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DOTTORATO DI RICERCA IN MEDICINA PRENATALE

CICLO DEL CORSO DI DOTTORATO

XIX

Titolo della tesi

PRODUZIONE SCIENTIFICA DURANTE IL DOTTORATO DI RICERCA IN MEDICINA PRENATALE

Dottorando

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A Riccardo

INTRODUZIONE

In questo elaborato finale per il Dottorato di Ricerca in Medicina Prenatale ho voluto raccogliere la produzione scientifica che ho portato a termine durante l'intera durata del Dottorato stesso.

Presento qui di seguito tutti i lavori editati a mezzo stampa e prodotti sotto l'egida del Dottorato in oggetto, omettendo gli atti congressuali (ove si eccettuino quelli prodotti su riviste indicizzate).

La produzione scientifica è relativa ai due campi di ricerca perseguiti nel corso degli ultimi 3 anni:

1. *Ruolo della plicometria ultrasonografica fetale nello sviluppo e nel miglioramento degli algoritmi di stima del peso fetale. Cambiamenti dei compartimenti corporei fetali in condizioni patologiche.*
2. *Epidemiologia delle trombofilie in una popolazione ostetrica e correlazione con gli esiti avversi della gravidanza.*

Le pubblicazioni inerenti al primo settore sono 4: la prima, in ordine di tempo, definisce sistematicamente la metodica della psicometria ecografia fetale e presenta le tavole di riferimento biometriche dei parametri plicometrici in gravidanza normale ed in gravidanza affetta da diabete gestazionale. Il lavoro in oggetto è stato pubblicato da Ultrasound Obst/Gyn (UOG) nel 2003. La seconda pubblicazione è del 2005, è stata editata sempre da UOG e mostra le differenze, in termini di tessuto sottocutaneo fetale, tra feti sani e feti con ritardo di crescita sin dall'inizio del terzo trimestre. La terza e la quarta pubblicazione in ambito di plicometria ultrasonografica fetale sono state prodotte su Journal of Obstetrics and Gynaecology Research (Elsevier) entrambe nel 2007, e trattano dell'introduzione dei parametrici plicometrici fetali negli algoritmi di stima del peso fetale. Inoltre in questi due ultimi lavori vengono prodotti due nuovi algoritmi per la stima del peso fetale: una formula matematica ed un modello tabulare a lettura visiva, per la predizione

del peso fetale a termine di gravidanza. Uno dei due lavori è in corso di stampa e viene qui presentato come manoscritto con lettera di accettazione da parte degli editori.

Le pubblicazioni inerenti al secondo ambito di ricerca da me seguito in corso di Dottorato riguardano gli studi di associazione tra condizioni di trombofilia ereditaria ed esiti avversi della gravidanza. La prima pubblicazione è del 2007 ed è stata editata su Journal of Obst/Gyn Research e mostra i risultati di uno studio condotto su due gruppi di pazienti gravide, con o senza patologie della gravidanza. In questo set di pazienti si andava a controllare l'associazione tra difetti trombofilici singoli ed esiti avversi. Anche il secondo lavoro è dello stesso tenore, ed è stato pubblicato si International Journal of Biomedical Sciences nel 2007. Anche in quest'ultima fatica si sono studiati i difetti trombofilici singoli, ma con approccio statistico differente.

Due lavori sono tuttora in mano ai referee: il primo rappresenta un ampio studio di associazione tra patologie della gravidanza su base microangiopatica e difetti trombofilici multipli (Am J Obst/Gyn, inviato, non ancora accettato) ed il secondo riguarda l'incidenza di difetti trombofilici in pazienti gravide con danno renale accertato (Acta scand, inviato). Di questi due ultimi lavori non mostro il manoscritto originale, in mancanza delle bozze di stampa.

Sempre in ambito di produzione letteraria, riporto anche alcuni case report pubblicati nello stesso periodo del Dottorato di Ricerca.

I primi tre case report trattano di patologie cordonali rare: un caso di trombosi segmentale dei vasi del funicolo; un caso di emorragia perivascolare del funicolo, ed un caso di cisti del piatto coriale. Il primo case report è completo di una revisione della letteratura riguardante la trombosi dei vasi del cordone ed è stato pubblicato nel 2003 su una rivista internazionale di medicina generale. Il terzo caso in realtà è stato esposto su una lettera all'editore di UOG.

Al di la delle mere note bibliografiche, questi tre case report rappresentano un interessante modo di approcciare alla letteratura scientifica. Tutti e tre questi lavori nascono dalla curiosità scaturita dall'osservazione di casi clinici gestiti personalmente nel corso della abituale e routinaria attività clinica assistenziale. La stessa attività di pratica clinica esce dall'ambito routinario se da essa si traggono spunti di studio e di approfondimento e se si desidera portare alla conoscenza degli altri operatori nello stesso settore casi che altrimenti rimarrebbero chiusi nell'oblio di cartelle cliniche accatastate.

E' recente la pubblicazione (2007) sul Journal of Ultrasound in Meidicne di un raro caso di gemelli torcosternopaghi a tipo Giano bifronte. Nella tesi riporto il lavoro con il ricco corredo iconografico ultrasonografico e post-natale.

E' poi presente un case report che tratta lo spinoso argomento della responsabilità professionale in ambito ostetrico e l'istituto della procedura riconvenzionale ed un lettera all'editore con note di tecnica di cervicometria ecografia. Entrambi questi ultimi lavori appartengono alla rivista ufficiale della Società Italiana di Ginecologia ed Ostetricia.

Sono persuaso che niente meglio di un lavoro pubblicato possa esprimere i risultati di una osservazione clinica o di un set di pazienti paragonabili per caratteristiche epidemiologiche.

Per questo motivo ho deciso di compilare la mia tesi riportando i lavori da me pubblicati durante il Dottorato in Medicina Prenatale. In realtà dietro ognuno di questi articoli c'è un lavoro intenso di risposta ai referee e di rielaborazione del manoscritto originario, anche più e più volte. Questa è senz'altro la parte più stimolante e più interessante di ogni pubblicazione e spesso comporta la preparazione di epistolari lunghi ed articolati che prolungano anche di molti mesi (o anni) l'uscita a stampa del proprio lavoro. Il più delle volte non si tratta di sterili discussioni dottrinali, ma di un costruttivo lavoro di revisione volto alla presentazione di un manoscritto più appetibile per la rivista ed i lettori.

Giovanni Larciprete

INDICE DELLE PUBBLICAZIONI RIPORTATE NELLA TESI DI DOTTORATO

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Fetal subcutaneous tissue thickness (SCTT) in healthy and gestational diabetic pregnancies

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KEYWORDS: fat mass; lean mass; ultrasound

ABSTRACT

Objective *To determine reference values of fetal subcutaneous tissue thickness (SCTT) throughout gestation in a healthy population and to compare them with those from a population of pregnant women with gestational diabetes under standard therapy.*

Methods *Three hundred and three women recruited from a high-risk pregnancy clinic were classified as being healthy (n = 218) or as having gestational diabetes (n = 85) on the basis of a negative or positive oral glucose tolerance test, respectively. They were enrolled into the cross-sectional study at 20 weeks' gestation. Ultrasound examinations were performed approximately every 3 weeks until delivery at term. The mid-arm fat mass and lean mass (MAFM, MALM), the mid-thigh fat mass and lean mass (MTFM, MTLM), the abdominal fat mass (AFM) and the subscapular fat mass (SSFM) were evaluated. Time-specific reference ranges were constructed from the 218 healthy women and a conventional Student's t-test was performed to compare SCTT values between the two study groups throughout gestation.*

Results *Normal ranges, including 5th, 50th and 95th centiles of the distribution, were generated for each SCTT parameter obtained in each of the two groups of women. Significant differences were found between the two study groups at 37–40 weeks' gestation for MTFM, at 20–22 and 26–28 weeks for MTLM, at 31–34 and 35–37 weeks for MAFM, at 26–28 and 38–40 weeks for SSFM, and at 39–40 weeks for AFM, the mean residual values always being greater in gestational diabetic women than they were in the group of healthy pregnant women.*

Conclusions *We provide gestational age-specific reference values for fetal SCTT. Fetal fat mass values, particularly in late gestation, are greater in women with gestational diabetes compared with healthy women. The reference values may have a role in assessing the influence of maternal metabolic control on fetal state.* Copyright © 2003 ISUOG. Published by John Wiley & Sons, Ltd.

INTRODUCTION

Fetal weight is commonly estimated using ultrasound-derived anthropometric measurements and population-based growth charts. The abdominal circumference is the most sensitive among the individual fetal parameters for the detection of fetal over-growth[1]. Nevertheless, both estimated fetal weight and abdominal circumference show a wide range of error (±10%) that could impact on clinical practice[2,3].

Fat content correlates directly with energy stores and fat and lean body mass are often used in the nutritional assessment of an individual. Fat constitutes 12–14% of normal birth weight yet has been demonstrated to account for 46% of its variance[4]. As such, ultrasound-generated estimates of fetal fat may be useful in the evaluation of fetal growth abnormalities.

Ultrasound-derived anthropometric measurements of fetal body composition have previously been obtained. Bernstein *et al.*[5] compared fat and lean body mass measurements in healthy fetuses across gestation and showed significant correlations with both birth weight and estimates of neonatal lean and fat mass. Galan *et al.*[6] reported that the reduced birth weight of a subset of North American newborns was the result of a reduction in fetal subcutaneous fat tissue and not in lean mass, highlighting

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the potential usefulness of longitudinal fetal subcutaneous ultrasound measurements for detection of differences in specific populations.

Gestational diabetes (GD) mellitus is the commonest metabolic disorder of pregnancy and is defined as 'varying degrees of glucose intolerance first recognized in pregnancy'[7]. The obstetric complications of GD are linked to the vaginal delivery of a large-for-gestational age fetus[8] and to the increased risk of late stillbirth[9]. The neonatal metabolic complications of GD, including hypoglycemia and hypocalcemia, are caused by fetal hyperinsulinemia which occurs as a consequence of maternal hyperglycemia[10]. In GD patients maternal glycemia and obesity appear to be independent contributors to the occurrence of fetal macrosomia, operative delivery, hypertension and thromboembolic disease[11]. Subsequently, GD appears to be associated with the development of diabetes in the mother and diabetes and obesity in the offspring[12].

There are conflicting guidelines for the management of GD patients, but there is agreement on the efficacy of dietary regimen and insulin therapy to reduce fetal size, and in particular fetal adiposity[13]. For these reasons we considered that the availability of reference values for fetal fat tissue measurements may be useful for clinical practice, in particular to identify excessive fetal fat deposition in normal and GD pregnancies.

The aims of this study were to construct reference ranges of fetal subcutaneous tissue thickness (SCTT) in a selected population of normal pregnancies, as determined by oral glucose tolerance test, and to compare these values with those from pregnant women with GD.

METHODS

Patient selection

This was a cross-sectional study in which high-risk patients at 20–22 weeks' gestation from our outpatient clinic were enrolled between January and December 2001. Considered as risk factors were: family history of diabetes mellitus (first-degree relative), body mass index ≥ 27 kg/m^2, glycosuria > 600 mg/L, previous delivery of a baby with birth weight ≥ 4500 g, previous GD, age ≥ 37 years, or polyhydramnios. Inclusion criteria were: singleton pregnancy, certain gestational age, and absence of fetal anomalies. Patients with twin pregnancies, Type I or II diabetes, chronic hypertension and fetal growth restriction were excluded. A total of 325 patients (180 in Rome, 145 in Florence) were initially recruited. Twenty-two were subsequently excluded, 19 for incomplete perinatal data and three due to the appearance of reduced fetal growth and oligohydramnios. The remaining 303 consisted of 218 healthy pregnant women on the basis of the oral glucose tolerance test, and 85 (28%) with GD.

At 24–28 weeks' gestation patients underwent the 'one-step approach' for the diagnosis of GD[14] and were given a 100-g oral glucose tolerance test according to the Expert Committee on the Diagnosis and Classification of Diabetes Mellitus[14] and the results were evaluated according to the National Diabetes Data Group's criteria[15]. On the basis of the glucose tolerance test the patients were classified as 'being healthy' (this group was used for construction of the normal reference ranges) or as 'having GD'.

Those meeting the criteria for the diagnosis of GD were started on a standard 1600-Kcal diet (50% carbohydrates). After 7–10 days of the dietary regimen, home monitoring of blood glucose was undertaken, assessing the peripheral blood glucose level with a commercially available glucose testing device (OneTouch Ultra, Lifescan, Milpitas, CA, USA) for a week to evaluate the efficacy of the therapy. The therapeutic goals were: preprandial blood glucose level ≤ 90 mg/dL and 2 h postprandial level ≤ 120 mg/dL[16]. When the daily pre- and postprandial blood glucose values were frequently > 100 mg/dL and > 120 mg/dL, respectively, short-term insulin therapy was added to the diet (Actrapid, Novo Nordisk, Bagsvaerd, Denmark) before breakfast, lunch and dinner[17]. Insulin doses were subsequently adjusted individually after a second blood glucose profile[17]. Eighteen of 85 (21.2%) patients had subcutaneous insulin treatment to re-establish the optimal levels of glycemia.

Sonography

Serial ultrasound examinations were performed approximately every 3 weeks until delivery at term. At each examination the patients underwent a detailed ultrasound scan with a commercially available Tecknos Esaote (Genova, Italy) ultrasound machine equipped with a 3.5- or 5-MHz probe (at the Fatebenefratelli Hospital, Tor Vergata University, Rome), or with an ATL HDI 5000 ultrasound machine (ATL, Seattle, WA, USA) with a 3.5-MHz transducer (at the Department of Gynecology, Perinatology and Human Reproduction, University of Florence, Florence).

Routine sonographic biometric parameters measured included head and abdominal circumference, and femur and humerus length. To obtain fat mass and lean mass, several measurements were assessed. We used the technique of Bernstein *et al.*[5] to measure the fat and lean body mass areas on axial ultrasound images of the mid upper arm and mid upper leg, and on the cross-sectional images of the abdomen and the subscapular field[5,18,19]. Briefly, mid-arm fat mass (MAFM, cm^2), mid-arm lean mass (MALM, cm^2), mid-thigh fat mass (MTFM, cm^2) and mid-thigh lean mass (MTLM, cm^2) were obtained as follows (Figure 1): a sagittal view of the long bone and extremity was obtained in the middle of the ultrasound screen at an angle of 0° to the transducer. The transducer was then rotated 90° in the middle of the long bone to obtain the axial view of the extremity. The fat mass (MAFM, MTFM) was measured by taking the total cross-sectional limb area and subtracting the central lean area (MALM, MTLM) that consisted of muscle and bone. The abdominal fat mass (AFM, mm)

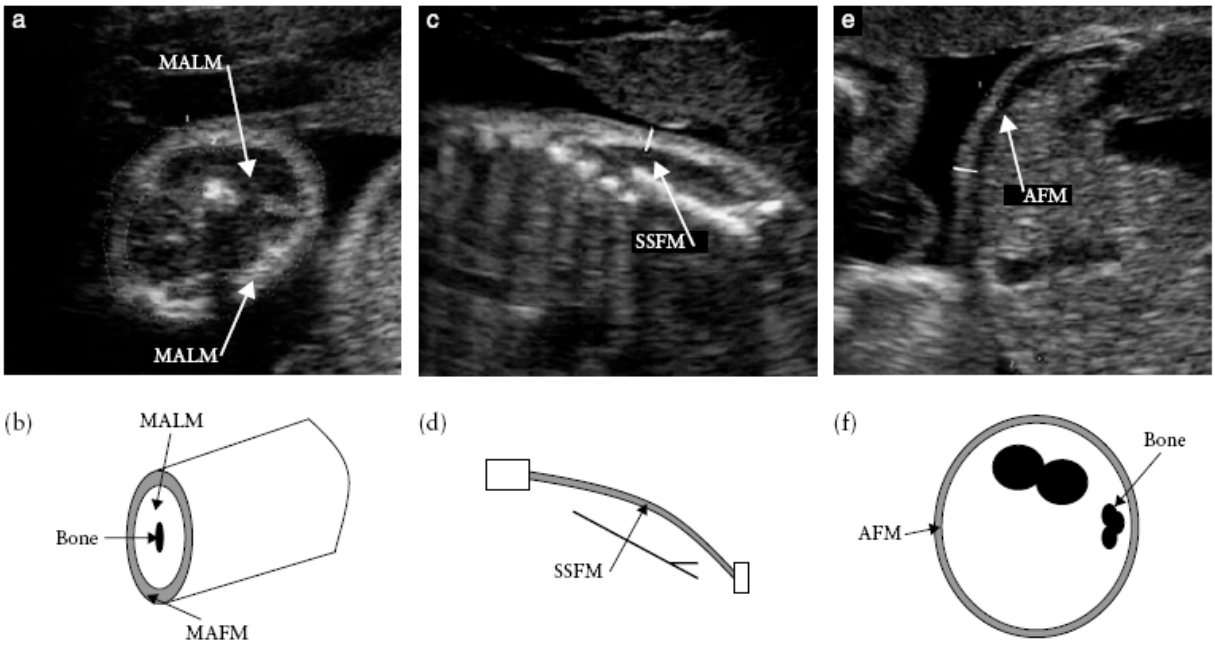

Figure 1 Ultrasound images (a, c, e) and schematic representations (b, d, f) showing how the different subcutaneous tissue thickness measurements are obtained. (a,b) Axial view of the fetal arm showing mid-arm fat mass (MAFM) and mid-arm lean mass (MALM) evaluation; the process is similar for mid-thigh fat and lean mass. (c,d) Evaluation of the subscapular fat mass (SSFM) measurement. (e,f) Evaluation of the abdominal fat mass (AFM).

was determined by measuring the thickness of the anterior abdominal subcutaneous tissue on the same axial image as that used for abdominal circumference measurement (Figure 1), as previously reported by Gardeil *et al.*[20]. Subscapular fat mass (SSFM, mm) was evaluated by taking the shoulder skin width perpendicularly to the bone at its lower end.

Reproducibility

The intra- and interobserver reproducibility was tested in 20 different images for the following SCTT parameters: MAFM, MTFM, MALM, MTLM, AFM and SSFM. Two operators (G.L. and E.P.), blinded to each other's and their own recordings, performed three measurements for each SCTT parameter. Precision was assessed as the coefficient of variation of each SCTT parameter (Table 1).

Statistical analysis

For every SCTT parameter time-specific reference ranges were computed, according to the procedure described by Royston[21]. Logarithmic transformations of the SCTT parameters were used to fit quadratic polynomial time-based curves. This was performed for all the parameters investigated with the exception of SSFM and MALM, for which the logarithmic parameter fit a linear time-based curve. We checked 'normality of model's residuals' and 'homoscedasticity' by means of normal plots and the associated test recommended by Royston[21].

Table 1 Variability of repeated ultrasound measurements of subcutaneous tissue thickness parameters

	Coefficient of variation (%)	
Parameter	*Intraobserver*	*Interobserver*
MAFM	8.4	10.2
MALM	7.5	9.7
MTFM	9.2	9.8
MTLM	5.7	7.0
SSFM	7.9	10.5
AFM	8.2	10.9

AFM, abdominal fat mass; MAFM, mid-arm fat mass; MALM, mid-arm lean mass; MTFM, mid-thigh fat mass; MTLM, mid-thigh lean mass; SSFM, subscapular fat mass.

To compare the healthy group with the GD group, we plotted the residuals for the GD pregnant women against gestational age (residuals computed using the estimated parameters previously obtained for the healthy group) and we checked for Gaussian distribution of residuals using normal plots and the corresponding statistical test proposed by Royston[21]. Then, since no trend with gestational age seemed to be present, we performed a classic *t*-test to compare mean differences of residuals between the two groups at each gestational age.

The study was approved by the institutional review boards at the Tor Vergata University of Rome and at the University of Florence and the local ethics committee of the Fatebenefratelli Hospital-Isola Tiberina of Rome approved the study protocol.

RESULTS

Characteristics of the study groups are summarized in Table 2. Pre-gestational body mass index and birth weight were greater among the group of GD patients.

Model-based reference ranges were generated for each fetal parameter obtained in the cross-sectional group of normal patients. Table 3 shows the 5th, 50th and 95th centiles of these distributions. Each parameter increased progressively with advancing gestational age, as expected in a group of normal fetuses in normal pregnant women.

Figure 2 shows time-specific estimated 90% reference ranges for SCTT parameters for both the normal and the GD groups. Because of the logarithmic transformation, the range is asymmetrical about the regression line and its width increases somewhat with gestational age.

Significant differences were found between the two study groups at 37–40 weeks' gestation for MTFM (11.86 ± 2.70 vs. 14.22 ± 3.52 cm^2, $P = 0.02$), at 20–22 and 26–28 weeks for MTLM (1.82 ± 0.42 vs. 3.00 ± 0.08 cm^2, $P = 0.04$ and 3.91 ± 0.74 vs. 4.29 ± 0.95 cm^2, $P = 0.05$, respectively), at 31–34 and 35–37 weeks for MAFM (4.13 ± 0.89 vs. 4.92 ± 1.13 cm^2, $P = 0.03$ and 5.13 ± 1.57 vs. 6.62 ± 1.70 cm^2, $P < 0.01$, respectively), at 26–28 and 38–40 weeks for SSFM (2.95 ± 0.52 vs. 3.24 ± 1.28 mm, $P = 0.03$ and 5.30 ± 1.41 vs. 6.73 ± 1.24 mm, $P < 0.01$, respectively), and at 39–40 weeks for AFM (6.18 ± 1.32 vs. 6.80 ± 0.89 mm, $P = 0.03$), the means of the GD residuals always being greater than those obtained from the healthy group of pregnant women.

Table 2 Characteristics of the studied populations

Characteristic	*Healthy group*	*GD group*	P*
n	218	85	
Age (years, mean ± SD)	27.4 ± 6.7	28.2 ± 4.5	NS
Prepregnancy body mass index (kg/m^2, mean ± SD)	24.3 ± 2.9	25.4 ± 3.2	0.01
Gestational age at delivery (weeks, mean ± SD)	39.0 ± 3.0	38.6 ± 2.6	NS
Birth weight (g, mean ± SD)	3283 ± 395	3481 ± 416	< 0.01

*Using *t*-test. NS, not significant; GD, gestational diabetes.

DISCUSSION

The first aim of the present study was to provide reference values for new parameters of fetal body composition as several studies support the role of fetal fat and lean mass assessment in the determination of normal fetal development[5,6,16].

Bernstein and Catalano examined the utility of ultrasound to measure subcutaneous fetal fat in the extremities. The use of a simple linear measurement of fat thickness across the extremity was found to be poorly reproducible, with an intraobserver coefficient of variation of 28%[18]. This appeared to be the result of a distortion in the proximal extremities resulting from external compression. The measurement of fat area in the proximal extremities has proved to be more reproducible. The coefficients of variation for the sonographic estimates of subcutaneous fat area in the present study compare reasonably with published coefficients of variation for the measurement of skinfold thickness in neonates[22].

Bernstein *et al.*[5] also reported that fetal fat and lean body mass have peculiar growth profiles and that, as a

Table 3 Reference ranges of subcutaneous tissue thickness parameters in the healthy pregnant group ($n = 218$)

		Gestational age (weeks)						
Parameter	*Percentile*	*20–22*	*23–25*	*26–28*	*29–31*	*32–34*	*35–37*	*38–40*
MAFM (cm^2)	5th	0.66	0.99	1.43	1.99	2.65	3.40	4.19
	50th	1.00	1.50	2.16	3.00	4.00	5.13	6.32
	95th	1.50	2.26	3.26	4.53	6.04	7.74	9.54
MALM (cm^2)	5th	0.80	0.98	1.20	1.46	1.78	2.18	2.66
	50th	1.23	1.50	1.84	2.24	2.74	3.34	4.08
	95th	1.89	2.31	2.82	3.44	4.20	5.13	6.26
MTFM (cm^2)	5th	0.88	1.53	2.45	3.64	5.02	6.40	7.56
	50th	1.33	2.30	3.70	5.50	7.57	9.66	11.41
	95th	2.00	3.48	5.58	8.30	11.43	14.57	17.22
MTLM (cm^2)	5th	1.27	1.91	2.73	3.67	4.66	5.59	6.34
	50th	1.82	2.75	3.91	5.27	6.69	8.03	9.11
	95th	2.61	3.94	5.62	7.56	9.61	11.54	13.08
SSFM (mm)	5th	1.39	1.61	1.86	2.15	2.49	2.89	3.34
	50th	2.20	2.55	2.95	3.42	3.95	4.58	5.30
	95th	3.49	4.04	4.68	5.41	6.27	7.25	8.40
AFM (mm)	5th	1.39	1.86	2.38	2.90	3.38	3.76	4.00
	50th	2.09	2.80	3.58	4.38	5.10	5.68	6.03
	95th	3.15	4.23	5.41	6.60	7.70	8.57	9.10

AFM, abdominal fat mass; MAFM, mid-arm fat mass; MALM, mid-arm lean mass; MTFM, mid-thigh fat mass; MTLM, mid-thigh lean mass; SSFM, subscapular fat mass.

