Title: Delayed villous maturation of the placenta – quantitative assessment in different cohorts.

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Running Head: Delayed villous maturation.

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Abstract:

OBJECTIVE: Placental villous maturation is maximal in the third trimester with an abundance of terminal villi. Delayed villous maturation (DVM) of the placenta is associated with chromosomal abnormalities, gestational diabetes and an adverse outcome. The aim of this study was to compare quantitative assessment of vasculo-syncytial membranes in cases of liveborn infants, perinatal deaths and controls.

METHODS: Cases were selected as following: (1). liveborn infants with a qualitative diagnosis of DVM (n=15); (2). controls matched for gestational age whose placentas did not have DVM (n = 15); (3) stillbirths (SB) / neonatal deaths (NND) showing DVM (n=13); and (4) stillbirths from autopsies where DVM was felt to be the cause of death (COD) (n=12). Vasculo-syncytial membranes (VSM) were counted in ten terminal villi in each of ten consecutive high power fields on three slides. Data analysis was carried out using SPSS.

RESULTS: Liveborn cases with DVM showed statistically significantly less VSM than controls (mean 1.01 vs. 2.42, $p < 0.0001$). The SB / NND group also showed significantly less VSM than the control group (mean 0.46 vs. 2.42, $p < 0.0001$) and less than the liveborn DVM group (mean 0.46 vs. 1.01, $p = 0.001$). The COD group was significantly different to the control group (mean 0.42 vs. 2.42, $p < 0.0001$) and the liveborn DVM group (mean 0.42 vs. 1.01, $p < 0.0001$) but not significantly different to the SB / NND group.

CONCLUSION: There is a quantitative reduction in vasculo-syncytial membranes in cases of DVM compared to controls.

Key Words / Phrases

Delayed villous maturation

Vasculo-syncitial membranes

Distal villous immaturity

Diabetes mellitus

Perinatal mortality

Introduction

Placental villous maturation is maximal in the third trimester with an abundance of terminal villi, defined by small calibre (40-100um), minimal stroma and abundant vasculo-syncytial membrane (VSM) formation¹.

Vasculo-syncytial membranes are composed of fetal capillary walls closely apposed to trophoblast basement membrane and a thin layer of trophoblast cytoplasm and facilitate optimal gas exchange (Figure 1). At term, terminal villi account for 40-50% of placental volume and 60% of the cross sectional area².

DVM is an entity whereby the maturation of the terminal placental villi does not occur normally, or takes place to a lesser degree that is normal for gestation. On low power the villi are enlarged with increased stromal cellularity and extracellular matrix. On higher power many capillaries are not peripherally located resulting in a decrease in vasculo-syncytial membranes. The villous trophoblast surrounding the villous appears thickened and hypercellular. (Figure 2). There are relatively few references to DVM in the literature. It is a placental cause of fetal death³ and is associated with chromosomal abnormalities⁴ and diabetes mellitus^{5,6}.

A risk of recurrence is also documented⁷. In a retrospective study, we have previously shown an association between DVM as defined qualitatively, and an adverse perinatal outcome⁸. We have also shown that DVM is not associated with antenatal ultrasound parameters⁶. The literature supports both qualitative and quantitative approaches to the diagnosis of DVM. The aim of this study was to perform a quantitative assessment of vasculo-syncytial membranes in DVM in cases of perinatal deaths and controls.

