OBSTETRICS

Down syndrome maternal serum screening in patients with renal disease

Alexandra Benachi, MD, PhD; Sophie Dreux, PhD; Sahar Kaddioui-Maalej, MD; Isabelle Czerkiewicz, MD; Fadi Fakhouri, MD, PhD; Eric Thervet, MD, PhD; Françoise Muller, PhD

OBJECTIVE: The objective of the study was to determine the value of maternal serum Down syndrome screening in patients affected by renal disease.

STUDY DESIGN: A study group of 54 pregnant women with renal diseases defined before pregnancy, was compared with a control group of 108 patients matched for maternal age, maternal weight, smoking status, and gestational age. Maternal serum markers (free β-human chorionic gonadotropin [hCG], total hCG, alpha-fetoprotein) expressed in multiple of median and maternal renal function markers (creatinine, β2-microglobulin, α1-microglobulin) were assayed.

RESULTS: The percentage of patients in the Down syndrome at-risk group (>1:250) using free β-hCG was significantly higher ($P < .02$) in the renal disease group (48%) than in the control group (12%). No significant difference was observed for total hCG (25% vs 15%).

CONCLUSION: Down syndrome screening using free β-hCG is not applicable in patients with renal disease whatever the maternal serum creatinine and can be used with caution when total hCG is used.

Key words: pregnancy, prenatal diagnosis, renal failure, trisomy 21

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The usual approach to prenatal screening for Down syndrome (DS) is to estimate a woman's risk of having a trisomy 21–affected pregnancy on the basis of factors such as maternal age, maternal serum markers, or first-trimester nuchal translucency measurement, using different combinations. Depending on the combinations, 60-90% of fetuses with DS can be detected with a 5% false-positive rate.[1-4]

Different confounding factors have been evaluated, such as maternal weight, maternal smoking status, twin pregnancies, a previous trisomy 21–affected child, and various adjustments, allow an appropriate screening. However, in patients affected by renal disease, abnormally high human chorionic gonadotropin (hCG) values have been described in case report or small series, but various issues remain unsolved, such as the relative modification of the DS markers, the creatinine cutoff above which DS screening should not be used, and the application of such screening in renal transplant recipients.[5-8]

The aim of the present study was to firmly establish the limits of DS maternal serum screening in a retrospective series of pregnant women presenting with renal diseases when free β-hCG or total hCG is used.

From the Department of Obstetrics and Gynecology (Drs Benachi and Kaddioui-Maalej), Hôpital Necker Enfants Malades, Assistance Publique Hôpitaux de Paris, Université Paris Descartes, the Department of Biochemistry, Hôpital Robert Debré, Assistance Publique Hôpitaux de Paris, Université Paris Ile de France Ouest (Drs Dreux, Czerkiewicz, and Muller), Department of Nephrology, Hôpital Necker Enfants Malades, Assistance Publique Hôpitaux de Paris (Dr Fakhouri), and Department of Renal Transplantation, Hôpital Necker Enfants Malades, Assistance Publique Hôpitaux de Paris (Dr Thervet), Paris, France.

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Reprints: Françoise Muller, PhD, Biochimie Hormonologie, Hôpital Robert Debré, 48, Bd Sérurier, 75019 Paris, France. francoise.muller@rdb.aphp.fr

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Materials and Methods

This retrospective study was conducted during the period 2000-2008 in patients who underwent routine second-trimester maternal DS screening. Informed consent was obtained for each patient. Institutional review board approval was obtained for this study (Comité d'Ethique de la Recherche en Obstétrique et Gynécologie 2008-021).

The study group comprised 54 pregnant women with renal disease (RD group). Twin pregnancies were excluded. The control group consisted of 108 serum samples randomly selected from the routine second-trimester maternal serum screening database, matched with the study group based on maternal age, maternal weight, smoking status, and gestational age. For both groups, patients older than 35 years were included, therefore leading to a high false-positive rate of DS risk calculation. Amniocentesis was performed at the parents' request in 10 patients of the RD group and in 13 of the control group. Fetal karyotyping was normal in all cases. No DS was observed at birth in both groups.

