Predictive Value of Nephelometric and High-Performance Liquid Chromatography Assays of Urine Albumin for Mortality in a High-Risk Aboriginal Population

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Background: Urine albumin assays by high-performance liquid chromatography (HPLC) yield greater values than immunoassays at lower albumin levels. We compared predictive values of albumin-creatinine ratios (ACRs) by these 2 techniques for mortality in Aboriginal people.

Study Design & Setting: This was a longitudinal study of 741 adults in a remote Aboriginal community who participated in a baseline health survey between 1992 and 1998 at ages ranging from 18 to 84 years (mean, 34 years). All natural deaths were documented on follow-up until 2006. Urine albumin concentrations were measured simultaneously by using both nephelometric and HPLC techniques on baseline urine samples retrieved from −70°C storage, as well as creatinine concentrations, and ACRs were derived. Age- and sex-specific tertiles of ACR were compiled. Cox regression analyses were used to evaluate the predictive value of ACR for natural deaths by ACR tertiles and again by *z* score changes in ACRs as continuous variables.

Results: Participants were followed up for a median of 11 years, during which a total of 119 natural deaths were documented. ACRs on baseline urine samples were greater by HPLC than immunoassay at lower ACR ranges, but were fairly concordant at levels greater than 100 mg/mmol. Levels of ACR by both techniques were strong predictors of death, but correlations of death with ACR tertiles and with ACR levels on a continuum were similar for the 2 techniques.

Limitations: The age- and sex-specific tertiles used might introduce some risk of bias in the assessment of predictive value. In addition, assays were performed on urine after more than 10 years of cold storage.

Conclusion: Despite different absolute values, this study did not show that ACR level by either technique was superior in predicting deaths.

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INDEX WORDS: Aboriginal people; albumin-creatinine ratio; natural deaths; Cox regression; immunoassay; high-performance liquid chromatography.

Australian Aboriginal people, especially those living in remote regions, have greater rates of all-cause mortality, cardiovascular death, and end-stage renal disease[1,2] compared with other Australians. The disparities are worse for younger age groups. For example, adult Aborigines in Northern Territory have mortality rates 2 to 4 times greater than non-Aboriginal Australians, whereas death rates of 25- to 44-years-olds are increased approximately 15-fold.[3]

High rates of end-stage renal disease in the Australian Aboriginal population are well documented, with the incidence in those in remote regions up to 30 times the national incidence for all Australians.[4] Albuminuria marks the underlying renal disease. In a study of a high-risk community, albuminuria, which was pervasive, not only marked all the future risk of renal death, but also predicted cardiovascular and nonrenal noncardiovascular deaths.[5-7] Albuminuria was estimated to contribute to 75% of the risk of all-cause natural death during the interval of observation.[1] Studies in other populations and ethnic groups have also documented the association of albuminuria with nonrenal death, as well with renal disease and renal death.[8-15]

Various immunochemical assays have been used to determine albumin concentration in urine, includ-

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ing nephelometry, immunoturbidometry, enzyme-linked immunosorbent assays, and radioimmunoassays. Recently a method using size-exclusion high-performance liquid chromatography (HPLC) has also been developed which detects additional fractions of intact albumin that conventional assays fail to detect.[16,17] In previous studies, the amount of albumin detected by HPLC was significantly greater than that determined by conventional immunoassays, with the most marked differences occurring at lower levels of albuminuria, especially at or less than the microalbuminuric range.[16,18-21] One study showed that cutoff values for albumin-creatinine ratio (ACR) for detecting cardiovascular disease and diabetes were significantly different between the 2 techniques.[22] It has been queried whether people with an ACR $<$ 3.4 mg/mmol (30 mg/g) by immunoassay, but $>$ 3.4 mg/mmol by HPLC are in a special category of risk for abnormal clinical features or mortality. A recent Australian report suggested that the HPLC assay could identify some people at increased risk of mortality that immunoassay would not detect.[23]

The present study is a longitudinal analysis in an Australian Aboriginal community. It aims to examine and compare predictive values of urine ACR for renal and nonrenal deaths by using albumin measurements by both immunoassay and HPLC techniques on the same urine specimens.