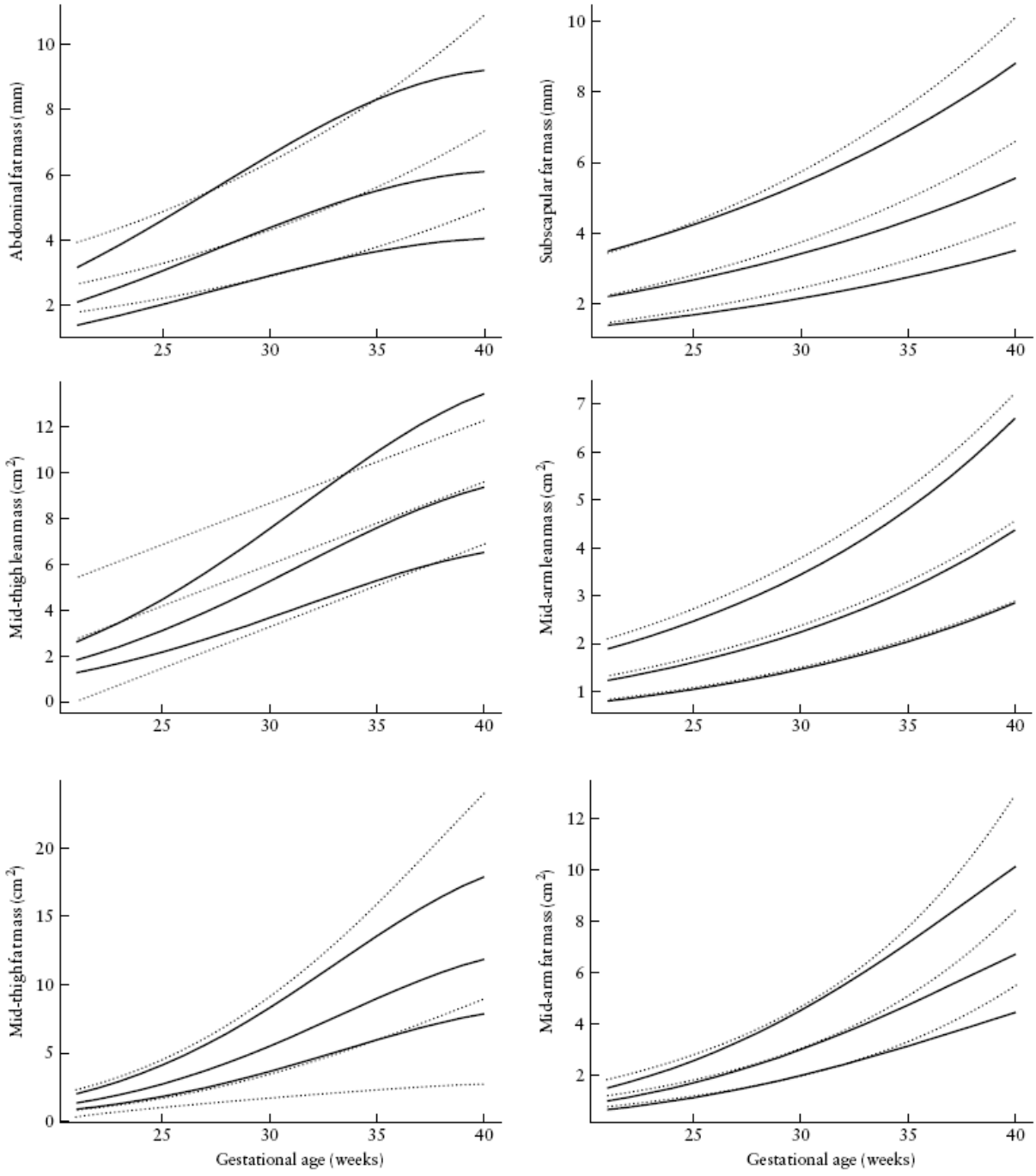

Figure 2 Gestation-specific reference ranges for the studied subcutaneous tissue thickness parameters within the healthy (solid lines) and the gestational diabetic (dotted lines) groups (groups identified on the basis of oral glucose tolerance test). The 5th, 50th and 95th centiles are shown in each case. The comparison between groups was performed with a classic *t*-test between residuals, per each gestational age, computed using only the normal reference ranges.

result of an accelerated rate of growth in late gestation, the measurement of fetal fat will provide a more sensitive and specific marker of abnormal fetal growth when compared with index values of lean body mass. An index of fat as a predictor of morbidity has been widely applied in neonates. In the growth-restricted neonate a low ponderal index (Rohrer's index of corpulence) has a stronger association with several specific morbidities than does birth weight[23].

Galan *et al.*[6] recently showed that sonography can be used to follow subcutaneous measurements longitudinally and to detect differences, and potentially disease

processes, in study populations. For these reasons we set out to determine SCTT parameters in a population of pregnant women with GD.

Bernstein and Catalano[24] reported that increased neonatal fat is associated with an increased risk of Cesarean delivery in infants born to mothers with GD. Moreover subcutaneous fat appears to be a stronger index of maternal glucose control than does the ambulatory glycemic profile[24,25]. Perinatal mortality and morbidity are increased among macrosomic fetuses from GD mothers compared with macrosomic fetuses from normal pregnant women[26]. Therefore the development of an index of fetal fat distribution could be clinically helpful.

Furthermore, in GD the relative risk of shoulder dystocia for a 4000-g fetus appears to be three or four times higher than that observed in a normal population[26]. Conventional ultrasound fetal biometry has limited value in these clinical situations: when the fetal weight is $> 90^{th}$ centile the measurement error may even reach 15%[27].

The second aim of our study was to determine fetal SCTT values for a GD population under standard treatment. The small study size, strict maternal metabolic control, and absence of severe macrosomia in the population are the main factors that must be taken into account when evaluating the reproducibility of these results. Although the normal reference values were obtained from a healthy normoglycemic population, few differences between the two study groups were found. This may be due to the strict control of the diabetic patients (diet, diet plus insulin, frequent blood glucose profiles). Moreover, we assume that GD fetuses receiving an excessive glucose load might show stable features of fat distribution that could be difficult to differentiate from normal fetuses. It is therefore intriguing that an increased MTLM in the GD fetuses was found before the definitive diagnosis of GD. This finding seems to suggest that, although the maternal metabolic maladaptation did not definitively occur at 20–22 weeks, the fetus was probably already affected, showing signs of an excessive availability of nutrients (higher MTLM). Yet the abnormal metabolic influence on the fetus was not evident through the traditional sonographic measurements.

It could be important to evaluate the potential impact of the SCTT on the correct estimation of birth weight. Several birth-weight prediction formulae[15] have been provided over the last 15 years; the ability of these formulae to predict fetal weight has always been associated with an error of at least 8–10%. The incorporation of SCTT measurements into existing formulae involving long bone (humerus and femur) and head and abdominal circumference measurements could reduce the amplitude of this error. In cases in which excessive or reduced growth affects primarily soft tissue deposits and not the conventional parameters used to assess fetal growth, the evaluation of SCTT could have a prominent role in the identification of mild nutritional fetal abnormalities.

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Intrauterine growth restriction and fetal body composition

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KEYWORDS: fetal body compartments; intrauterine growth restriction; subcutaneous tissue thickness

ABSTRACT

Objective *To assess the differences in fetal body compartments between fetuses with normal growth and those with reduced intrauterine growth, during the third trimester, through ultrasonographic determination of subcutaneous tissue thickness (SCTT).*

Methods *Twenty-eight patients were enrolled into this case control study carried out at 30–31 weeks' gestation. Two study groups were matched for maternal age and pregestational body mass index: controls* (n = *14) and intrauterine growth-restricted (IUGR) fetuses* (n = *14). Routine ultrasound-derived biometric parameters (head circumference, abdominal circumference, femur length and humerus length) were measured. Additionally, the mid-arm fat mass and lean mass (MAFM and MALM), the mid-thigh fat mass and lean mass (MTFM and MTLM), the abdominal fat mass (AFM) and the subscapular fat mass (SSFM) were measured. The Mann–Whitney* U*-test and Student's* t*-test were used to compare the two groups.*

Results *The abdominal circumference and the humerus were significantly smaller in IUGR fetuses than in controls. Most of the SCTT values were different in the two groups. The SSFM (3.6 ± 1.1 vs. 2.6 ± 0.7 mm;* P = *0.011), the AFM (5.1 ± 0.7 vs. 4 ± 1 mm;* P = *0.01), the MAFM (3.5 ± 0.9 vs. 2.2 ± 0.8 cm^2;* P < *0.01) and MALM (2.1 ± 0.4 vs. 1.7 ± 0.5 cm^2;* P = *0.029) were all significantly greater in fetuses with normal development compared to those with growth restriction.*

Conclusions *During the third trimester, SCTT (with the exception of MTFM and MTLM) is reduced in fetuses with IUGR. Furthermore, MALM is lower in growth-restricted fetuses, confirming that the parameters measured in this study are affected in IUGR fetuses. Our findings indicate that specific changes in fetal body compartments occur as a result of chronic metabolic impairment.*

INTRODUCTION

Fetal weight can be measured ultrasonographically using estimated fetal anthropometric measurements and population-based growth charts. Estimated fetal weight (EFW) is commonly used as an index of fetal growth and is generally calculated through a combination of parameters that include, amongst others, abdominal circumference. Nevertheless, both the EFW and abdominal circumference show a wide range of variation that could potentially impact on clinical practice[1,2]. Error in EFW may be as high as 25%[3] and results from technical measurement errors, as well as the assumptions that fetal density is constant throughout gestation and is independent of the fetal pathological processes that alter normal muscle/fat ratios[4,5].

Fat content correlates directly with energy stores. Fat mass and lean body mass are often used in the nutritional assessment of the individual. Fat constitutes 12–14% of birth weight and has been shown to account for 46% of the variation noted in neonatal weight[6]. Consequently, ultrasound-generated estimates of fetal fat may be useful in the evaluation of fetal growth abnormalities. Several authors have previously used ultrasonography to assess anthropometric measurements of fetal body composition[4,5]. For instance, Bernstein *et al*[5] compared fat and lean body mass measurements in healthy fetuses across gestation and demonstrated significant correlations with birth weight and estimates of neonatal lean and fat mass.

Recently (2004), Deter stated the importance of assessing fetal growth not on the basis of single

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anatomical variables, such as birth weight or abdominal circumference, but rather using the novel concepts of individualized growth assessment and the Prenatal Growth Profiles, in which growth is assessed with each fetus serving as its own control[7]. This is a more precise approach, since fetal growth potential is in part linked to demographic-, age- and women-specific variables[7]. Utilization of fetal soft tissue measurements was discussed in this study[7] and in a previous study by the same author[8].

Reference values for fetal subcutaneous tissue thickness (SCTT) parameters have been recently reported in a 300-subject longitudinal ultrasound study. Fetuses at 22 weeks' gestation were studied from mothers who had healthy pregnancies compared to women with gestational diabetes. When the growth profiles from the two populations were compared, a 'fat-free mass index' using the mid-thigh lean mass (MTLM) was shown to be an effective means (even at 22 weeks' gestation) of differentiating between normal fetuses and those with diabetic mothers. Other conventional ultrasound measurements have been shown to be limited in their ability to differentiate between normal and affected fetuses at 22 weeks' gestation[9].

Moreover, Marconi *et al*[10] have shown that in intrauterine growth-restricted (IUGR) fetuses there is a rapid breakdown of proteins, indicating that the 'fat-free mass' is affected in IUGR fetuses. Furthermore, these authors have shown that maternal leucine infusions result in a lower fetal–maternal leucine enrichment ratio when compared to normal fetuses. This finding is further verified in animal experiments in which fetal amino acid infusions are able to prevent fetal growth restriction[11]. Conversely, Galan *et al*[4] have reported that the reduced birth weight of a subset of North American newborns was due to a reduction in fetal subcutaneous fat tissue and not lean mass. Despite this apparent contrast, this work highlights the potential value of the ultrasonographic evaluation of fetal skinfold thickness in the detection of differences between specific populations.

Ambiguity in these results has been the motivation for our investigations on various tissue compartments of the fetus. The main aim of the present study was to assess the differences in the fetal compartments between normal and growth-restricted fetuses during the third trimester, through the use of ultrasonographic measurements of various SCTT parameters.

METHODS

Population

The study was approved by the Institutional Review Boards at Tor Vergata University of Rome. Patients were enrolled from the authors' outpatient high-risk pregnancy clinic at 30–31 weeks' gestation during the period January–December 2002. Two study groups, the control and growth-restricted groups, were matched for age and pregestational body mass index (BMI). The control group consisted of primigravida women with a healthy singleton pregnancy, while the growth-restricted group consisted of primigravida women with a singleton pregnancy with the ultrasound-determined diagnosis of IUGR[12], as having a fetal abdominal circumference < 5th centile for gestational age by local reference values, an estimated fetal weight < 10th centile for gestational age and an umbilical artery pulsatility index of more than 2 SDs above the gestational mean compared to local reference values. Additional inclusion criteria for fetuses in this study were specific gestational age, absence of fetal chromosomal abnormalities and normal fetal anatomy.

Gestational age was calculated from the first day of the last menstrual period and confirmed by either a first- or second-trimester ultrasound scan. When the ultrasound-determined gestational age differed from that calculated from the last menstrual period by > 7 days in the first trimester, or by > 10 days in the second trimester, the ultrasound-determined gestational age was used.

Ultrasonography

Patients underwent a conventional ultrasound scan with the commercially available unmodified Teknos Esaote Ultrasound Machine (ESAOTE S.p.a. Headquarters, Genova, Italy), at the Fatebenefratelli Hospital, Tor Vergata University in Rome, using a 3.5-MHz probe. Routinely obtained biometric parameters included the head and abdominal circumferences and femur and humerus lengths.

To obtain fat mass and lean mass, several measurements were assessed as previously described by Valensise *et al*[13]. We used a similar technique to that of Bernstein *et al*[5] to measure the fat and lean body mass areas on axial ultrasound images in the mid-upper arm and mid-upper leg regions and the cross-sectional images of the abdomen and the subscapular field[5,14,15]. Briefly, the mid-arm fat mass (MAFM), mid-arm lean mass (MALM), mid-thigh fat mass (MTFM) and mid-thigh lean mass (MTLM) (all measurements in cm^2) were obtained using a longitudinal view of the long bone and extremities at an angle of 0° to the transducer. The transducer was then rotated 90° at the middle of the long bone to obtain an axial view of the extremity. The fat mass (MAFM, MTFM) was measured by taking the total cross-sectional limb area and subtracting the central lean area (MALM, MTLM) that consisted of muscle and bone (Figure 1a, 1b).

The abdominal fat mass (AFM, in mm) was determined by measuring the thickness of the anterior abdominal subcutaneous tissue on the same axial image from which the abdominal circumference is obtained (Figure 1c) as previously described by Gardeil *et al*[16].

The subscapular fat mass (SSFM, in mm) was evaluated (Figure 1d) by measuring the SCTT perpendicularly at the lower apex of the flat bone, in a sagittal section, as described by Valensise *et al*[13].

The reproducibility and precision of SCTT measurement have been previously reported[9]. All measurements were carried out by the same investigator.

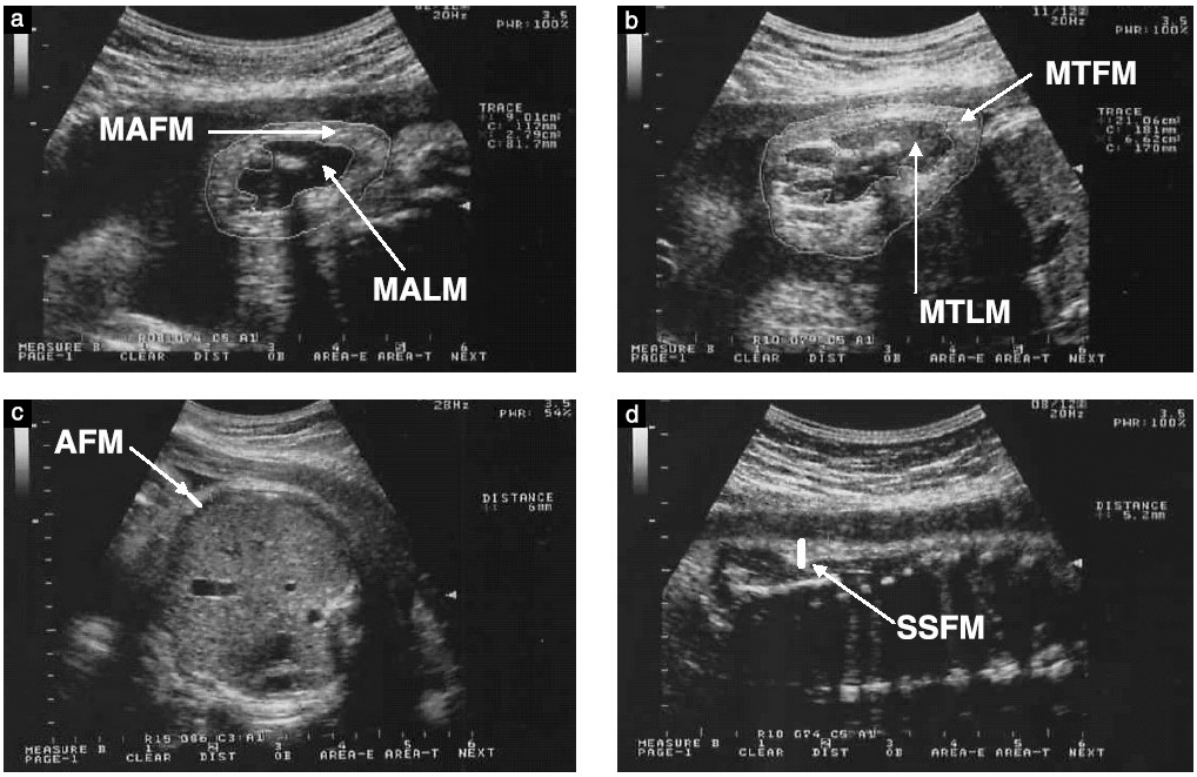

Figure 1 Axial views of the extremities showing (a) the mid-arm fat mass (MAFM) and mid-arm lean mass (MALM) and (b) the mid-thigh fat mass (MTFM) and mid-thigh lean mass (MTLM). Ultrasound scans illustrating how (c) abdominal fat mass (AFM) and (d) subscapular fat mass (SSFM) measurements are made.

Statistical analysis

The study compared two groups, where the anticipated difference in means is 1 and the anticipated SD is 0.5. The sample size required for an $\alpha = 0.05$ and a power of 95% was eight cases per group. The sample size required for an $\alpha = 0.01$ and a power of 95% was 11 cases per group. Power analysis was performed according to Sokel and Rolf[17].

We compared the conventional ultrasound parameters and the SCTT parameters of the two study groups using the non-parametric Mann–Whitney *U*-test since, to the best of our knowledge, it is more powerful than its parametric equivalent.

Table 1 Characteristics of the study groups

Characteristic	*Normal fetuses* (n = 14)	*IUGR fetuses* (n = 14)	*Mann–Whitney P-value*
Age (years)	28.4 ± 3.5	29.2 ± 1.3	0.330
Pregestational BMI	26.8 ± 2.1	27.1 ± 2.3	0.642
GA at study time (weeks)	31 ± 2	30 ± 2	0.513
GA at delivery (weeks)	39 ± 1	35 ± 3	< 0.01
Birth weight (g)	3425 ± 301	1797 ± 559	< 0.01
Birth weight percentile	63.8 ± 18.6	9.4 ± 0.7	< 0.01

Values represent mean ± SD. BMI, body mass index (weight/height^2); GA, gestational age; IUGR, intrauterine growth restriction.

RESULTS

The characteristics of the study groups are summarized in Table 1. The gestational age at delivery was significantly different for the two groups: 39 ± 1 weeks' gestation for normal fetuses vs. 35 ± 3 weeks' gestation for growth-restricted fetuses ($P < 0.01$). The birth weights and their percentiles were lower in growth-restricted fetuses than in normal fetuses ($P < 0.01$) (Table 1).

Nine of 14 (64%) patients with growth-restricted fetuses developed gestational hypertension, two (14.3%) had pre-eclampsia and three (21.4%) women had normal blood pressure values. Gestational hypertensive and pre-eclamptic patients were classified using the International Society for the Study of Hypertension in Pregnancy criteria[18].

The abdominal circumference was significantly smaller in growth-restricted fetuses than in normal fetuses ($P < 0.01$) as was humerus length ($P = 0.036$). Head circumference and femur length were not significantly different between the two study groups (Table 2).

Most of the SCTT values were significantly smaller in the growth-restricted fetuses at the study time (30–31 weeks' gestation) when compared to the normal

Table 2 Differences in the conventional ultrasound parameters in normal and growth-restricted fetuses

Conventional ultrasound parameters	*Normal fetuses (n = 14)*	*IUGR fetuses (n = 14)*	*Mann–Whitney P-value*
Femur (cm)	5.8 ± 0.6	5.4 ± 0.4	0.055
Humerus (cm)	5.1 ± 0.5	4.8 ± 0.3	0.036
Abdominal circumference (cm)	26.8 ± 2.3	23.0 ± 2.6	< 0.01
Head circumference (cm)	28.1 ± 2.0	27.3 ± 1.8	0.175

Values represent mean ± SD. IUGR, intrauterine growth restriction.

fetuses. Table 3 summarizes these soft tissues parameters. The results show that the SSFM, AFM, MAFM and MALM were statistically lower in growth-restricted fetuses than in fetuses with normal intrauterine growth.

DISCUSSION

The importance of the evaluation of fetal SCTT has been the subject of an increasing number of studies in recent years. Bernstein *et al*[5] have previously shown that ultrasound can be used to measure subcutaneous fetal fat in the extremities. The variations noted when using ultrasonographic estimates of subcutaneous fat in fetuses do compare relatively reasonably with the variations observed in the measurement of skinfold thickness in neonates[19].

It is clear from clinical practice that the routine use of ultrasonographically measured parameters is valuable in the assessment of gestational age. These parameters are reasonably accurate in part due to their resistance to environmental influence. However, it is this quality that makes them less well suited for the identification of fetal growth abnormalities. When compared to all the routinely measured parameters, abdominal circumference is the most sensitive when detecting growth abnormalities. Bernstein *et al* reported that fetal fat and lean body mass have unique growth profiles and that, as a result of an accelerated rate of growth in late gestation, the measurement of fetal fat may provide a more sensitive and specific means of identifying abnormal fetal growth when compared with index values of lean body mass[5].

Table 3 Differences in the fetal body compartments in normal and growth-restricted fetuses

SCTT parameters	*Normal fetuses (n = 14)*	*IUGR fetuses (n = 14)*	*Mann–Whitney P-value*
AFM (mm)	5.1 ± 0.7	4.0 ± 1.0	0.010
SSFM (mm)	3.6 ± 1.1	2.6 ± 0.7	0.011
MAFM (cm^2)	3.5 ± 0.9	2.2 ± 0.8	< 0.01
MALM (cm^2)	2.1 ± 0.4	1.7 ± 0.5	0.029
MTFM (cm^2)	5.1 ± 1.6	4.2 ± 1.0	0.278
MTLM (cm^2)	4.2 ± 1.1	3.7 ± 1.8	0.100

Values represent mean ± SD. AFM, abdominal fat mass; IUGR, intrauterine growth restriction; MAFM, mid-arm fat mass; MALM, mid-arm lean mass; MTFM, mid-thigh fat mass; MTLM, mid-thigh lean mass; SCTT, subcutaneous tissue thickness; SSFM, subscapular fat mass.

The evaluation of fetal growth should be made on an individualized basis, derived from the concept that fetal growth is a complex process that can be adversely affected in various ways, in different individuals[7]. Therefore, individualized growth assessment provides a comprehensive and integrated evaluation of fetal growth. It corrects for differences in age and growth potential, the two primary confounding variables of growth assessment. This new method takes into consideration the concept that soft tissues undergo early changes in abnormal growth conditions such as IUGR or macrosomia[7].

Several studies have taken into account the assessment of the SCTT in pregnant women suffering from gestational diabetes and treated by dietary means or through the use of insulin[20]. Bernstein and Catalano[21] showed that increased neonatal fat (independent of birth weight) was associated with a significant increase in the risk of birth by Cesarean section of infants of women with gestational diabetes. From both the scientific literature and clinical practice, the use of 'fat-index' as a predictor of morbidity has been widely used in neonates. Additionally, Whitelaw[22] has demonstrated that subcutaneous fat is a more accurate indicator of maternal glucose control than is birth weight in infants of diabetic mothers.

We have previously provided reference ranges for soft tissue measurements in fetuses of normal mothers and those with gestational diabetes[9], and the present study aimed to identify whether similar changes in the fetal SCTT measurements were observed in IUGR fetuses[9,13]. Walther *et al* have shown that a low ponderal index (Rohrer's index of corpulence) is more strongly associated with neonatal growth restriction than is birth weight, emphasizing further the importance of studying the fetal body composition in pathological conditions[23]. In a longitudinal ultrasound assessment of fetal subcutaneous measurements, Galan *et al*[4] reported a reduction in fat mass in growth-restricted fetuses; however, lean body mass measurements were not changed. In the present study we observed that most of the SCTT measurements (excluding the MTFM and MTLM) are decreased in growth-restricted fetuses at 30 weeks' gestation. Therefore, we conclude that 'fat mass' is significantly reduced in fetuses that develop IUGR. Interestingly, the MALM measurement was also found to be decreased in growth-restricted fetuses suggesting that the 'lean mass' may also be affected in IUGR.

The differences noted in the present study compared to those of Galan *et al*[4] could be in part attributable to the different gestational age at which the measurements were performed in the studies and could also be due to population differences including the fact that the mothers in the Galan *et al* study had lived at high altitude.

We speculate that in adverse environmental conditions (i.e. chronic hypoxia, maternal hypertension or other pathological states) there may be a reduction in fetal energy stores (notably the fatty tissue), which may explain the reduction in fat mass noted in the present study. It

is possible that the reduction in muscle mass observed may be due to muscle breakdown in order to provide a further source of energy in this adverse situation. In other words, even muscles and lean tissue undergo damage with loss of substrates. This finding could be due to diversion of incoming energy sources to direct energy production instead of energy storage as usually occurs in the late third trimester.

Conventional ultrasound-measured parameters such as the abdominal circumference do provide insight as to whether a fetus is growth-restricted or not, and the abdominal circumference has been shown to decrease with progression of IUGR. However, the present findings suggest that use of the SCTT may provide insight into the specific changes that occur in the various fetal compartments of the growth-restricted fetus. Furthermore, we propose that future use of SCTT measurements may provide an opportunity to quantify and locate the effects of various novel treatments such as administration of maternal amino acid infusions[11] on the growth-restricted fetus.

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Ultrasound-determined fetal subcutaneous tissue thickness for a birthweight prediction model

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Abstract

Aim: The aim of this study was to explore a birthweight prediction model using ultrasound determined tissue thickness (SCTT) parameters.

Methods: We measured routine ultrasonographic biometric parameters and in addition, fetal SCTT in 201 healthy singleton pregnancies. Mid-arm fat and lean mass, mid-thigh fat and lean mass, subscapular fat mass and abdominal fat mass (AFM) were measured in order to calculate a birthweight prediction model. Ultrasound measurements were analyzed using an 'ANOVArepeated measures model'. The growth rate (β-slope) of the selected parameters was computed and the correlation coefficient with the birthweight and the Kendall rank correlation tau, were calculated.

Results: From the ultrasound determined SCTT parameters, only abdominal circumference (AC), AFM, and MTLM showed a statistically significant trend. The β-slope of mid-thigh lean mass was excluded since it exhibited significant correlation with the β-slope of AFM. The final regression model could be calculated as: birthweight (gr.) = intercept + α_1(AFM β-slope) + α_2(AC β-slope), where α_1, α_2 represent regression coefficients.

Conclusions: We provide a graphical birthweight prediction model for clinical practice using conventional and specific ultrasound measurements of fetal subcutaneous tissue thickness. This model is based upon an overall analysis of the ultrasound estimated body components.

Key words: birthweight prediction model, fetal skinfolds, subcutaneous tissue.

Introduction

Fetal weight is commonly evaluated using ultrasonographically estimated fetal anthropometric measurements and population-based growth charts. The abdominal circumference is the most sensitive among the individual fetal parameters for the detection of fetal over-growth.[1] Estimated fetal weight (EFW) is commonly used as an index of fetal growth and is calculated using a combination of parameters including abdominal circumference. Nevertheless, both EFW and abdominal circumference show a wide range of error (±10%) that may impact clinical practice.[2,3]

Fat content correlates directly with energy stores. Fat mass and lean body mass is often used in the nutritional assessment of the individual. Fat constitutes 12% to 14% of birth weight and has been demonstrated to account for 46% of the variance noted in neonatal weight.[4] Therefore, ultrasound generated estimates of fetal fat may be useful in the evaluation of fetal growth abnormalities.

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Several authors have previously used ultrasonography to assess anthropometric measurements of fetal body composition. Bernstein *et al.*[5] compared fat and lean body mass measurements in healthy fetuses across gestation and showed a significant correlation with both birthweight and estimates of neonatal lean and fat mass. Galan *et al.*[6] reported that the reduced birth weight of a subset of North-American newborns was the result of a reduction in fetal subcutaneous fat tissue rather than lean mass, suggesting that longitudinal fetal subcutaneous ultrasound measurements for detection of differences in specific populations may be potentially useful.

