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Thrombophilic risk factors for placental stillbirth

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ABSTRACT

Objectives: To define the characteristics of placental stillbirth and the possible contribution of thrombophilic risk factors.

Study design: A prospective cohort study was performed. Women diagnosed with antenatal stillbirth (>20 weeks) of singleton pregnancies between 2006 and 2008 were referred postpartum for evaluation. Maternal risk factors, fetal, placental and cord abnormalities, and a detailed thrombophilia screening, including inherited and acquired thrombophilia, were evaluated. Fetal autopsy and placental pathology were encouraged.

Placental stillbirth was defined as death of a normally-formed fetus with evidence of intrauterine fetal growth restriction, oligohydramnios, placental abruption and/or histological evidence of placental contribution to fetal death. Pregnancy characteristics and thrombophilia profiles were compared between placental and non-placental stillbirth cases.

Results: Sixty-seven women with stillbirth comprised the study group. Placental stillbirth was evident in 33/67 (49.3%). Significantly more women with placental stillbirth were nulliparous, when compared with non-placental stillbirth women (21/33 vs. 9/34, $p = 0.002$). Mean gestational age was lower for placental, compared with non-placental stillbirth (31.1 ± 6.1 weeks vs. 33.9 ± 4.8 weeks, $p = 0.04$), as was birth weight. Thirty-six of the 67 women (53.7%) tested positive for at least one thrombophilia. The prevalence of maternal thrombophilia was higher for placental stillbirth women (63.6%), and even higher (69.6%) for women after preterm (<37 weeks) placental stillbirth. Factor V Leiden and/or prothrombin G20210A mutation were much more prevalent in placental versus non-placental stillbirth women (OR 3.06, 95% CI 1.07–8.7).

Conclusions: Placental stillbirth comprises a unique subgroup with specific maternal characteristics. Maternal thrombophilia is highly prevalent, especially in preterm placental stillbirth. This may have implications for the management strategy in future pregnancies in this subgroup.

1. Introduction

Antenatal stillbirth is one of the catastrophic events in obstetrics. The incidence is estimated at 6–7 per 1000 live births [1], and is similar to the incidence of all combined neonatal deaths. Stillbirth occurs 6–10 times more often than sudden infant death syndrome [2]. The causes and associations of stillbirth are many and not well defined, and encompass fetal, cord, maternal and placental associations. Possible fetal associations include fetal structural abnormalities, especially cardiac abnormalities, fetal chromosomal anomalies, intrauterine fetal infection, feto-maternal hemorrhage, etc. Umbilical cord associations include cord accidents such as cord prolapse and true knots in the cord. Maternal associations include infection, obesity, smoking and extremities in maternal age – both young (<20 years) and advanced (>40 years). Intrauterine fetal growth restriction (FGR) is one of the more common associations with antenatal fetal death, with the small-for gestational age fetus (>3 standard deviations below the mean) having an incidence of stillbirth >10 times higher than that of the appropriate-for gestational age fetus [3]. Placental insufficiency may result in FGR, and typical findings include histological evidence of placental thrombosis and/or infarcts. Gardosi et al. [4] demonstrated that fetal growth restriction may account for up to 42% of unexplained stillbirth cases when customized individual growth potential is used.

Inherited and acquired thrombophilic disorders are the subject of much research in the recent literature. Maternal thrombophilias have been widely investigated for their association with various pregnancy complications including fetal loss, with variable results [5–9]. Nevertheless most researchers, including several metaanalyses [10,11], agree on the increased prevalence of several thrombophilic risk factors in the setting of adverse pregnancy outcomes. Even with these many associations it is difficult to

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attribute a specific cause to a case of stillbirth, and up to 50% of stillbirth cases remain unexplained [12].

Our aim in the present study was to describe the distribution and associations of prothrombotic thrombophilic disorders within a cohort of stillbirth cases, with specific focus on placenta-related stillbirth.

2. Methods

2.1. Study design and evaluation protocol

Women diagnosed at Sheba Medical center, Israel, with antenatal stillbirth of singleton pregnancies after 20 weeks gestation, between January 2006 and December 2008, were prospectively referred to the stillbirth clinic at 6–8 weeks postpartum, for further evaluation. A detailed medical history was obtained in each case with emphasis on factors known to be associated with antenatal fetal death, including high BMI, socioeconomic status, maternal age, smoking, hypertension, diabetes, and previous thromboembolic events, either personal or familial. A detailed obstetric history was also obtained with emphasis on previous pregnancy losses, their timing and causes, as well as an outline of the index pregnancy, including first trimester and follow-up blood tests for toxoplasma, rubella, cytomegalovirus, and herpes viruses (TORCH), results of prenatal screening tests including nuchal translucency and maternal serum screening, ultrasound anomaly scanning in the first and second trimesters, fetal echocardiogram, and karyotyping, where available. Fasting glucose, 50 g glucose challenge testing (GCT) and 100 g oral glucose tolerance testing (OGTT) results were obtained, if available, as well as gestational age at diagnosis, birth weight, obstetrical complications and mode of delivery. Furthermore, at the time of delivery all women were encouraged to agree to a complete fetoplacental autopsy, and if they declined (due to religious beliefs or personal preference), placental histopathology was recommended. In addition, all women were sent for a repeat TORCH screening, parvovirus serology testing, fasting glucose levels and a complete thrombophilia screening evaluation.

