Urinary microalbumin/creatinine ratios: reference range in uncomplicated pregnancy

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ABSTRACT

During uncomplicated pregnancy, the development of proteinuria is accepted as a poor prognostic sign and is associated with increasing maternal and perinatal mortality and morbidity. Physiological proteinuria increases with increasing gestation and one of its largest constituents is albumin. The reference range for the (micro)albumin/creatinine ratio (ACR) has not been described for normal pregnancy. This prospective cross-sectional study describes the gestation-specific 95% reference ranges for urinary microalbumin concentration, creatinine concentration and ACR in uncomplicated pregnancy. There is a significant increase (P < 0.016) in the ACR in the third trimester. The mean difference is 0.091 mg of albumin/mmol of creatinine (95% confidence interval, 0.014–0.168). Our results describe the first well-defined gestation-specific 95% reference range for a point-of-care measurement of the ACR. These data are essential if such testing is to be employed in antenatal care.

INTRODUCTION

Proteinuria gradually increases throughout pregnancy and is the result of selective glomerular filtration and non-selective (proximal tubule) re-absorption. Although the glomerulus is relatively impermeable to albumin, it is known that in non-pregnant women, albumin filtration of 500–600 mg/day occurs (i.e. a filtrate concentration of 3 mg/l). Levels of 5 mg/100 ml in the first and second trimesters, and 10 mg/100 ml in the third trimester are normal, and levels may reach 300 mg/day in a normal pregnancy in the third trimester [1]. Values in excess of 15 mg/100 ml or 300 mg/day are usually associated with either pre-eclampsia or underlying renal disease.

Microalbuminuria is defined as urinary excretion of albumin that is persistently above normal, although below the sensitivity of conventional semi-quantitative test strips. In the non-pregnant population it reflects glomerular, or less commonly, tubulo-interstitial dysfunction, and is considered only after structural abnormalities and infection of the renal tract have been excluded.

The classification of microalbuminuria has been hampered by a lack of consensus as to the preferred method of urine collection, the quantitative expression of albuminuria [2], considerable postural and diurnal variation in subjects [3], and possible ethnic differences [4]. In a non-pregnant diabetic population, an early morning urine (EMU) specimen with an (micro)albumin/creatinine ratio (ACR) of > 3 mg/mmol reliably predicts an overnight excretion of 300 μg/min. If such a ratio is greater than 1 mg/mmol, then it is mandatory to collect a 24-h sample of urine to quantify the albuminuria [5,6]. It is apparent that urinary albumin excretion can be 10–20 times higher than normal, without being detected by conventional dipstick or laboratory tests.

Key words: albumin/creatinine ratio, microalbuminuria, pregnancy, reference range.
Abbreviations: ACR, (micro)albumin/creatinine ratio; EMU, early morning urine.
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As such, using sensitive immunoassays, several groups have defined the normal reference range for a non-pregnant population [5]; however, similar studies in uncomplicated pregnant populations are inconsistent, confounded by differing methods of sample collection and methodologically flawed. None of these studies used statistical methods that are appropriate for the analysis of data that are time-dependent. Therefore, none of the previous studies are sufficiently accurate for either research or clinical application. Since it has been suggested that there is a phase of microalbuminuria that precedes overt proteinuria, it may be that its detection could allow the earlier prediction of pre-eclampsia [7].

It is accepted that there is a need for accurate reference ranges to allow correct interpretation of results. We therefore undertook this study to describe the gestation-specific reference range for microalbumin excretion measured at point-of-care in uncomplicated pregnancy.

METHODS

This was a prospective cross-sectional study of Caucasian women attending the antenatal clinic of Leicester Royal Infirmary, a large teaching hospital in the U.K. The study had local ethical committee approval and each woman gave informed consent. Subjects were asked to bring a fresh, mid-stream first void EMU sample to the antenatal clinic for testing. The study was pragmatic in design with no dietary or exercise restrictions, and testing was performed at a point-of-care that was designed to reflect clinical practice.