Recently, Deter stated the importance of assessing the fetal growth not on the basis of single anatomical variables, such as birthweight or abdominal circumference, but instead using the novel concept of the individualized growth assessment (IGA) and the Prenatal Growth Profiles, in which growth is assessed whereby each fetus serves as its own control.[7] This is a more precise approach, since fetal growth potential is in part linked to demographic-, age- and women-specific variables.[7]

The reference ranges for the fetal subcutaneous tissue thickness (SCTT) during pregnancy have been reported elsewhere and the methodology of the standard ultrasound measurement technique has been provided.[8]

The aims of this study were to develop a model to explore the relationship between the growth of the fetal subcutaneous tissues and the birthweight. Additionally, we attempted to further define the current estimated fetal weight formulas published. We propose that the availability of longitudinal reference values for fetal fat and lean tissue measurements may be useful in the assessment of both normal and abnormal pregnancies.

Materials and Methods

Population

Pregnant patients between 20 and 22 weeks gestation were enrolled from the high risk outpatient obstetrics ultrasound clinic (Fatebenefratelli Hospital, Italy) between January and December 2002. The study was approved by the Institutional Review Boards of Tor Vergata University and Fatebenefratelli Hospital-Isola Tiberina (Rome, Italy).

Inclusion criteria were: singleton and healthy pregnancy, gestational age, absence of fetal anomalies, absence of oligo- or polyhydramnios. Ultrasound scans were then performed between 26 and 37 weeks' gestation.

Ultrasonography

Serial ultrasound measurements were performed every three weeks until delivery (collecting 4 ultrasound scans per patient). At each study time point, patients had a complete conventional ultrasound (US) scan using a Teknos Esaote Ultrasound Machine (ESAOTE S.p.A. Headquarters, Genova, Italy) with a 3.5 or 5 MHz probe.

Study times were as follows: 26–27 weeks, 29–30 weeks, 32–33 weeks and 35–37 weeks.

Routine ultrasonographic biometric parameters included head circumference, abdominal circumference, and femur and humerus length.

To obtain fetal fat mass and lean mass, several measurements were assessed. We used the technique previously described by Bernstein *et al.*[5] to measure the fat and lean body mass areas on axial ultrasound images of the mid upper arm and mid upper leg, and on the cross-sectional images of the abdomen and the subscapular field.[9–11] Mid-arm fat mass (MAFM, cm^2), mid-arm lean mass (MALM, cm^2), mid-thigh fat mass (MTFM, cm^2) and mid-thigh lean mass (MTLM, cm^2) were obtained as follows: a longitudinal view of the long bone and extremity in the middle of the ultrasound screen was performed with an angle of 0 degrees to the transducer. The transducer was then rotated in the middle of the long bone, 90 degrees to obtain the axial view of the extremity. The fat mass (MAFM, MTFM) was measured by taking the total cross-sectional limb area and subtracting the central lean area (MALM, MTLM) that consisted of muscle and bone.

The abdominal fat mass (AFM, millimeters, mm) was determined by measuring the thickness of the anterior abdominal subcutaneous tissue on the same axial image from where the abdominal circumference was measured in a similar manner to that previously reported by Gardeil *et al.*[11] Subscapular fat mass (SSFM, mm) was evaluated by taking a sagittal ultrasound view of the fetal shoulder and measuring the skin width perpendicularly to the bone at its lower end.

To assess the growth trends of SCTT parameters, the US measurements were analyzed using the ANOVA repeated measures model, testing three types of patterns: linear, quadratic and cubic. We observed that the studied US parameters followed a significantly linear trend between 26 and 37 weeks' gestation overall.

Thus, for every subject and every SCTT parameter, we estimated the 'β-slope', which represents the mean linear growth rate between two consecutive study intervals (e.g. AC-β-slope = 2.5 cm means that the abdominal circumference increased 2.5 cm between two consecutive US-scans).

To explore the relationship between the β-slope of the SCTT parameters with respect to the birthweight, we used a stepwise procedure and the β-slopes of AFM, MTLM and AC were selected as significant explanatory variables.

The β-slope of MTLM was excluded from the statistical model because of its significant correlation with the β-slope of AFM (i.e. to avoid multicollinearity) and due to the small group of high outliers (five subjects with β-slopes of AFM >15 mm).

To account for the 'multicollinearity problem', i.e. the fact that some explanatory variables could be significantly correlated among them and for this reason some of them should be excluded from the final regression model, we used the stepwise approach. In stepwise regression, the computer program finds the explicative variable (i.e. the variable used to explain the dependent one) with the highest correlation with the dependent variable (birthweight); it then tries each of the remaining explicative variables in a multiple linear regression until it finds the two variables with the highest R square; then, it tries all of them until it finds the combination of three with the highest R square. The overall R square gets bigger as variables are added; ideally, of course, nearly the 100% of variance should be explained. But a cut-off is used: a variable enter the model only if a significant F change ($P < 0.05$) is obtained. As a consequence, if two variables are highly correlated, only one will enter in the model with a stepwise approach. Following this approach, MALM, MTFM and SSFM were automatically excluded from the final model.

An overview of the fetal subcutaneous tissue thickness measurements, in relation to their accuracy and reproducibility have been reported elsewhere.[8]

Statistical analysis

Multiple linear regression analysis was used to explore the relationship between the β-slope of fetal subcutaneous tissue thickness values across gestation and birthweight. The β-slope was computed on SCTT variables during the course of pregnancy and represented the increasing rate for each studied parameter. The ANOVA repeated measures model was previously used to assess the type of growth trend of SCTT measurements.

Results

Birthweight prediction model developmental procedure

Initially we enrolled 231 patients, however, 23 were excluded due to incomplete perinatal data, 7 patients were excluded for the subsequent appearance of oligohydramnios, and 201 healthy pregnant patients were used for final evaluation. Characteristics of the study group are summarized in Table 1. Summary values for birth weight distribution of the study population are shown in Table 2.

Birthweight distribution was not a true normal distribution since there was a group of low outliers, i.e. the first 5% of the fetal measurements were in neonates who then went onto have a low birth weight (<2680 g) and there is a group of high outliers, i.e. 3% of observations have a high birth weight (>4000 g).

Figure 1 shows the relationship of the β-slopes of the AFM and the AC with respect to birthweight, with superimposed estimated regression lines. The correlation coefficient was: $r = 0.22$; $P = 0.03$.

Table 3 provides the results of the fitted model; the final form of the regression model is the following:

$$\text{birthweight (gr.)} = \text{intercept} + \alpha_1(\text{AFM } \beta\text{-slope}) + \alpha_2(\text{AC } \beta\text{-slope}),$$

where α_1, α_2 represent regression coefficients shown in Table 3.

The multiple R-Squared of the model is quite low (multiple R-Squared: 0.13, F-statistic: 6.8, the P-value is 0.002) probably due to the absence of other possible explanatory variables.

The graphical view of the model is shown in Figure 2. The picture resembles a 'palette' scheme and seems to be a practical birthweight prediction tool. In fact, by simply matching the AC and AFM β-slopes of

Table 1 Characteristics of the studied population

n.	201
Mean age (Years)	27.4 ± 6.7
Body mass index	24.3 ± 2.9
Primigravidae (%)	45
Gestational age at delivery (weeks)	39.0 ± 3.0

Values in mean ± SD or percentage.

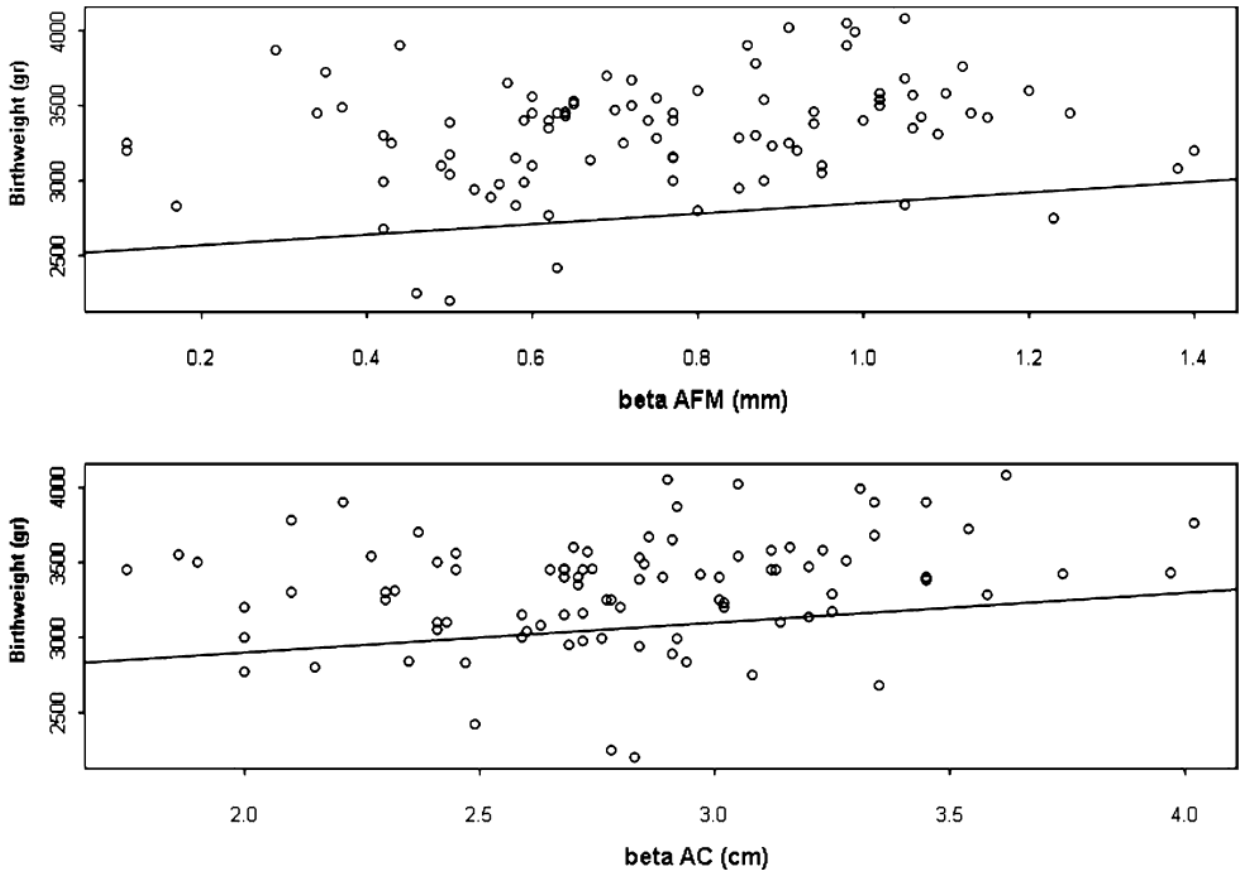

Figure 1 The upper picture represents the regression analysis between birthweight and abdominal fat mass beta-slope. x-axes: b-slope for abdominal fat mass (AFM); y-axes: birthweight, with superimposed estimated regression lines. The lower picture represents the same analysis between birthweight and abdominal circumference beta-slope. x-axes: b-slope for abdominal circumference (AC); y-axes: birthweight, with superimposed estimated regression lines.

Table 2 Birthweight distribution in the study group

Number	Minimum	5th Centile	1st Quartile	Median	Mean	3rd Quartile	95th Centile	Maximum
201	1790	2680	3050	3380	3283	3500	3900	4260

Values in g.

a studied fetus on the y and x-axes, we can obtain a color level. We can analyze the obtained color from the palette scheme which can generate an estimation of the level of fetal growth. For example, if we were to measure an AC β-slope of 4 cm and an AFM β-slope of 1 mm after two consecutive US scans performed with three weeks interval (gestational age ranging from 26 to 37 weeks), then we would find that value is within the sky-blue region and thus representative of fetal growth which would be approximately 3600 g at term gestation.

Table 3 Linear regression model results

Coefficients	Value	Standard error	*t*-value	p(t)
Intercept	2500	243.63	10.26	0.000
α_1	350	129.23	2.71	0.008
α_2	198.8	78.09	2.54	0.013

α_1 represents the regression estimated coefficient for the β-slope of the abdominal fat mass (AFM). α_2 represents the regression estimated coefficient for the β-slope of abdominal circumference (AC).

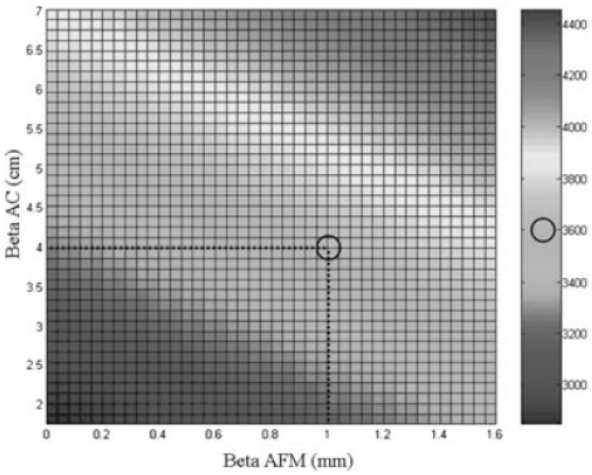

Figure 2 Schematic representation of the correlation between birthweight, beta-slope of abdominal circumference (AC) and beta-slope of Abdominal Fat Mass (AFM). AFM β-slope in mm; AC β-slope in cm; birthweight in gr. An estimation of the fetal growth level can be checked on the right column after the AC and AFM β-slopes have been cross-matched on the y and x-axes, respectively. E.g. If we obtain from a patient an AC β-slope of 4 cm and an AFM β-slope of 1 mm after two consecutive ultrasound scans (in the 26–37 weeks interval), the term gestation birthweight should approximately be of about 3600 g (circled in the column on the right).

Discussion

Several studies support the valuable role of intrauterine fat and lean body mass assessment in the determination of the correct fetal development. Reference charts for the fetal skinfolds have previously been provided.[8]

We speculate that the ability to measure the US parameters representative of subcutaneous tissues and to compare individual measurements with the reference values will be of assistance in the identification of intrauterine growth abnormalities.

Bernstein *et al*. previously examined the utility of ultrasonography to measure subcutaneous fetal fat in the extremities. Their use of a simple linear measurement of fat thickness was not easily reproducible, with an intraobserver coefficient of variation of 28%.[5,9] This appeared to be the result of a distortion in the proximal extremities resulting from external compression. The measurement of fat area in more proximal extremities has proven to be more reproducible. The coefficients of variation for the ultrasonographic estimates of subcutaneous fat areas has been proven to compare adequately with published coefficients of variation for the measurement of skinfold thickness in neonates.[8,12]

The majority of routine ultrasonographic parameters are valuable in the assessment of gestational age and are not influenced significantly by environmental factors, however it is this same quality which makes them less well suited for the identification of growth abnormalities. Abdominal circumference, which demonstrates a linear growth profile when compared with the bony tissue US parameters is the most sensitive of the individual ultrasonographic variables in detecting growth abnormalities. Bernstein *et al*. reported that fetal fat and lean body mass have unique growth profiles and that, as a result of an accelerated rate of growth in late gestation, the measurement of fetal fat will provide a more sensitive and specific marker of abnormal fetal growth when compared with index values of lean body mass.[5]

Galan *et al*.[6] recently showed that ultrasonography can be used to follow subcutaneous measurements longitudinally and to detect differences, and potentially disease processes, in study populations. The use of a 'fat index' as a predictor of morbidity has been widely used in neonates. In the growth-restricted neonate a low ponderal index (Rohrer's index of corpulence) has a stronger association than the simple birthweight, with several specific morbidities.[13]

This work attempts to assist and further define the sonographical quantification in the derivation of fetal weight by adding new information pertaining to the fetal soft tissues.

It is important to evaluate the potential impact of the subcutaneous tissue thickness (SCTT) on the correct estimation of the birthweight. Several birthweight prediction formulas[14] have been published over the last 15 years, however these formulae have consistently predicted birthweight with a margin of 8–10% error. The inclusion of the studied SCTT parameters into the existing birthweight prediction algorithms may reduce this degree of error.

Our US measurements at 3, 4 and 5 weeks showed that the shortest study interval required to pick up a difference in US measurements was 3 weeks. Therefore we choose a 3-week interval between 2 US assessments in this study. The aim of this study was to create a reproducible 'model' rather than a birthweight prediction algorithm.

From this study, we propose that utilization of the β-slope evaluation process where a 'color of fetal growth' is identified and combined with the beta-slope

of AC and AFM provides an easy method to visualize 'dynamic' growth. It may be more pertinent to add data from the soft tissue evaluation to the abdominal circumference, head and long bone data. In some circumstances, addition of the soft tissue data may be more accurate to detect decreased or increased fetal growth and recent work has shown that SCTT may have a role in the identification of mild nutritional fetal abnormalities.[15]

At present we do not propose that this model be used in routine scans but rather should be considered in patients who demonstrate fetal growth abnormalities (macrosomia, gestational diabetes or fetal growth restriction) which may help guide clinical practice and provide information about SCTT.

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COULD THE BIRTHWEIGHT PREDICTION MODELS BE IMPROVED BY ADDING FETAL SUBCUTANEOUS TISSUES?

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COULD THE BIRTHWEIGHT PREDICTION MODELS BE IMPROVED BY ADDING FETAL SUBCUTANEOUS TISSUES?

ABSTRACT

Objectives. The aims of the study were: 1) to compare the accuracy of standard ultrasonic algorithms in the estimation of fetal weight; 2) to test two new algorithms in order to improve the global performance of birthweight prediction by adding in the fetal subcutaneous tissue thickness.

Methods. We enrolled 398 patients who were between 34-42 weeks' gestation. Routine ultrasonographic biometric parameters as well as subcutaneous tissue thickness ultrasound parameters were measured. Correlation matrices between US parameters, in order to evaluate the degree of multicollinearity between these parameters were computed prior to develop a stepwise multiple regression birthweight (BW) predictive model.

Results. Contributions of single ultrasound measurements in predicting BW were examined, by fitting Log-transformed BW versus single US measurement; we found that the mid-thigh tissue area was able to significantly improve the performance of BW prediction when added to the other standard US measurements. We derived two new algorithms which appeared to be better at predicting BW. Furthermore there was a lower minimum absolute estimation error noted when compared to other reported formulas.

Conclusions. Our algorithms showed the usefulness to add the mid-thigh tissue area evaluation in BW prediction with respect to the other reported algorithms based on routine ultrasound biometric parameters.

COULD THE BIRTHWEIGHT PREDICTION MODELS BE IMPROVED BY ADDING FETAL SUBCUTANEOUS TISSUES?

INTRODUCTION

Fetal weight is commonly evaluated through the use of both ultrasound derived estimated fetal anthropometric parameters and population-based growth charts. Among the individual fetal measurements, it is the abdominal circumference, which is the most sensitive indicator in the detection of fetal over-growth (1). Estimated fetal weight (EFW) is commonly used as an index of fetal growth and is generally computed through a combination of parameters, which also include abdominal circumference. However, it is important to note that both EFW and abdominal circumference may show a wide margin of error between 10% - 25% which may impact significantly on clinical practice (2-4).

This margin of error may be attributable to factors such as technical errors which may occur whilst carrying out the procedure. Other contributing factors may include the assumption that the body composition of the fetus remains the same throughout the gestational period and that the composition remains the same in some fetal pathologies which in actual fact may alter the normal muscle to fat ratios (5, 6).

Since fat content correlates directly with energy stores, the fat mass and lean body mass are often used in the nutritional assessment of an individual. Fat constitutes 12-14% of birth weight and has been shown to account for variations noted in neonatal weight (7). Consequently, ultrasound-generated estimates of fetal fat may be useful in the evaluation of fetal growth abnormalities. Several authors have used ultrasonography as a means in assessing anthropometric measurements of fetal body composition (5,6). Bernstein et al (6) compared fat and lean body mass measurements in healthy fetuses throughout gestation and showed significant correlations between birth weight and neonatal estimates of lean and fat mass.

Recently, two works from the same research group (7, 8) stated the importance of assessing the fetal growth not on the basis of conventional ultrasound parameters, but rather using the novel concepts of individualized growth assessment (IGA) linked with the fetal mid-**limbs** soft tissues evaluation (fractional arm volume, and **fractional thigh volume**), allowing earlier detection and improved monitoring of soft tissue abnormalities that can occur in fetuses with growth disturbances.

Nahum et al investigated the accuracy of more than 20 ultrasonic algorithms for the estimation of term fetal weight within an 82 patients study population, and found there to be a variability in the accuracy of algorithms (9). But this study examined only nondiabetic pregnant women and reported the absolute value of prediction errors which does not account for both positive and negative bias within the observations (9).

Dudley provided a more systematic review of four databases of fetal weight estimation models evaluating 11 different methods and even including the three dimensional ultrasound scan (fetal limb cross-sectional

measurements) (10). The author stated that the accuracy of EFW is compromised by large intra- and inter-observer variability. He recommended averaging of multiple measurements, improvements in image quality, uniform calibration of equipment, careful refinement of measurement methods, to improve the accuracy of fetal weight estimation (10).

Many new algorithms have begun to utilize soft tissue parameters in order to improve the birthweight prediction process.

Recently a clear method to ultrasonographically detect fetal soft tissue parameters was shown and reference values for fetal subcutaneous tissue thickness (SCTT) in several fetal compartments have been reported in a large 300-subject longitudinal ultrasound study (11). The cited work had other two interesting issues: reference ranges for fetal soft tissues were performed also for gestational diabetic pregnant women and the variability (intra- and inter-observer coefficient of variation) was reported for repeated measurements (11).

The authors concluded their paper speculating that "*the incorporation of SCTT measurements into existing formulae involving long bone (humerus and femur) and head and abdominal circumference measurements could reduce the amplitude of the birthweight estimation errors*" (11).

On this basis, the aims of our present study were to compare the accuracy of standard ultrasonic algorithms in the estimation of fetal weight. Additionally, we wished to test two new algorithms (derived from a multiple linear regression), in the estimation of fetal weight, with the aim of improving the global performance of the process of 'birthweight prediction' through the addition of soft tissue parameters.

METHODS

Patient selection

This was a cross-sectional study in which we enrolled 398 patients who had been admitted for delivery (either via spontaneous delivery or cesarean section) at any gestational age. The study was approved by the Institutional Review Boards at Tor Vergata University of Rome and was conducted under written informed consent.

The patients were admitted via the department of Obstetrics and Gynecology at Fatebenefratelli Hospital Isola Tiberina (Rome, Italy) throughout the period of January-December 2003. The inclusion criteria were: 1) singleton pregnancy, 2) confirmed gestational age, and 3) the absence of fetal anomalies. Women with twin pregnancy and type 1 diabetes were excluded from the study.

Ultrasound scans were performed at the admission, taking both the routine conventional and the SCTT parameters in the same session, by the same operator.

Two operators (G.L. and G.D.P.) performed the US measurements, both for conventional or SCTT parameters.

Gestational age was calculated from the first day of the last menstrual period and confirmed by either a first- or second-trimester ultrasound scan. When the ultrasound determined gestational age differed from that calculated from the last menstrual period by >7 days in the first trimester, or by >10 days in the second trimester, the ultrasound-determined gestational age was used.

Ultrasonography

A conventional ultrasound scan was carried out on the patients using the unmodified Teknos Esaote Ultrasound Machine (ESAOTE S.p.a. Headquarters, Genova, Italy) with a 3.5 MHz probe. The routine ultrasound biometric parameters collected included head circumference, abdominal circumference, femur and humerus length. To calculate the fat mass, several measurements were undertaken. We used a method previously described to measure the fat mass area using axial ultrasound images of the mid upper arm and mid upper leg, and on the cross-sectional images of the abdomen and the subscapular field (6,12,13). Briefly, **mid-arm tissue area** (**MATA**, squared centimeters, cm^2) and **mid-thigh tissue area** (**MTTA**, cm^2) were obtained using a longitudinal view of the long bone and extremity. The ultrasound **scan** was performed with an angle of 0 degrees to the transducer. The transducer was then rotated in the middle of the long bone, 90 degrees to obtain the axial view of the extremity. The fat mass (**MATA, MTTA**) was

calculated by subtracting the central lean tissues i.e. muscle and bone from the total cross-sectional area of the limb.

The abdominal tissue thickness (ATT, millimeters, mm) was determined by measuring the thickness of the anterior abdominal subcutaneous tissue on the same axial image from which the abdominal circumference is obtained (Figure 1), as previously reported (14). Subscapular tissue thickness (SSTT, mm) was also evaluated. The intra- and inter-observer reproducibility was similar to that described previously (11) for each SCTT parameter.

Statistical analysis

The study sample was divided into 4 main groups: Group A: who had a normal pregnancy, Group B: those with gestational diabetes, Group C: those mothers with an intrauterine growth restricted (IUGR) fetus and a small group of women with type 1 diabetes mellitus that were excluded from the analysis.

In order to test for demographic and gestational differences between the groups, we compared their characteristics in terms of age, pre-pregnancy body mass index and gestational age at delivery using the ANOVA model. Subsequently, we considered the body weight (BW) distribution across group (a dependent variable in the final regression model), and we tested for differences in BW between Groups A, B and C, through the use of both the ANOVA model and also through the post-hoc comparison between pairs of diagnostic groups.

The gaussian shape of the US measurements distribution has been evaluated by means of Kolmogorov-Smirnov normality test (all p values>0.05). A MANOVA model (Multivariate Analysis of Variance), was used to test if the US measurements, used as dependent variables in the model, were significantly different across groups, the three-levels between-subjects factor. To investigate for the presence of multicollinearity, i.e. a significant level of pairwise correlation between the US variables (a critical aspect to evaluate prior to build the final multiple regression model to estimate birthweight), Pearson correlation matrices between US measurements were computed for the entire study sample.

In order to reliably compare performances of BW prediction equations in two independent samples, we splitted our database of 392 patients into two samples, S1 and S2, using a computer generated pseudo-random selection. Measurements from 25% of patients (98 patients) formed sample group-S2, and the remaining 75% of patients formed S1 (294 patients). We used S1 to derive “our” equations.

To examine contributions of single ultrasound measurements in predicting BW, equations were derived by fitting log-transformed birthweight versus individual US derived measurements in S1 (**Table 1**). Log-transformation of the birthweight has been used after evaluation of the Box-Cox linearity plots, in fact when

performing the linear fit of BW against single US measurements the appropriate transformation to both improve the fit and minimizing the error sum of squares was the logarithmic one, a finding also consistently with the literature on this topic.

To produce the BW predictive multiple linear regression model, a 'stepwise' approach has been used in order to select the best group of 'explicative' variables (the independent variables in the regression model, where the dependent one is the variable to be explained, i.e. the BW). This procedure takes into account the existing interactions between explicative variables.

In stepwise regression, the computer program finds the explicative variable with the highest correlation with the dependent variable; it then tries each of the remaining explicative variables in a multiple linear regression until it finds the two variables with the highest R square; then, it tries all of them until it finds the combination of three with the highest R square, and so on. The cut-off used is that a variable enter the model only if a significant F change ($p<0.05$) is obtained. As a consequence, if two variables are highly correlated, only one will enter in the model with a stepwise approach.

