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Research Paper

Age-Related Patterns in Urinary Biomarkers Using Urine Dipstick Analysis

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Abstract: The urinary system's primary functions are to eliminate waste from the body, regulate blood volume and pressure, control electrolyte and metabolite levels, and maintain blood pH. Aging significantly impacts the urinary system, often reducing the efficiency of its organs. As individuals age, the urinary tract undergoes various changes that can affect its function and are reflected in urine diagnostic parameters. In a study conducted with 100 individuals in Durres, Albania, the sample consisted of 46% women and 56% men. The results revealed that 15% tested positive for proteinuria, 45% for ketones, 5% for high glucose levels, and 6% for nitrites. Statistical analysis indicated a significant relationship between high glucose levels in urine and age ($\chi^2 = 0.203^*$, $p = 0.043$), suggesting that glucose levels may increase with age. Additionally, there was a negative relationship between urine pH and age ($p < 0.001$), indicating that pH levels tend to decrease as people get older. These findings underscore the importance of monitoring urine diagnostic parameters in aging populations. Urine reagent strips proved to be a valuable tool in this study, offering quick and efficient results and allowing for the detection of a wide range of chemical parameters. This approach provides essential insights into the functional changes occurring in the urinary system with age.

Key words: *Urinary system, diagnostic parameters, urine analysis, aging.*

Introduction

The urinary tract functions as the body's filtration system, removing urine, which consists of waste and excess fluid. For normal urination, all components of the urinary tract must work together in the proper sequence. Additionally, the urinary system plays a crucial role in regulating the volume, acidity, salt concentration, and chemical composition of blood, lymph, and other body fluids.

Aging significantly affects the urinary system, as the efficiency of its organs diminishes over time. The population of individuals over 65 is growing rapidly in developed countries, leading to an increase in elderly patients diagnosed with kidney damage and nephrosclerosis (O'Sullivan *et al*., 2017). Excess protein in the urine indicates that the kidney filters, known as glomeruli, are not functioning correctly and are allowing protein to leak into the urine. Damage to the glomeruli results in conditions such as nephritis or glomerulonephritis, which become more likely with age (Sureshkumar *et al*., 2003).

Urinalysis is a diagnostic test that examines the visual, chemical, and microscopic characteristics of urine. It may involve a series of tests to detect and measure various compounds in a single urine sample. This test is essential for assessing kidney function, identifying potential infections, and monitoring chronic conditions. Healthcare providers commonly use urinalysis to assess and monitor conditions like liver disease, kidney disease, and diabetes, as well as to diagnose urinary tract infections (UTIs). The urine reagent strip test, or dipstick test, is used to evaluate the chemical properties of a urine sample. Lab technicians use dipsticks with chemical pads that change color when exposed to specific substances (Kutter 2000). Regular urinalysis can be a valuable tool in preventive healthcare, allowing for the early detection of abnormalities before they develop into more serious conditions. By analyzing changes in urine composition, healthcare professionals can gain insights into an individual's overall health and tailor treatment strategies accordingly.

Material and Methods

In this study, urine samples from 100 individuals (both women and men aged 15 to 90 years recommended by the doctor for urine analysis) from the city of Durres, Albania, were analyzed. The samples were collected and analyzed from April to May 2023 at the "Medical Care Lami" medical laboratory in Durrës, in collaboration with the Department of Biology, Faculty of Natural Sciences,

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University of Tirana. The entire procedure adhered to the EFLM European Urinalysis Guideline 2023 (Kouri *et al*., 2023).

Participants were instructed to collect a urine sample, preferably the first morning urine, in a sterile container. They were advised to avoid alcohol, coffee, tea, smoking, and vigorous exercise for 24 hours prior to sample collection. This protocol was designed to minimize potential confounding factors that could affect the accuracy of the results. After the urine samples were delivered, the necessary analyses were performed.

Urine samples were analyzed using Labstrip U11 Plus test strips (77 Elektronika), which measure 11 parameters: bilirubin, urobilinogen, ketones, ascorbic acid, glucose, protein, blood, pH, nitrite, leukocytes, and specific gravity. For reading the Labstrip U11 Plus urinalysis strips, the DocUReader 2 Pro analyzer was used. Although visual interpretation using a color chart is an alternative method, the DocUReader 2 Pro was employed in this study to ensure higher accuracy. This choice was made to reduce the potential for human error and improve the reliability of the results.

The data on urinary chemical parameters obtained from the Labstrip U11 Plus tests were processed using IBM SPSS (Statistical Package for Social Sciences) version 16 for Windows. To assess the relationship between urinary parameters and age, the chi-square test and Pearson correlation test were used, with a significance level set at $p < 0.05$. These statistical methods were chosen to identify any significant trends or correlations between age and the various urinary parameters measured.