Methods:

This is a prospective nested cohort study. The study was approved by the hospital's Ethics Committee. Liveborn cases were those with a diagnosis of DVM identified from the pathology database from 2000-2007 (n=15). Controls were the next delivery on the database matched individually based on gestational age (n=15). Another group was identified from the study cohort of stillbirths (SB) and neonatal deaths (NND) showing DVM on placental histology (n=13). A further group was identified from the hospital perinatal autopsy database where DVM was felt to be the cause of death (COD) (n=12). The diagnosis of DVM as a cause of death accounted for approximately 3% of autopsy cases >500g over this period, and was made in cases >35 weeks in whom no other cause was found at full autopsy examination. All cases were >35 weeks gestational age. Gross pathological details of placental examination including cord pathologies (long cord >85cm, short <40cm, abnormal insertions (marginal, velamentous) and thin cord <0.8cm diameter) were noted. Clinical details were obtained from the autopsy report. The evaluation of mothers following stillbirth or neonatal death included assessment of glycosylated haemoglobin, the result of which was included in the autopsy report.

The mid portion of placental parenchyma was assessed in three sections from each placenta. Ten consecutive high power fields were evaluated and vasculo-syncytial membranes counted in each of ten terminal villi in each field. A vasculo-syncytial membrane was that part of the villus where the fetal vessel lumens and the intervillous space were separated by only a thin layer

composed of villous trophoblast cytoplasm, endothelial cytoplasm and their respective basement membranes.⁷.

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 17.0 (SPSS, Chicago, IL, USA). The data was non-normally distributed and therefore the non-parametric Mann Whitney U test was used for comparison of group means. Statistical significance was set at 0.05.

Results:

Liveborn cases with DVM showed statistically significantly less VSM than controls (mean 1.01 vs. 2.42, $p < 0.0001$).

The SB / NND group also showed significantly less VSM than control group (mean 0.46 vs. 2.42, $p < 0.0001$) and less than the liveborn DVM group (mean 0.46 vs. 1.01, $p = 0.001$).

The COD group was significantly different from the control group (mean 0.42 vs. 2.42, $p < 0.0001$) and from the liveborn DVM group (mean 0.42 vs. 1.01, $p < 0.0001$) but not significantly different from the SB / NND group.

Diabetes mellitus was documented in two of the DVM case group and three of the control group. No diabetics were identified in the SB / NND or COD groups.

No chromosomal abnormalities were documented in the DVM liveborn group.

The SB / NND group showed one Trisomy 21 (Down's syndrome) and three Trisomy 18 (Edward's syndrome) cases while the COD group had one infant with Trisomy 21 (Down's syndrome).

Ten (77%) of the SB / NND group had autopsies performed. Following autopsy and placental analysis congenital abnormalities were the cause of death in six cases, with two of fetomaternal haemorrhage. There were two unexplained (one dichorionic twin) and one each of non-immune hydrops, fetal thrombotic vasculopathy and cord accident. Sixteen of the twenty cases (80%) that underwent autopsy examination showed maceration. Nine cases showed severe maceration, six cases showed moderate and one case, mild maceration.

Discussion:

This study has shown that there is a spectrum of severity of DVM present in at risk clinical groups, with the groups of neonatal deaths and stillbirths showing the fewest VSM. The placentae from cases of stillbirth from both the SB / NND and COD groups had significantly lower numbers of VSM when compared with placentae from live born infants showing DVM. This supports the opinions in the literature regarding the clinical relevance of DVM.

Stallmach reported a 5.7% incidence of DVM in 15,415 cases, with 2.3% of cases DVM associated with stillbirth⁷. We have confirmed this association, reporting an incidence of 8.6% of intra-uterine death and 3.7% of neonatal death⁸. While DVM accounts for a relatively small percentage of total perinatal

loss (4%⁹), it may recur. The potential for recurrence is reported at 5.4%⁷ a finding supported by the current study in which two patients (5%) had recurrent DVM. Detailed placental examination is an integral part of the investigation of stillbirth and neonatal death^{3,9,10} and the diagnosis of DVM should be actively considered in cases of apparent unexplained stillbirth. The effects of DVM are not confined to stillbirth or NND, and may play a role in other adverse outcomes: marked distal villous immaturity was reported in two of 158 cases of placentas from term infants with cerebral palsy¹¹.