To have a benchmark, among the huge number of published birthweight prediction equations, we selected six formulas widely used in the current literature on this topic (9, 15) (**table 2**). Two of the selected algorithms were from Hsieh (16), two from Hadlock (17) and two from Warsof (18). A statistical comparison of errors distributions has been obtained by means of a repeated measures ANOVA model, both in S1 and S2, where in the within-subjects contrasts section the first six equations error distributions has been tested against the proposed ones **(19). A repeated-measures model is one in which multiple measurements on the same subject comprise the replicate data. In the present application, we aimed to test the null hypothesis of no difference between the equation error distributions. Since there were not independent samples of women but instead each subject has been tested with the different equations, and there is consequential relationship among the data in each row, being errors of the BW estimation equations on the same subject, the within-subjects contrasts have to be used. It has to be noted that, in the case of absolute estimation errors, to evaluate the within-subjects contrasts , we used the Greenhouse-Geisser correction on the resulting p-values that do not require compound symmetry. Moreover, to further check the results obtained, we applied to these data a nonparametric repeated-measures analysis of variance, the Friedman's test, that do not require neither normality or homoscedasticity characteristics.**

All statistical analyses were performed using SPSS 13 for Windows.

RESULTS

Ultrasound **scan** was performed at 34-42 weeks' gestation and the mean interval between ultrasonographic assessment and delivery was 3 days (ranging from 6 days to 1 day before delivery.

From our sample, 329 women (82.7%) had a normal pregnancy (group A), 41 women (10.3%) were diagnosed with gestational diabetes (group B) and 22 women (5.5%) had an ultrasound derived diagnosis of intrauterine growth restriction (IUGR) (Group C) (18). Diagnosis of IUGR was based on a fetal abdominal circumference <5th **percentile** for gestational age by local reference values, an estimated fetal weight <10th **percentile** for gestational age and an umbilical artery pulsatility index of more than 2 SDs above the gestational mean compared to local reference values (20). Six women with type 1 diabetes mellitus were excluded from our study group and so a total of 392 were studied. Gestational diabetes was diagnosed using the criteria outlined by the National Diabetes Data Group (21,22).

Birthweights of greater than 4000 g were considered as macrosomic (23) and 23 women from group A (7%) and 4 women from group B (9.7%) had macrosomic fetuses. The characteristics of the study groups are summarized in **table 3**. The percentiles of the birthweight distribution and descriptive statistics for birthweight across the three diagnostic groups are reported in **table 4.**

The birthweight (BW) distributions for the normal and gestational diabetic (GD) groups **were similar until** the 75th percentile, however at higher birthweight values **a divergence was observed.** Group C showed consistently lower BW values with respect to the other groups. BW appeared to be significantly affected by diagnosis, (ANOVA $F[2,389]=32.37$, $p<0.0001$); in particular, from the post-hoc and contrasts results, birthweight resulted significantly affected by diagnosis of intrauterine growth restriction (IUGR) (group C versus group A, and group C versus group B, $p<0.0001$). **The difference between Group A and B were not statistically significant.**

All US measurements were normally distributed (Kolmogorov-Smirnov normality test, *p* values>0.05), therefore parametric techniques has been used in the successive statistical analysis.

In the MANOVA model the US measurements, considered as dependent variables, were significantly different across groups (results shown in table 5). Only the sub-scapular tissue thickness (SSTT) and the mid-arm tissue area (MATA) were not significantly different across diagnostic groups. Using the control group (group A) as the reference category, we can see that the group C (IUGR) is significantly lower for in all the US parameters measured excluding the SSTT, MATA and the abdominal tissue thickness (ATT). Comparisons between Group A and B were not significant for all US parameters except for the abdominal tissue thickness.

Correlations between ultrasound measurements

In order to assess the multicollinearity level between explicative variables, bivariate correlations between ultrasound measurements were computed (data not shown). To summarize results obtained, significant correlation (Pearson correlation coefficient p value<0.05) were found between: biparietal diameter (BPD) with abdominal circumference (AC), femur with head circumference (HC); moreover, AC correlated with HC and mid-thigh tissue area (MTTA). HC was associated with humerus; sub-scapular tissue thickness (SSTT) with mid-arm tissue area (MATA), mid-thigh tissue area (MTTA) and abdominal tissue thickness (ATT); finally, MTTA correlated with ATT.

Given this complex situation of pairwise associations, the stepwise approach to select the most representative subset of US variables, maximally uncorrelated, was used in the multiple regression model to predict BW, as explained in the above method section.

Comparison of benchmark formulas for predicting BW

Simple linear regression models, varying from linear to cubic interpolations, selected on the basis of the best fitting of the log BW data in terms of R square, produced a clear indication that the mid-thigh tissue area (MTTA) could significantly improve performance in BW prediction.
This finding was confirmed using the method of stepwise model construction, that selected as the best subset of explicative variables the product of AC and BPD plus the MTTA measurement.
Taking into account the significant differences in BW distribution across the three groups considered, we decided to include the diagnostic group information in a separate birthweight prediction model. Therefore, two separate formulae were derived: Larciprete (a), considering only the stepwise selected ultrasound measurements, and Larciprete (b), with the stepwise selected ultrasound measurements plus the diagnostic group. These are as follows:

- Larciprete (a): Log EFW=3.030+0.001448 x (AC x BPD)+0.002099 x **MTTA**
- Larciprete (b): Log EFW=3.008+0.00138 x (AC x BPD)+0.002140 x **MTTA** + 0.02123 x DIAG

where DIAG was coded as: 1=IUGR; 2=Normal; 3=GD.

These two equations showed a fitting performance (R^2) comparable to the best R^2 obtained with the benchmark equations, and the minimum absolute estimation errors with respect to the other formulas reported **in table 2** (Table 6).
When the benchmark equations error distributions has been tested against the two proposed ones all contrast *p* values were<0.05 in sample S1 indicating a significant mean lower error for our algorithms for the three types of error distributions reported in table 6 (E, AE and PE); **having obtained the same results with the Friedman's test for the absolute errors distributions, for sake of synthesis in table 6 the repeated-measures ANOVA Greenhouse-Geisser corrected p-values have been reported in the AE columns.**
Some of the contrasts in sample S2, marked with a cross in table 6, indicated no significant difference, i.e. a similar error distribution to the benchmark equations in those cases.

CONCLUSIONS

The routine use of ultrasonographic measured parameters has been demonstrated to be of clinical benefit in the assessment of gestational age. Ultrasound derived parameters are usually resistant to external influence such as environmental factors, which account in part for their accuracy. However, it is also this same characteristic which reduces their suitability in the identification of fetal growth abnormalities. From the parameters discussed it is the measurement of the abdominal circumference which has been shown to be most sensitive in the detection of growth abnormalities (1, 2, 6).

Over the last 30 years, ultrasonographic fetal biometry is often assumed to be more accurate than clinical methods in the estimation of fetal weight. This is largely due to the presumption that ultrasonographic measurements of multiple linear and planar dimensions of the fetus provide sufficient parametric information to create an accurate algorithmic reconstruction of the three-dimensional fetus with varying tissue density. Thus correspondingly, a large number of studies have attempted to create 'best-fit' fetal biometric algorithms which can predict birth weight on the basis of obstetrical ultrasonographic measurements. Numerous studies have recently challenged the accuracy of these ultrasonographic birth weight estimations and have concluded that ultrasonography may be no more accurate in the prediction of birth weight than clinical palpation or even maternal self-estimations of fetal weight (24, 25, 26). Furthermore some studies suggest that quantitative assessment of maternal characteristics may be as accurate as obstetric ultrasonography in birthweight prediction (27, 28). Therefore, to date the most accurate method for the prediction of term birth weight has yet to be elucidated.

A study by Nahum et al (9) assessed twenty commonly used fetal US biometric algorithms in the accuracy in the prediction of term fetal weight (9). The equations were based on various combinations of the fetal measurements including the AC, BPD, and femur length (FL). The accuracy of US derived fetal weight predictions were quantified in each case through calculation of its correlation with actual birthweight, the mean absolute error, the mean absolute percentage error, and the percentage of birthweights which predicted to within ± 10% to 15% of the actual birthweight. Comparison among the equations that used the fetal AC and BPD indicated that algorithms of Hadlock et al (17), Warsof et al (18), Jordaan et al (29) and Hsieh (23) were more accurate and had a comparable predictive accuracy than many other described algorithms (Shepard and Vintzileos) (29, 30).

Nahum et al (9) stated that the ultrasonographic algorithms that were based exclusively on the measurement of the fetal AC proved to be as accurate as the other classes of equations which had been based on multiple standard ultrasonographic fetal measurements. Furthermore, they observed that fetal AC at term was the most accurate of all four measurements studied with respect to the birthweight, suggesting that AC may be the most predictive measure in the estimation of birthweight at term. Thus in the prediction

of birthweight of a normal well-dated singleton fetus, other measurements other than AC may be superfluous. However, factors such as suboptimal fetal position, oligohydamnios, anterior placentation, and maternal obesity may confound and reduce the accuracy of ultrasonic birth-weight estimates.

It is important to remember that despite the wide degree of error that is associated with ultrasonic estimates of term fetal weight, both the technology dependent and labour-intensive nature of ultrasonic estimates may foster a false sense of reassurance among obstetric practitioners as to the projected weight of an individual fetus. This may sometimes result in the potential overuse or lack of obstetric interventions concerning both the timing and mode of delivery of a fetus, which may be potentially detrimental.

Recently, Deter (2004) stated the importance of assessing the fetal growth not on the basis of single anatomical variables, such as birthweight or abdominal circumference, but rather using the novel concepts of individualized growth assessment (IGA) and the Prenatal Growth Profiles, in which growth is assessed whereby each fetus serves as its own control (31). This is a more precise approach, since fetal growth potential is in part linked to demographic and age-specific variables (31). The 'individualized' evaluation of fetal growth has been derived from the concept that fetal growth is a more complex process that can be adversely affected in various ways, in different individuals (31). Therefore, the IGA (Individualized Growth Assessment), provides a comprehensive and integrated evaluation of fetal growth, correcting for differences in age and growth potential which are two primary confounding variables of growth assessment.

Deter shows that third trimester growth trajectories for a specific parameter are predicted from sonographic data obtained during the second trimester of pregnancy (31).

This new method takes into consideration the concept that soft tissues undergo early changes in abnormal growth conditions such as IUGR or macrosomia (31).

Lee et al (32) introduced the fractional thigh volume as a new soft tissue parameter for fetal growth evaluation, defining its relationship to menstrual age and developing individualized growth standards, thus applying the soft tissues to the IGA model. Moreover, they added the fractional arm volume, a soft tissue parameter, to their research (***7***).

These concepts highlight the importance of the evaluation of fetal subcutaneous tissue thickness (SCTT) in the assessment of fetal growth. Bernstein and Catalano used the ultrasound approach to measure subcutaneous fetal fat in the extremities and noted variations in SCTT comparable to that noted in skin fold thickness measurements in neonates (6, 33). Additionally, they report that fetal fat and lean body mass have unique growth profiles and an accelerated rate of growth is noted in late gestation. Therefore the measurement of fetal fat may well provide a more sensitive and specific means of identifying abnormal fetal growth when compared with index values of lean body mass (6).

Our previous study has provided us with the gestational reference ranges for fetal soft tissues in both normal and gestational diabetic mothers (11). We propose that adding the SCTT parameters to the

conventional ultrasound algorithms may help with the individualization approach in birthweight prediction, following the “Deter paradigm”. From our work, we describe higher birthweights in fetuses from normal and diabetic women when compared to women with growth restricted fetuses. The similarities between the normal and diabetic mothers may be attributed to the dietary regimen undertaken by the diabetic patients.

From the conventional US parameters, we have seen no real differences between normal and diabetic patients except for the abdominal skin fold which was greater in fetuses from diabetic mothers. In terms of the growth restricted fetuses, both conventional and SCTT ultrasound parameters were lower with the exception of MATA and HC which correlate with findings from our previous work (11).

Generally, an absence of differences among groups for most SCTT parameters was seen.

Using the proposed algorithms we have found lower mean errors, mean absolute errors and percentage errors when compared to those algorithms which are currently used (Hsieh, Hadlock and Warsof) (16, 17, 18). **Nonetheless, the quite low fitting performance for the relationship between individual SCTT parameters and birth weight has to be cited. Small differences were noted in systematic and random estimation errors when the results using weight estimation functions with a SCTT parameter are compared to the results using weight estimation functions without such parameters.**

Our algorithms showed the usefulness to add the mid-thigh tissue area evaluation in birthweight prediction with respect to the other reported algorithms.

But our findings need to be further clarified since we used functions from the literature in a new sample, without determining sample-specific coefficients, and this behaviour frequently gives poorer results than were obtained in the original studies.

From this viewpoint, the aid of fetal soft tissues should be carefully evaluated in the future.

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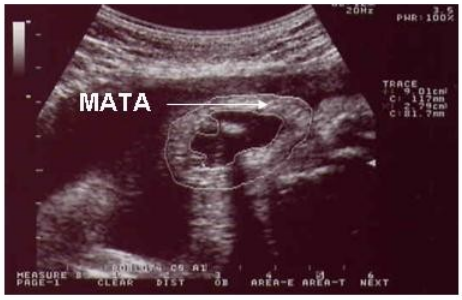
Figure 1



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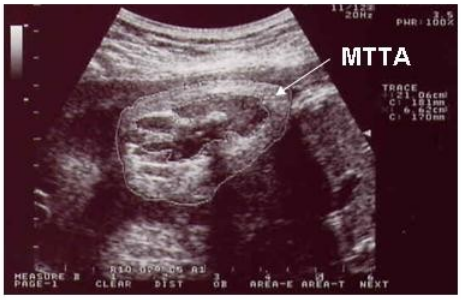

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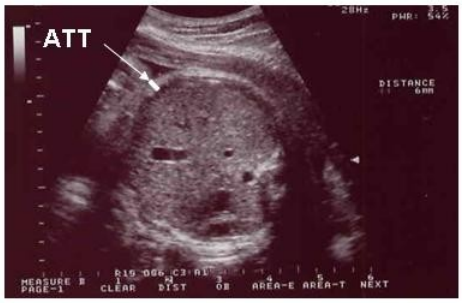

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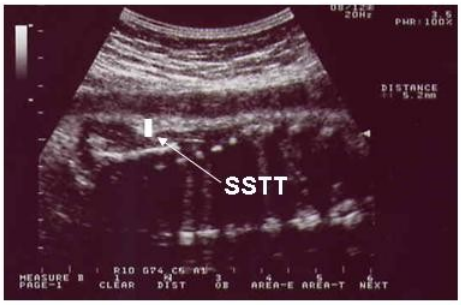

d

Figure 1. The *a* and *b* pictures represent the ultrasound scan showing the axial view of the extremities (MATA=mid-arm tissue area and MTTA=mid-thigh tissue area, respectively). The *c* picture represents the way to evaluate the abdominal tissue thickness (ATT) and the *d* picture shows the subscapular tissue thickness (SSTT) measurement.

Table 1. Single-measurement regression models derived from S1.

model	equation order	regression coefficients (intercept; betas)	R^2
EFW by AC	Quadratic	2.3221; 0.0516; -0.0005	0.59
EFW by BPD	Linear	2.7266; 0.0851	0.26
EFW by F	Cubic	4.6043; -0.2569; 0.002	0.17
EFW by SSTT	only cubic term	3.4922; 0.00008	0.06
EFW by HC	Quadratic	0.5008; 0.1597; -0.0021	0.27
EFW by humerus	Linear	3.0792; 0.0662	0.11
EFW by MATA	Linear	3.4470; 0.0095	0.08
EFW by MTTA	Linear	3.4113; 0.0082	0.19
EFW by ATT	only cubic term	3.4878; 0.00008	0.07

MATA=mid-arm tissue area. MTTA=mid-thigh tissue area. SSTT=subscapular tissue thickness. ATT=abdominal tissue thickness. EFW=estimated fetal weight. AC=abdominal circumference. BPD=biparietal diameter. F=femur length. HC=head circumference.

Table 2. Birthweight prediction formulas commonly used in literature

Hsieh (a) [15]	Log_{10} EFW= 5.6541 x 0.001 x AC x BPD-1.5515 x 0.0001 x (AC^2) x BPD +1.9782 x 0.00001 x (AC^3) + 5.2594 x 0.01 x BPD + 2.13153
Hsieh (b) [15]	Log_{10} EFW= 9.4962 x 0.001 x AC x BPD – 0.1432 x F – 7.6742 X 0.0001 x AC x (BPD^2) + 1.7450 x 0.001 x (BPD^2) x F + 2.7193
Hadlock (a) [16]	Log_{10} EFW=1.335-0.0034 x AC x F + 0.0316 x BPD + 0.0457 x AC+0.1623 x F
Hadlock (b) [16]	Log_{10} EFW=1.326-0.00326 x AC x F + 0.0107 x HC + 0.0438 x AC + 0.158 x F
Warsof (a) [17]	Log_{10} EFW=-1.8367+0.092 x AC-0.000019 x (AC^3)
Warsof (b) [17]	Log_{10} EFW= -1.599 + 0.144 x BPD + 0.032 x AC-0.000111 x ((BPD^2) x AC)

EFW=estimated fetal weight. AC=abdominal circumference. BPD=biparietal diameter. F=femur length. HC=head circumference. The Warsof algorithms express the BW in kilograms.

Table 3. Characteristics of the studied populations

	Normal patients Group A	GD patients Group B	IUGR patients Group C	ANOVA and Student-Newman-Keuls test
N.	329	41	22	
Age (years)	27.4±6.7	28.2±4.5	29.2±1.3	N.S.
Pre-pregnancy Body Mass Index (Kg/m^2)	24.3±2.9	25.4±3.2	27.1±2.3	P<0.05 C vs A, C vs B, B vs A
Gestational age at delivery (Weeks)	39.0±3.0	38.6±2.6	35.0±3.0	P<0.05 A vs C, B vs C

GD: gestational diabetes; IUGR: intrauterine growth restriction; N.S.: not significant.
Values in mean ± SD

Table 4. Percentiles of birth-weight distribution and descriptive statistics across the three diagnostic groups

	Normal patients Group A	GD patients Group B	IUGR patients Group C	ANOVA and Student-Newman-Keuls test
BW percentiles (weighted average, g.)				
5^{th}	2750	2752	1756	
10^{th}	2860	2804	1931	
25^{th}	3050	3005	2565	
50^{th}	3260	3280	2650	
75^{th}	3510	3575	2785	
90^{th}	3800	4028	2920	
95^{th}	4130	4195	30777	
Mean BW (g.)	3303	3335	2599	P<0.05 B vs C, A vs C
SD	402	427	322	

GD: gestational diabetes; IUGR: intrauterine growth restriction; N.S.: not significant; BW: birth-weight; SD: standard deviation.

Table 5. Descriptive statistics for the ultrasound measurements within the three study groups (mean ± SD)

	Normal patients Group A n. 329	**GD patients Group B** n. 41	**IUGR patients Group C** n. 22	**MANOVA Contrasts Results P value** Group B vs Group A	Group C vs Group A
Conventional US Parameters					
BPD (cm)	9.25 ± 0.36	9.11 ± 0.27	8.88 ± 0.33	0.187	0.000*
AC (cm)	34.16 ± 2.25	34.50 ± 2.58	30.78 ± .1.99	0.395	0.000*
Femur (cm)	7.23 ± 0.37	7.09 ± 0.26	6.91 ± 0.27	0.157	0.000*
HC (cm)	33.78 ± 1.44	33.47 ± 1.33	32.03 ± 1.54	0.522	0.000*
Humerus (cm)	6.51 ± 0.30	6.47 ± 0.33	6.33 ± 0.36	0.381	0.009*
SCTT parameters					
SSTT (mm)	5.69 ± 1.47	6.34 ± 1.44	5.51 ± 1.60	0.307	0.574
MATA (cm^2)	6.65 ± 1.74	6.92 ± 1.50	6.38 ± 1.90	0.264	0.513
MTTA (cm^2)	12.00 ± 3.07	13.30 ± 3.47	10.19 ± 2.94	0.321	0.009*
ATT(mm)	6.18 ± 1.39	6.78 ± 1.27	5.74 ± 1.33	0.029*	0.151

SCTT=subcutaneous tissue thickness. MATA=mid-arm tissue area. MTTA=mid-thigh tissue area. SSTT=subscapular tissue thickness. ATT=abdominal tissue thickness. MANOVA= multivariate analysis of variance. *p<0.05

Table 6. Comparison of benchmark formulas, our formulas in estimating fetal weight in S1 and S2.

Formula	R^2		E(g) (mean ± SD)		AE(g) (mean ± SD)		PE (%) (mean ± SD)	
	S1	S2	S1	S2	S1	S2	S1	S2
Hsieh (a)	0.64	0.63	56.78±331.93	29.51±302.27	253.65±221.01	223.83±203.99	**1.72±9.97**	**1.02 ±8.69^{+}**
Hsieh (b)	0.64	0.64	48.08±325.10	30.61±297.80	248.71±214.32	231±188.94	**1.47±9.81**	**0.99±8.70^{+}**
Hadlock (a)	0.62	0.60	56.96±318.68	40.20±296.54	244.61±211.59	231.08±188.68	**1.90±9.61**	**1.53±8.70**
Hadlock (b)	0.61	0.60	49.80±327.44	14.08±297.75^{+}	247.40±219.74	222.02±197.55	**1.65±9.93**	**0.79±8.68^{+}**
Warsof (a)	0.56	0.46	188.87±323.76	170.67±351.44	293.96±232.17	319.73±222.78	**6.13±10.20**	**5.76±10.48**
Warsof (b)	0.64	0.64	-95.24±310.25	-110.26±288.14	256.35±198.52	228.70±206.03	**-2.90±9.32**	**-3.30±8.28**
Larciprete (a)	0.64	0.65	-9.85±257.86*	-15.34±250.12**	194.59±169.09*^	170.38±182.91**^	**0.31±7.95***	**0.18±7.58****
Larciprete (b)	0.66	0.68	-9.43±252.57*	-11.03±244.03**	192.28±163.64*^	165.82±178.55**^	**0.29±7.73***	**0.29±7.38****

EFW=estimated fetal weight; ABW=actual birth weight; E (mean error): EFW-ABW; AE (mean absolute error): |EFW-ABW|; PE (percentage error): (EFW-ABW)x100/ABW. *;** $p<0.05$ within-subject contrasts with respect to benchmark equations; $^{+}$within-subject contrasts p value>0.05 w.r.t. our two proposed equations. **^Greenhouse-Geisser corrected p values.**

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Single inherited thrombophilias and adverse pregnancy outcomes

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Abstract

Introduction: Inherited thrombophilia is believed to be a multiple gene disease with more than one defect. We aimed to determine the association between single thrombophilic patterns and a variety of pregnancy diseases.

Methods: 284 pregnant women were recruited for the present study and were divided in two groups: A group (176 controls) and B group (108 cases). Patients belonging to the B group had one of the following: severe pre-eclampsia, hemolysis, hepatic enzymes increase, hypertension and low platelet count (HELLP) syndrome, gestational hypertension, fetal growth restriction, intrauterine death, abruptio placentae and disseminated intravascular coagulopathy. To detect methylenetetrahydrofolate reductase (MTHFR) A1298C, MTHFR C677T, factor V Leiden, PAI-1, mutant prothrombin G20210A, an inverse hybridization technology was used. Plasma homocysteine, antithrombin (AT) III and protein S were determined. A modified functional activated protein C resistance was detected.

Results: MTHFR C677T and hyperhomocysteinemia were more prevalent than other thrombophilias. Deficiency in AT III was significantly linked with pre-eclampsia (relative risk 0.88; 95% CI 0.83–0.94). Activated protein C resistance (APCR) was significantly related to the abruptio placentae (relative risk 0.71; 95% CI 0.61–0.82).

Comments: Apart from the linkage between AT III deficiency and the occurrence of pre-eclampsia, and apart from the increased risk of abruptio placentae in pregnant women with altered APCR, we obtained findings in contrast with some of the published literature. In our case series, no association of pre-eclampsia with factor V Leiden or with prothrombin gene mutation was found.

Key words: pregnancy, thrombophilias.

Introduction

Inherited thrombophilia is believed to be a multiple gene disease with more than one defect, which explains why some women with thrombophilia never have a thrombotic event while others have complications. This condition is generated by specific point mutations (single-nucleotide polymorphism) including the factor V Leiden mutation (G1691A Factor V), the methylenetetrahydrofolate reductase (MTHFR) mutations (C677T MTHFR and A1298C MTHFR), the G20210A prothrombin (G20210A PTR) gene mutation, and the plasminogen activator inhibitor-1 mutant genotype (PAI-1 5G/5G). Other thrombophilias

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include deficiencies in antithrombin III (AT III), protein S, protein C, resistance to the activated protein C (APCR) and elevated homocysteinemia.[1]

Thrombophilias have been recently explored as a cause of placental thrombosis, severe pre-eclampsia/eclampsia, hemolysis, hepatic enzymes increase, hypertension and low platelet count (HELLP) syndrome, placental abruption, intrauterine growth restriction, unexplained stillbirth and recurrent miscarriage.[2]

The link between thrombophilia and certain pathologies of pregnancy may be an inadequate feto-placental circulation.[3]

The early pathogenesis of pre-eclampsia may be related to the normal spiral arteries failure to adapt by trophoblastic migration, leading to reduced placental blood flow. In late pregnancy, failure of trophoblast invasion of the fetoplacental vessels is found in normotensive and hypertensive pregnancies complicated by fetal growth restriction. Subsequently, widespread vascular endothelial cell dysfunction is thought to mediate the vasospasm and manifest as hypertension, which is a central feature of established pre-eclampsia.[4]

Kupferminc *et al.*,[3] showed further evidence of inherited and acquired thrombophilias, by means of a study led on Israeli women with serious pregnancy complications compared with healthy pregnant women.

Kupferminc also produced a recent review,[5] in which the topic is fully explained with a complete overview on the aspects of thrombophilia and adverse pregnancy outcomes. We undertook the present study believing that the more methods we find to control the pregnancy course, the more we will be able to reduce maternal and fetal damage.

The aim of the present study was to determine to what extent single inherited thrombophilias are associated with adverse obstetric complications correlated with fetoplacental insufficiency, such as pre-eclampsia, HELLP syndrome, gestational hypertension (GH), fetal growth restriction (FGR), intrauterine death (ID), abruptio placentae, and disseminated intravascular coagulopathy (DIC).

Methods

Patients selection

Since September 2002 until May 2005, pregnant women coming to our Obstetrics and Gynecology tertiary care unit of Fatebenefratelli Isola Tiberina (Rome) were enrolled consecutively in the present study at discharge from the hospital, ranging from 2 to 8 days after delivery. All these women delivered at the Department of Obstetrics and Gynecology of Fatebenefratelli Hospital, Isola Tiberina, Rome. Patients were retrospectively scheduled in two study groups, according to the absence (Group A, Controls) or presence (Group B, Cases) of any of the cited adverse pregnancy outcomes correlated with fetoplacental insufficiency such as severe pre-eclampsia, HELLP syndrome, GH, FGR, ID, abruptio placentae and DIC.[5]

Gestational age was calculated from the first day of the last menstrual period and confirmed by either a first- or second-trimester ultrasound scan. When the ultrasound determined gestational age differed from that calculated from the last menstrual period by >7 days in the first trimester, or by >10 days in the second trimester, the ultrasound-determined gestational age was used.