Results

In our sample of 100 individuals, 46 are women and 54 are men. The age distribution reveals that 47% of the participants are under the age of 40, while 53% are over 40. Notably, 53% of the sample are over this age range where there is a claimed higher probability of abnormalities such as proteinuria due to impaired kidney filtration. To investigate this, we grouped the data into five distinct age categories, as illustrated in Table 1. Our analysis shows that 15% of the individuals tested positive for proteinuria, with the highest prevalence observed in the age group over 73 years.

We applied the Pearson correlation test to assess the linear relationship between age and the presence of proteins in the urine. The test results yielded a p-value of 0.096, indicating that there is no statistically significant correlation between age and proteinuria at the 0.05 significance level. This indicates that although proteinuria appears more frequently in older age groups, the association between age and the occurrence of proteinuria is not statistically significant in our sample. Further studies with larger sample sizes might be needed to explore this potential association more thoroughly.

Table 1. Variation in proteinuria levels among different age groups

Age groups (years)	**N (%) Normal level of proteins <20 mg/dL**	**N (%) Abnormal level of proteins >20 mg/dL**	**Total**
<28	25 (86.2)	4 (13.8)	**29**
29-43	19 (95.0)	1 (5.0)	**20**
44-58	18 (90.0)	2 (10.0)	**20**
59-73	16 (84.2)	3 (15.8)	**19**
>73	7 (58.3)	5 (41.7)	**12**
Total	**85**	**15**	**100**

Urine pH changes with increasing age, making it an important parameter to assess in this study. Table 2 illustrates the distribution of urine pH across different age groups of the participants. It is evident from the data that older individuals tend to have lower pH levels compared to their younger counterparts.

Specifically, 57% of the sample exhibited a pH of 5, indicating an acidic environment. In contrast, 30% of the individuals had a pH in the range of 5.5-6, which is generally considered the optimal pH level for urinary health. The prevalence of acidic pH in a significant portion of the sample suggests that age-related changes in urine pH could be a noteworthy factor in assessing overall health.

To explore the relationship between age and pH, a Pearson correlation test was conducted. The results confirmed a significant negative correlation between age and urine pH ($p < 0.001$). This finding indicates that as age increases, urine pH tends to decrease, meaning older individuals are more likely to have lower (more acidic) pH levels.

These results underscore the importance of considering urine pH in conjunction with age when evaluating health status. Lower urine pH may be associated with various health conditions or metabolic changes, making it crucial for healthcare providers to integrate this information with other clinical data to make informed decisions. Regular monitoring and assessment of urine pH could be beneficial in identifying potential health issues early and managing them effectively.

Table 2. Variation in urine pH among various age groups

	pH					
Age groups (years)	**5.0**	**5.5**	**6.0**	**6.5**	**7.0**	**Total**
<28	8	8	3	5	5	29
29-43	10	4	4	2	0	20
44-58	17	0	3	0	0	20
59-73	16	2	1	0	0	19
>73	6	3	2	1	0	12
Total	**57**	**17**	**13**	**8**	**5**	**100**

Table 3 below illustrates the distribution of ketones levels across different age groups of the participants. From the results obtained, it is noted that 45% of the individuals of this sample are positive in terms of the high presence of ketones in the urine, while 55% of the individuals are negative. Chi-square test was used to test whether there is a relationship between ketones and age. From the data we obtained, it turned out that there is no significant relationship between ketones and age (p= 0.068, α=0.05).

Table 3. Variation in ketones levels among various age groups

Age groups (years)	**N (%) Normal level of ketones <5 mg/dL**	**N (%)Abnormal level of ketones >5 mg/dL**	**Total**
<28	12 (41.4)	17 (58.6)	**29**
29-43	11 (55.0)	9 (45.0)	**20**
44-58	13 (65.0)	7 (35.0)	**20**
59-73	9 (47.4)	10 (52.6)	**19**
>73	10 (83.3)	2 (16.7)	**12**
Total	**55**	**45**	**100**

Figure 1 illustrates the distribution of glucose levels within the study sample. Out of 100 individuals, 95 exhibit normal glucose values, while 5 individuals (5% of the sample) have elevated glucose levels. To analyze the association between glucose levels and age, a Chi-square test was conducted. The results reveal a Chi-square statistic (χ^2) of 0.203, with a p-value of 0.043. Given the significance level (α) of 0.05, the p-value is less than α, indicating a statistically significant relationship between glucose levels and age. The significant p-value (0.043) suggests that there is a meaningful relationship between glucose levels and age in this sample. This finding implies that age is likely associated with variations in glucose levels, highlighting the importance of considering age as a factor in glucose level assessments and potentially guiding targeted interventions for individuals based on their age group.

