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CLINICAL ARTICLE

Prospective prenatal serum screening for Down syndrome in Venezuela

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Abstract

Objective: To assess the usefulness of triple-marker screening for Down syndrome in Venezuela. *Method:* Maternal serum concentrations of alpha fetoprotein (AFP), beta human chorionic gonadotropin (β-hCG), and unconjugated estriol (uE3) were measured weekly in 3895 women from the 15th to the 20th week of pregnancy. Population-specific likelihood ratios were determined and used to calculate the risk of fetal Down syndrome for each pregnancy. *Results:* The median multiple of the median values for AFP, β-hCG, and uE3 concentrations were 0.69, 2.10, and 0.67 for the affected pregnancies. The likelihood ratio for a positive result was 1:19. The detection and false-positive rates were 69.23% and 5.8%. *Conclusion:* These findings were consistent with reported data and therefore confirmed triple-marker serum screening as effective and suitable for prenatal care in Venezuela. Latin American governments and Health Agencies should recommend offering this screening method to all pregnant women.

1. Introduction

Prenatal screening for Down syndrome has evolved from a risk assessment based on maternal age to an approach that also uses maternal serum concentrations of specific analytes. Abnormal maternal serum levels of alpha fetoprotein (AFP), human chorionic gonadotropin (hCG or, its fraction-free subunit β-hCG), and unconjugated estriol (uE3) during the second trimester of pregnancy have been shown to be associated with Down syndrome [1,2]. For each pregnancy, the separate probabilities derived from the serum levels of each of the 3 markers and the risk associated with maternal age generate a compound probability for a fetus to be affected with Down syndrome. If the screening result is positive, the pregnant woman is offered an amniocentesis [3]. The screening strategy requires strict quality control and audit.

There have been tremendous advances in prenatal diagnosis over the past decades, and triple-marker maternal

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serum screening for Down syndrome has become an established part of obstetric practice in developed countries. However, data from Latin American countries are insufficient to achieve consensus regarding implementation of prenatal screening for Down syndrome. The objective of this study was to determine whether triple-marker maternal serum screening for Down syndrome would be useful in Venezuela pregnant women.

2. Materials and methods

We analyzed the results of a 10-year prospective study of triple-marker serum screening involving 3859 consecutive pregnant Venezuelan women at the Medical Genetics Unit of the University of Zulia in Maracaibo, Venezuela, between January 1998 and December 2007.

The exclusion criteria were the following: multiple pregnancy, structural fetal malformations, chromosomal abnormalities detected at previous karyotyping, pregnancy loss at any time, stillbirth, or death in the neonatal period. Information on pregnancy outcome was obtained partly from karyotyping and partly from information collected from phone enquiries from the women themselves or their attending obstetrician.

The results of 854 of the women were excluded for a lack of follow-up information, and those of another 28 women because their offspring had congenital abnormalities (n = 13) or chromosomal abnormalities other than trisomy 21 (n = 15). There were 13 serum samples from women whose pregnancies were affected by trisomy 21. Of these, 2 were excluded when assessing the AFP-related parameters of the trivariate risk for Down syndrome because an etiology known to increase the serum level of AFP was present (ie, neural tube defect, abdominal wall defect, or gastrointestinal congenital malformations).

The offspring of the remaining 2964 women was euploidic and the results of the triple-marker serum screening for these women were used for control (Fig. 1). All women in the control group were of mestizo background and none had diabetes mellitus.

Maternal serum concentrations of AFP, β-hCG, and uE3 were measured in the control and study groups. Maternal serum samples were collected from the 15th to the 20th week (included) of gestation. Sera were kept at 4 °C until measurements were made, within 48 hours. The Microparticle Enzymatic Immunoassay (Abbott Laboratories, Abbott Park, IL, USA) was used to measure AFP and β-hCG concentrations. The coefficient of variation was 2.8% within and between assays for AFP, and it was 1.8% within assays and 4.3% between assays for β-hCG. The Fluorescent Polarizing Immuno Assay (Abbott Laboratories) was used to measure uE3 concentration. The coefficient of variation was 5.9% both within and between assays.