Inclusion criteria to be recruited within Group B were: HELLP syndrome, defined as severe pre-eclampsia complicated with hemolysis (total bilirubin >1.2 mg/dL, lactic dehydrogenase >600 U/L), hepatic enzymes increase (aspartate aminotransferase >70 U/L) and thrombocytopenia (platelets count <100 000/mm^3);[2] FGR, defined as neonatal weight <10° percentile;[2] placental abruption assessed clinically on the basis of antepartum uterine tenderness and vaginal bleeding and confirmed by inspection of the placenta at delivery;[2] ID defined as the delivery of a dead fetus after 24 weeks' gestation;[2] GH defined as an increase of systolic blood pressure of 30 mmHg or higher and/or increase of diastolic blood pressure of 15 mmHg or higher from average values before 20 weeks' gestation. In case of unknown prior values, two readings of 140/90 mmHg or higher on two different occasions more than 4 h apart, after 20 weeks' gestation;[6] DIC was defined according to Levi M *et al.*[7] The definition of pre-eclampsia was based on recommendations of the Consensus Report of the American Working Group on High Blood Pressure in Pregnancy and of the Working Group of the German Society of Obstetrics and Gynecology.[6] Diagnostic criteria were as follows:

1 Increase of systolic blood pressure of 30 mmHg or higher and/or increase of diastolic blood pressure of 15 mmHg or higher from average values before 20 weeks' gestation. In case of unknown prior values, two readings of 140/90 mmHg or higher on two different occasions more than 4 h apart.
2 Proteinuria defined as protein excretion of 0.3 g or more in a 24-h specimen or repeated dipsticks of 30 mg/dL (which correlates to 1+ dipsticks in

commercial kits) in two random urine specimens collected more than 4 h apart.

3 Onset of first symptoms beyond 20 weeks' gestation from last menstrual period and regression of symptoms after 6 weeks postpartum.

4 Absence of pre-existent hypertension, proteinuria, and edema, as well as diabetes, chronic kidney, hepatic, or vascular disease.

Pregnancies with fetal congenital anomalies and women with chronic hypertension, diabetes mellitus and pre-existing renal disease have been excluded from the recruitment. A peripheral venous blood sample has been taken from every recruited case. Women with combined thrombophilic defects were excluded from recruitment.

Patients with MTHFR (any type) mutant genotype (homo/heterozygous) or hyperhomocysteinemia (more than 12 μmol/L) were invariably instructed to ingest folates daily until delivery,[8] following an internal departmental treatment protocol.

Laboratory assay

To detect MTHFR A1298C, MTHFR C677T, factor V Leiden, PAI-1, mutant prothrombin G20210A, a commercially available kit was employed following the Manufacturer procedures (FV-Protrombina, MTHFR C677T/A1298C, HPA1a/b, APOB; Nuclear Laser Medicine, AC007, Milan, Italy).

Briefly, an inverse hybridization technology was used: DNA from EDTA plasma samples (maintained at –20°C) was isolated after lysis by means of a GenXtract resin as reported in the kit instructions. A subsequent simultaneous DNA amplification (multiplex) by Taq polymerase and amplification mix was obtained with 35 cycles in a thermocycler (GeneAmp PCR System 2400; Perkin Elmer, Milan, Italy).

The amplified fragments were hybridized on a membrane with different allele-specific probes linked to Biotin. Biotin is subsequently revealed by streptavidine-conjugated alkaline phosphatase exposed to an appropriately colored substrate. This colorimetric reaction allows detection of the presence of mutations.

Plasma homocysteine determinations were done by a commercially available kit used following the manufacturer's instructions: Axis Homocysteine EIA (Axis-Shield Diagnostics Ltd, Dundee, Scotland, UK). Intra-CV and inter-CV were less than 6% and 12%, respectively. The normal reference range was 5–12 μmol/L and sensitivity = 1 μmol/L.

Antithrombin III, amydolytic and immunologic (Behring, Marburg, Germany) and total and free (ELISA; Diagnostica Stago, Asniéres, France) protein S antigen were determined in all subjects, as reported in previous studies.[9,10]

Inter- and intra-assay coefficients of all the variables never exceeded 8.0 and 5.0%, respectively.

A modified functional activated protein C resistance was detected using factor V-deficient plasma (Coatest activated protein C resistance-V; Chromogenix, Goteborg, Sweden), as previously described.[11]

Informed consent to use the data for this work was obtained by the outpatients included in this study as approved by the ethical Committee of the FBF Hospital.

Statistical analysis

Homozygous mutations were taken into consideration for final conclusions.

Student's *t*-test was used to assess differences between groups; the Cochran-Mantel-Haenszel method was used for adjusted (personal and family medical history, past obstetrical anamnesis) relative risk estimation and 95% CI calculations. An alpha of 0.05 or less was considered to be statistically significant. All statistical analyses were performed using SPSS 13 for Windows.

Results

Characteristics of the study groups are summarized in Table 1. There were no differences between the two groups regarding maternal age and pregravidic BMI, but significant differences were noted in the gestational age at delivery (39 ± 2 weeks vs. 36 ± 3 weeks, $P = 0.065$), for the birthweights (3325 ± 398 g vs. 2770 ± 458 g, $P = 0.044$) and for the gestational weeks at enrolment (39 ± 2 weeks vs. 29 ± 7 weeks, $P = 0.037$).

At the beginning of the study, 300 pregnant women were enrolled. Sixteen were excluded: 10 had double or triple thrombophilic patterns, three had chronic hypertension, one patient had a fetus with congenital hearth abnormalities, two patients had type 1 diabetes mellitus. Therefore, the 284 subjects enrolled for the final study were divided in two study groups according to the absence (Group A, 176 subjects) or presence (Group B, 108 subjects) of any of the cited adverse pregnancy outcomes correlating with fetoplacental insufficiency such as pre-eclampsia, HELLP syndrome, GH, FGR, ID, abruptio placentae and DIC. Pregnancy outcomes

Table 1 Characteristics of the study groups. Student's *t*-test comparison

	Group A $n = 176$	Group B $n = 108$	*P*-values
Age (years)	33.2 ± 5.1	33.6 ± 3.2	0.424
Pregravidic BMI	24.4 ± 3.1	25.2 ± 2.8	0.189
GA at delivery (weeks)	39 ± 2	36 ± 3	0.065
Birthweight (g)	3325 ± 398	2770 ± 458	0.044
GA at enrolment (weeks)	39 ± 2	29 ± 7	0.037

BMI, body mass index; GA, gestational age.

Table 2 Distribution of the normal and pathological pregnancy outcomes

Outcome	*n*	Frequency (%)
Normal pregnancies	176	61.97
Intrauterine death	5	1.76
Gestational hypertension	25	8.88
Pre-eclampsia	7	2.46
HELLP syndrome	14	4.92
Fetal Growth Restriction	39	13.73
Abruptio placentae	16	5.63
DIC	2	0.70

HELLP, hemolysis, hepatic enzymes increase, hypertension and low platelet count syndrome; DIC, disseminated intravascular coagulopathy.

Table 3 Distribution of the thrombophilic patterns

Outcome	*n*	Frequency (%)
AT III deficiency	12	4.44
S protein deficiency	1	0.37
Activated C-Prot. Res.	46	17.03
Hyper-HCY	18	6.66
MTHFR C677T		
+/–	91	33.70
+/+	63	23.33
MTHFR A1298C		
+/–	2	0.74
+/+	5	1.85
G20210A PTR		
+/–	1	0.37
+/+	2	0.74
G1691A factor V		
+/–	14	5.18
+/+	2	0.74
PAI-1 (5G/5G)		
+/–	10	3.70
+/+	7	2.59

AT III, antithrombin III; MTHFR, methylenetetrahydrofolate reductase; PAI, plasminogen activator inhibitor; G20210A PTR, single-nucleotide polymorphism of prothrombin; G1691A factor V, factor V Leiden; hyper-HCY, hyperhomocysteinemia.

are described in Table 2, with FGR the most representative adverse pregnancy outcome of our series.

The occurrence of the single thrombophilic genotypes had a wide variation and the incidence of MTHFR C677T heterozygous or homozygous pattern and the appearance of hyperhomocysteinemia were greater than observed for the other thrombophilic subtypes (Table 3). In our series, we didn't observe cases with MTHFR mutations (whichever) combined with hyperhomocysteinemia.

Relative risks of adverse pregnancy outcomes were computed for women with thrombophilic patterns and are summarized in Table 4, which shows, for each adverse pregnancy outcome, the number of affected subjects with a single thrombophilic mutation with the relative risk and 95% confidence intervals.

Deficiency in AT III was found to be significantly linked to the occurrence of pre-eclampsia (six cases with ATIII deficiency over 7, with a relative risk 0.88; 95% CI 0.83–0.94). ACPR was found to be strictly related to the abruptio placentae (11 cases over 16 with ACPR, relative risk 0.71; 95% CI 0.61–0.82).

We didn't find an increased incidence of adverse pregnancy outcomes in subjects with S protein deficiency, hyperhomocysteinemia, C677T or A1298C MTHFR homozygous mutation, G20210A prothrombin homozygous mutation, G1691A factor V homozygous mutation, PAI-1 homozygous mutation.

Comments

As pre-eclampsia is associated with vascular and endothelial damage, which in turn is linked to coagulation problems, congenital thrombophilias may play an important role in this pathology.

For blood fluidity and wall repair, the placental vessels, like those at other sites, must maintain equilibrium between the procoagulant and anticoagulant mechanisms: an imbalance may lead to placental infarction on one or both of the placental sides.[12]

Table 4 Relative risk of adverse pregnancy outcomes in women with thrombophilic patterns

Outcome	ID	GH	PE	HELLP	FGR	Abruptio	DIC
			Cases with single mutation Relative Risk (95% CI)				
AT III deficiency	0	2	6	0	2	0	2
	–	0.64 (0.35–0.75)	0.88 (0.83–0.94)*	–	0.64 (0.42–0.81)	–	0.57 (0.76–1.06)
S protein deficiency	0	0	0	0	1	0	0
	–	–	–	–	0.65 (0.46–0.75)	–	–
Act. C-Prot. Res.	1	4	0	3	12	11	0
	0.72 (0.35–0.86)	0.87 (0.75–0.92)	–	0.65 (0.31–0.81)	0.81 (0.61–0.97)	0.71 (0.61–0.82)*	–
Hyper-HCY	0	3	0	2	6	2	0
	–	0.71 (0.56–0.84)	–	0.88 (0.78–0.94)	0.67 (0.45–0.76)	0.54 (0.35–0.75)	–
C677T MTHFR (+/+)	1	4	1	2	9	3	0
	0.81 (0.43–0.91)	0.54 (0.37–0.67)	0.71 (0.60–0.86)	0.84 (0.74–0.96)	0.76 (0.67–0.91)	0.58 (0.39–0.71)	–
A1298C MTHFR (+/+)	0	2	0	0	3	0	0
	–	0.91 (0.57–0.97)	–	–	0.70 (0.59–0.91)	–	–
G20210A PTR (+/+)	0	0	0	1	1	0	0
	–	–	–	0.81 (0.73–0.91)	0.75 (0.63–0.86)	–	–
G1691A factor V (+/+)	0	1	0	0	1	0	0
	–	0.63 (0.53–0.81)	–	–	0.68 (0.54–0.75)	–	–
PAI-1 (5G/5G) (+/+)	1	3	0	1	2	0	0
	0.86 (0.63–0.93)	0.81 (0.71–0.94)	–	0.72 (0.60–0.84)	0.68 (0.56–0.75)	–	–

*$P < 0.05$. AT III, antithrombin III; MTHFR, methylenetetrahydrofolate reductase; PAI, plasminogen activator inhibitor. Act C-Prot. Res, activated C-protein Resistance; Abn. Factor VIII, abnormal factor VIII; G20210A PTR, single-nucleotide polymorphism of prothrombin (PTR); G1691A factor V, factor V Leiden; hyper-HCY, hyperhomocysteinemia.

A reason for this finding may be that the low pressure intervillous blood flow in the presence of a maternal hypercoagulable state might trigger fibrin deposition in the placenta and cause placental infarcts that might incite development of severe disease. This could also be an explanation for the observation that women with pre-eclampsia and thrombophilia had higher rates of FGR compared with women with pre-eclampsia but without thrombophilia. According to the placental physiopathologic studies, a trophoblastic invasion defect during the first phase of the pregnancy and a normal placental neoangiogenic failure indicate an early predetermination of pregnancy outcome. When the defect is massive and premature, abortion may occur during the first trimester; whilst after week 20, pathologies like pre-eclampsia, FGR and ID occur.[2]

The mechanism is thought to be thrombophilia related fetoplacental insufficiency due to a compromised vascular support system.[13]

The factor V Leiden mutation results from a substitution of adenine for the normal guanine at the 1691 position of the factor V gene. As a result, factor V becomes resistant to cleavage by activated protein C. The factor V heterozygote is present in approximately 5.2% of white Americans and in 1.2% of African-Americans.[14] In our series it was not linked to any adverse pregnancy outcome as a single homozygous gene mutation.

Protein S deficiency is an autosomal dominant disorder that exposes the fetus to an increased risk of thromboembolism.

Protein S is a vitamin K-dependent plasma protein cofactor that is necessary along with activated protein C cofactor to inactivate factors Va and VIIIa and control the balance between coagulation and anticoagulation. Sanson *et al*.[15] showed that the relative risk of abortion and stillbirth per pregnancy for women with protein S, protein C, and antithrombin deficiencies was 2.0 times greater (95% CI 1.2–3.3) than in non-deficient women. Again, in our series, the S protein deficiency did not lead to any of the studied adverse pregnancy outcomes, but the small number of cases needs to be taken into consideration.

Another significant cause of thrombophilia in pregnancy is APCR.[16] Inherited APCR is an autosomal dominant disorder and is one of the most common forms of inherited thrombophilic disorders, with a prevalence of 5% in the general population.[17,18] In the majority of cases, APCR is due to a point mutation in the factor V gene, which prevents protein C from inactivating active factor V.[16] In our series, this thrombophilia had a frequency of 18.3% and was significantly linked to the abruptio placentae, being present in 10 of 16 cases.

Inherited hyperhomocystinemia is another cause of thrombophilia that results from genetic defects in methionine and homocysteine metabolism, which leads to recurrent venous thrombosis.

The human MTHFR gene, which is located on chromosome 1p36, belongs to the proposed candidate loci for pre-eclampsia.[19] The MTHFR gene is critical in the metabolism of homocysteine because the reaction catalyzed by MTHFR is a rate-limiting step in the folate cycle and can be affected by an individual's folate status.

A common missense mutation at nucleotide 677, which substitutes a valine for an alanine residue, has been associated with increased circulating levels of homocysteine caused by decreased enzyme activity in C677T homozygotes and heterozygotes.[20] Hyperhomocystinemia can induce vascular injury, increase platelet consumption, and can result in thrombosis caused by increased oxidative stress. Clinically, hyperhomocystinemia caused by the C677T mutation has been implicated in premature cardiovascular disease,[21] venous thrombosis,[22] and more recently in adverse pregnancy outcomes, especially pre-eclampsia.[19] However, the majority of follow-up studies failed to reconfirm a significant disease association with pre-eclampsia.[23–25] Because the frequency of the C677T allele underlies significant population-specific differences, it was proposed that variations in the relative contribution of disease alleles in different populations might explain the discrepant results of previous studies on MTHFR and pre-eclampsia.[26] This topic could explain why in our series we didn't observe any significant association between MTHFR homozygous mutations and adverse pregnancy outcomes, despite the high incidence of thrombophilias in our study population.

Recently, a second common mutation in the MTHFR gene has been described; an adenine-to-cytosine substitution at base 1298 (A1298C). This mutation also results in decreased MTHFR activity but is not associated with higher plasma homocysteine concentration or lower plasma folate concentration.[27]

Hernandez-Diaz[28] and Glanville[29] have recently showed a strong link between MTHFR mutations, hyperhomocysteinemia and both pre-eclampsia and intrauterine growth restriction.

In our series neither the C677T MTHFR nor the A1298C MTHFR was associated with adverse

pregnancy outcomes. Apart from the above cited considerations,[26] we also speculate that the oral folate supplementation could decrease the incidence of both hyperhomocysteinemia and adverse pregnancy outcomes in these patients.

Plasminogen activators (PA) induce changes in the fibrinolytic system that convert plasminogen to plasmin. During pregnancy, the anti-clotting activity of PA is kept in check by two plasminogen activator inhibitors, one of which (PAI-1) is endothelial cell related and the other (PAI-2) is produced in placental tissue.[30]

The concentrations of both PAI-1 and PAI-2 increase during pregnancy[31] to ensure hemostasis during labor and delivery. The mutant PAI-1 5G/5G homozygous genotype may be involved in the development of intrauterine growth restriction (IUGR) as well as pre-eclampsia.[32,33] Even in this case, we were unable to detect any correspondence between this thrombophilic pattern and adverse pregnancy outcomes.

Presumably, a similar increase in coagulation may be responsible for the increased incidence of miscarriage and fetal demise seen in protein C, S and ATIII deficient patients.[34,35]

Gerhardt *et al.* presents a case-control design, studying 97 women with severe pre-eclampsia in previous pregnancies and 277 normal women, to assess hereditary risk factors of venous thrombosis as risk determinants for severe pre-eclampsia. In this research, the onset of severe pre-eclampsia was significantly earlier in women with the G20210A prothrombin gene mutation (24.5 weeks vs. 30.1 weeks, $P = 0.046$) and in women with the PAI-15G-5G genotype (25.7 weeks vs. 30.8 weeks, $P = 0.024$), showing that these risk factors do not induce the pathomechanism but accelerate the course of pre-eclampsia.[36]

In a recent prevalence study on 200 patients, the G20210A single-nucleotide polymorphism of prothrombin was shown to be unrelated to recurrent spontaneous abortions, whereas factor V Leiden, along with APCR, and the combination of both, were seen in women with idiopathic recurrent pregnancy loss, suggesting a close link to this pathology.

A part from the link between AT III deficiency and the occurrence of pre-eclampsia, we obtained findings in contrast with Gerhardt *et al.*[36] In our case series, we had no association of pre-eclampsia with Factor V Leiden or with prothrombin gene mutation.

Tranquilli *et al.* stated that multiple thrombophilic factors carry a major additional risk of adverse maternal and fetal outcomes and correlate well with maternal mal-adaptation to pregnancy.[2] But our study deals with single thrombophilias and is poorly comparable with others. Moreover, it is important to underline that in our study we had no subject with recurrent spontaneous miscarriages because our study population was enrolled within a tertiary care unit and not within an outpatient clinic.

The main result of our research was that, excluding multiple gene mutation, single thrombophilias have to be carefully taken into consideration, but without generating unjustified fears about pregnancy.

Further studies are needed to check the link between thrombophilic gene mutations and adverse pregnancy outcomes, such as recurrent miscarriages and deep venous thrombosis. Eventually, we believe that increasing the number of the studied population and introducing the analysis of multiple gene mutations may help us in adding a more comprehensive contribution to this promising research field. Some years ago, these arguments begun to circulate in the published literature with great interest. After a few years, the increasing number of contrasts on this topic leads us to be more careful when dealing with thrombophilias.

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Thrombophilias and Pregnancy Complications: A Case-Control Study

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ABSTRACT

Inherited thrombophilia is believed to be a multiple gene disease with more than one defect. We wanted to determine the association between single thrombophilic patterns and a variety of pregnancy diseases. 301 pregnant women were recruited for the present case-control study and were divided into two groups: A group (176 controls) and B group (125 cases). Patients belonging to the B group had one of the following: severe preeclampsia, HELLP syndrome, gestational hypertension, fetal growth restriction (FGR), intrauterine death, abruptio placentae, placenta previa, disseminated intravascular coagulopathy (DIC) and preterm labour. To detect MTHFR A1298C, MTHFR C677T, Factor V Leiden, PAI-1, Mutant Prothrombin G20210A, an inverse hybridization technology was used. Plasma homocysteine, Antithrombin III and protein levels S were determined. A modified functional activated protein C resistance was assayed. MTHFR C677T and hyperhomocysteinemia were more numerous than other thrombophilias. Deficiency in AT III was significantly linked with preeclampsia (Pearson Index and p value: 0.131 and 0.022, respectively) and disseminated intravascular coagulopathy (Pearson Index and p value: 0.138 and 0.016 respectively). Activated Protein C resistance was related to abruptio placentae (Pearson Index and p value: 0.159 and 0.005 respectively). Apart from the linkage between AT III deficiency and the occurrence of preeclampsia and disseminated intravascular coagulopathy, we obtained findings in contrast to some literature. In our case series, no association of preeclampsia with Factor V Leiden or with prothrombin gene mutation was found.

Keywords: thrombophilias; pregnancy; Antithrombin III deficiency; preeclampsia; disseminated intravascular coagulation

INTRODUCTION

Inherited thrombophilia is believed to be a multiple gene disease with more than one defect, which explains why some women with thrombophilia never have a thrombotic event, whereas others have complications. This condition is generated by specific point mutations (single-nucleotide polymorphism) including the factor V Leiden mutation (G1691A Factor V), the methylenetetrahydrofolate reductase (MTHFR) mutations (C677T MTHFR and A1298C MTHFR), the G20210A prothrombin (G20210A PTR) gene mutation, and the plasminogen activator inhibitor-1 mutant genotype (PAI-1 5G/5G). Other thrombophilias incude deficiencies in antithrombin III (AT III),

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protein S, protein C, resistance to the activated protein C (APCR) and elevated homocysteinemia (1).

Thrombophilias have been recently explored as cause of placental thrombosis, severe preeclampsia/eclampsia, HELLP syndrome, placental abruption, intrauterine growth restriction, unexplained stillbirth and recurrent miscarriage (2).

The link between thrombophilia and certain pathologies of pregnancy may be an inadequate feto-placental circulation (3).

Kupferminc et al. (3), showed a further evidence of inherited and acquired thrombophilias, by means of a study on Israeli women with serious pregnancy complications compared with healthy pregnant women.

AIM OF THE STUDY

The aim of the present study was to determine to what extent single inherited thrombophilias are associated with adverse obstetric complications correlated with uteroplacental insufficiency such as preeclampsia, HELLP syndrome, gestational hypertension, fetal growth restriction (FGR), intrauterine death, abruptio placentae, placenta previa, disseminated intravascular coagulopathy (DIC) and preterm labour.

METHODS

Patients' selection

Since September 2002 until May 2005, pregnant women coming to our Obst/Gyn tertiary care unit of Fatebenefratelli Isola Tiberina (Rome) were enrolled consecutively in the present prospective case-control study, at the entrance. All these women delivered at the Department of Obstetrics and Gynecology of Fatebenefratelli Hospital, Isola Tiberina, Rome. Patients were scheduled in two study groups, according to the absence (Group A, Controls) or presence (Group B, Cases) of any of the cited adverse pregnancy outcomes correlated with uteroplacental insufficiency such as severe preeclampsia, HELLP syndrome, gestational hypertension, fetal growth restriction (FGR), intrauterine death, abruptio placentae, placenta previa, disseminated intravascular coagulopathy (DIC) and preterm labour.

Inclusion criteria to be recruited within the Group B were: HELLP syndrome, defined as severe preeclampsia complicated with hemolysis (total bilirubin >1.2 mg/dl, lactic dehydrogenase >600 U/l), hepatic enzymes increase (aspartate aminotransferase >70 U/l) and thrombocytopenia (platelets count <100,000/mm3) (2); fetal growth restriction (FGR), defined as neonatal weight <10° percentile (2); placental abruption assessed clinically on the basis of antepartum uterine tenderness and vaginal bleeding and confirmed by inspection of the placenta at delivery (2); intrauterine death (ID) defined as the delivery of a dead fetus after 24 weeks of gestation (2); gestational hypertension (GH) defined as an increase of systolic blood pressure of 30 mmHg or higher and/or increase of diastolic blood pressure of 15 mmHg or higher from average values before 20 weeks' gestation. In case of unknown prior values, two readings of 140/90 mmHg or higher on two different occasions more than 4 hours apart (4); preterm labour (PL) defined as delivery before 37 weeks'gestation; previa, defined when the chorionic plate lies just upon the inner uterine orifice after 30 weeks' gestation (ultrasound transvaginal scan); disseminated intra-vascular coagulopathy (DIC) was defined according to Levi M et al (5); definition of preeclampsia was based on recommendations of the Consensus Report of the American Working Group on High Blood Pressure in Pregnancy and of the Working Group of the German Society of Obstetrics and Gynecology (4). Diagnostic criteria were as follows:

- Increase of systolic blood pressure of 30 mm Hg or higher and/or increase of diastolic blood pressure of 15 mm Hg or higher from average values before 20 weeks' gestation. In case of unknown prior values, two readings of 140/90 mm Hg or higher on two different occasions more than 4 hours apart, is considered diagnostic of preeclampsia
- Proteinuria is defined as protein excretion of 0.3 g or more in a 24-hour specimen or repeated dipsticks of 30 mg/dL (which correlates to 1 + dipsticks in commercial kits) in two random urine specimens collected more than 4 hours apart.
- Onset of first symptoms beyond 20 weeks' gestation from last menstrual period and regression of symptoms after 6 weeks postpartum.
- Absence of preexistent hypertension, proteinuria, and edema, as well as diabetes, chronic kidney, hepatic, or vascular disease.

Pregnancies with fetal congenital anomalies and women with chronic hypertension, diabetes mellitus and preexisting renal disease were excluded from the recruitment. A peripheral venous blood sample was taken from every recruited case. Women with combined thrombophilic defects were excluded from the recruitment.

Patients with MTHFR (any type) mutant genotype (homo/heterozygous) or hyperhomocysteinemia (more

than 12 mol/L) were invariably instructed to ingest folates daily until delivery (6), following an internal departmental treatment protocol.

Laboratory assay

To detect MTHFR A1298C, MTHFR C677T, Factor V Leiden, PAI-1, Mutant Prothrombin G20210A, a commercially available kit was employed following the manufacturer procedures (FV-Protrombina, MTHFR C677T/A1298C, HPA1a/b, APOB; Nuclear Laser Medicine, AC007, Milan, Italy).

Briefly, an inverse hybridization technology is used: DNA from EDTA blood samples (maintained at –20°C) was isolated after lysis by means of a GenXtract resin, as reported in the kit instructions. A subsequent simultaneous DNA amplification (multiplex) by Taq polymerase and amplification mix was obtained with 35 cycles in thermocycler (GeneAmp PCR System 2400 of the Perkin Elmer, Milan, Italy).

The amplified fragment was hybridized on a membrane with different allele-specific probes linked to Biotin. Biotin is subsequently revealed by streptavidine-conjugated alkaline phosphatase exposed to appropriate coloured substrate. This colorimetric reaction allows the detection of mutations.

Plasma homocysteine determinations were done by a commercially available kit (Axis Homocysteine EIA, Axis-Shield Diagnostics Ltd, The Technology Park, and Dundee DD2 1Xa, UK) used following the manufacturer's instructions: Intra-CV and inter-CV were less than 6% and 12%, respectively. The normal reference range is 5-12 μmol/L and the sensitivity is 1 μmol/L.