Fetal growth restriction (FGR) was defined as birth weight less than the 10th percentile adjusted for gestational age and gender according to locally derived tables [13].

Thrombophilia testing was performed on peripheral blood samples collected for this purpose. Factor V Leiden [14], C677T MTHFR polymorphism [15], and prothrombin G20210A mutation [16,17] were investigated using PCR methods as previously described. Protein C, free Protein S, antithrombin (AT), circulating anticoagulant, anticardiolipin antibodies and beta-2 glycoprotein 1 antibodies were determined using appropriate commercially available kits. Anticardiolipin antibodies (aCL) were tested by an enzyme immunoassay in a Power Wave X340 Microplate Scanning Spectrophotometer (Bio-Tek Instuments, Inc., Vinooski, Vermont, USA) using Varelisa cardiolipin antibodies (Pharmacia Diagnostics, Freiburg, Germany). Normal values for aCL-IgG were 0–18 U/mL, and for aCL-IgM 0–10 U/mL. Lupus anticoagulant (LA) was tested in a Sysmex coagulometer (CA-1500) by two methods: (1) LAC-Dilute Russell's Viper Venom test (DRVVT) (Life Diagnostics, Frenchs Forest NSW, Australia), normal ratios being 0.9–1.3 and up to 1.6 in patients on warfarin therapy, (2) LAC-APTT test (Diagnostica Stago, Asnieres, France) with normal ratios being 0.9–1.55 and up to 1.7 in patients on warfarin therapy. In order to be considered positive, lupus anticoagulant (LAC-APTT and LAC DRVVT), anticardiolipin IgG and IgM antibody levels and Beta-2 Glycoprotein 1 IgG and IgM antibody levels were measured at least twice, at least 3 months apart.

As the rates of thrombophilia vary significantly between different populations, we used for the sake of comparison a previously published study by Salomon et al. [9] detailing the distribution of thrombophilic disorders in a cohort of 637 healthy nulliparous, low-risk pregnant women tested at our medical center. Information from that study includes rates of the Factor V Leiden mutation, Prothrombin G20210A mutation, C677T MTHFR polymorphism, lupus anticoagulant and anticardiolipin antibodies.

Trained pathologists performed fetal and placental pathology analyses. A detailed investigation for fetal anomalies that may explain fetal demise was performed, as well as a detailed placental analysis. Multiple placental infarcts, clusters of avascular villi, and evidence of villous dysmaturity were considered to contribute to in utero fetal death.

2.2. Establishing possible cause of death

We based our classification of cause of death on the risk factor classification by Fretts et al. [18]. Factors associated with fetal death were classified as the fetal or maternal conditions that were most likely to have initiated the 'process' that resulted in death. When two possible explanations for stillbirth were identified, both were listed.

'Placental stillbirth' was defined as fetal death associated with at least one of the following: FGR, placental abruption, and/or evidence of placental contribution to fetal death (multiple placental infarcts, clusters of avascular villi, or villous dysmaturity), and with no known fetal structural abnormalities. Cases were classified as 'unexplained' if no clinical or laboratory abnormalities could explain the death.

Institutional research ethics board approval for this study was granted.

2.3. Statistical analysis

Statistical analysis was performed with SigmaStat 1.0 software (Jandel Engineering Ltd., Linslade, Bedfordshire, UK). Categorical data were compared using the Pearson chi-square test and Fisher exact test, as appropriate. Odds ratios and 95% confidence intervals were calculated when appropriate and considered significant if the confidence interval excluded unity. Continuous variables were compared using Student's *t*-test when data were normally distributed and the Mann–Whitney rank sum test when not normally distributed. A p-value <0.05 was considered statistically significant.

3. Results

A total of 67 women with antenatal stillbirth of singleton pregnancies were referred for evaluation at the stillbirth clinic, and completed the evaluation protocol. Mean maternal age was 30.7 ± 5.4 years, with 2/67 (3%) over the age of 40, and 1/67 (1.5%) under the age of 20. Mean gestational age at diagnosis of stillbirth was 32.5 ± 5.6 weeks. 30/67 (48%) women were nulliparous at the diagnosis of stillbirth.

A total of 27 (40.3%) women in the study group consented to pathological evaluation of pregnancy products, 24 of fetus and placenta and 3 of placenta only.

The incidence and distribution of maternal medical risk factors, fetal and cord abnormalities, placental and unexplained stillbirth is presented in Table 1. Within this cohort of women with stillbirth, 33/67 (49.3%) women had evidence of placental stillbirth, and were termed the "placental stillbirth subgroup". Of the 33 women after placental stillbirth, 22 had evidence of intrauterine growth restriction (most with either pre-eclampsia and/or HELLP syndrome and/or abnormal Doppler flow parameters prior to fetal demise), 12 had evidence of large or multiple placental infarcts,