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Prevalence of isolated non-albumin proteinuria in the US population tested for both, urine total protein and urine albumin: An unexpected discovery

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ABSTRACT

Background: Isolated non-albumin proteinuria (NAP) is a condition when urine total protein concentrations are elevated without elevation of urine albumin. The prevalence of NAP in the US population tested for both, urine total protein and albumin was assessed in this study.

Methods: The database of a US nationwide laboratory network was queried for test results when random urine albumin was ordered together with urine total protein and also when timed 24-hour urine albumin was ordered together with urine total protein. The total prevalence of NAP in the US population tested for both, urine total protein and albumin was calculated for patient groups having normal and low-normal urine albumin (random and timed) with elevated and severely increased urine total protein (random and timed). Also, the prevalence of NAP was calculated for patients with normal urine albumin to assess the probability of missing proteinuria if only urine albumin is measured.

Results: The prevalence of NAP in the random samples group was 10.1% (15.2% for females and 4.7% for males). Among patients with normal random albumin, there were 20.0% (27.3% of females and 10.7% of males) patients with NAP. The prevalence of NAP in the timed samples group was 24.6% (29.8% for females and 18.5% for males). Among patients with normal timed urine albumin, there were 36.2% (40.0% of females and 30.8% of males) patients with NAP. There was a strong positive association with female gender and NAP in most patients groups.

Conclusions: Testing for only urine (micro)albumin can miss up to 40% of females and 30.8% of males with gross proteinuria.

1. Introduction

Measurement of proteinuria during routine urinalysis is one of the most commonly ordered laboratory tests. It can be performed as an initial qualitative or semi-quantitative screen using dipstick or performed quantitatively on a chemistry analyzer using a random or a timed 24-hour urine collection specimen. The random urine specimen type is gaining more popularity among ordering physicians and their patients due to sample collection convenience and reported frequent inaccuracy and imprecision of 24-hour timed collections [1,2].

Approximately 40% of protein, normally excreted with urine, is a high-molecular-weight albumin (about 65,000 Da). About 20% is of low-molecular-weight such as light chains of immunoglobulins (about 20,000 Da), and the remaining approximately 40% is made up of Tamm-Horsfall mucoproteins secreted by the distal tubule (about 90,000 Da) [1]. Proteins cross the renal glomerular barriers in inverse proportion to their size and negative charge. Proteins with a molecular weight of <20.000 Da pass easily. Passage of albumin with a negative charge and molecular weight of 65,000 Da or more is restricted under normal conditions. The smaller proteins are then mostly reabsorbed at the proximal tubule, and only negligible amounts are normally excreted [1].

Initial positive dipstick protein test may be observed in up to 17% of physical examinations [1]. However, <2% of those patients may have serious disorders [1]. Most benign causes of proteinuria include fever, intense exercise, dehydration, stress, and acute illness/inflammation.

Abbreviations: NAP, non-albumin proteinuria; CKD, chronic kidney disease; ACR, albumin-to-creatinine ratio; PCR, total protein-to-creatinine ratio; AER, albumin excretion rate; PER, total protein excretion rate; SD, standard deviation; eGFR, estimated glomerular filtration rate.

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Urine dipstick protein tests are more sensitive to albumin than to globulins, hemoglobin, Bence-Jones Proteins, or mucoproteins and a negative result do not rule out the presence of these other proteins. Also, dipstick tests may produce falsely positive results due to interferences [1,3].

There are three major types of proteinuria: glomerular, tubular, and overflow. Glomerular proteinuria is the most common form and results in primary loss of albumin. It is a hallmark of diabetic nephropathy and the measurement of albumin in urine is now recommended for the staging of chronic kidney disease (CKD). The remaining two other major types of proteinuria, however, may not be detected by the measurement of urine albumin alone. Tubular proteinuria can occur from tubulointerstitial disease and decreased reabsorption of low-molecular-weight proteins. Tubular diseases include hypertensive nephrosclerosis and nephropathy caused by nonsteroidal anti-inflammatory agents. Overflow proteinuria occurs when there is an overproduction of low-molecular-weight proteins that exceeds the ability of the proximal tubules to reabsorb those proteins. The most common cause is overproduction of Bence-Jones Proteins in multiple myeloma, monoclonal gammopathy, lymphoma, and leukemia [1].