Antithrombin III, amydolytic and immunologic (Behring, Marburg, Germany) and total and free (ELISA; Diagnostica Stago, Asniéres, France) protein S antigen were determined in all subjects, as reported elsewhere (7, 8).

Inter- and intra-assay coefficients of all the variables never exceeded 8.0 and 5.0%, respectively.

A modified functional activated protein C resistance was detected using factor V-deficient plasma (Coatest activated protein C resistance-V; Chromogenix; Goteborg, Sweden), as previously described (9).

Informed consent to utilize the data for this work was obtained by the outpatients included in this study. The study was approved by the ethical committee of the Fatebenefratelli Hospital.

Statistical analysis

For a power of 95% and a type I error of 0.01 we needed 100 patients per group, and, for a power of 90% and a Type I error of 0.001 we needed 110 patients (10). Homozygous mutations were taken into consideration for final conclusions.

Student T test was used to assess differences between groups and Pearson correlation 2-tailed method was used to check correlations between thrombophilic patterns and the occurrence of adverse pregnancy outcomes.

RESULTS

Characteristics of the study groups are summarized in table 1. There were no differences between the two groups regarding maternal age and pregravidic BMI (Body Mass Index), but significant differences were noted about the gestational age at delivery (39 ± 2 weeks vs 35 ± 4 weeks, p=0.045), for the birthweights (3325 ± 398 g vs 2560 ± 256 g, p=0.034) and for the gestational weeks at the enrollement (39 ± 2 weeks vs 26 ± 8 weeks, p=0.024).

Three hundred eighteen pregnant women were enrolled in the present study. Seventeen out of them were excluded: 10 had double or triple thrombophilic patterns, 3 had chronic hypertension, 1 patient had a fetus with congenital cardiac abnormalities, 3 patients had type 1 diabetes mellitus. Therefore the 301 subjects enrolled for the final study were divided into two study groups according to the absence (Group A, 176 subjects) or presence (Group B, 125 subjects) of any of the cited adverse pregnancy outcomes correlated with uteroplacental insufficiency such as preeclampsia, HELLP syndrome, gestational hypertension, fetal growth restriction (FGR), intrauterine death, abruptio placentae, placenta previa, disseminated intravascular coagulopathy (DIC) and preterm labour. Pregnancy outcomes are described in table 2, being the fetal growth restriction the most representative adverse pregnancy outcome of our series.

Table 1. Characteristics of the study groups. Student t-test comparison

	Group A n. 176	Group B n. 125	p-values
Age (years)	33.2 ± 5.1	34.7 ± 4.1	0.124
Pre-pregnancy BMI	24.4 ± 3.1	25.0 ± 3.3	0.235
G.A. at delivery (weeks)	39 ± 2	35 ± 4	0.045
Birthweight (g)	3325 ± 398	2560 ± 256	0.034
G.A. at enrollment (weeks)	39 ± 2	26 ± 8	0.024

G.A., gestational age.

Table 2. Distribution of the normal and pathological pregnancy outcomes

Outcome	N.	Frequency (%)
Normal pregnancies	176	58.80
Intrauterine death	5	1.64
Gestational hypertension	25	8.22
Preeclampsia	7	2.30
HELLP syndrome	14	4.60
Fetal Growth Restriction	39	12.82
Abruptio placentae	16	5.26
Previa	10	3.28
DIC	2	0.65
Preterm labour	7	2.43

The occurrence of the single thrombophilic genotypes had a wide variation and the incidence of MTHFR C677T heterozygous or homozygous pattern and the appearance of hyperhomocysteinemia were greater than that observed for the other thrombophilic subtypes (Table 3). In our series we didn't observe cases with either MTHFR mutations combined with hyperhomocysteinemia.

Correlations between thrombophilic patterns and adverse pregnancy outcomes are summarized in table 4, showing, per each adverse pregnancy outcome, the number of affected subjects with a single thrombophilic mutation, with the relative Pearson correlation coefficient and significance.

Table 3. Distribution of the thrombophilic patterns

Outcome	*N.*	*Frequency (%)*
AT III deficiency	12	4.15
Protein S deficiency	1	0.34
APCR	53	18.33
Hyper-HCY	20	6.92
MTHFR C677T		
+/-	91	31.48
+/+	69	23.87
MTHFR A1298C		
+/-	2	0.69
+/+	5	1.73
G20210A PTR		
+/-	1	0.34
+/+	2	0.69
G1691A factor V		
+/-	14	4.84
+/+	2	0.69
PAI-1 (5G/5G)		
+/-	10	3.46
+/+	7	2.42

AT III, antithrombin III; PAI, plasminogen activator inhibitor; MTHFR, methylenetetrahydrofolate reductase; G20210A PTR, single-nucleotide polymorphism of prothrombin (PTR); G1691A factor V, factor V Leiden; hyper-HCY, hyperhomocysteinemia; APCR, activated protein C resistance.

Deficiency in AT III was found to be significantly linked with the occurrence of preeclampsia (Six of seven cases with ATIII deficiency, Pearson Index and p value: 0.131 and 0.022, respectively) and disseminated intravascular coagulopathy (Two of two cases with ATIII deficiency, Pearson Index and p value: 0.138 and 0.016, respectively). Activated Protein C resistance was found to be strictly related to the abruptio placentae (Eleven of sixteen cases with Activated protein C Resistance, Pearson Index and p value: 0.159 and 0.005, respectively).

We didn't find an increased incidence of adverse pregnancy outcomes in subjects with protein S deficiency, hyperhomocysteinemia, C677T or A1298C MTHFR homozygous mutation, G20210A Prothrombin homozygous mutation, G1691A factor V homozygous mutation, PAI-1 homozygous mutation.

COMMENTS

Since preeclampsia is associated with vascular and endothelial damage, which in turn is linked to coagulation problems, congenital thrombophilias may play an important role in this pathology.

For blood fluidity and wall repair, the placental vessels, like those at other sites, must maintain equilibrium between the procoagulant and anticoagulant mechanisms: an imbalance may lead to placental infarction on one or both of the placental sides (11).

The factor V Leiden mutation results from a substitution of adenine for the normal guanine at the 1691 position of the factor V gene. As a result, factor V becomes resistant to cleavage by activated protein C. The factor V heterozygote is present in approximately 5.2% of white Americans and in 1.2% of African-Americans (12). In our series it was not linked to any adverse pregnancy outcome, as a single homozygous gene mutation.

Protein S deficiency is an autosomal dominant disorder that exposes the fetus to an increased risk of thromboembolism. Protein S is a Vitamin K-dependent plasma protein cofactor that is necessary along with activated protein C cofactor to inactivate factors Va and VIIIa and control the balance between coagulation and anticoagulation. Sanson et al. (13) showed that the relative risk of abortion and stillbirth per pregnancy for women with protein S, protein C,

Table 4. Correlations between thrombophilic patterns and adverse pregnancy outcomes

Outcome	*ID*	*GH*	*PE*	*HEELP*	*FGR*	*PL*	*Abruptio*	*Previa*	*DIC*
	Cases with single mutation Pearson correlation 2-tailed significance								
AT III deficiency	0	2	6	0	2	0	0	0	2
	-0.035	-0.034	0.131	0.002	0.012	-0.042	0.052	-0.050	0.138
	0.541	0.551	0.022	0.972	0.837	0.468	0.366	0.383	0.016
Protein S deficiency	0	0	0	0	1	0	0	0	0
	-0.024	-0.055	-0.028	-0.041	-0.016	-0.028	-0.043	-0.034	-0.015
	0.679	0.337	0.623	0.481	0.786	0.623	0.45	0.555	0.794
APCR	1	4	0	3	12	3	11	4	0
	-0.028	-0.066	-0.034	-0.048	0.010	-0.034	0.159	-0.041	-0.018
	0.622	0.253	0.558	0.402	0.868	0.558	0.005	0.481	0.756
Hyper-HCY	0	3	0	2	6	0	2	2	0
	0.046	-0.016	-0.050	0.089	0.110	-0.050	-0.026	-0.060	-0.026
	0.424	0.785	0.386	0.122	0.056	0.386	0.647	0.298	0.122
C677T MTHFR (+/+)	1	4	1	2	9	2	3	4	0
	-0.061	0.039	-0.084	-0.041	0.131	-0.084	-0.023	-0.041	0.044
	0.286	0.493	0.144	0.478	0.022	0.144	0.695	0.477	0.448
A1298C MTHFR (+/+)	0	2	0	0	3	0	0	0	0
	-0.032	-0.041	-0.038	0.033	0.070	0.146	-0.017	-0.046	-0.020
	0.576	0.476	0.506	0.563	0.224	0.011	0.762	0.425	0.725
G20210A PTR (+/+)	0	0	0	1	1	0	0	0	0
	-0.027	-0.063	-0.032	0.016	-0.002	-0.032	0.009	0.108	-0.017
	0.638	0.275	0.576	0.775	0.971	0.576	0.872	0.059	0.767
G1691A factor V (+/+)	0	1	0	0	1	0	0	0	0
	-0.038	-0.089	-0.046	-0.065	0.029	-0.046	0.102	-0.001	-0.024
	0.505	0.122	0.428	0.257	0.611	0.428	0.077	0.985	0.675
PAI-1 (5G/5G) (+/+)	1	3	0	1	2	0	0	0	0
	0.005	-0.035	-0.054	-0.016	0.018	-0.054	-0.054	0.007	-0.029
	0.934	0.54	0.350	0.779	0.755	0.350	0.348	0.906	0.620

AT III, antithrombin III; PAI, plasminogen activator inhibitor; MTHFR, methylenetetrahydrofolate reductase. APCR, activated C-protein Resistance; G20210A PTR, single-nucleotide polymorphism of prothrombin (PTR); G1691A factor V, factor V Leiden; hyper-HCY, hyperhomocysteinemia.

and antithrombin deficiencies was 2.0 times greater (95% CI 1.2–3.3) than in non-deficient women. Again, in our series, the S protein deficiency did not lead to any of the studied adverse pregnancy outcomes, with the exclusion of abortion, not studied in this case-series.

Another significant cause of thrombophilia in pregnancy is activated protein C resistance (APCR) (14). Inherited APCR is an autosomal dominant disorder and is one of the most common forms of inherited thrombophilic disorders, with a prevalence of 5% in the general population (15, 16). In the majority of cases, APCR is due to a point mutation in the factor V gene, which prevents protein C from inactivating active factor V (14). In our series this thrombophilia had a frequency of 18.3% and was significantly linked to the abruptio placentae, being present 10 of 16 cases.

Inherited hyperhomocystinemia is another cause of thrombophilia that results from genetic defects in methionine and homocysteine metabolism, which leads to recurrent venous thrombosis. The human methylenetetrahydrofolate reductase (MTHFR) gene, which is located on chromosome 1p36, belongs to the proposed candidate loci for preeclampsia (17). The MTHFR gene is critical in the metabolism of homocysteine because the reaction catalyzed by MTHFR is a rate-limiting step in the folate cycle and can be affected by an individual's folate status.

A common missense mutation at nucleotide 677, which

substitutes a valine for an alanine residue, has been associated with increased circulating levels of homocysteine caused by decreased enzyme activity in C677T homozygotes and heterozygotes (18). Hyperhomocystinemia can induce vascular injury, increase platelet consumption, and can result in thrombosis caused by increased oxidative stress. Clinically, hyperhomocystinemia caused by the C677T mutation has been implicated in premature cardiovascular disease (19), venous thrombosis (20), and more recently in adverse pregnancy outcome, especially preeclampsia (17). However, the majority of follow-up studies failed to reconfirm a significant disease association with preeclampsia (21, 22, 23). Because the frequency of the C677T allele underlies significant population-specific differences, it was proposed that variations in the relative contribution of disease alleles in different populations might explain the discrepant results of previous studies on MTHFR and preeclampsia (24). This could explain why in our series we didn't observe any significant association between MTHFR homozygous mutations and adverse pregnancy outcomes, despite the high incidence of this thrombophilias in our study population.

Recently, a second common mutation in the MTHFR gene has been described, an adenine-to-cytosine substitution at base 1298 (A1298C). This mutation also results in decreased MTHFR activity but is not associated with higher plasma homocysteine concentration or lower plasma folate concentration (25).

Hernandez-Diaz (26) and Glanville (27) have recently showed a tight linkage between MTHFR mutations, hyperhomocysteinemia and both preeclampsia and intrauterine growth restriction.

In our series nor the C677T MTHFR neither the A1298C MTHFR was associated with adverse pregnancy outcomes. Apart from the above cited considerations (24), we also speculate that oral folate supplementation could decrease the incidence of both hyperhomocysteinemia and adverse pregnancy outcomes in these patients.

Plasminogen activators (PA) induce changes in the fibrinolytic system that convert plasminogen to plasmin. During pregnancy, the anticlotting activity of PA is kept in check by two plasminogen activator inhibitors, one of which (PAI-1) is endothelial cell related and the other (PAI-2) is produced in placental tissue (28).

The concentrations of both PAI-1 and PAI-2 increase during pregnancy (29) in order to ensure hemostasis during labor and delivery. The mutant PAI-1 5G/5G homozygous genotype may be involved in the development of intrauterine growth restriction (IUGR) as well as preeclampsia (30, 31). Even in this case, we were unable to detect any association between this thrombophilic pattern and adverse pregnancy outcomes.

Presumably, a similar increase in coagulation may be responsible for the increased incidence of miscarriage and fetal demise seen in protein C, S and ATIII deficient patients (32, 33).

Gerhardt et al presents a case-control study of 97 women with severe preeclampsia in previous pregnancies and 277 normal women, to assess hereditary risk factors of venous thrombosis as risk determinants for severe preeclampsia. In his research, the onset of severe preeclampsia was significantly earlier in women with the G20210A prothrombin gene mutation (24.5 weeks vs. 30.1 weeks, P=0.046) and in women with the PAI-15G-5G genotype (25.7 weeks vs. 30.8 weeks, P=0.024), showing that these risk factors do not induce the pathomechanism but accelerate the course of preeclampsia (34).

In a recent prevalence study on 200 patients, the G20210A single-nucleotide polymorphism of prothrombin was shown to be unrelated to recurrent spontaneous abortions, whereas factor V Leiden, along with APCR, as well as combination of both, were seen in women with idiopathic recurrent pregnancy loss, suggesting a close linkage with this pathology.

Apart from the linkage between AT III deficiency and the occurrence of preeclampsia and disseminated intravascular coagulopathy, we obtained findings in contrast with Gerhardt A et al. In our case series, we had no association of preeclampsia with Factor V Leiden or with prothrombin gene mutation. The mechanism of impact of single inherited thrombophilias on abruptio placentae surely deals with microangiopathy, a common pattern found within the thrombophilic population, but it is far to be demonstrated.

Our study deals with single thrombophilias and is poorly comparable with others. Moreover, it is important to underline that in our study we had no subject with recurrent spontaneous miscarriages because our study population was enrolled within a tertiary care unit and not within an outpatient clinic.

We enrolled subjects beyond the statistically recommended numbers necessary to reach strong evidence.

The main result of our research was that, excluding multiple gene mutation, single thrombophilias have to be carefully taken into consideration, but without generating unjustified fears about pregnancy.

Further studies are needed to check the linkage between thrombophilic gene mutations and adverse preg-

nancy outcomes.

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Case Study

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Absent end diastolic flow in umbilical artery and umbilical cord thrombosis at term of pregnancy. Case report and review of the literature

Authors' Contribution:
A Study Design
B Data Collection
C Statistical Analysis
D Data Interpretation
E Manuscript Preparation
F Literature Search
G Funds Collection

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Summary

Background: Meconium staining of the fetus and placenta is associated with increased neonatal mortality and asphyxia. Very often it is unclear whether the discharge of meconium is a cause or an effect of fetal distress. In the available literature there are no large epidemiological studies of pregnancy outcome with meconium-related lesions, even though this could be useful to improve our state of knowledge on this topic.

Case Report: A case of umbilical cord vascular necrosis is described. A severely asphyxiated infant was delivered at 39 weeks' gestation by cesarean section due to alarming results of fetal heart rate monitoring and rupture of membranes with meconium-stained amniotic fluid. There was no meconium aspiration. We report a review of 15 similar cases. In the whole series, a linkage between umbilical cord vascular necrosis and evidence of remote meconium discharge always seems to be detectable. The pathophysiological mechanism is unknown.

Conclusions: It is still not clear why only a tiny percentage of cases with meconium-stained amniotic fluid develop umbilical cord lesions and poor pregnancy outcome. Further investigations are needed to explain why some meconium-stained newborns suffer severe neurological and other damage even without meconium aspiration.

key words: meconium • umbilical cord • fetal distress

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BACKGROUND

Meconium staining of the fetus and placenta is associated with increased neonatal mortality and asphyxia. Very often it is unclear if the discharge of meconium is a cause or an effect of fetal distress.

The available literature contains no large epidemiological studies of pregnancy outcome with meconium-related lesions. The appearance of meconium-induced thrombosis in placental and umbilical cord vessels has previously been reported [1]. The lesions do not occur if meconium is present in the amniotic cavity for less than 16 hours [1]. Experimental findings support the hypothesis that infra-amniotic meconium diffuses into umbilical cord and placental vessels and produces vasoconstriction [1,2]. Pickens et al. postulated the presence of a vasoactive meconium peptide, able *in vitro* to counteract serotonin action on cord vessels [3]. Further evidence in support of this hypothesis would explain why some meconium-stained newborns suffer severe neurological and other damage even without meconium aspiration.

CASE REPORT

On May 13, 1999, GL, a 31-year-old primigravida, was admitted to the Sant'Eugenio Hospital (Tor Vergata University, Rome) at 39 weeks' gestation, with leakage of meconium-stained amniotic fluid and the absence of fetal movements.

The pregnancy course had been normal up to that time.

The tochocardiographic trace (Figure 1A) suddenly seemed to be alarming, showing a sinusoidal pattern, without a recognizable fetal heart rate (FHR) baseline, with a deep reduction of variability. Doppler evaluations of the umbilical artery showed an increased PI with no end diastolic flow (Figure 1 B,C). A cesarean section was performed on an emergency basis due to acute fetal distress.

A severely asphyxiated, 3635 g male newborn was delivered, who required immediate transfer to neonatal intensive care as soon. The neonatal blood pH was 7.20. He had no cardiac activity and was intubated. Adrenaline was administered into the trachea. After 6

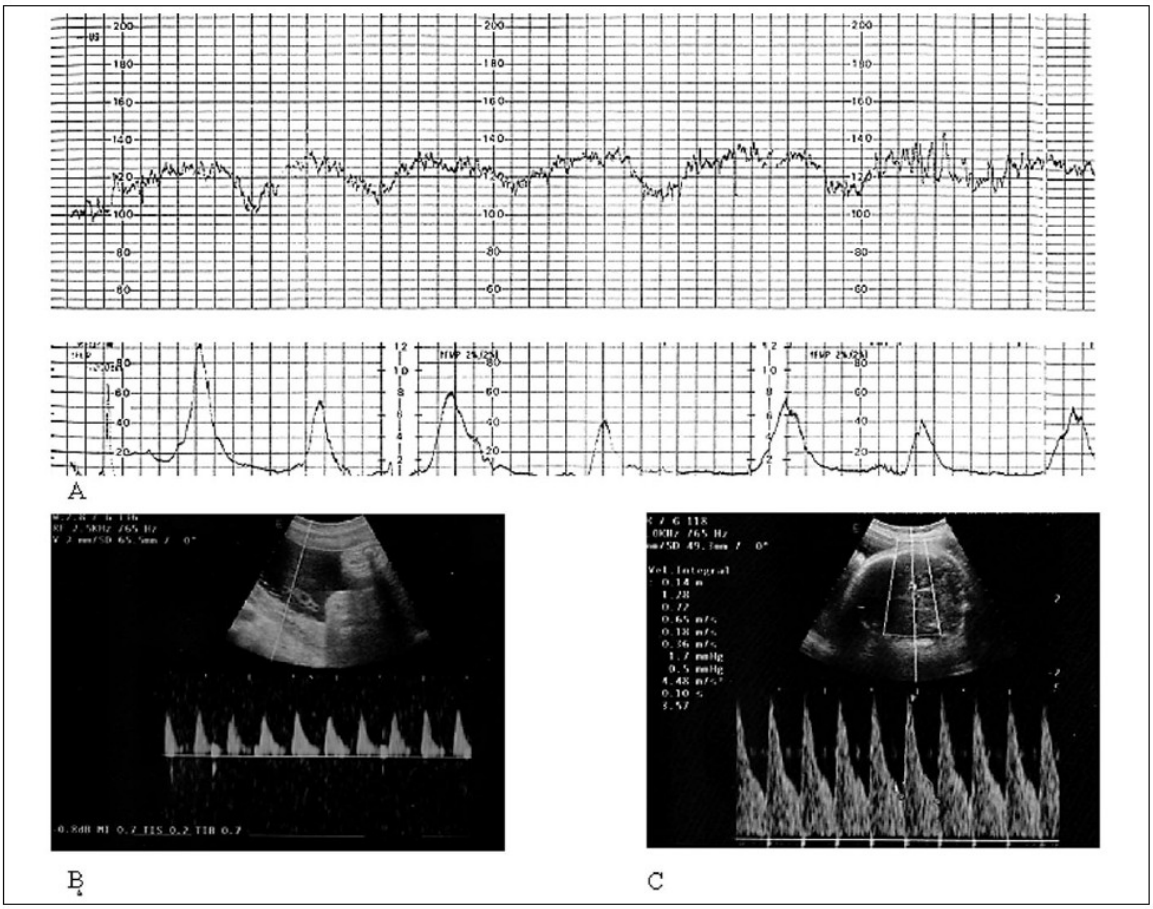

Figure 1. A) Alarming fetal heart rate monitoring: FHR ranging from 100 beats per minute (bpm) to 130 bpm; base-line not available; sinusoidal pattern, reduced variability (<5 bpm). B) Doppler evaluation of umbilical artery and C) central cerebral artery.

minutes, slow spontaneous cardiac activity began. There were no spontaneous or stimulated movements. Severe respiratory distress appeared, with acidosis, hypoxia and cyanosis. Generalized seizures occurred.

The fetal surface of the placenta and membranes were deeply green-colored and stained with meconium. The 43-cm umbilical cord was strongly green-colored. Thrombi were seen within the cord vessels at gross examination of the slide with fixed specimen (Figure 2). Light microscopic analysis showed both arterial and venous thrombosis in the umbilical cord (Figure 3), acute inflammation surrounding the vessels, and meconium-laden macrophages. Focal pigment deposits were observed in hematoxylin and eosin-stained slides. Bilirubin was observed in the macrophages, between umbilical vascular myocytes and in the Wharton's jelly. Bacterial cultures of the placenta and membranes were negative.

Newborn magnetic resonance showed hypoxic lesions of the basal nuclei and thalamus. The baby was discharged from the hospital after 10 days. Now, 2 years later, he is severely tetraplegic and needs continuous assistance. His coagulation (C and S protein included) was regular both at delivery and 1 year later.

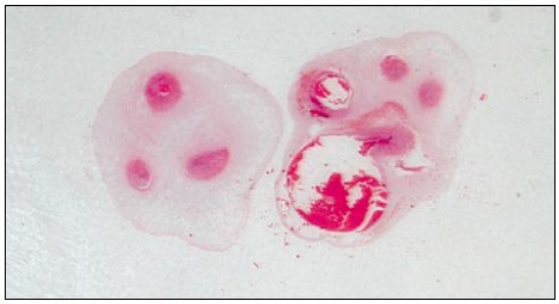

Figure 2. Gross examination of a cord section at the level of the segmental lesion: thrombi inside the vessels.

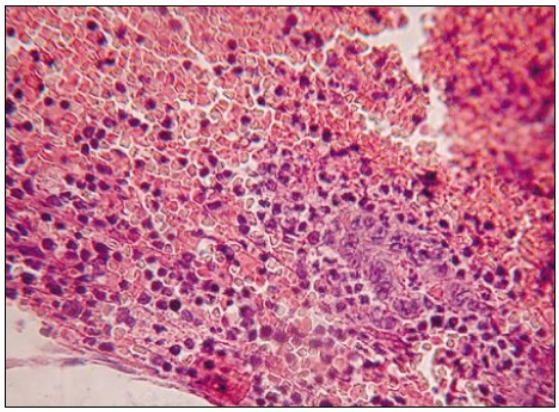

Figure 3. Thrombi, with nucleated cells, inside the umbilical vein and arteries. Macrophages with bilirubin near the vessels, within the Warthon's jelly.

DISCUSSION

Partial or complete thrombosis in the placental surface vessels is common and frequently associated with fetal demise. Thrombosis of the cord vessels also occurs, but is observed much less frequently. The veins are involved much more commonly than the arteries.

Thrombosis of the cord is a rare complication of pregnancy, occurring in about 1 in 1500 placentas, while in high-risk pregnancies the risk may be as high as 1 in 250 placentas [4]. Venous thrombosis occurs alone in approximately 70% of cases, venous and arterial thrombosis in approximately 20% of cases, and arterial thrombosis alone in 10%. As with a single umbilical artery, there is apparently a strong association with perinatal mortality and morbidity as the result of the selection of cases retrospectively, but prospective data do not reveal such an association [4].

Arterial thromboses in the placental surface vessels or in the cord are very uncommon, but when they do occur, they are often fatal, with a stillbirth rate of 90% [4]. Their cause is much less well understood than that of venous obliteration. There has been some speculation about protein C deficiency [5].

Venous thrombi, in contrast, are observed quite frequently by a careful examiner of the placenta. Their principal causes are obstruction by long and often heavily spiraled umbilical cords, velamentous cord insertion and acute chorioamnionitis. Surface vessel thrombosis occasionally accompanies maternal diabetes, but this has now become uncommon, since diabetes is much better controlled. Much more commonly, venous thrombosis occurs with excessively long (or short) umbilical cords, within prolapsed cord, or after nuchal entanglement or stricture. The thrombi are recognized by their yellowish discoloration, their immobility when being 'stroked' and the frequent adjacent membrane discoloration found around them, due to hemolysis.

Venous thrombosis may commence when a membranous vessel in a velamentous cord insertion is injured by the fetus. Although it has been suggested that such thrombi can embolize to the fetus, confirmation of this in published reports is hard to find [5].

Thrombosis of the cord veins is also seen in the cord entanglement of monoamnionic twins and in tightening knots of the umbilical cord.

Placentas and cords with thromboses may be meconium-stained, and the question then arises whether the thrombosis was present before the meconium discharge occurred. Alternatively, it is possible that meconium causes vascular insults with resultant mural thrombosis. The discharge of meconium before parturition can stain the amniotic fluid and fetal membranes, but generally speaking this is not regarded as a significant medical event. The cause of meconium discharge is complex. The incidence of meconium-stained gestational tissues may be as high as one-fifth of deliveries [6].

Table 1. Clinical features of 15 cases with umbilical cord vascular thrombosis (UCVT).

