Feasibility of using paper impregnated with urine instead of liquid urine for assessing ovarian activity

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Abstract

Background Ovarian activity should ideally be assessed by serial non-invasive methods that require simple procedures for sample collection and storage. Measurement of urinary oestrone-3-glucuronide and pregnanediol-3 α -glucuronide is a non-invasive method available for assessment of ovarian activity, but transport of large numbers of urine samples is cumbersome and samples need to be stored frozen. An alternative sample collection, transport and storage procedure that is easier to handle and requires no or minimal cold storage facilities will particularly benefit studies in which ovulatory activity needs to be assessed in field settings.

Objective To evaluate the feasibility of using paper impregnated with urine as an alternative to liquid urine for the measurement of oestrone-3-glucuronide and pregnanediol-3 α -glucuronide concentrations in the assessment of ovarian activity.

Methods Urine samples collected daily throughout regular menstrual cycles were stored as liquid urine at -20°C, and as paper impregnated with urine, in the refrigerator for 3 to 12 months or at room temperature for 1 to 6 months. Oestrone-3-glucuronide and pregnanediol-3 α -glucuronide concentrations were measured in these urine samples by enzyme immunoassay. Values obtained were correlated using Spearman's correlation test.

Results The pattern of oestrone-3-glucuronide and pregnanediol-3 α -glucuronide concentrations estimated using paper impregnated with urine followed that of liquid urine in all storage conditions used. Values obtained by two methods correlated significantly ($p < 0.001$ to 0.0001) though the paper impregnated with urine gave slightly higher values.

Conclusions Paper impregnated with urine can be used to facilitate sample collection, transport and storage of urine when oestrone-3-glucuronide and pregnanediol-3 α -glucuronide measurements are required in a large number of serial samples to assess ovarian activity.

Introduction

Ovarian activity reflects the reproductive status of a woman and its assessment ideally requires serial monitoring

of either biochemical variables or imaging. The biochemical method, which requires serial estimation of hormones or their metabolites in body fluids is reliable. Although the estimation of hormone levels in serum or plasma seems ideal, venepuncture becomes a drawback when several measurements are required. The development of assays suitable to measure hormones or their metabolites in other body fluids such as urine and saliva (1, 2, 3) has helped overcome this problem. Still, a large number of samples need to be collected and frozen until assayed. This requires adequate cold storage facilities with uninterrupted power supply. Difficulties experienced in a previous study which involved hormone metabolite estimation in a large number of urine samples of postpartum women to assess return of fertility (4) led us to validate an alternative method of storing urine for assessing metabolites of ovarian steroids.

Previous studies in the UK showed that paper impregnated with urine and stored refrigerated yield results similar to frozen liquid urine when metabolites of oestrogen and progesterone are estimated (S. Sufi, unpublished observations). The feasibility of this method had not been assessed in tropical humid environments. Storage of paper impregnated with urine at room temperature has not been studied either. The present study assessed the feasibility of using paper impregnated with urine for the measurement of metabolites of oestrogen and progesterone.

Methods

Early morning urine samples were collected daily during apparently normal menstrual cycles from 6 regularly menstruating women. Indicator pH paper (range 5.2 – 7.2 BDH, BDH Laboratory Supplies, Poole, Dorset, UK) was dipped in urine until the two coloured squares absorbed urine by capillary action. These were dried at room temperature and stored in airtight bags. Two sets were kept at room temperature (28°C), one set for a period of 1 month, the other for 6 months. Four sets were kept in a refrigerator (4°C) one each for 3, 6, 9 and 12 months. Liquid urine samples were stored frozen at -20°C until assayed. Oestrone-3-glucuronide and pregnanediol-3 α -glucuronide levels were measured in liquid urine and in pH paper impregnated with urine by enzyme immunoassay technique using WHO matched reagents purchased from

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Immuno-metrics Ltd (Fulham, London, UK). Sensitivity of the oestrone-3-glucuronide and pregnanediol-3 α -glucuronide were 0.1 nmol/l and 1 nmol/l respectively; and interassay coefficients of variation were 6.9% and 7.7% for low-dose; 5.7% and 4.4% for medium-dose; and 2.8% and 3.1% for high-dose quality control pools.

Before the measurement of hormone metabolite concentrations using paper impregnated with urine, an elution step was carried out. Each coloured square of the pH paper containing dried urine was cut off and placed at the bottom of an assay tube. Five hundred μ l of assay buffer was added to each tube and the tubes were incubated at 37°C for 90 minutes. It was estimated that each coloured square of pH paper absorbs approximately 5 μ l of urine, thus giving a 1:100 dilution when eluted in 500 μ l of assay buffer. These tubes were used straight away for oestrone-3-glucuronide assays as the recommended dilution for this assay was 1:100. However, further dilution with an equal quantity of assay buffer was necessary for pregnanediol-3 α -glucuronide assays as the recommended dilution was 1:200. Pieces of pH paper were removed from the assay tubes and the solution was used for enzyme immunoassay. All the samples were assayed in duplicate. All samples collected during one menstrual cycle were included within a single assay to minimise assay variation.