The distinct condition when there is no elevation in urine albumin, but the total urine protein concentrations are exceeding the established thresholds is described as isolated non-albumin proteinuria (NAP) [4]. NAP is defined (using clinical practice guidelines thresholds) as albumin-to-creatinine ratio (ACR) <3 mg/mmol with total protein-to-creatinine ratio (PCR) >14.9 mg/mmol when measured in the same specimen [4,5]. Some researchers use more strict stratification and also exclude subjects with "high-normal" ACR between 1 and 3 mg/mmol. In the latter case, the NAP will include only subjects with ACR <1 mg/mmol and PCR >14.9 mg/mmol [4]. The prevalence of NAP in different populations of CKD patients and the United Kingdom (UK) primary care patients was reported in a number of recent studies and all used random urine samples test results [4,6–9]. However, to our knowledge, there were no studies reporting the prevalence of NAP in the US population that is routinely tested for proteinuria, prevalence of NAP with severely increased proteinuria (PCR >50 mg/mmol) [5], and probability of missing the diagnosis of proteinuria when only urine albumin is measured in this population. Additionally, no such data is available for timed 24-hour albumin excretion rate (AER) and total protein excretion rate (PER). This study is intended to fill this gap. For the timed samples group we defined NAP as AER <30 mg/24 h with PER >149 mg/24 h, and NAP without "high-normal" AER as AER <10 mg/24 h with PER >149 mg/24 h (see definitions in Table 1).

Table 1
Definitions of patient result categories according to proteinuria thresholds.

Patient result categories	Proteinuria thresholds
All random NAP results	ACR <3 mg/mmol with PCR > 14.9 mg/mmol
Random NAP without "high-normal" ACR results	ACR <1 mg/mmol with PCR > 14.9 mg/mmol
All random NAP with severely increased PCR results	ACR < 3 mg/mmol with PCR > 50 mg/mmol
Random NAP without "high-normal" ACR with severely increased PCR results	ACR <1 mg/mmol with PCR > 50 mg/mmol
All timed NAP results	AER < 30 mg/24 h with PER > 149 mg/24 h
Timed NAP without "high-normal" AER results	AER < 10 mg/24 h with PER > 149 mg/24 h
All timed NAP with severely increased PER results	AER < 30 mg/24 h with PER > 500 mg/24 h
Timed NAP without "high-normal" AER with severely increased PER results	AER < 10 mg/24 h with PER > 500 mg/24 h

2. Materials and methods

2.1. Participants

The patient test results for ACR when it was ordered together with PCR on the same specimen (total of 89,757 pairs), 24-hour AER when it was ordered together with 24-hour PER on the same specimen (total of 16,946 pairs), and corresponding patient's age and gender were queried from the US national database of the Laboratory Corporation of America network of 20 regional clinical laboratories located across the entire United States over the period between May 01, 2014 and April 30, 2016. This population cannot be considered as a general population but rather may be called "clinically indicated US population" because of the fact that this is a population that was routinely tested for proteinuria using both, urine total protein and urine albumin tests, and therefore represents the selection bias. The laboratory locations used for the combined test data pool are presented in the Supplemental Data Table 1. All patient demographic data were de-identified and no results were excluded from the initial test query. The protocol for this study was determined to be exempt under existing regulations by an Institutional Review Board. The summary statistics for the test results used in this study are presented in Table 2.

2.2. Laboratory methods and statistical analysis

All test results originated from random or timed 24-hour urine collection samples received for testing at all laboratories in the network. All laboratories were standardized to the same FDA-cleared instruments and reagents, and were operated according to the manufacturer's specifications as well as to standardized internal standard operating procedures. The following methods were used for the generation of results included in this study: urine albumin (immunoturbidimetric method), urine total protein (benzethonium chloride turbidimetric method), and urine creatinine (Jaffe colorimetric method) - all on Roche cobas c701 (Roche Diagnostics, Indianapolis, IN, USA). Patient samples were handled according to the manufacturer's instructions prior to testing. The detailed description of the LabCorp analytical method performance and sample handling is presented in the Supplemental Data Table 2. All statistical calculations were performed using the QuickCalcs software package (GraphPad Software, Inc., La Jolla, CA, USA). Patient groups stratification was based on urine albumin and total protein concentration cutoffs described in clinical practice guidelines and published studies [4,5].

3. Results

The prevalence of laboratory test results that would meet the definition of NAP (ACR <3 mg/mmol with PCR >14.9 mg/mmol), NAP without patients with "high-normal" ACR (ACR <1 mg/mmol with PCR >14.9 mg/mmol) in the US selected population (females, males, and total) served by our reference laboratories is presented in Table 2. Additionally, we assessed the prevalence of patients with severely increased PCR >50 mg/mmol for both groups of NAP patients. We also calculated the prevalence of patients (females, males, and total) with normal timed 24-hour AER < 30 mg/24 h who had elevated timed 24-hour PER > 149 mg/24 h as well as severely elevated PER > 500 mg/24 h, and without patients with "high-normal" AER (AER <10 mg/24 h who also have elevated PER > 149 mg/24 h) as well as severely elevated PER >500 mg/24 h. Next, we analyzed the prevalence of NAP among patients with normal ACR and AER as well as among patients with normal ACR and AER but without "high-normal" ACR and AER. All this data, together with the distribution of genders, mean ages, and ages standard deviations (SD) in those distinctive patient groups are presented in Table 2. The associations with female gender and NAP for random and timed samples groups are presented in Table 3. The statistical