Case no.	Authors	Fetal distress	Hours of MSAF	Consistency of MSAF	Cord artery pH	Gestational age at delivery (Wk)	Birth weight (g)	Birth lenght (cm)	Meconium below cords
1	Altshuler G et al. [1]	No	NI	NI	NI	40	2650	46.0	Yes
2	Altshuler G et al. [1]	Yes	>1.2	Thick	7.14	40	2540	45.0	NI
3	Altshuler G et al. [1]	Yes	>2.5	Thick	7.13	39	3550	50.0	Yes
4	Altshuler G et al. [1]	Yes	NI	Thick	NI	41	2425	47.5	Yes
5	Altshuler G et al. [1]	Yes	>2.0	Thick	7.13	40	2950	50.0	No
6	Altshuler G et al. [1]	Yes	>4.5	Thick	7.17	41	3610	53.5	No
7	Altshuler G et al. [1]	Yes	>26.0	Thick	7.01	42	4440	54.0	Yes
8	Altshuler G et al. [1]	No	>5.0	NI	7.18	42	2790	49.0	No
9	Altshuler G et al. [1]	No	>10.0	Fluid	7.18	40	3289	50.0	No
10	Altshuler G et al. [1]	No	NI	Thick	NI	40	3850	54.0	No
11	Sienko A et al. [8]	NI	NI	NI	NI	18	114	20.0	NI
12	Sienko A et al. [8]	NI	NI	NI	NI	15	62	18.0	NI
13	Sienko A et al. [8]	NI	NI	NI	NI	29	1240	35.0	NI
14	Sienko A et al. [8]	Yes	NI	NI	NI	30	1890	32.5	NI
15	Lurie S et al. [9]	Yes	NI	NI	6.80	42	2800	NI	NI

Case no.	Authors	Color of membranes and placenta	Fetal Outcome	Placental weight (g)	Pathological findings	Placental amnion balloon change
1	Altshuler G et al. [1]	Green-gray	Low weight, gastroschisis	600	Large Placenta	Yes
2	Altshuler G et al. [1]	Green-gray	Fetal distress	400	NI	Yes
3	Altshuler G et al. [1]	NI	Fetal distress, low weight	663	Placentomegaly	Yes
4	Altshuler G et al. [1]	Green-brown	Fetal distress,	500	NI	Yes
5	Altshuler G et al. [1]	Green-gray	Fetal distress,	484	NI	Yes
6	Altshuler G et al. [1]	Green-tan	Fetal distress,	440	Placenta wide and thin	Yes
7	Altshuler G et al. [1]	Green-yellow	Fetal distress, meconium aspiration syndrome	685	Large Placenta ,UCVT, umbelical cord ulcers	Yes
8	Altshuler G et al. [1]	Green-yellow	Small head circumference	550	NI	Yes
9	Altshuler G et al. [1]	Dark tan-green	Small head circumference	485	UCVT, umbelical cord ulcers	Yes
10	Altshuler G et al. [1]	Green	NI	475	Placenta wide and thin	No
11	Sienko A et al. [8]	Grey-Brown	Fetal death	88	UCVT, avascular villi, infarction	NI
12	Sienko A et al. [8]	Grey-Brown	Fetal death	82	UCVT, Placental ischemic changes	NI
13	Sienko A et al. [8]	Tan-Brown	Fetal death	NI	UCVT, necrotic decidua	NI
14	Sienko A et al. [8]	Brown-green	Fetal growth restriction	216	UCVT, chronic ischemic changes of villi	NI
15	Lurie S et al. [9]	NI	Fetal distress, need for intubation	700	UCVT and cord maceration and detachment	No

MSAF – meconium stained amniotic fluid;
NI – no information

How this phenomenon influences fetal well-being is unclear. Nonetheless, several reports suggest that meconium is associated with injury to the fetal brain [2], and with the reduction of umbilical cord glycogen levels [6]. Meconium-induced vasoconstriction has been demonstrated *in vitro* [1,2,7].

Altshuler et al. first made the remarkable observation of a characteristic lesion of umbilical cord blood vessels induced by meconium toxicity. They observed an intensely meconium-stained placenta in which the staining had penetrated deeply into the membranes and also into the umbilical cord. There it had caused necrosis of the walls of the umbilical vessels [1].

Criteria for the diagnosis of chronic meconium staining include one or more of the following: positive clinical history, grossly meconium-stained placental appearance, histopathologic amniotic epithelial balloon degeneration, and meconium-laden macrophages in deep subamniotic connective tissue [1].

We collected the few cases of cord thrombosis and protracted meconium staining reported in the literature (Table 1), and in the entire series there always seems to be a detectable linkage between umbilical cord vascular lesions and evidence of remote meconium discharge. The lesion is usually segmental, and the Wharton's jelly is filled with macrophages. There may be an intimal accumulation of polymorphonuclear leukocytes.

Altshuler described 10 cases in a retrospective study [1]. The color of the amniotic fluid varied from green to brown, and meconium below the cords was detected in

4 cases. The author suggested that a minimal meconium exposure of 16 hours was needed for this vascular injury to occur [1].

Sienko described 4 cases of umbilical cord vascular thrombosis with meconium-stained amniotic fluid [8], showing a generally poor perinatal outcome (two abortions between 18 and 20 weeks' gestation, one fetal death and a small-for-gestational age liveborn delivered by cesarean section because of repetitive variable deceleration).

Lurie described a case of complete intrapartum detachment of umbilical cord at an area of maceration and vascular thrombosis associated with meconium-stained amniotic fluid: the delivered infant was severely asphyxiated [9]. Only in this case is there a description of the fetal monitoring results, showing fetal tachycardia (170/minute), with good variability [9].

The color of the amniotic fluid varied in this series from green to gray or brown, and generally was not correlated to the degree of fetal damage. Moreover, there was no correlation between the consistency of the meconium-stained amniotic fluid and the fetal cord artery pH. Nevertheless, Sienko suggests that the degree of meconium-associated risks and the color of meconium correlate with the time the fetus has been exposed to potent soluble biological components [8]. He also observed that the most clinically significant meconium does not have a greenish color, and that protracted placental staining, i.e. longer than 6 hours of fetal and placental exposure to meconium, progressively manifests as a greenish tan, muddy brown, and light tan color [8].

Birthweight was generally normal if compared with gestational age, leading us to believe that umbilical cord damage is an acute feature, caused by an acute, though undetermined event.

The etiology of meconium toxicity affecting the umbilical cord is unknown, but meconium-stained amniotic fluid is a constant associated finding in the described cases of umbilical cord vascular lesions [10].

Innocuous pregnancy complications may cause some fetuses to discharge meconium, which may become hazardous, independently of aspiration. In our review, meconium aspiration was not always detectable.

We know that chronically meconium-stained fetuses may ultimately suffer cerebral palsy and other devastating disorders [2].

CONCLUSIONS

Even meconium-induced umbilical cord damage seems to be a meaningful, detrimental lesion. The pathophysiological mechanism remains unknown, and at the present state of our knowledged it cannot be explained why only a tiny percentage of cases with meconium- stained amniotic fluid develop umbilical cord lesions and poor pregnancy outcome.

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Umbilical Cord Segmental Hemorrhage and Fetal Distress

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ABSTRACT

We describe an unexplained case of umbilical cord segmental hemorrhage linked with meconium-stained amniotic fluid. A severely asphyxiated infant was delivered at term by Caesarean section. There were poor prognostic signs on fetal cardiotocography with rupture of membranes with meconium-stained amniotic fluid. The pathophysiologic mechanism in this case is still unknown, even if we argued a possible role of the umbilical cord shortness.

Keywords: umbilical cord; amniotic fluid; fetal distress

CASE

On February 2, 1999, a 31-year-old woman, gravida 1 at 39 weeks' gestation, was admitted to Fatebenefratelli Hospital (Tor Vergata University, Rome) with fetal tachycardia (170 beats/minute, figure 1), little variability in fetal heart rate from cardiotocography (amplitude range of 5 beats/minute), increased decelerations (lasting 5 minutes) and rupture of membranes. Thereafter, an ultrasound scan was performed, which showed an absence of end-diastolic flow of the umbilical artery (UA), abnormal ductus venosus (DV) flow and reduced cerebral vascular resistances (figure 1). From the clinical history, we found that the patient had had a 6-hour history of strongly brown stained and highly viscous amniotic fluid loss.

Within 25 minutes of admission, a Caesarean section was performed for acute fetal distress, since a spontaneous vaginal delivery was not imminent. A severely asphyxiated male newborn was delivered, weighing 3020 grams. He had low Apgar scores (4 and 5 at 1 minute and 5 minutes respectively) with an umbilical arterial pH of 6.9. The neonate did not breath spontaneously and was promptly intubated and ventilated and transferred to the neonatal intensive care unit where 5 days of supportive management was required.

The placenta weighed 650 g and cord measured 34 cm. There was uniform meconium staining of the umbilical cord and the fetal membranes. Serial cross-sections of the blood vessels failed to disclose any evidence of thrombi, kinking or torsion. The umbilical cord was in part black-colored in a tract measuring 5 cm (Figure 2). Gross examination of the fixed specimen clearly demonstrated an infarction of the cord (Figure 3). On light microscopy, an umbilical cord segmental hemorrhage was noted, with prominent fetal red blood cells seen around the vessels (Figure 4), fitting the Wharton's jelly in the perivascular space, by a 10 cm length (Placental Pathology). All microbiological cultures of the placenta and membranes were negative.

Both mother and neonate were discharged from hospital after 10 days without further complications. The neonate was followed up and remains in good health after 10 months of delivery.

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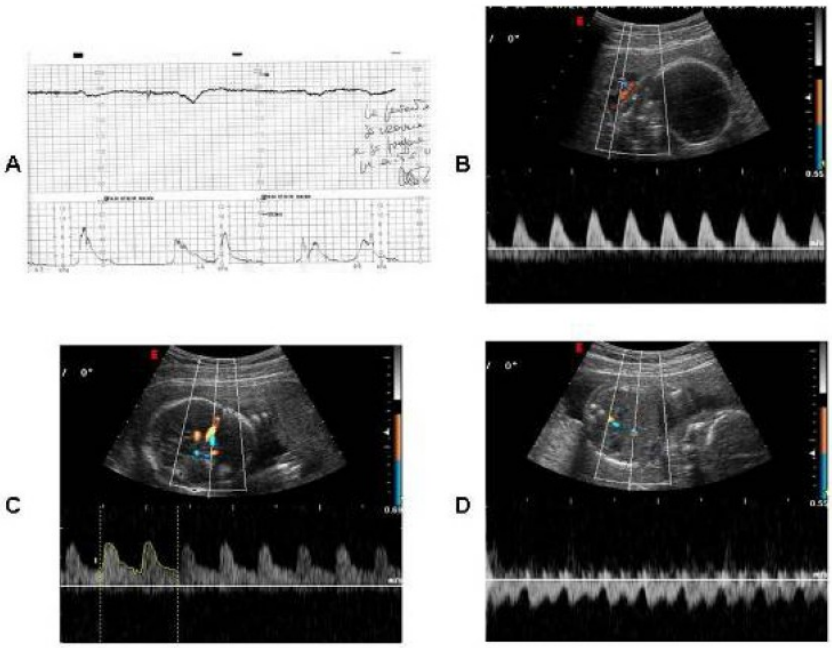

Figure 1. A. Fetal heart rate monitoring: decreased variability and tachycardia. B. Pulsed Doppler evaluation of the umbilical artery showing absence of the end-diastolic flow. C. Pulsed Doppler waveform of the ductus venosus at 39 weeks' gestation showing reversed end-diastolic blood flow (during atrial contraction). D. Doppler sonography of the middle cerebral artery showed reduced resistance.

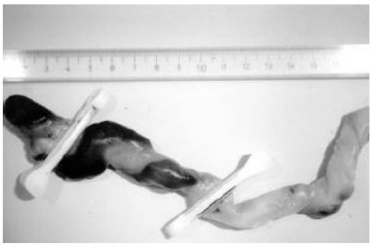

Figure 2. Umbilical cord specimen: 10 cm black-colored tract following the vessels with a spiral shape.

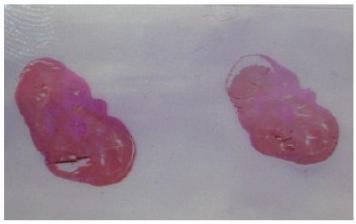

Figure 3. Gross examination of the slide with the fixed and colored specimen, showing hemorrhage surrounding and pressing the umbilical cord vessels.

COMMENT

Bleeding from the vessels of the cord is usually the result of obstetric trauma either at the time of delivery or occasionally as a result of puncture during diagnostic amniocentesis (2).

The vessels at the velamentous insertion are at particular risk during delivery as they lay unprotected. Additonally, a very short umbilical cord may be torn or avulsed during the second stage of labor. In general, haemorrhage into the substance of the cord is usually venous in origin, although if extensive it may surround all three vessels and result in compression, with the most impact seen on venous flow.

The incidence of true antepartum hematomas is low: 1 in 5505 according to Dippel (3). In general, it is difficult to be certain that the cord hemorrhages found on routine examination of the placenta are significant, since the majority will be the result of handling during labor. The lesions are usually segmental, measuring up to 10 cm in length though up to 42 cm of cord have been described.

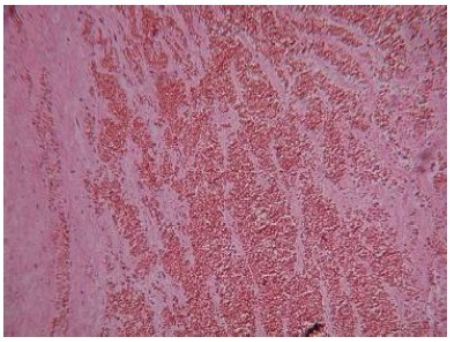

Figure 4. Light microscopy. Fetal red blood cells into the stroma. Vessels' walls are squeezed by the outstanding infarction.

Beside trauma, other etiological factors include inflammation, abnormal vessel structure, varicosities, syphilis and idiopathic calcification may be involved.

Previously, Feldberg et al (4) described a spontaneous haematoma of the umbilical cord where no pathologic lesion was found within the vessels. However, they speculated that an extremely short cord (14 cm) may have contributed to the vessel rupture. A prolonged deceleration discovered during a routine nonstress test led to emergency Caesarean section, with delivery of a healthy neonate.

In our case the cord was short too, leading us to follow this aetiologic factor, like described elsewhere (5, 6)

Gregora and Lai (7) showed that fetal heart rate monitoring and ultrasound is critical in the management of such a rare disease, following the observations made by Feldberg. From our case, we hypothesize that the occurrence of a late deceleration during non- stress cardiotocography is the epiphenomenon of an extremely rare event, rather than a practical and effective diagnostic tool to detect such a detrimental lesion. However, it is important to mention that previous authors have also highlighted the importance of poor prognostic signs on fetal heart rate when managing fetuses with cord damage (5, 6).

The mortality associated with this umbilical lesion is high and up to fifty percent usually die (8, 9). From this, it is clear that umbilical cord puncture should always be undertaken with extreme care when unexplained intrauterine death has occurred following amniocentesis. In our case, it appeared that none of the previously described etiological factors were involved. Additionally, no trauma occurred during labor.

Only the mild shortness of the umbilical cord could be taken into account as a possible determining factor for such a rare case.

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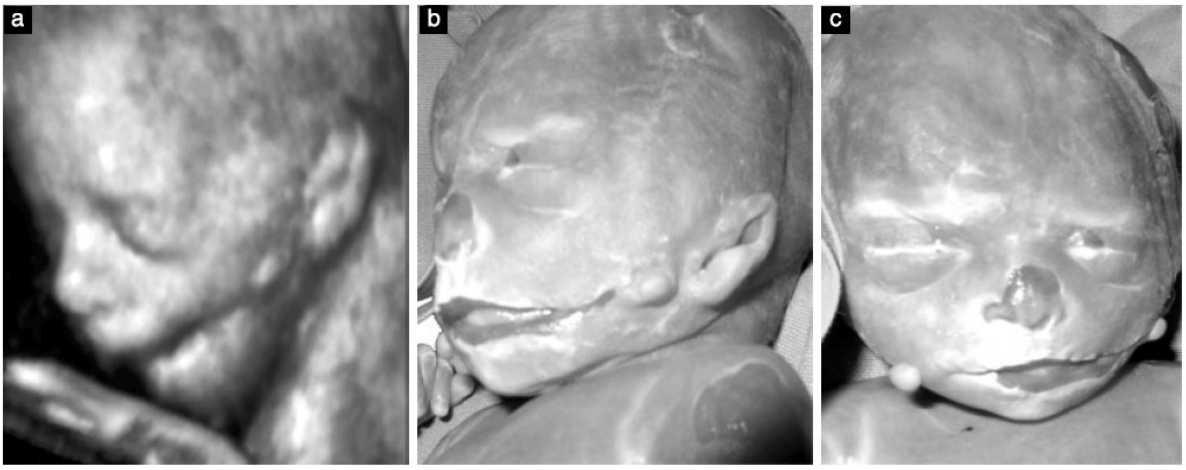

Figure 2 Surface rendering of the fetal face confirming left macrostomia and skin tag. (a) Notice the sunken appearance of the cheek. (b and c) The image is compared with the appearance of the fetus after termination of pregnancy.

lips and alveolar ridge is well visualized with a standard 2D scan. However, the lateral part of the fetal face is not equally accessible on both sides, and therefore it is particularly difficult with a 2D approach to compare the two sides and identify asymmetry. In our own case, the standard 2D sonographic views of the face, the profile and the upper lips, did appear normal. Although a lateral cleft was suspected, it was not until a 3D view of the face was obtained that the anomaly was fully appreciated.

Prenatal diagnosis of bilateral Tessier cleft number 7 in the third trimester has been previously reported[6,7]. To our knowledge, this is the first time that a unilateral cleft has been diagnosed, and at mid-gestation. We stress the excellent correlation between the antenatal 3D sonogram and the postmortem appearance of the fetus.

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SUPPLEMENTARY MATERIAL ON THE INTERNET

The following material is available from the Journal homepage:
http://www.interscience.wiley.com/jpages/0960-7692/suppmat (restricted access)

Videoclip S1 Three-dimensional rotational clip demonstrating the lateral facial cleft and a skin tag.

Choroid plate cyst: not such a rare finding

De Leon-Luis *et al.*, in a previous issue of this Journal, have reported that the placental surface cyst with contents less echogenic than the surrounding amniotic fluid is a unique ultrasonographic feature[1]. We observed a choroid plate cyst that was seen for the first time at 30 weeks' gestation during a routine ultrasound examination in a 32-year-old, primigravid Italian woman. The cyst measured about 4 cm in diameter (Figure 1) and was located in the amniotic fluid, close to the placental surface. Movement by the patient during the examination did not cause a shift in position of the cyst, leading us to speculate that

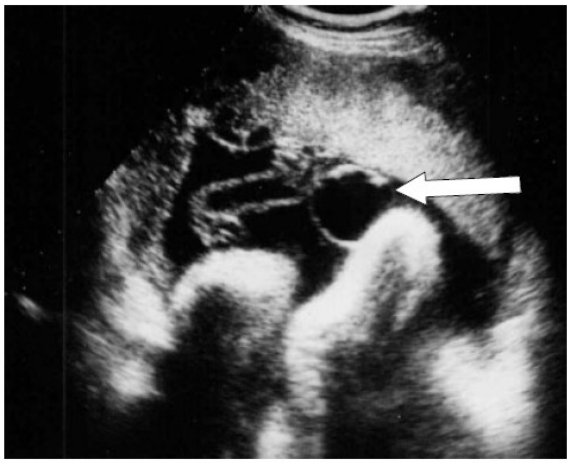

Figure 1 Ultrasound image showing a fluid cyst (arrow) in the amniotic fluid at 30 weeks' gestation.

it was attached to the choroid plate. A previous scan at 22 weeks had not shown any placental cyst. Fetal karyotyping performed at 16 weeks' gestation provided a normal result.

The umbilical cord and associated blood velocity waveforms were normal. Color Doppler imaging of the cyst excluded a vascular content. Fetal growth assessment was normal and the patient was informed that most placental surface cysts are associated with normal pregnancy outcomes[2]. The two subsequent ultrasound examinations at 33 and 37 weeks that were performed to monitor the cyst, showed no change in dimension, position or echogenicity. Spontaneous vaginal delivery occurred at term of a 3500-g male infant with Apgar scores of 9 and 10 at 1 and 5 min, respectively. Pathological evaluation showed a normal disc-shaped 620-g placenta, with a normal trivascular cord with a length of 48 cm and a diameter of 1.7 cm. On the fetal surface of the placenta there was a fluid encapsulated cyst, measuring $4 \times 3 \times 3$ cm, located 4 cm from the cord insertion (Figure 2). Both the mother and the newborn had an uneventful discharge from the hospital after a normal 24-h postpartum recovery.

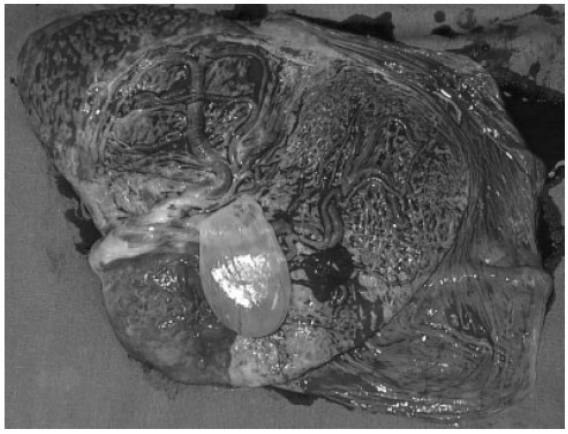

Figure 2 Gross examination of the placenta revealing a surface encapsulated fluid-filled cyst.

As exemplified by Brown *et al.*[2], choroid plate cyst is not a rare phenomenon. Counseling to reassure the patient should be tailored accordingly.

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Symmetric Cephalthoracopagus "Janiceps" Twins: Sonographic Features

To the Editor: Conjoined twins can be classified on the basis of the site of the union; thus, 3 main types can be described: ventral union, dorsal union, and rarer forms of union. A ventral union is characterized by the fusion of the 2 embryos on the ventral side (eg, the abdomen). Dorsal union twins are joined on the dorsal aspect (eg, the vertebral column or occipital bone). Twins conjoined at the head and chest are called cephalothoracopagus twins. The cephalothoracopagus variety called "Janus" is characterized by the presence of 2 opposite faces, which are composite structures, half of which belonging to 1 twin and half to the other. We present the sonographic and anatomic features in a case of Janiceps twins.

A 33-year-old pregnant woman underwent her first ultrasound scan at 22 weeks' gestation, with the diagnosis of joined twins. For ethical and religious reasons, she decided to avoid first-trimester screening procedures for fetal abnormalities (both structural and chromosomal). Sonographic features are described, with the help of the corresponding necroscopic characteristics (Figures 1 and 2). The twins were joined at the head and chest. The fusion of the cranial parts was symmetric. The single head showed 2 composite faces, 1 on each side (Figures 1, A and B, and 2, B and C). An enlargement of the cranial vault was noted. It is important to note that each face was well shaped, and the head resulted in a composite structure belonging half to 1 twin and half to the other. The fusion of the 2 heads involved the occipital regions, which were undetectable. The sphenoidal bone represented the center of the mass of the single head and was a deformed structure shared by the twins. One face had a common structure including a nose and mouth (proboscis), and the oral cavities were also complex and deformed because of the presence of a septum (Figures 1D and 2C). Two completely independent vertebral columns diverged inferiorly. A single common trachea was observed (Figure 1D). A fused thorax with 2 distinct hearts was seen. Although the heads were joined occipitally, the bodies were linked frontally, from the neck to the chest and the superior abdominal wall (Figure 2A). The arms and legs of the twins were well formed and independent (Figure 2A). The twins were female and had a common umbilical cord, rising from the abdominal wall at the midline, just before the separation of the 2 bodies (Figure 1C).

At 33 weeks' gestation, because of preterm labor and polyhydramnios, the patient underwent a cesarean delivery. The twins died of acute respiratory failure soon afterward. Their weight was 4790 g, and a common umbilical cord rose from the lower abdominal wall (Figure 2A). No magnetic resonance imaging was performed during pregnancy or postmortem. An autopsy was avoided, following the last wish of the parents.

According to the "fission" hypothesis, this phenomenon occurs at the blastula stage, between the 13th and 15th days after fertilization. It has recently been proposed that conjoined twins result from secondary fusion of 2 separate embryos.[1]

Like all monozygotic twins, conjoined twins are always the same sex, both male or both female. It is estimated that 70% of conjoined twins are female.[2]

The subset of cephalothoracopagus twins is particularly interesting given its features at the midline.[3] Chen et al[4] reported a similar case but moreover displayed the shared circulation between the 2 separate hearts. Iura and coworkers[5] conducted a Doppler velocimetric study of cephalothoracopagus twins during the course of pregnancy. Even in that, case, 2 hearts were seen. In both of these cases, polyhydramnios was present. Unfortunately, our case lacked first-trimester imaging.

It is important to note that in symmetric cephalothoracopagus janiceps twins, the face is well shaped but is shared by the twins and is therefore a composite structure belonging half to 1 twin and half to the other. In our case, the heads were joined at the occipital region, as they rotated 90° in opposite directions on their own thorax, thus resulting in 2 complete faces, located on the 2 opposite sites of a common head.

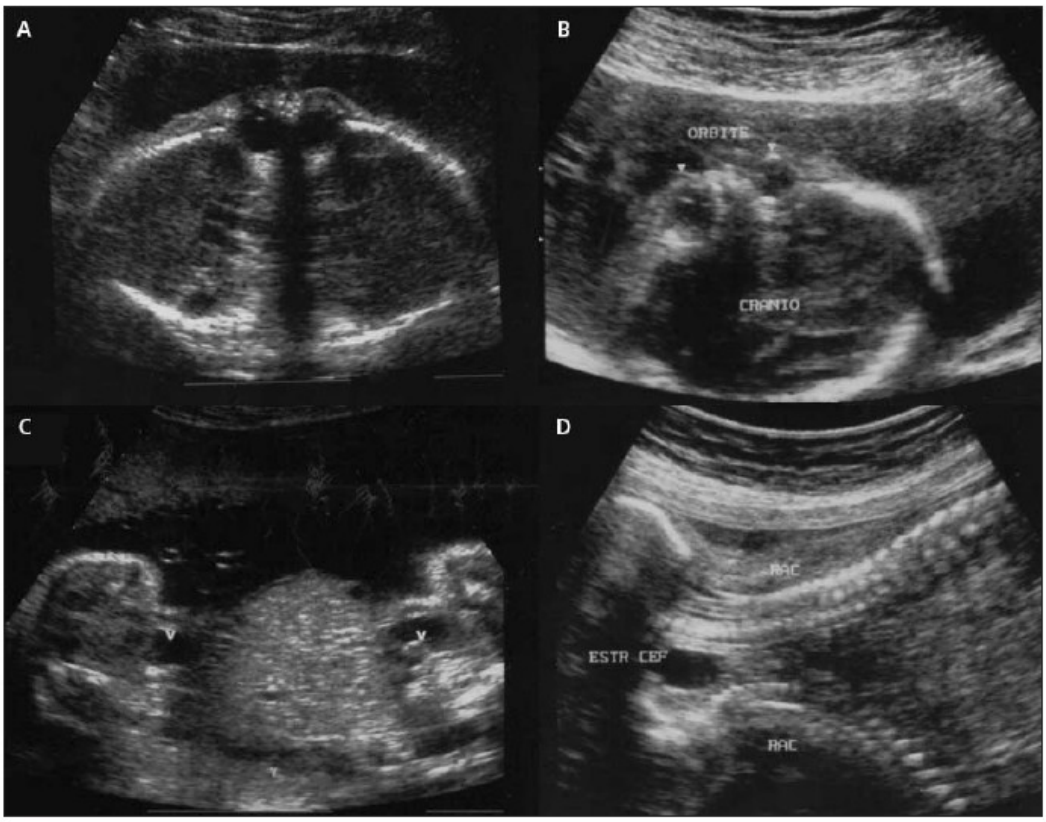

Figure 1. Sonographic features of the joined cephalothoracopagus twins. **A** and **B**, Joined heads with tight orbits and fused brain structures. **C**, Separate bladders. **D**, Joined cervical spines with 1 tracheal pouch in the middle.