French DS screening policy relies on second-trimester maternal serum marker screening (gestational age between 14

TABLE 1
Comparison of median values of parameters between renal disease and control groups

Parameters	Renal disease group (n = 54), median (extremes)	Control group (n = 108), median (extremes)	*P*
Age, y	31 (23-42)	31 (23-41)	NS
Maternal weight, kg	62 (45-144)	61 (42-113)	NS
Gestational age at sampling, wks and d	15.3 (14.1-24.6)	15.3 (14.1-25)	NS
Creatinine, μmol/L	84 (34-329)	49 (22-69)	< .0001
Urea, mmol/L	6 (3.1-12.9)	3.2 (1.7-5.7)	< .0001
α1-Microglobuline, mg/L	28.3 (3.9-126.3)	15.4 (1.2-46)	< .0001
β2-Microglobuline, mg/L	2.33 (1.24-9.87)	1.28 (0.96-2.63)	< .0001
Total protein, g/L	68 (55-79)	69 (60-88)	NS
AFP, MoM	1.07 (0.44-1.92)	0.96 (0.41-1.99)	NS
Free β-hCG, MoM	2.13 (0.32-32.8)	1.04 (0.18-13.3)	< .0001
DS risk calculation, 1/[a]	270 (9-10,000)	1321 (5-10,000)	< .0001
Total hCG, MoM	1.40 (0.3-9.5)	1.10 (0.2-7.1)	.01
DS risk calculation, 1/[b]	1132 (30-10,000)	1350 (16-10,000)	NS

AFP, alpha-fetoprotein; *β-hCG,* β-human chorionic gonadotropin; *FPR,* false-positive rates; *MoM,* multiple of the median; *NS,* nonsignificant.

[a] Based on the combination of free β-hCG, AFP, and maternal age; [b] Based on the combination of total hCG, AFP, and maternal age.

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and 18 weeks of amenorrhea) and is strictly regulated. First trimester is actually not available. Routine second-trimester maternal serum screening was based on free β-hCG and alpha-fetoprotein (AFP) (Dualkit, AutoDelfia, Life cycle software; PerkinElmer, Turku, Finland). Results were expressed in multiple of median (MoM). In the present study, 2 DS risks were calculated, 1 based on the combination of DS risk because of maternal age, AFP MoM, and free β-hCG MoM and the other risk using total hCG MoM instead of free β-hCG. A cutoff of 1/250 at sampling was used for both risks.

Of the 54 samples, 44 were available (kept frozen at −40°C), allowing determination of total hCG for DS screening (AutoDelfia; PerkinElmer) and maternal serum markers of renal failure including urea (DiaSys, Condom, France), creatinine (Creatinine-2Enzy; Siemens, Tarrytown, NY), β2-microglobulin (Olympus, Hamburg, Germany), α1-microglobulin (Roche Hitachi, Mannheim, Germany), and total protein (Protein2; Siemens) as a reference marker. When the sample was not available, maternal serum creatinine measured in the month of the serum screening was taken into account.

Renal diseases before pregnancy were defined by proteinuria greater than 0.5 g per 24 hours and/or glomerular filtration rate (GFR) (estimated using the modification of the diet in renal disease [MDRD] formula) less than 60 mL/min.[9] The etiologies of renal diseases were as follows: polycystic renal disease (n = 8); uropathy with reflux (n = 7); glomerulonephritis (n = 6); lupus nephritis (n = 6); Berger disease (n = 4); rheumatoid purpura (n = 4); nephrotic syndrome (n = 4); diabetic nephropathy (n = 3); glomerular nephropathy (n = 2); tubular interstitial granulomatosis nephropathy (n = 3); nephronophthisis (n = 2); nephroangiosclerosis (n = 1); Alport syndrome (n = 1); and unknown (n = 3).