METHODS

Study Population

Between 1992 and 1998, a total of 955 people 18 years and older in an Australian Aboriginal community living in a remote area in the Northern Territory of Australia participated in a screening program for chronic diseases. Of them, 756 had sufficient stored urine for retrieval, allowing ACR assays by means of immunoassay and HPLC. Complete data for all variables, including body mass index, blood pressure, and diabetes, were available for 741, who are included in this analysis.

The profiles of this community, including ACR by means of immunoassay, were described previously.[3] Participants were followed until death, the start of dialysis therapy, or the censor date of April 30, 2006. Deaths were recorded from community and hospital records. The principal causes of deaths were coded as natural or unnatural. Unnatural deaths, which include acute intoxications, accidents, drowning, suicide, homicide, and so on, were excluded from analysis in this study. Natural deaths were categorized into renal deaths and nonrenal deaths, as described previously.[3] Renal death refers to participants who started on dialysis therapy for end-stage renal disease or died with chronic renal failure without dialysis therapy. Nonrenal death includes those with primary or underlying cardiovascular disease death as well as other natural deaths, excluding renal death.

Measurements of Baseline Characteristics

Height, weight, and blood pressure were measured using standard procedures. Glucose levels and other parameters were measured as described elsewhere.[24-27]

Some diagnostic criteria used in this study were as follows: (1) diabetes includes those known to be diabetic before the baseline survey and/or who had a fasting glucose level of $\geq$ 126.1 mg/dL ($\geq$7.0 mmol/L) or 2-hour glucose level of $\geq$ 200 mg/dL ($\geq$11.1 mmol/L) or random glucose level of $\geq$ 200 mg/dL ($\geq$11.1 mmol/L); (2) obesity indicates a body mass index of $\geq$ 30 kg/m^2; and (3) hypertension indicates diastolic blood pressure of $\geq$ 90 mm Hg and/or systolic blood pressure of $\geq$ 140 mm Hg.

At the baseline survey, urine samples were collected. Within 48 hours of collection and storage at 4°C, immunoreactive albumin was measured by immunonephelometry (Beckman Instruments, Brea, CA). Aliquots of 10 mL of urine were stored at −70°C. In 2004, samples were retrieved and thawed, and albumin was measured on the same urine specimen by both rate nephelometry with the Beckman Array (Beckman/Coulter, Sydney, Australia; interassay coefficient of variation $<$ 3%) and by HPLC, using an Agilent Zorbax GF-250 column (4.6 $\times$ 250 mm; 4 Um; Agilent Technologies, Forest Hill, Victoria, Australia; and Agilent/Hewlett Packard 1100 HP system with UV-visible detector; Hewlett Packard, Waldbronn, Germany). Urinary creatinine was measured by the modified kinetic Jaffé reaction (Olympus AU600 Autoanalyzer; interassay coefficient of variation, 2%), and applied to derive ACRs from albumin levels by both assays. Other measurements included serum cholesterol, high-density lipoprotein cholesterol, triglycerides and creatinine.

Urine ACRs by immunoassay on frozen and thawed specimens showed good correlation with those measured on the same specimens at baseline before freezing, as described in a separate report, indicating that little immunologic activity was lost by the freezing and thawing process[28] (Nicol et al; unpublished data, 2008).

Statistical Analysis

Baseline characteristics across the different tertiles of ACR by the 2 techniques were compared by analysis of variance for continuous variables and χ^2 test for categorical variables. Statistical significance is defined as $P < 0.05$ (2 tailed).

Several approaches were used to compare the predictive values for death of ACR levels by the 2 methods. We compared deaths by categories of ACR and also evaluated the associations of outcomes with ACR over a continuum.

In the first analysis of death by ACR categories, survival of people in 3 groupings of lower levels of albuminuria were compared: those who had urine ACRs $<$ 3.4 mg/mmol by both techniques, those with ACR $<$ 3.4 mg/mmol by immunoassay but $\geq$ 3.4 mg/mmol by HPLC, and those with ACR of $\geq$ 3.4 mg/mmol but $<$ 34 mg/mmol (300 mg/g) by both techniques.