Table 4 below illustrates the distribution of nitrinuria cases across different age groups of the participants, when 94% of individuals in the sample were negative and 6% positive. Any degree of pink color in the urine is interpreted as a positive nitrite test representing the presence of 105 or more organisms per milliliter. Using the Pearson correlation test, we analyzed whether there is a significant relationship between nitrites and the age of individuals. From the data obtained, it appears that there is no relationship between them *(p*=0.668).

The specific gravity (SG) in this sample has a range of normal values, we have a minimum value of 1015 and a maximum value of 1035 (Figure 2). The value 1030 has the highest frequency, with 46 individuals out of 100 total. To test whether between age and SG the Pearson correlation test was used, p=0.325. From this data we conclude that there is no relationship between these two variables. All other parameters in our sample of 100 individuals, which were tested with LabStrip U11 including bilirubin, urobilinogen, ascorbic acid, blood and leukocytes, were normal (for urobilinogen) or negative (for bilirubin, ascorbic acid, blood and leukocytes).

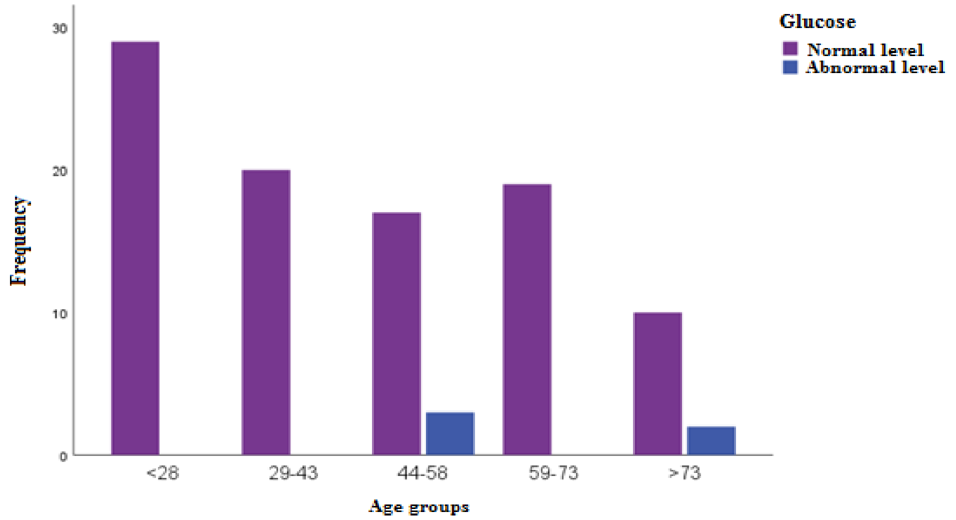

Figure 1. Variation in glucose levels among different age groups

Table 4: Prevalence of Nitrinuria Across Various Age Groups

Age groups (years)	N (%) Negative for nitrites	N (%) Positive for nitrites	Total
<28	27 (93.1)	2 (6.9)	**29**
29-43	18 (90.0)	2 (10.0)	**20**
44-58	20 (100.0)	0 (0.0)	**20**
59-73	19 (100.0)	0 (0.0)	**19**
>73	10 (83.3)	2 (16.7)	**12**
Total	**94**	**6**	**100**