To estimate renal function, guidelines specifically exclude interpretation of the MDRD and Cockroft-Gault formulas in

TABLE 2
False-positive rates in maternal serum Down syndrome screening using free β-hCG or total hCG

Variable	FPR, % (free β-hCG)	FPR, % (total hCG)
Control group	12 (13/108)	15 (15/100)
Total renal disease group	48 (26/54)[a]	25 (10/40)
Renal disease subgroups		
Creatinine <125 μmol/L	44.6 (21/47)[a]	20 (7/35)
Creatinine <100 μmol/L	38.5 (15/39)[a]	20 (6/30)
Creatinine <80 μmol/L	33.3 (8/24)[a]	22 (4/18)

Risk was calculated combining maternal age, multiple of the median free β-hCG (or total hCG) and alpha-fetoprotein.
β-hCG, β-human chorionic gonadotropin; *FPR,* false-positive rates.

[a] Significant difference when compared with control group.

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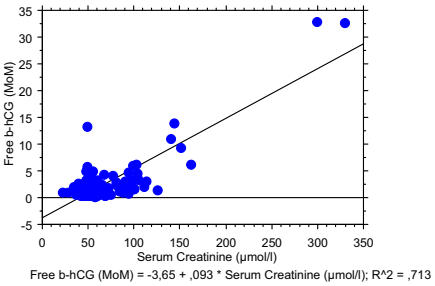
FIGURE 1
Maternal serum creatinine and free b-hCG MoM correlation



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pregnant women; therefore, renal function was evaluated based on serum creatinine using a 125 µmol/L cutoff as proposed by Shemesh et al[10] and Perrone et al.[11] Comparisons were performed using the Mann-Whitney test and χ^2 for percentages.

RESULTS

Table 1 presents the population of the RD group vs the control group for all studied parameters. As expected, no significant difference was observed for matched criteria, and a significant difference was observed for markers of maternal renal failure between the 2 groups.

A significant difference was observed between the RD group and controls for MoM free β-hCG and total hCG, but no difference was noted for MoM AFP.

A correlation was observed in the RD group between maternal serum creatinine and MoM free β-hCG ($r = 0.744$; $P < .01$) or MoM total hCG ($r = 0.70$; $P < .01$) (Figures 1 and 2). When another maternal renal failure marker (α1-microglobulin, β2-microglobulin) was used, the correlation with MoM free β-hCG was similar ($r = 0.63$; $P < .01$ and $r = 0.78$; $P < .01$, respectively).

False-positive rates (FPRs) are presented in Table 2. Using free β-hCG, FPR was 48% in the RD group vs 12% in the control group, a significant difference ($\chi^2 = 5.05$). Using total hCG, FPR was 25% in the RD group vs 15% in the control group, a nonsignificant difference ($\chi^2 = 1.39$) Different maternal serum creatinine cutoffs were tested (125 µmol/L, 100 µmol/L, and 80 µmol/L) to analyze the FPRs. For risks calculated using free β-hCG, the difference between the control group and the RD group was significant whatever the cutoff, therefore excluding the possible use of DS maternal serum markers in patients with renal disease, whatever the maternal serum creatinine. For risks calculated using total hCG, the difference between the control group and the RD group was nonsignificant whatever the cutoff.

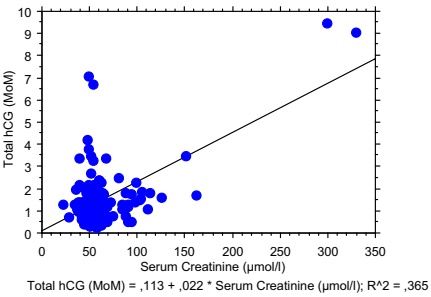
COMMENT

In this study we observed that DS maternal serum screening using free β-hCG is inappropriate for patients with renal disease because of an FPR significantly higher in the renal disease group (48%) than in the control group (12%). This high percentage is due to a significant difference in MoM free β-hCG (2.13 MoM vs 1.04 MoM). Even if we observed a correlation between the degree of renal failure evaluated by maternal serum creatinine level and free β-hCG ($r = 0.74$), no maternal serum creatinine cutoff allowed to reach a nonsignificant difference in DS FPR. Increased levels of maternal serum free β-hCG have been previously noted in studies with evidence of maternal renal disease, but these were small series and no firm conclusions could be drawn.[5-8]