All women recruited were asked to produce a single first void EMU sample for analysis; they were then followed-up until delivery. Women were not recruited if they had a history of pre-existing hypertension, renal disease, proteinuria or diabetes, pre-eclampsia complicating a previous pregnancy or were non-Caucasian. They were excluded if they developed hypertension (blood pressure > 140/90 mmHg), significant proteinuria (> 0.3 g of protein/day) or gestational diabetes before or after recruitment, or if they delivered before 36 completed weeks of pregnancy.

Each voided sample was tested at point-of-care on the DCA® 2000 (Bayer Corporation, Elkhart, IN, U.S.A.). All samples were tested on the morning of the visit to clinic. This new system for estimating microalbumin levels utilizes a cartridge system and a 40-µl sample of urine. It employs an immunoturbidimetric assay for albumin (calibrated against the reference preparation for proteins in human serum CRM470) in the presence of polyethylene glycol, with the resultant complexes increasing the turbidity. The colorimetric assay for creatinine is based on the coloured complex that is produced when creatinine forms a complex with 3,5-dinitrobenzoic acid. Both assays are monitored at 531 nm and the test takes 7 min to complete. The intra-assay and inter-assay coefficients of variation are reported as being within the range 0.5–5% for microalbumin and 2–8% for ACR (in non-pregnant populations) [8]. We have found the device to have similar intra-assay and inter-assay coefficients of variation in pregnancy [9].

Gestation-specific 95% centiles were obtained for all three measurements using the methods of Royston and Wright [10,11]. These provide curves of the median and the standard deviation for the outcome of interest, and thus enable the 95% centiles to be calculated as a function of time. Creatinine and ACR were subjected to a logarithmic transformation prior to modelling. The means of each transformed measure, as a function of gestation, were modelled using fractional polynomials [12], which enable complex smooth curves to be fitted to the data, if appropriate. The standard deviation of the outcome measures, as a function of gestation, was also investigated by fitting fractional polynomials to the scaled absolute residuals. Normality was then assessed via a normal plot of the standardized normal deviates. No adjustment for skewness in the standardized normal deviates was necessary.

The analysis of the microalbumin data was complicated by the fact that lower bound truncation occurred at 5 mg/l, with 28% (125/447) of women having microalbumin levels recorded as 5 mg/l. With this level of truncation, the normality assumption is violated and the estimate of the standard deviation would be reduced, thus leading to an underestimate of the 95% centile. Therefore, in order to calculate the 95% centile for microalbumin, a multiple imputation procedure was used [13]. In this procedure, 20 new datasets were created where any women who had a microalbumin value of 5 mg/l were assigned a new value that was sampled from a uniform distribution within the range 0.1–5.0 mg/l. Each of these datasets was transformed to normality using a square root transformation and then analysed using the methods described above. The results of each analysis were then combined to achieve the equations for the median and 95% centile.

The above methods depend upon the transformation to normality being adequate. A good fit was assessed using histograms, normal probability plots and the Shapiro–Wilk statistic. In all cases, the normality assumption after transformation was reasonable.

RESULTS

A total of 482 women from the antenatal population participated in this study. Of these, 22 women developed hypertension (with or without proteinuria) and two developed persistent proteinuria alone. Three women developed gestational diabetes and eight gave birth before 36 weeks. These women were excluded, leaving a study...
population of 447. Of these, 118 women were between 5 and 14 weeks of gestation, 158 were between 15 and 28 weeks and 171 were between 29 and 40 weeks. The women had an average age of 28 years (range 18–43 years) and 56% of them were primiparous.

