18 $\pm$ 201858.77	
Alcohol consumption (%)		
No (81)	503340.87 $\pm$ 830748.31	0,023*
Yes (19)	207793.84 $\pm$ 262595.72	
Smoking status (%)		
No (74)	551050.71 $\pm$ 854376.12	0,003*
Yes (25)	145637.64 $\pm$ 242958.96	

4. Discussion

Urine is often sampled from patients participating in clinical studies. Biological homeostasis occurs in humans, but little is known about the variability between genders of components found in urine. It is important to define the biochemical and cellular variance within a normal population before scientific or clinical conclusions are made regarding different pathophysiologies. This study investigates the variability of selected urine biochemical and cellular factor in a group of 100 healthy men and women and was conducted to determine the effect of gender on these factors. To our knowledge, this is the first report on dipstick urine analysis screening among asymptomatic university students and staff in Lebanon.

Leukocyturia and hematuria was one of the most common abnormality found in our studied group. This was contrasted to other studies as in Egypt and Nigeria where proteinuria was the most common positive finding [9,10]. These two studies showed abnormalities among primary school children group.

A positive finding in our study was nitrituria that accounted for 3% of the studied group compared to the 1.5% in the Nigerian screening [11]. Urinary nitrites are produced by bacterial breakdown of dietary nitrates. The reliability of the nitrite test for urinary tract infections has been investigated by several workers, most of whom concluded that false positive results were rare and that the test had a higher specificity for urinary tract infections [12]. Specificity of urinary nitrite test is about 98% [13,14].

Our results showed more abnormalities among females groups than in boys. This was similar to the results of the Nigerian study [15], but contrasted with the results of the Egyptian study which showed that age and sex had no impact in the results of the screening done [9]. The finding of higher leucocytes and nitrites among females could be a reflection of higher rates of asymptomatic bacteriuria in the females of the studied group. Many studies showed that bacteriuria is 30 times more in females than in males, attributed to the fact that female have short urethra which predisposes them to ascending bacterial infection [15,16].

The results showed that the presence of bilirubin, urobilinogen, the ketone bodies and blood in the urine of women and men donors has no significant effect on the number of urine viable cells. However, the presence of glucose and proteins in urine increase significantly the number of urine living cells. Glucose is normally filtered by the glomerulus and fully reabsorbed in the proximal tubule. The increase of the level of glucose in the urine (glucosuria) in diabetic donors can result in most cases by an elevation of blood glucose, this high level of glucose can alter the appearance of podocytes (epithelial cells the glomerular filtration barrier facing the urinary space). The high level of glucose makes the filtration membrane permeable to glucose and proteins of large size, which explains the high rate of protein in the urine. Furthermore, studies have been shown

that exposure of glomerular cells at a high level of glucose may increase the synthesis of proteins of the extracellular matrix and growth factors. These factors can affect the viability cell, which explains the increase in the number of urine living cells in samples with glucose and proteins.

Our results showed that cigarettes, alcohol and theine consumption decrease significantly the number of urine viable cells. It's known that consumption of alcohol permanently alter renal filtration membrane, increasing urine volume by producing disturbances in electrolyte levels, which can affect cell viability in the urine.

5. Conclusions

In conclusion, urinary biochemical and cellular composition of a healthy population showed a distribution related to a significant extent to gender. We suggest that routine urinalysis should be part of screening of students at school and universities in Lebanon, and that further follow-up should be offered to determine the exact etiology of any abnormal finding.

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