When total hCG is used instead of free β-hCG in DS risk calculation, the difference in FPR between the control and RD groups was smaller (15% vs 25%) and not significant. However, because of a significant difference in MoM values (1.10 vs 1.40), maternal serum DS screening using total hCG must be used with caution.

To estimate renal function, because of the numerous disadvantages of using filtration markers, the Cockcroft-Gault formula and MDRD equations are widely used as indirect estimates of renal function. However, because during pregnancy maternal adaptation is characterized by substantially increased GFR, usually 50% above the prepregnancy value, the guidelines specifically exclude interpretation of the MDRD and Cockcroft-Gault formulas in pregnant women.[12] As recommended, we based

FIGURE 2
Maternal serum creatinine and total hCG MoM correlation



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renal function evaluation in pregnant women on serum creatinine.

Little is known regarding the reasons for the raised maternal serum free β-hCG levels in patients with impaired renal function. Two major pathophysiological mechanisms can be hypothesized. The first mechanism is related to chronic hypertension associated with renal failure complicated by vasculopathy, which may cause placental hypoxia. Increased maternal serum hCG levels may be due to reduced perfusion in the intervillous circulation of the placenta, with subsequent hypoxia and increased hCG. Meuris et al[13] demonstrated that hypoxia stimulates the formation of trophoblastic tissue and therefore increases the production of hCG, which enters the maternal circulation. Because AFP is not of placental origin but of fetal liver origin, the normal AFP values we observed in patients with renal disease support this hypothesis.

The second mechanism is a decreased renal clearance of the hCG because of impaired renal function.[14] This prompted us to assess the relation between free β-hCG and different renal function markers. A significant correlation was observed between free β-hCG and creatinine and β2-microglobulin and α1-microglobulin.

These correlations are therefore in accordance with the second hypothesis, which relates high hCG values in renal failure patients to decreased hCG clearance. The molecular weight of the studied molecules can explain these differences. Total hCG is a heterodimeric glycoprotein hormone of placental ori-

gin (55 KDa). The 2 subunits, alpha (22 KDa) and beta (34 KDa), are not covalently linked. This hormone exists in many forms.[15] The β-subunit is dissociated from the α-subunit and is first degraded into a nicked free β-subunit (missing the C-terminal peptide) and subsequently converted in the maternal kidneys to β-core fragment, the final degradation product.[7]

The whole molecule is the major form found in serum and the β-core fragment is the major form in urine.[15] It has been demonstrated that when purified hCG is infused into human, only 21.7% is excreted in urine, the remaining 78.3% being taken up and processed mainly by the kidneys.[16] The difference observed in MoM between total hCG (1.40) and free β-hCG (2.13) could be explained by the difference in molecular weight of the molecule (55 and 34 KDa, respectively).

The similarity in MoMs for free β-hCG, α1-microglobulin, and β2-microglobulin (2.13, 1.83, and 1.82, respectively) and in molecular weight (34, 27, and 11.7 KDa, respectively) favors the hypothesis of a low free β-hCG clearance in patients with renal failure. The absence of difference in MoM AFP could be explained by its high molecular weight (72 KDa).

In conclusion, maternal serum DS screening based on free β-hCG is not suitable. In such patients, DS screening should be based on maternal age and first-trimester nuchal translucency measurement.[17] When not available, second-trimester maternal serum screening using total hCG can be a help to reassure a non–at-risk patient. For an at-risk patient, a second-trimester genetic scan screening would allow DS risk calculation.[18] ■

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