YMJ

Re: Microalbuminuria in Normal Korean Children

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Dear Sir,

We read with much interest the article of Kwak, et al.[1] The authors report normal reference values of urinary albumin excretion (expressed as an albumin/creatinine ratio) in a normal population of normal children/adolescents aged 1 month to 19 years old. Detection, evaluation and treatment of microalbuminuria (MA) has recently become an extremely important health issue, and screening of MA is now included in several national and international recommendations/guidelines for hypertension (HTN)[2] and diabetes mellitus (DM)[3] management. The efficacy of early detection of albuminuria/proteinuria is further substantiated by powerful and effective treatment with angiotensin-converting enzyme inhibitors or angiotensin receptor blockers, which have been shown to delay progression towards end-stage renal disease[4] and decrease cardiovascular morbidity/mortality.[5] Providing pediatricians with much needed reference values for urine albumin excretion is therefore a laudable exercise. Epidemiologic studies concerning urinary albumin excretion in children are scarce, and most of them are conducted in subjects with pre-existing DM, obesity, or chronic kidney disease.[6-8] Very few epidemiological studies have been performed in normal children.[9-12] However, before adopting these newly published reference values, we would like to make the following comments in regards to some aspects of the study, more specifically patient descriptions, results reporting and statistical analysis.

Selection of healthy subjects for the elaboration of reference intervals for a given measure is known to be fraught with difficulties. Subjects with a disease (overt or subclinical) can be hidden in the studied population and modify the distribution of the values for the measure in question.[13] Kwak, et al. enrolled children in a busy outpatient clinic of a University Hospital in Korea.[1] Children were reported as "healthy", and although the authors excluded subjects with overt proteinuria, DM, renal disease or acute infection (all conditions known to cause MA/proteinuria), the exact reason for their visit was not stated. MA is also known to be associated with HTN,[14] obesity,[15] and DM/metabolic syndrome,[16] all conditions which were not specifically mentioned in the paper. The authors should have reported if children presented HTN, obesity or metabolic syndrome, all factors that could impact the presence of MA. Low body mass index (BMI) should have also been reported, as a (very) low muscular mass could alter creatinine excretion,[17] hence the reason for the use of the albumin/creatinine ratio, although creatinine excretion was found to be within normal limits in patients with Duchenne Muscular Dystrophy.[18]

Pre-analytical procedures should be described in more detail, given the potential (albeit small) impact of freezing urine on its albumin concentration.[19] It is not clear

how albuminuria (RIA? HPLC?) and creatinine (compensated Jaffé technique? enzymatic method?) were measured with the Toshiba 200 FR Neo, and this should be specified in order to increase the reproducibility of the results in subsequent studies. The authors use the Schwartz formula for estimation of the glomerular filtration rate. The newly revised Schwartz formulas have recently been shown to be more accurate, and should now be used for children aged one year and above with a deemed normal renal function.[20]

Reference values should be subjected to careful statistical treatment, including partitioning, inspection of the distribution, identification and elimination of outliers.[21,22] The authors use parametric statistical methods (mean and standard deviation) to define the reference intervals. This is in contrast with the current recommendations of the International Federation for Clinical Chemistry (IFCC) who recommended the use of non-parametric methods,[13] although both methods may produce similar estimates of the percentiles. In this study, no data on the (assumed) normal distribution of the results or outliers identification were provided. The log transformation of spot urine MA/creatinine ratios might indeed indicate a non-Gaussian distribution of the results. However, there was no mention of how outliers were evaluated and eventually eliminated. Deletion of outliers can improve the characteristics of data distribution. While no statistical evaluation of outliers has the ability to predict outliers in every situation, there are several methods that are recommended, such as the Dixon/Reed method, which has been used extensively.[21,23] Partitioning for subgroups according to age in pediatric studies should be based on strong statistical methods as well.[24,25] It is not clear how age partitioning was performed in this study. Whenever possible, sample size targets should be determined at the start of the study.[26]

In summary and once again, the authors provided us with one of the few studies to describe reference values for albumin excretion in a population of healthy children, and they should be congratulated for that. However, given the abovementioned limitations, further research is needed, with studies including a larger number of well-defined healthy participants (the IFCC guidelines for establishing reference intervals recommend 120 reference subjects per subgroup or partition,[13]) which will allow us to obtain reference intervals with more robust statistical methods (non-parametric methods often give narrower reference intervals than those calculated by parametric statistics).[26,27] Reference intervals calculated from small samples should be applied cautiously.[26,27]

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AUTHOR'S REPLY

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We thank you for your review and comments on our article.

This study was conducted on 352 healthy children (185 males and 167 females) who visited the pediatric department at Konkuk University Hospital between July 2007 and March 2010. The patients with overt proteinuria, renal diseases, diabetes mellitus, and acute infectious diseases were excluded. Actually, most of the subjects were follow-up visit patients who were discharged with acute illnesses such as acute gastroenteritis, acute pharyngitis or viral pneumonia. We checked that they had no fever and no hypertension.[1] Children with metabolic disease or other conditions that could potentially impact the presence of microalbuminuria were excluded. In this study, we measured the subjects' height, weight, blood pressure, and serum creatinine. The z-scores of the heights and weights were obtained using the LMS method and compared to 2007 Korean growth charts.[1]

Although there were 7 children with more than 97 percentile of body mass index (BMI), we did not observe any significant difference in microalbumin/creatinine ratio.

Random morning urine samples were stored in a refrigerator without freezing and processed within one hour. Urine microalbumin and creatinine were examined by Toshiba 200 FR Neo (Toshiba Medical System Co., Tokyo, Japan) using the Jaffe technique. Glomerular filtration rates were obtained using the Schwartz formula.[2] Although the newly revised Schwartz formula, which was based on height, serum creatinine, cystatin C, blood urea nitrogen, and gender, have recently been shown to be more accurate, we could not use this formula because serum cystatin C was not fully obtained.[3,4] As you mentioned, the original Schwartz formula could overestimate glomerular filtration rate (GFR), and further studies using the new equation are required.

In this study, we divided subjects into five groups based on their age: from one to 12 months (Group 1), 12 to 28 months (Group 2), 29 to 48 months (Group 3), 4 to 6 years (Group 4), and 7 to 19 years (Group 5). The International Federation for Clinical Chemistry (IFCC) guidelines for establishing reference intervals recommend 120 reference subjects per subgroup or partition, if using the classical non parametric protocol.[5] And IFCC recommended evaluation and elimination of outliers using 'Dixon/Reed one-third rule'.[6,7] However, it was difficult to collect 120 samples for each age group in this study, which was conducted at a single university hospital. And as you know, studies performed in children are known to have significant biological variability because during the childhood years, rapid and significant changes occur during growth and development.[8] It was difficult to choose a partitioning method for subgroups, despite the availability of a few statistical approaches such as the Harris-Boyd method.[9] In our study, we considered physiological changes according to age, and observed statistically significant differences in value among subgroups. GFR increases gradually to mature values when linear growth is completed during adolescence. Rhodin, et al.[10] reported that GFR is predicted to be 90% that of adult GFR at 1-year postnatal age, and demonstrate a consistent relationship from early prematurity to adulthood. Yap, et al.[11] divided subjects into 2 subgroups, from neonate to 2 years and 2 to 4 years, and presented the data according to age. So we decided to subdivide the subjects, aged 1 month to 19 years old, into five groups considering these physiological factors.

In conclusion, although this study was performed at a single center and has limitations, it presented data describ-

ing a reference range for microalbuminuria in healthy children. Further multicenter studies including a larger number of population is needed to determine the reference values for spot urine microalbumin/creatinine ratios in normal children.

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