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LETTERS TO THE EDITOR

First trimester prenatal diagnosis of trisomy 21 in discordant twins using fetal nuchal translucency thickness and maternal serum free β-hCG and PAPP-A

Screening for trisomy 21 in twin pregnancies poses a serious clinical, ethical and moral dilemma (Reynolds, 1995), leading some authors to advocate that such screening should be discouraged (Wald *et al*., 1997). Nevertheless, algorithms have been developed (Wald *et al*., 1991; Spencer *et al*., 1994) for biochemical screening in twins during the second trimester which will allow detection of approximately 50% of cases when one twin is unaffected and the other affected (discordant twins). This theoretical model has been shown to work in routine screening practice (Verdin *et al*., 1997).

Significant interest is now focussed on moving screening for trisomy 21 into the first trimester, when a combination of fetal nuchal translucency, maternal serum free β-hCG and PAPP-A have been shown to identify 90% of cases of trisomy 21 (Spencer *et al*., 1999) and 90% of other chromosomal anomalies (Tul *et al*., 1999; Spencer *et al*., 2000a, b, c). Sebire *et al*. (1996a) showed that fetal nuchal translucency could be used to screen both concordant and discordant twins for trisomy 21; and, more recently, data on the distribution of maternal serum biochemical markers has led to the development of a combined algorithm for screening twins in the first trimester (Spencer, 2000). Here we present details of our first case of twins in which trisomy 21 was prospectively detected and diagnosed.

CASE REPORT

A 35-year-old woman presented at the OSCAR clinic (Spencer *et al*., 2000, submitted) at Harold Wood Hospital for routine obstetric booking and prenatal screening at 12 + 1 weeks by menstrual dates. There was no history of any previous pregnancy. As part of her prenatal screening, blood was analysed for free β-hCG and PAPP-A prior to transabdominal ultrasonography for gestational dating, nuchal translucency thickness and anomaly scan. Ultrasound examination revealed a dichorionic twin pregnancy. Fetus 1 had a nuchal thickness of 2.6 mm with a crown–rump length of 51.5 mm, and Fetus 2 had a nuchal thickness of 2.0 mm with a crown–rump length of 59.1 mm. These NT measurements were equivalent to 1.86 and 1.43 MoM respectively. The maternal serum free β-hCG was 188.4 IU/l which when corrected for twins (Spencer, 2000) was equivalents to 1.86 MoM in a singleton pregnancy. Similarly, the maternal serum PAPP-A of 3.39 IU/l was corrected to 0.64 MoM.

The estimated risk for trisomy 21 based on maternal age was 1 : 208. The risk increased to 1 : 120 when maternal age was combined with maternal serum biochemistry. Based on nuchal thickness and maternal age, Fetus 1 had a risk of 1 : 46 and Fetus 2 had a risk of 1 : 212. When combined together the maternal age/fetal nuchal thickness/maternal serum biochemistry risk for Fetus 1 was 1 : 27 and for Fetus 2 was 1 : 122.

The patient was counselled as to the possible options, including invasive testing and the possibility of embryo reduction should one of the fetuses be chromosomally abnormal, and she chose to have chorionic villus sampling. This was carried out transabdominally under ultrasound guidance and samples were obtained from both placentas. Karyotyping by both PCR and conventional culture indicated that Fetus 1 was a female with trisomy 21 and that Fetus 2 was a normal female. After further counselling, the patient chose to have embryo reduction, which was carried out by ultrasound-guided injection of potassium chloride in the chest of the affected fetus at 12 weeks of gestation. The pregnancy with the single live fetus is progressing normally.

Although alone maternal serum biochemistry can indicate the presence of an increased risk of trisomy 21 and might be expected to identify a little over 50% of cases, it cannot identify the specific twin affected. Nuchal translucency, however, in addition to being a more specific marker for trisomy 21 than any biochemical parameter, can specifically identify the twin at increased risk. This increased nuchal thickness can also then be used to differentiate between the two fetuses when diagnostic procedures and fetal reduction are carried out. Furthermore, the estimated risk can also be used in deciding whether to carry out first trimester chorionic villus sampling or second trimester amniocentesis (Sebire *et al*., 1996b).

This case serves to illustrate the value of combining fetal nuchal translucency and maternal serum biochemistry and that such combinations can be successfully used to screen for trisomy 21 in twins discordant for this anomaly.

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Prenatal screening for Down syndrome: pre-analytical precautions

We want to focus on precautions which need to be made at the pre-analytical stage of prenatal screening for Down syndrome.

Biochemical screening for Down syndrome, which is now well established, is based on assaying several serum markers—Human chorionic gonadotrophin (hCG) or its free β-fraction, alpha-fetoprotein (AFP) and possibly oestriol (Bogart *et al.*, 1987; Wald *et al.*, 1988; Spencer, 1991). This screening test is currently conducted in the second trimester of pregnancy between 15 and 17 weeks of amenorrhoea and starts in the first trimester. The threshold determining whether a woman belongs to a population at an increased risk of Down syndrome is currently set at 1/250. Beyond this threshold in France, costs for additional diagnostic examinations are covered by the state.

In France, there are 61 approved laboratories for carrying out screening tests. As a result, most sera are sent to the testing laboratory and where they are subjected to an important pre-analytical treatment. Of particular important are: (1) reliable identification of the patient; and (2) the stability of the molecules to be assayed.

A source of potential identification error is introduced while the serum of the primary tube is decanted into a secondary tube. A reversal of identities may occur at this stage with serious consequences.

The result of the AFP, hCG and particularly free β-fraction assays must not be modified by the storage conditions from the moment when the blood specimen is collected to when the assay is conducted (Spencer *et al.*, 1993; Cuckle and Jones, 1994). This is particularly true for the hCG β-subunit, which is produced by the splitting of the total hCG. Bacterial contamination during the treatment of the sample has been blamed as the possible cause of an artificial increase in the concentration of this molecule (Kardana and Cole, 1997).

In a first study (Muller *et al.*, 1999), we demonstrated that transport, as such, did not have any significant difference on the screening test results. In this study, we wanted to check whether the two remaining pitfalls (identity error and splitting due to contamination) could be prevented by collecting the specimen in a sterile tube without any anticoagulant and with separating gel, centrifuging within 2 h and by leaving the tube stoppered until it reached the testing laboratory. While the first problem (identity error) is solved in an obvious way by removing the secondary tube, it was necessary to ensure that there was no difference in the assays.

Two similar laboratories at respective distances of 50 and 500 km sent us a total of 100 double specimens for screening tests for Down syndrome—one conventional test on a decanted (D) dry tube and the other on a non-decanted (ND) tube as described above. The two specimens were assayed in our laboratory systematically in the same test series. All the specimens were collected using Becton–Dickinson® vacutainer tubes. The specimens were transported by mail with no special precautions within 24–72 h (maximum).

The two molecules assayed were the free β-hCG fraction AFP, with the EGG-Wallac® duo technique