Gestational age was estimated from the date of the last menstrual period and, in 89% of cases, by ultrasound measurement of the biparietal diameter. In less than 1% of cases the date of the last menstrual period and the ultrasound result were divergent, and the ultrasound estimate was then selected. To correct for gestational variations, all values for the biochemical markers were converted to multiples of the median (MoM) values using the weekly median values obtained from the control group for the same gestational age.

A multiple linear regression analysis taking into account maternal weight, presence of a chronic condition such as insulin-dependent diabetes mellitus, use of tobacco, use of alcohol, use of medications, use of illicit drugs, gravidity, parity, and previous pregnancy loss showed a significant inverse relationship ($P<0.05$) between maternal weight and the serum concentrations of the 3 markers, expressed as MoM. The regression functions between maternal weight and the serum concentrations of AFP, β-hCG, and uE3 were estimated separately. Then, all the MoM values were adjusted according to maternal weight in kilograms using the following equations: adjusted AFP MoM = observed AFP/exp (0.4915 − 0.0076 × maternal weight); adjusted hCG MoM = observed hCG/exp(0.3955 − 0.0061 × maternal weight); and adjusted uE3 MoM = observed uE3/exp(1.0816 − 0.0012 × maternal weight), where *exp* indicates exponential.

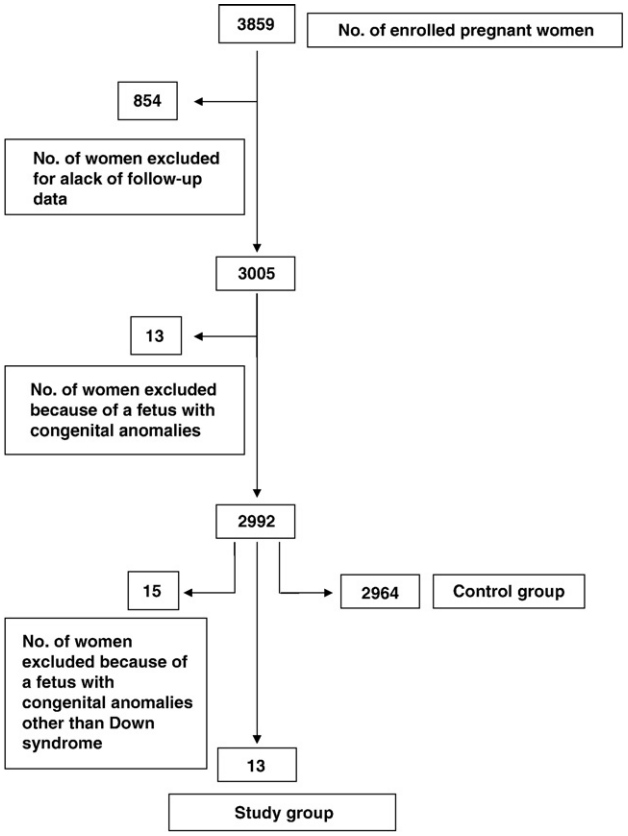

Figure 1 Flow chart of the prospective study.

Calculation of the trivariate risk of Down syndrome using biochemical markers was performed as reported elsewhere [4]. Likelihood ratios (LRs) were determined from the Gaussian distribution curves of the values for the 3 markers' concentration in the study and control group. These LRs were multiplied by the *a priori* age-related risk for fetal Down syndrome. Logistic regression analysis was performed to verify the reliability of the Gaussian model. A risk of 1 in 270 was the cut-off to distinguish pregnancies at low or high risk for fetal Down syndrome. For each marker the means, standard deviations, and correlation coefficients, all based on logarithmic MoM values, were determined separately for the affected and unaffected pregnancies to calculate the performance of the screening in the second trimester.

The performance of this second-trimester, trivariate biochemical screening was analyzed by examining the detection rate, the false-positive rate, and the specificity of the tests. Data analyses were performed using GraphPad Prism for