From a taxonomic viewpoint, the anatomic variety we found should be considered symmetric cephalothoracopagus twins, Janus type, orthogonal variety.

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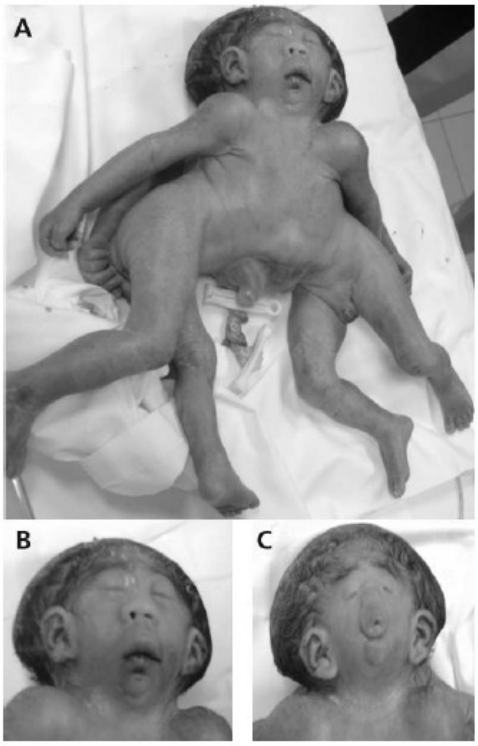

Figure 2. Necroscopic characteristics of the joined twins. **A**, Janiceps twins observed on 1 side. **B** and **C**, Each face resulted in a composite structure belonging half to 1 twin and half to the other. The fusion of the 2 heads involved the occipital regions. The presence of a proboscis and the absence of a mouth and lips are shown in **C**.

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CASE REPORT

Pitfalls in transabdominal sonography of the cervix

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ABSTRACT

Pitfalls in transabdominal sonography of the cervix

We will deal with the case of a clinical mistake occurred during a transabdominal ultrasound scan of the third trimester, where a shortened cervix was misdiagnosed, thus leading to the conclusion that higher preterm labour risk might be expected. When checked by transvaginal US, the cervix appeared within the normal ranges for that gestational age. We will discuss the limits of both the techniques.

Key words: ultrasounds, cervix, pre-term labour.

SOMMARIO

Errori valutativi in corso di ecotomografia transaddominale della cervice uterina in gravidanza

Descriviamo il caso di una errata valutazione della lunghezza cervicale, avvenuta nell'ambito di un'ecotomografia transaddominale del terzo trimestre. L'erronea percezione di una marcata riduzione della cervice veniva prontamente smentita da un ulteriore, necessario controllo ecotomografico transvaginale, che metteva in evidenza normali parametri cervicali. Si discutono i limiti delle due tecniche ecotomografiche.

Parole chiave: ecografia, cervice, parto pre-termine.

INTRODUCTION

Transvaginal ultrasound of the cervix is commonly used to diagnose cervical incompetence and has become the basis of the clinical management of preterm labour risk [1, 2]. The sonographic criteria for the diagnosis of cervical incompetence included dilation of the internal os, prolapse of the membranes into the endocervical canal, shortening of the distal cervical segment, and exacerbation of these findings that are associated with transfundal pressure [3, 4]. In 1999 Berghella et al. carried out a detailed analysis of the cervical parameters with regards to the increased risk of preterm labour [5]. Since then the transvaginal midsagittal ultrasound scan has appeared to be the most reliable and feasible means to check the cervix. Sonographic measurement of the cervical length and funneling from the 22nd to the 24th week of gestation have proved to be useful as far as the prediction of the risk of subsequent spontaneous delivery before the 33rd week of gestation is concerned [6]. The great importance of this topic has been recently highlighted, as a whole number of Ultrasound in Obstetrics and Gynaecology had the cervical assessment as their lead theme [7, 8]. Nonetheless, no agreement has been reached so far between clinicians as to the definition of the unique role the transvaginal route has in the US examination of the cervical parameters.

CASE REPORT

A peculiar case was dealt with in our Fatebenefratelli Isola Tiberina Ultrasound Outpatient Clinic, where A.B., a healthy low risk primigravida, underwent a complete transabdominal ultrasound scan at the 28th week of gestation. The examiner did not find any foetal abnormality and the biometry seemed adequate to the patient's gestational age. Just before the end of the US, the membranes seemed to have swollen into a dilated and shortened cervix (Fig. 1). Therefore, the patient was performed a transvaginal US, which showed that the cervical

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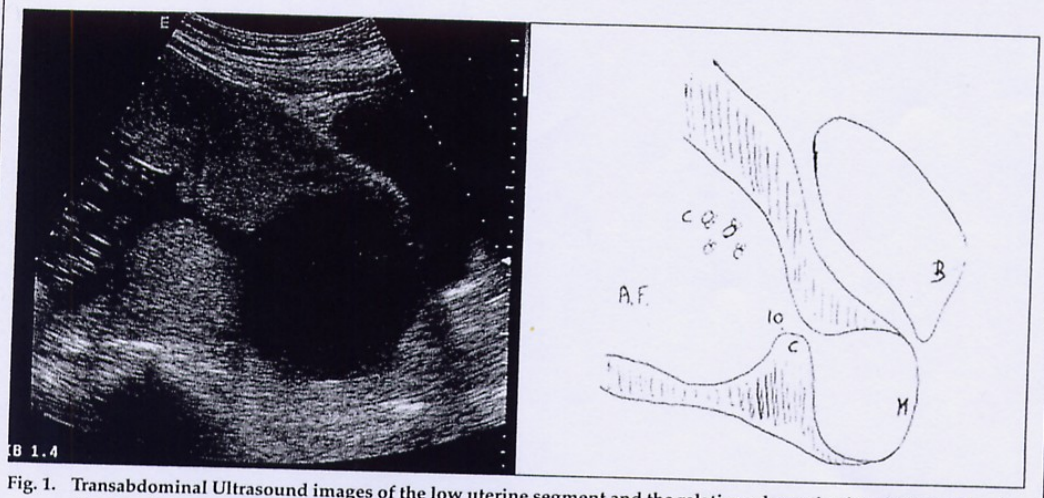

Fig. 1. Transabdominal Ultrasound images of the low uterine segment and the relative schematic picture illustrating the visualized structures. C=cord vessels, AF=amniotic fluid, M= membranes, B=bladder, IO=internal cervical os.

length was 45 mm and neither funneling nor swelling of the membranes was detected. The inner cervical os was closed both at rest and under pressure, and the inferior uterine segment was "8"-shaped due to a myoma in the posterior wall of the inferior segment (Fig. 2).

As far as the evaluation of the transabdominal US is concerned, it is to be pointed out that the hour-glass constriction does not look exactly like a cervix. Firstly, it is too high (above the fundus of the bladder), secondly, there is no evidence of cervical glands, which is an essential prerequisite when recognizing the cervix transabdominally. Moreover, the contracted appearance might not be that of a fibroid, but of a local uterine contracture, which is not uncommon in the early second trimester. Finally, if analyzed with close attention, the cervix can be seen below the membranes; furthermore, the cervical canal is also visible, as it is in the correct place at

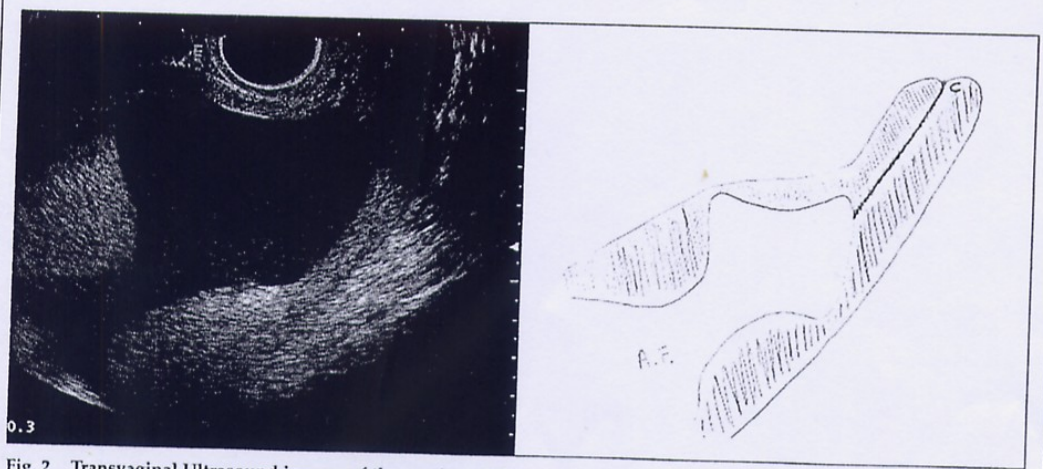

Fig. 2. Transvaginal Ultrasound images of the cervix and the relative schematic picture illustrating the visualized structures. C=cervix, AF=amniotic fluid.

the junction of the bladder base and fundus. Clearly, the transvaginal scan appears to be effective and more direct, leaving the clinician with almost no doubt about the true cervical characteristics. The difference in the two diagnosis is to be taken under consideration, as cystic lesions of the vagina are relatively common and usually represent benign conditions; whereas, a vaginal cyst may be an embryological derivative, an ectopic tissue or a urological abnormality. History, physical examination and radiological imaging, including voiding cystourethrogram (not within pregnant women) and magnetic resonance imaging, are useful for the diagnosis [9].

In conclusion, it can be maintained that, despite the wide variety of results on that issue (8), transvaginal ultrasound has proved to be an objective method of diagnosing cervical incompetence, whereas transabdominal scan is likely to fail. Thus, whoever still has doubts about this issue should bear in mind never to check the cervix "from above"!

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LETTERA ALL'EDITORE

Il caso della costola rotta che rotta non è. La spensierata accusa di malpractice e la domanda riconvenzionale

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Presentiamo un incredibile caso di richiesta di risarcimento venuto alla nostra conoscenza.

Non citerò nomi né di pazienti, né di avvocati, né di medici, né della struttura coinvolta, persuaso come sono che queste circostanze oramai non hanno più luogo né tempo, ma rappresentano semplicemente l'attuale contesto sociale e mediatico in cui il ginecologo (ed il medico in generale) si trova ad esprimere la propria professionalità.

CASO CLINICO

La paziente, signora MPI, è stata ricoverata il 14 dicembre 2004 per prodromi del travaglio di parto, alle ore 12.05, a 41 settimane di gestazione.

In anamnesi patologica remota si annoverano laserterapia per lesione cervicale da HPV (1999), i comuni esantemi infantili, appendicectomia e tonsillectomia.

L'anamnesi ostetrica pregressa evidenzia una condizione di poliabortività, con due precedenti aborti, uno nel 2000 ed uno nel 2003 (quest'ultimo diagnosticato e trattato presso la stessa struttura fatta oggetto di richiesta di risarcimento).

Al momento del ricovero le condizioni fetali e materne erano giudicate buone.

Il parto si è verificato il 15 dicembre alle ore 1.50 della notte, per via vaginale. Il tracciato cardiotocografico si è mostrato regolare per tutta la durata della prima fase del travaglio (periodo dilatante). Lo stesso tracciato ha evidenziato, in fase espulsiva inoltrata (quindi a fine travaglio), lievi decelerazioni ripetitive, considerate quali segno d'attenzione per un rapido espletamento del parto, configurando la diagnosi di sofferenza fetale. In altre parole, il tracciato cardiotocografico, alla fine del travaglio, segnalava l'opportunità di agevolare l'espletamento del parto in tempi rapidi, al fine di evitare l'insorgenza di una vera sofferenza fetale. Si è deciso pertanto di accelerare l'espulsione fetale tramite manovra di Kristeller, con conseguente rapido espletamento del parto e neonato in perfetta salute (Apgar score 9/10), di 3600 g, di sesso fenotipico maschile.

Il post-partum aveva decorso regolare. Nei successivi giorni di puerperio la paziente lamentava dolori a livello toracico, di frequente riscontro in donne sottoposte a manovra di Kristeller.

La radiografia del torace, prontamente richiesta dal reparto di degenza, evidenziava un regolare quadro radiografico toracico, senza alterazioni di sorta.

La paziente veniva giudicata dimissibile il 18 dicembre.

Ottobre 2006 (due anni dopo il parto!), lettera dallo studio legale M&Co con richiesta risarcimento per gravi danni alla salute della signora arrecati dalla frattura costale. Ma, come già detto e come facilmente si dimostrerà, la frattura costale non ha mai avuto luogo.

CONSIDERAZIONI

La manovra di Kristeller è procedura ampiamente descritta in letteratura e su libri di testo (*Pescetto, Moracci-Berlingeri, Candiani*) ed è nota per essere manovra cui ricorrere in stato di necessità (*1994. Gerin, Medicina legale e delle assicurazioni, pag. 395* sullo stato di necessità) e non scevra di rischi. Trattandosi di una spremitura *ab estrinseco*, è a tutti noto che la

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manovra di Kristeller può provocare traumi a carico delle pareti vaginali (sottoposte ad una eccessiva *vis a tergo*) e traumi alle pareti toraciche direttamente interessate dall'esercizio della pressione (1). Tuttavia, come dicevamo, a questa manovra si ricorre in casi di necessità, laddove, a condizioni permittenti (vale a dire nella fase conclusiva del secondo periodo del parto, detto periodo espulsivo), gli strumenti a nostra disposizione mettano in evidenza una condizione di sofferenza fetale. Se la diagnosi di sofferenza fetale avviene al termine del periodo espulsivo, con parte presentata prossima all'*outlet*, il modo più rapido di risolvere il caso clinico è senza ombra di dubbio la manovra di Kristeller (oppure l'applicazione di ventosa e/o di forcipe, con gli ulteriori rischi cui si sottopongono madre e feto).

E proprio di questo stiamo parlando.

Nel caso in oggetto si è svolto un regolare travaglio di parto (testimoniato dalla diligente stesura delle curve cervimetriche e del partogramma), al termine del quale il tracciato cardiotocografico, sino a quel momento regolare, ha dato segni di sofferenza fetale, prontamente riconosciuti dal sanitario scrivente, e prontamente risolti tramite manovra a tutti nota in campo ostetrico. La prova che il travaglio ed il parto siano stati gestiti *lege artis* risiede nelle condizioni neonatali alla nascita: eccellenti.

Il principio di necessità dovrebbe essere parimenti noto a medici ed avvocati. Non v'è chi non veda che, se nel tentativo di rianimare un paziente con arresto cardiorespiratorio, si produca una lesione toracica nel corso delle manovre rianimatorie (rianimazione cardiorespiratoria), o si produca una frattura dentaria in corso di rapida intubazione, le lesioni riportate dal paziente critico non siano da ascrivere all'operato del sanitario ma al momento di concitazione che rende necessaria la messa in opera di manovre risolutive ma a volte traumatiche. Le stesse manovre, ancorché traumatiche, sono tuttavia in grado di risolvere la situazione di urgenza.

Nel caso in oggetto si verifica la stessa fattispecie di cui sopra, ma ciò che viene notato da paziente e relativi legali non è già la pronta reazione del sanitario ad uno stato di sofferenza fetale potenzialmente lesivo per la salute futura del nascituro, ma il trauma toracico riportato dalla paziente. La stessa paziente, che usciva dall'ospedale dopo 3 giorni dal parto, in buona salute e con un referto radiografico assolutamente negativo (senza cioè diagnosi di frattura costale), portava a casa un figlio perfettamente sano, dopo la precedente esperienza di due pregressi aborti consecutivi.

La dedizione, le capacità tecniche, la prontezza (erano le ore 1.50 del mattino), la tempestività non vengono riconosciute tali perché, come si legge nella lettera dello studio legale chiamato a tutela della paziente, la loro assistita ha riportato "*gravi danni alla salute*". È forse la paziente uscita con un pneumotorace da frattura costale scomposta? Recava forse il referto radiologico una diagnosi di frattura costale? Sicuramente, alla dimissione, la paziente era in buono stato di salute stando ai parametri in possesso dei sanitari e la radiografia in duplice proiezione del torace non evidenziava alcuna frattura costale (né di una né di più costole). E, ovviamente, tutto questo era ben riportato in cartella clinica ma è stato altresì ampiamente sottovalutato dai legali della paziente.

La professione dell'ostetricia prevede il ricorso a tecniche che spesso possono avere effetti collaterali non trascurabili, ma accettabili se si considera il risultato finale. Lo stesso taglio cesareo reca in se risvolti potenzialmente lesivi della persona. Si pensi allo sviluppo di cicatrici deturpanti (cheloidi), di complicanze perioperatorie, di lesioni vescicali. Non per questo ci si astiene dal compiere atti medici di questo genere.

Ma la continua vessazione cui siamo sottoposti, il sempre crescente contenzioso medico-legale, le rutilanti richieste di risarcimento anche a sproposito, stanno minando seriamente la serietà e la serenità con cui siamo chiamati a svolgere la nostra professione.

Costringere il medico ad avere sempre più ossessivamente un occhio al risvolto medico-legale nuoce *in primis* agli utenti (presi in carico da medici paralizzati dal contenzioso medico-legale) e poi alla nostra categoria.

Ma ciò che più spiace è notare che troppo spesso, come in questo caso, l'evento "parto" venga vissuto come occasione di potenziale

rivalsa sul sistema sanitario nazionale e o sul sistema assicurativo. Ogni occasione è buona per richiedere un risarcimento pecuniario.

In questo caso specifico emerge anche la assoluta incauta leggerezza con cui giurisperiti intentano azioni legali senza aver valutato attentamente ciò che è diligentemente riportato in cartella clinica.

Per osare una richiesta di risarcimento di tale fatta si deduce che in questa occasione si siano ignorate molto leggermente troppe circostanze:

- la dinamica dei fatti;
- le motivazioni del ricorso alla manovra di Kristeller;
- la natura e gli aspetti tecnici di tale manovra;
- i documenti presenti in cartella clinica;
- lo stato di necessità che connotava la vicenda.

Quest' ultimo punto rende ancora più incredibile l'accaduto.

In altre parole questo è il classico caso di scuola in cui l'eventuale lesione accidentale subita dal paziente (lesione di cui ad oggi non abbiamo notizia e che comunque non era stata evidenziata dagli accertamenti radiografici posti in essere al momento del puerperio proprio per verificare l'eventuale presenza di fratture costali) non è ascrivibile al sanitario che agiva in stato di necessità, operando al meglio per evitare danni ben più gravi quali una tetraplegia da sofferenza fetale.

Purtroppo queste richieste risarcitorie presentano anche un triste risvolto economico.

È infatti inutile ricordare che da gesti inconsulti di tale risma derivano innumerevoli problemi per il medico sottoposto a tale vessazione. Le polizze assicurative disdicono immediatamente la copertura assicurativa, il premio assicurativo aumenta automaticamente, inizia il pellegrinaggio doloroso alla ricerca di una nuova compagnia assicurativa, il tutto con conseguente perdita di tempo e, soprattutto, di denaro. Tempo e denaro sottratti alla vita privata. Inoltre l'immagine del professionista viene sicuramente offuscata da simili circostanze.

Fortunatamente si comincia timidamente ad assistere a sporadici casi in cui il convenuto (il ginecologo) ricorra alla domanda riconvenzionale nei confronti dell'attore (paziente e relativo collegio legale) nell'ambito del medesimo processo, al fine di ottenere un provvedimento a sé favorevole, diverso e indipendente dal rigetto totale o parziale della domanda dell'attore; il codice di procedura civile disciplina i tempi e i modi di proposizione della domanda riconvenzionale nel processo ordinario di cognizione.

Proporre a propria volta una richiesta di risarcimento ove il contenzioso mosso dalla paziente risulti infondato (art. 96 C.P.C., lite temeraria) sembra essere, ad oggi, l'unica via, sebbene lenta e faticosa, per salvaguardare noi e la nostra professione.

Ove, in futuro, passi per definitivo il concetto secondo cui il medico, chiamato in causa, opti immediatamente per la domanda riconvenzionale e, in caso di proscioglimento, si veda riconosciuto a propria volta un risarcimento per i danni subiti (economici, d'immagine, morali, ecc.), siamo persuasi che certa faciloneria nell'attribuire colpe per errori medici possa finalmente cedere il passo ad analisi attente e meditate dell'operato dei sanitari.

Chiedere a nostra volta un risarcimento alla paziente che ci ha vessato con un ingiusto processo sembra essere l'unico deterrente in grado di assicurare una boccata di fresco respiro alla nostra categoria.

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OP11.10
Laparoscopy in women with previous abdominal open surgery. Safety of the ultrasound preoperative evauation of the subumbilical field

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Objectives: To determine the feasibility of preoperative ultrasound evaluation of the umbilical region of patients with previous open abdominal surgery undergoing laparoscopy.
Methods: Prospective, consecutive study. Tertiary referral center. Twenty-five women undergoing operative laparoscopy with previous open surgery (Group A) and 22 women undergoing laparoscopy as first surgery (Group B). Shortly before laparoscopy, dynamic ultrasound evaluation of the umbilical field was undertaken with a 7.5-MHz microconvex probe, with the help of two ECG pounches (Burton TM, Genova, Italy), by slightly pulling the skin and moving the abdominal wall under the probe. Abdominal wall tissue thickness (AWTT, mm), urachus to peritoneum thickness (UTP, mm), and the 'sliding layers' sign between bowels and peritoneum were evaluated at the same time, as described elsewhere. The Mann–Whitney *U*-test was used for nonparametric measurements.
Results: There were no differences between the two study groups as regards to BMI and anthropometric characteristics. AWTT did not show significant differences between the groups. The UTP was shorter in Group A (1.5 ± 0.3 mm vs. 3.5 ± 0.9 mm, $P = 0.002$). Lack of the 'sliding layers' sign was observed in 16 out of 25 patients in Group A and in one out of 22 patients in Group B and all of these patients were observed to have subumbilical fibrous adhesions during laparoscopy.
Conclusions: In patients suspected to have subumbilical adhesions, 'open' techniques seem to be more suitable and safer. The urachus appears to be a feasible and sure peritoneal landmark for the first trocar insertion. Regardless of body mass index, the UTP and the

PRODUZIONE SCIENTIFICA DURANTE IL DOTTORATO DI RICERCA IN MEDICINA PERINATALE

ABSTRACT

Nell'elaborato finale del Dottorato di Ricerca in Medicina Prenatale ho seguito principalmente due rami di ricerca clinica:

1- La ricerca sull'utilizzo e lo sviluppo della plicometria ultrasonogrfica fetale
2- Lo studio delle associazioni tra mutazioni (o condizioni) trombofiliche e l'insorgere di patologie della gravidanza.

Nella prima applicazione ho studiato alcuni dei nuovi parametri plicometrici fetali proposti in letteratura negli ultimi anni. Ho implementato la tecnica di misura, contribuendo alla sua standardizzazione. In seguito ho prodotto tavole biometriche di riferimento nelle successive epoche di gravidanza, sia in condizioni di normalità, sia in pazienti con diabete gestazionale. Ho condotto uno studio sui cambiamenti dei compartimenti corporei fetali in caso di ritardo di crescita. Ho inoltre studiato il potenziale beneficio (in termini di accuratezza diagnostica) dell'uso dei parametri plicometrici fetali nei preesistenti modelli di stima del peso fetale. Da ultimo ho prodotto un algoritmo per la predizione del peso alla nascita, introducendovi anche uno dei parametri plicometrici da me studiati. I risultati della mia ricerca in questo campo vengono esposti in 4 articoli editati su stampa internazionale dal 2003 al 2007, proprio in corso del Dottorato di Ricerca in oggetto.

Nella seconda applicazione ho studiato l'associazione tra trombofilia ed esiti avversi della gravidanza. I due lavori presentati nel seguente documento hanno timbro epidemiologico e mostrano in comune lo stesso set di pazienti, ma differente analisi statistica. In questo attuale campo la mia ricerca sta procedendo. Due manoscritti sono in coso di valutazione e trattano dell'associazione tra trombofilia ed alterata funzione renale in gravidanza.

Sempre nella stessa tesi di Dottorato di Ricerca mi preme mostrare alcuni case report relativi ad interessanti casi clinici occorsi alla mia visione nel corso di studi in oggetto, ed aventi come tema principale la patologia cordonale.

Da ultimo presento gli atti del congresso ISUOG (International Society of Ultrasounds in Obst/Gyn) riguardanti nuove tecniche ecografiche di monitoraggio per interventi endoscopici in pazienti infertili.

SCIENTIFIC PRODUCTION DURING THE PERINATAL MEDICINE PHELLOWSHIP

ABSTRACT

The results and conclusive reports of my Phellowship in Perinatal Medicine are enclosed within the final manuscript of the following thesis.

My main research application fields were:

1- The study of fetal skinfolds by means of ultrasound evaluation.

2- The study of the impact of thrombophilias in pregnancy.

My former research field dealed with the study of the fetal subcutaneous tissues and their detection by ultrasounds. I spent part of my Phellowship analyzing whether these innovative ultrasound parameters could add more informations to the worldwide used birthweight estimating conventional algorithms. Thus, the targets of my research in this first field were: to analyze the behaviour of fetal compartments in different physiological or pathological gestational conditions; to analyze the usefulness of the fetal skinfolds in ameliorating pre-existing birthweight estimating formulae; to build-up a mathematical model to predict term neonatal weight as a result of an integration between traditional and innovative fetal US parameters.

The sum of my findings is elucidated in 4 published papers edited from year 2003 to year 2007.

The latter research field deals with the correlation between connatal thrombophilias and the occurrence of adverse pregnancy outcomes. In fact by now I can show the result of this research by presenting two works that share the same setting with a different statistical viewpoint. The research is going on and other 2 manuscripts are away to be evaluated by the referees.

Moreover, within the same document I show some clinical cases published as case reports (with or without a review), most of them dealing with umbilical cord pathologies.

Eventually I bring also some proceedings from Congresses from the International Society of Ultrasound in Obstetrics and Gynaecology, also edited by the ISUOG official magazine.

PAROLE CHIAVE

Plicometria fetale, ecografia ostetrica, peso fetale, algoritmi di crescita, tabelle di riferimento, biometria, trombofilie, ritardo di crescita, preclampisa, omocisteina, cordone ombelicale, flussimetria

KEY WORDS

Fetal skinfolds, ultrasound, fetal weight, birthweight prediction, referral charts, thrombophilias, intrauterine growth restriction, preclampsia, omocysteinemia, umbilical cord, Doppler velocimetry.