The urine volume of a healthy subject is variable. This could alter the concentration of solutes in the urine without any change in their absolute levels. Hence concentration of urinary analytes is expressed in terms of urinary creatinine, as creatinine excretion in health is relatively constant as this is an accurate estimation of a urinary analyte concentration (5). Urinary creatinine concentrations were measured in liquid urine and in paper impregnated with urine in the samples using the alkaline picrate method (6). Six small squares of pH paper strips dipped in each sample of urine and dried previously were eluted by incubating with 3 ml distilled water at 37°C for 90 min to obtain 3 ml of urine diluted to 1:100 for the creatinine assay. In the case of liquid urine, 30 μ l of urine was made up to 3 ml with distilled water to obtain the 1:100 dilution prior to assay. Oestrone-3-glucuronide and pregnanediol-3 α -glucuronide concentrations were expressed in terms of creatinine concentration.

Spearman's correlation test was used to correlate values obtained with liquid urine and impregnated paper.

Results

Measurement of hormone metabolites in urine samples stored at 4°C for 3, 6, 9 and 12 months and at room temperature for 1 and 6 months in the form of impregnated paper yielded cyclical patterns similar to those obtained with frozen liquid urine. Cyclical patterns of oestrone-3-glucuronide and pregnanediol-3 α -glucuronide levels obtained using paper impregnated with urine and stored at 4°C for 12 months and liquid urine are shown in Figures 1A and B. The values obtained by paper impregnated with urine seem

to be slightly higher than the values obtained by liquid in all the instances. But the values obtained by two methods correlated significantly (Table 1).

Figure 1. Oestrone-3-glucuronide (A) and pregnanediol-3 α -glucuronide (B) concentrations measured using paper impregnated with urine and stored at 4°C for 12 months (filled squares) and from liquid urine (blank squares) during a normal menstrual cycle. Oestrone-3-glucuronide and pregnanediol-3 α -glucuronide levels have been expressed in terms of urinary creatinine levels.

Table 1. Correlation of hormone metabolite concentrations obtained by using liquid urine and paper impregnated with urine stored at room temperature and at 4°C for varying periods of time

	Oestrone-3-glucuronide <i>r</i> value	Pregnanediol-3 α -glucuronide <i>r</i> value
Room temperature (28°C)		
1 month	0.905**	0.962**
6 months	0.813**	0.876**
Refrigerator (4°C)		
3 months	0.794*	0.930**
6 months	0.874**	0.919**
9 months	0.946**	0.964**
12 months	0.863**	0.969**

* $P < 0.001$, ** $P < 0.0001$

Creatinine concentrations obtained by two methods also correlated significantly ($r = 0.801$; $p < 0.001$) though paper impregnated with urine yielded higher values.

Discussion

These results show that excretory patterns of oestrone-3-glucuronide and pregnanediol-3 α -glucuronide are similar when these are estimated using liquid urine and paper impregnated with urine confirming that paper impregnated with urine can be used in their determination. This will facilitate handling of large numbers of samples. Furthermore, it is possible that papers impregnated with urine can be stored in a refrigerator or even at room temperature in airtight bags for fairly long periods of time, eliminating or minimising the need for cold storage facilities. This is particularly important if samples are being collected in the field. The use of paper strips will also prevent the difficulties faced in transporting samples to laboratories, as air-dried strips can be sent even through mail whereas liquid urine may require refrigerated transport.

The use of impregnated paper in the determination of hormone metabolite levels showed higher values when compared to values obtained with liquid urine. Although it was estimated that each square of pH paper absorbs about 5 μ l of urine, the higher values of hormone metabolites obtained using impregnated paper suggest that each square absorbs a higher volume of urine. Higher creatinine values obtained with impregnated paper strengthens this possibility. Discrepancies in the absolute values obtained with paper impregnated with urine and liquid urine do not pose problems as it is the pattern of oestrone-3-glucuronide and pregnanediol-3 α -glucuronide levels that are important in detecting ovarian activity.

Our study shows that paper impregnated with urine stored at room temperature or in a refrigerator can be used as an alternative for liquid urine even in humid tropical countries to measure oestrone-3-glucuronide and pregnanediol-3 α -glucuronide concentrations in assessing ovarian activity, facilitating collection, transport and storage of samples. This method will particularly benefit research programs involving field sample collection in developing countries.

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