Table 1 Values for each marker and risks for trisomy 21

Karyotype	Gestational age, wk	Maternal age, y	AFP, MoM	β-hCG, MoM	uE3, MoM	Estimated risk
47,XY+21	17	29	0.78	2.05	0.87	1:372
47,XY+21	18	29	0.71	1.88	0.73	1:308
47,XX+21	15	30	0.83	1.98	0.69	1:300
48,XYY+21	17	31	2.15	1.96	0.34	1:270
47,XY+21	17	35	0.71	3.32	0.75	1:56
46,XX+t(14;21)	18	38	2.31	1.87	0.84	1:300
47,XX+21	18	38	0.71	2.32	0.67	1:31
46,XX+t(21;21)	18	39	0.67	2.15	0.62	1:21
47,XY+21	19	39	0.61	2.01	0.67	1:30
47,XX+21	17	40	0.67	2.30	0.66	1:20
47,XY+21	15	41	0.68	1.58	0.64	1:30
47,XX+21	16	42	0.69	1.97	0.74	1:16
47,XY+21	15	43	0.63	1.97	0.53	1:6

Abbreviation: AFP, alpha fetoprotein; β-hCG, beta human chorionic gonadotropin; MoM, multiples of the median (MoM) values for the same gestational age in the control group; uE3, unconjugated estriol.

Windows XP, version 4. (GraphPad Software, San Diego, CA, USA). The Kolmogorov-Smirnov test was used to test the normal distribution of the analytes and Pearson correlation coefficients were used to assess the correlations among the various indices. $P<0.05$ was considered statistically significant.

The study protocol was approved by the local institutional review committee and informed consent was obtained on the first visit to the medical geneticist of our unit.

3. Results

Of 28 cases of chromosomal aberrations detected by amniocentesis there were 13 cases of trisomy 21, 11 resulting from meiotic nondisjunction and 2 from Robertsonian translocation, one homologous and the other heterologous (Table 1). The median MoM values were 0.69 (95% CI, 0.66 to 0.74; range 0.61–0.83) for AFP; 2.10 (95% CI, 1.85 to 2.35; range 1.58–3.32) for β-hCG and 0.67 (95% CI, 0.59 to 0.75; range 0.34–0.87) for uE3 in the study group. They were 1.03 for AFP, 1.02 for β-hCG, and 0.97 for uE3 in the control group, and the differences were significant for each marker ($P<0.001$ by the Mann-Whitney test in affected pregnancies with Down syndrome and normal pregnancies.

The distributions of the AFP, β-hCG, and uE3 MoM values on the logarithmic transformation fit a normal Gaussian distribution in both groups, and the Kolmogorov-Smirnov test showed no deviation from linearity at the 0.01 probability level. The means and standard deviations of Gaussian distributions of the logarithmic MoM values and the Pearson correlation coefficient for each marker are shown in Table 2. No correlations were found between the logarithmic distributions of AFP MoM and β-hCG MoM, AFP MoM and uE3 MoM, or of β-hCG MoM and uE3 MoM in either group. No significant correlations were found between maternal age and AFP, β-hCG, or uE3 either in the study group ($r=-0.22$, $P=0.47$; $r=-0.06$, $P=0.85$; or $r=-0.11$, $P=0.71$, respectively or in the control group ($r=0.03$, $P=0.70$; $r=-0.03$, $P=0.69$, and $r=-0.02$, $P=0.79$).

The detection and false-positive rates were 69.2% (9 of 13) and 5.8% (173 of 2992), respectively. The specificity was

Table 2 Gaussian distribution of the logarithmic MoM values and Pearson correlation coefficients for each marker in pregnancies unaffected and affected with trisomy 21

Marker	Unaffected Pregnancies		Affected Pregnancies	
MOM values				
	Mean	Standard deviation	Mean	Standard deviation
AFP	0.01053	0.05762	−0.1664	0.03101
β-hCG	0.002529	0.01466	0.3142	0.07972
uE3	0.003733	0.03005	−0.1844	0.1121
Pearson correlation coefficients				
	r	*P* value	*r*	*P* value
AFP plus β-hCG	0.33	<0.05	0.35	0.29
β-hCG plus uE3	0.03	<0.05	0.30	0.32
AFP plus uE3	0.06	<0.05	0.60	0.05

Abbreviation: AFP, alpha fetoprotein; β-hCG, beta human chorionic gonadotropin; MoM, multiples of the median (MoM) values for the same gestational age in the control group; uE3, unconjugated estriol.