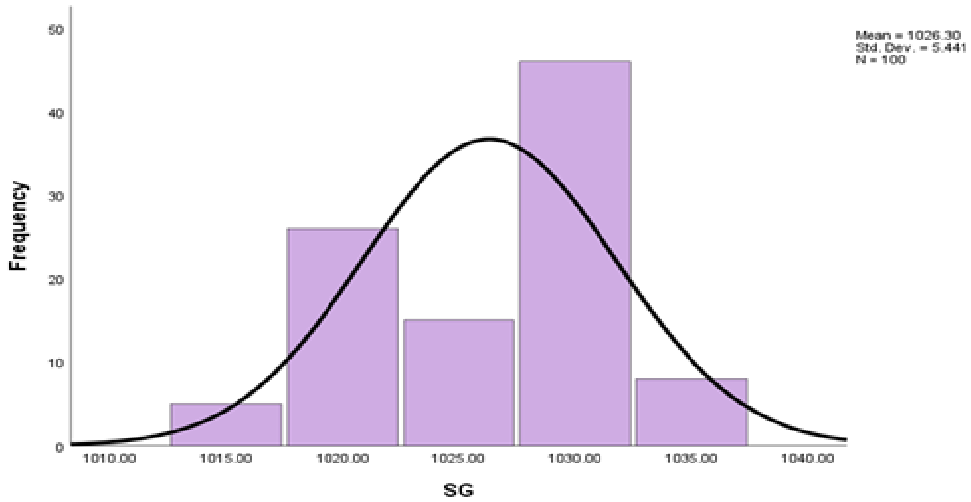

Figure 2. Frequency of SG values of the simple

Discussion and Conclusion

In our study, the presence of elevated protein levels in urine was found in 15% of cases. This aligns with the range reported in other studies, which varies from 14.1% to 24.9% (Parker *et al*., 2020; Rosenstock *et al*., 2018). Proteinuria, or the presence of abnormal amounts of protein in urine, serves as an early indicator of chronic kidney disease (CKD). The prevalence of CKD in Europe is reported to range from 3.3% to 17.3% (Brück *et al*., 2016). While CKD can occur with normal protein levels in urine, proteinuria can also be an early sign of other conditions such as diabetes, hypertension, and cardiovascular diseases.

Our study also identified a negative correlation between urinary pH levels and age, indicating that as individuals age, urine pH tends to decrease, becoming more acidic. Highly acidic urine can result from several conditions including acidosis, uncontrolled diabetes, diarrhea, starvation, dehydration, and respiratory diseases associated with carbon dioxide retention and acidosis. Some studies suggest that low urine pH might signal chronic kidney disease, heart failure, metabolic abnormalities, or other disorders (Nakanishi *et al*., 2012; Kraut & Madias, 2016).

In our sample, 45% of individuals exhibited high levels of ketones in urine. Ketoneuria can be a useful screening tool to estimate the ketonic state of the body. Elevated ketone levels may indicate a risk of ketoacidosis. Factors contributing to elevated ketone levels include prolonged fasting, a low carbohydrate diet, certain medications, excessive alcohol intake, and dehydration. Studies have associated ketone presence in urine with metabolic health and weight (Kim *et al*., 2020) and with aerobic exercise (Han & Jeo, 2020).

Glucose levels in urine are considered normal in small amounts. However, glucose concentrations exceeding 25 mg/dL in random fresh urine samples are indicative of a pathological condition known as glucosuria. In our sample, 3% exhibited glucosuria. A significant relationship was observed between high glucose levels in urine and age (p=0.043). Elevated glucose in urine may suggest conditions such as diabetes, gestational diabetes, or certain kidney disorders, which are more prevalent in older adults (Kalyani & Egan, 2013).

The presence of nitrites in urine, found in 6% of our sample, is generally associated with bacterial infections in the urinary tract. Nitrite testing is based on the ability to convert nitrate to nitrite, with production linked to certain bacteria such as members of the Enterobacteriaceae family. It is important to note that not all bacteria reduce nitrates, so a negative test does not rule out infection (Franz & Harl, 1999).

Specific gravity (SG) measures the kidneys' ability to regulate water content and excrete waste. It plays a crucial role in diagnosing conditions affecting urine water content. SG values can vary based on fluid intake: a low SG (around 1.001) may be normal with high water consumption, while a high SG (above 1.030) may be normal with low fluid intake. Insufficient water intake has been linked to health conditions such as renal insufficiency and cardiovascular issues (Clark, 2011; Dmitrieva, 2022).

Diagnosing urinary parameters is crucial for identifying potential disorders based on abnormal values. Abnormal findings may indicate various conditions, some of which could be serious. It is essential to study these parameters in conjunction with physiological mechanisms and overall kidney function. The results from such analyses should assist in differentiating between normal samples and those requiring further examination for possible renal or genitourinary tract disorders. Positive samples may warrant additional investigations such as microscopic examination, chemical analysis, or bacteriological tests (Oyaert & Delanghe, 2019).

Urine specimen analysis serves as a rapid, first-line screening method in a multi-test workflow. Due to its wide availability and ease of use, it provides clinicians and patients with prompt point-of-care information. While visual inspection of urine samples might be subject to subjective color interpretation, automated analyzers offer more reliable results (White *et al*., 2011; Rowell, 1998).

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Compliance with Ethical Standards Ethical responsibilities of Authors: *The author has read, understood, and complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors".*

Conflict of Interest: *The authors declare that they do not have any conflict of interest.*

Change of Authorship: *The author has read, understood, and complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors and is aware that with minor exceptions, no changes can be made to authorship once the paper is submitted.*

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