**Microalbumin**

The mean level of the square root of microalbumin was found to be linearly associated with gestation. A linear relationship was also found between the standard deviation of the square root of microalbumin and gestation. The standard normal deviates appeared to be normally distributed. The median level of microalbumin as a function of gestation can be obtained using the following equation

$$\text{Micro}_{\text{median}} = [2.349 + (0.01195 \times g)]^2$$

where $g$ is the gestation period in weeks. The 95% centile is determined using the formula

$$\text{Micro}_{95} = [3.464 + (0.0275 \times g)]^2$$

The median and 95% centile are plotted in Figure 1.

**Creatinine**

After the logarithmic transformation, no evidence of an association between mean creatinine levels and gestation was found. Similarly, there was no evidence of an association between the standard deviation of creatinine and gestation. There was no evidence of non-normality. Thus, the median level of creatinine was estimated to be 8.51 mmol/l, with the 95% centile being 17.08 mmol/l. This is plotted in Figure 2.

**ACR**

The mean log(ACR) was associated with gestation, but the standard deviation of log(ACR) was found to be independent of ACR. The standard normal deviates appeared to be normally distributed. The median ACR level as a function of gestation can be obtained using the following equation

$$\text{ACR}_{\text{median}} = \exp[-0.0898 - (0.02512 \times g^3) + (0.02086 \times g^3 \times \ln g)]$$

with the 95% centile being obtained with the equation

$$\text{ACR}_{95} = \exp[0.5223 - (0.02512 \times g^3) + (0.02086 \times g^3 \times \ln g)]$$

The median and 95% centile are plotted against gestation in Figure 3. It can be seen that the mean ACR is fairly constant until the final trimester.

Table 1 compares the mean ACR in the first and second trimesters with that in the third trimester. The mean difference is 0.091 (95% confidence interval, 0.014–0.168; $P = 0.016$).
**Table 1** Statistical comparison of albumin/creatinine ratios in the third trimester with those in the first and second trimesters

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>Mean</th>
<th>S.D.</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st/2nd</td>
<td>264</td>
<td>0.931</td>
<td>0.359</td>
<td>0.091 (0.014–0.168)</td>
</tr>
<tr>
<td>3rd Trimester</td>
<td>183</td>
<td>1.022</td>
<td>0.436</td>
<td></td>
</tr>
</tbody>
</table>

**DISCUSSION**

This study provides the first methodologically correct description of the reference range for ACRs in uncomplicated pregnancy. Previous studies to describe the excretion of albumin in normal and hypertensive pregnancies were confounded by differing methods of sample collection, although all use sensitive radioimmunoassays allowing the detection of microalbumin levels. These studies are summarized in Table 2.

The rise in albumin excretion that we report in the third trimester of pregnancy is consistent with the findings of others. The advantage of using the ACR to quantify albumin excretion is that it allows a single urine void to be tested. Several studies have used 24-h collections of urine to measure total albumin excretion. Lopez-Espinoza et al. [14] report increased albuminuria in the third trimester, compared with the first and second, with persistence until 1 week postnatally; this was also reported by Taylor and Davison [15] and Hayashi et al. [16]. In what was methodologically the most accurate study prior to this, Higby et al. [17] reported a reference range for 24-h total urinary albumin excretion, which is also seen to rise in the third trimester.

Wright et al. [18], using 2-h collections, found no change in the albumin excretion rate compared with non-pregnant women, although they did find that the ACR was higher at all stages in pregnancy than it was in the non-pregnant state. They also found the ACR was higher at 36 weeks compared with 14 weeks. Similar changes in the ACR have been described in smaller longitudinal studies, although these have not been produced with a gestation-specific reference range and have used random urine samples [19]. These studies suggest that the ACR may allow spot urine samples to be used to quantify albuminuria accurately and this may have implications for the screening for pre-eclampsia in antenatal care.

Not all previously reported studies have agreed on albumin excretion patterns. Misiani et al. [20] found both urinary albumin excretion and ACRs to be reduced compared with non-pregnant and post-natal values. Armstrong et al. [21] found no differences between ACRs in normal pregnancy and those of non-pregnant women; however, they used only random un-timed urine samples, which makes comparisons with other studies
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REFERENCES


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