Table 3 False-positive and detection rates of triple-marker screening for Down syndrome according to maternal age [a]

Maternal age, y	All screened pregnant women	Pregnancies with a risk ≥ 1:270	All pregnancies with trisomy 21	Pregnancies with a risk ≥ 1:270 and trisomy 21	Percentage of detection
<30	1403 (46.68)	37 (2.63)	2 (0.14)	0	0
≥30–34	776 (25.81)	28 (3.61)	2 (0.26)	1	50
≥35–37	471 (15.67)	52 (11.04)	1 (0.21)	1	100
≥38	355 (11.84)	65 (18.31)	8 (2.25)	7	85.70
Total	3005	182 (6.05)	13 (0.43)	9	69.23

[a] Values are given as number (percentage) unless otherwise indicated.

94.2% (2819 of 2992) and the likelihood ratio (ie, the odds of being affected given a positive result) was 1:19.

4. Discussion

Triple-marker serum screening was developed to identify pregnant women whose risk of having a fetus with trisomy 21 is sufficiently high to be offered fetal karyotyping. It is now part of routine prenatal care in developed countries, but to the best of our knowledge the present serum screening program is the first reported study in Latin-American pregnant women.

For accurate interpretation of the results, reliable and precise estimates of the maternal serum markers are needed for the design and implementation of prenatal biochemical screening programs. Median values were monitored throughout the study and updated when necessary. In our study with Venezuelan women, the serum levels of the 3 markers fitted a Gaussian distribution similar to the distributions seen for white or Asian women both in normal pregnancies and in pregnancies affected by Down syndrome [4–7]. Nine of 13 (69.23%) cases of fetal Down syndrome were identified using a cut-off risk for fetal Down syndrome of 1:270, with a false-positive rate of 5.8%.

The detection and false-positive rates varied with maternal age in our study (Table 3). The detection rate was about 25% for pregnant women younger than 35 years, with a false-positive rate of 2.98%. For pregnant women older than 35 years, the detection and false-positive rates were of 88.88% and 14.16%, respectively. Thus, the sensitivity of second-trimester maternal serum screening was much higher for women of older maternal age in our population, a finding that has also been reported by others [1,8–10]. Although the screening's main target are pregnant women younger than 35 years, the screening is also of great importance for pregnant women 35 years or older. Still, the proportion of women older than 35 years was significantly higher in our study group than in the general population of pregnant Venezuelan women. This effect could be due to an ascertainment bias in our population.

Two fetuses with Down syndrome had *de novo* Robertsonian translocations. Serum concentrations of β-hCG and uE3, expressed as MoM, were similar in these pregnancies and other pregnancies with trisomy 21 ($P > 0.05$ by the Mann-Whitney test). The MoM value for AFP was higher in one case, however. This fetus (46,XX + t[21;21]) also had omphalocele, esophageal atresia, and a tracheo-esophageal fistula, which could have caused the increase in the AFP level, affecting its detection by marker serum screening. Saller et al. [11] found AFP, β-hCG, and uE3 MoMs to be similar in pregnancies with fetal trisomy 21 resulting from meiotic nondisjunction and those resulting from Robertsonian translocation, which suggests that Down syndrome from both causes will be detected in a similar percentage of cases.

Of the 3 markers usually used in maternal serum screening programs, hCG has clearly been shown to provide the best predictive value for fetal Down syndrome. A retrospective analysis demonstrated the usefulness of assessing free β-hCG levels in the identification of pregnancies with Down syndrome [12]. Assessing β-hCG is preferred to assessing intact or total hCG because, statistically, the Mahalanobis distance (ie, the number of standard deviations between the mean levels in affected and unaffected pregnancies) is more discriminatory [13]. Although the β-hCG MoM value was the best discriminating marker between unaffected and affected pregnancies in this study, the dual-marker combination β-hCG/AFP was also a good discriminator. However, in one fetus, trisomy 21 could be not ascertained by bivariate function (β-hCG and AFP), but was detected by trivariate function (β-hCG, AFP, and uE3). Since the false-positive rate did not increase when uE3 was added to calculate the risk of fetal Down syndrome, we recommend using the triple-marker rather than the double-marker screening.

In conclusion, the detection rate, false-positive rate, and specificity in this report are consistent with those reported in other studies, thus confirming the triple-marker serum screening as a suitable and efficient part of prenatal care. Governments and Health Agencies should recommend and support its implementation in routine prenatal care in Latin America.

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