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COMMENTARY

Maternal Urine Screening for Down Syndrome: Past Studies and Future Perspectives

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Antenatal screening for Down syndrome is currently based on multiple maternal serum and ultrasound markers. Replacing serum by urine would have obvious practical advantages and this prompted a series of urinalysis studies in the mid-late 1990s. Earlier studies had found third trimester 24-h total estrogen (tE) to be low in Down syndrome [1] as is second trimester serum unconjugated estriol (E_3), but the main focus was now 'spot' urine human chorionic gonadotropin (hCG). The intact hCG molecule comprises two covalently bound subunits (α and β) which is cleared from the blood and metabolized principally to the β-core molecule (hCGβcf) in urine [2].

There were reasons to believe that in Down syndrome urinary hCGβcf might be increased to a greater extent than serum hCG or free β-hCG. Deactivation of hCG occurs in blood when a major receptor binding loop on the β subunit is 'nicked' causing more rapid dissociation [3], and it was suggested that nicking occurs more in Down syndrome [4]. Another hCG species of interest was hyperglycosylated hCG which is also known as invasive trophoblast antigen (ITA).

All together 18 studies reported urinary levels in Down syndrome pregnancies on hCGβcf, six on free-α, free-β or intact hCG, one using an assay that measured both hCGβcf and hCG, and six on ITA. In addition, one study tested tE and five total E_3. Overall this body of work established that urinary products of both hCG and estrogen are markers of Down syndrome. For example, in a meta-analysis of nine second trimester studies the average urinary level of hCGβcf was 3.7 multiples of the gestation specific median (MoM) compared with 2.0 MoM for maternal serum hCG and 2.3 MoM for free β-hCG [5]. In the first trimester urinary levels hCGβcf are also raised but to a much less extent. When all the hCG species are measured in the same samples, ITA appears to be the most discriminatory [6].

Whilst these results are significant, there is substantial heterogeneity between the published studies, probably due to differences in assay method, study design, and the integrity of urine samples during transport and storage. Moreover, individual variability is higher for spot urine marker levels than for the original molecule in serum, which impedes discriminatory power in screening. This variability is in part due to no uniformity in the time of voiding so that concentrations were expressed per mmol creatinine determined by the Jaffe method which is believed to correct for the daily variations in fluid output. Since creatinine clearance from the blood into the kidney tubule is primarily by glomerular filtration and believed to be independent of urinary flow, the variation in creatinine concentration in a given urine sample should be primarily due to the volume of fluid being excreted.

However, the creatinine concentration is only approximately related to urine concentration and may not be the best measure in all circumstances: the assumed independence of excretion rate from urine flow has been questioned; changes in lean body mass alter the total mass excreted; and renal function changes during pregnancy.

Standardizing the sampling protocol so that, for example, only early morning mid-stream specimens are collected is unlikely to be effective. Another approach is to seek more specific and reproducible measures of urinary concentration. In one study, three methods of normalizing

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the level of hCGβcf for variable urine concentration were compared: high performance liquid chromatography measurement of creatinine, osmolarity and optical density [7]. But none were found to be an improvement over the Jaffe method.

As a consequence of these uncertainties, the concept of using urine to screen for Down syndrome has not been developed further. This could change with advances in matrix assisted laser desorption ionisation (MALDI), time of flight (ToF), and mass spectrometry (MS) instrumentation. This approach has already been used to identify variation in urinary hCGβcf glycosylation by analyzing molecules in the high-resolution range of 3000–5000 m/z [8, 9] which might have clinical application during pregnancy [10]. With the advanced instrumentation it is possible to examine urinary molecules in the range 6000–15,000 m/z without the need of any purification. This has now been applied to Down syndrome screening [11].

Samples taken at 12–17 weeks gestation were tested in 18 Down syndrome and 83 unaffected pregnancies. Spectral data was normalized and at 12–14 weeks the eight cases demonstrated an additional peak at 11,000–12,000 m/z and a corresponding reduction in intensity at 6000–8000 m/z. The ratio of the normalized values at these two ranges completely separated the cases and controls. The spectral pattern was similar in 10 cases at 15–17 weeks but there was overlap with three cases within the normal range.

Another approach is to apply a different MS technology. Zwitterionic hydrophilic interaction liquid chromatography (ZIC-HILIC) ion trap (IT)-ToF MS has been developed for metabolomic investigation of pregnancy urine [12, 13]. In a shotgun metabolomic analysis of urine samples from 23 Down syndrome and 93 unaffected pregnancies the method identified more than 300 significant ions [14]. When multivariate modeling was used to combine the ions all but three of the cases were detected with no false-positives.

These preliminary studies indicate that a mass spectrometry approach has the potential for a rapid, robust, and affordable Down syndrome screening test based on maternal urine. Combining the series, including all gestational ages, the overall Down syndrome detection rate was 85% for a 0% false-positive rate. More work will be needed to confirm that the screening performance will be higher than the established tests based on maternal serum and ultrasound markers. It also remains to be seen how performance compares with noninvasive prenatal testing (NIPT) using maternal plasma cell-free (cf)DNA, which was a Down syndrome detection rate exceeding 99% and false-positive rate under 0.5% [15]. Technical limitations such as a turnaround time exceeding a week, a high test failure rate and high cost preclude immediate implementation of routine cfDNA testing a public health setting, although costs are reducing all the time and methods are improving.

Compliance with Ethical Standards

Conflict of interest None.

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