As with many placental pathologies, the diagnosis of DVM may be approached in a qualitative or a quantitative fashion. Stallmach's method of assessing ten terminal villi in ten high power fields in three sections of parenchyma was the method used in this study⁷. Other authors have adopted a qualitative approach using mild, moderate and severe DVM⁵ or a mean number of VSM less than the 10th centile¹². The incidence of DVM reported in the literature is variable, ranging from 5.7% - 7.7%^{3,7}. With a qualitative approach, we reported DVM in approximately 7% of placentas analysed in our institution⁸. In the current study, qualitative assessment within this cohort, recorded as mild, moderate or severe correlated poorly with quantitative mean values (Figure 3). While a linear relationship might have been anticipated, assessment of villous maturity by experienced pathologists shows inter-observer variation¹⁰. Different grades of DVM were present in every clinical group in the current study. However, cases diagnosed as DVM on a quantitative basis all had a mean VSM count < 1.69. We interpret these

findings as highlighting the importance of any grade of DVM found on placental examination.

Despite a trend to lower VSM means between liveborn DVM, SB/NND and COD groups, there was no statistically significant difference. It may be that a decision to attribute the death to DVM only in the absence of another cause disguises its role in a perinatal loss. DVM is a likely mechanism of death in serious congenital anomalies such as trisomy 18. Each year, we see DVM in some otherwise normal infants who unexpectedly require aggressive resuscitation at birth and who, we feel, represent “near miss” stillbirths. With increased recognition of DVM in larger cohorts and with a focus on separate assessment of the mechanism of death from the overall diagnosis¹³, any difference between these two groups may become clearer.

DVM is associated with diabetes mellitus, with rates of DVM in diabetic placentas ranging from 81%⁵ to 16.6%⁸. Hyperglycemia and hyperinsulinaemia of diabetic pregnancies may be one of several mechanisms by which DVM occurs, but in a previous study we did not identify a difference between glycosylated haemoglobin or fructosamine values in pre-gestational diabetic patients with or without DVM⁶. Two of our liveborn DVM group (13%) and four of our control group (26%) had a diagnosis of diabetes mellitus (either gestational or pre-pregnancy). However, not all placentas from diabetic pregnancies will have DVM, and controls were not selected such as to exclude diabetic patients. Diabetes is not a homogenous disease¹⁴.

Chromosomal abnormalities in particular trisomy 13, 18 and 21 are associated with DVM⁴. There is an increased perinatal loss with trisomy, and DVM may be a mechanism by which this occurs. Rates of DVM in trisomic placentas are reported at 60%⁴ with two villous patterns noted histologically; villi with few vessels and the other pattern showing focal hypervascular areas containing abnormal vessels^{4,15}. While our study included three cases of trisomy 18 and two of trisomy 21, we did not note the two villous patterns.

Hypercoiling of the umbilical cord is also associated with DVM¹². Hypercoiling is associated with a range of adverse fetal outcomes¹⁶, but methods of assessment of the coiling index vary¹⁷. Because of the time scale over which this study took place, coiling was not uniformly recorded. Other cord pathologies were evenly distributed across all groups. (Data not shown).

Maceration is often viewed as a major problem in histologic assessment, but its presence does not preclude the diagnosis of DVM. Nine of 16 cases (56%) in the current study showed severe maceration. We noted frequent separation of trophoblast from the villous stroma in these cases, and quantitative assessment was more time consuming, but not impossible to conduct. We found that quantitation as described above took approximately twenty minutes per case to perform. The extent of maceration did not correlate with either the qualitative grade of DVM or the mean VSM scores (data not shown). Two cases of neonatal death showed moderate and severe DVM respectively, refuting the suggestion that severe DVM is purely an epiphenomenon of maceration.

Various aetiologies for the pathogenesis of DVM have been suggested including increased levels of placental growth factors as seen in diabetes and maternal obesity, vascular changes secondary to hypercoiling of the umbilical cord and chromosomal abnormalities ¹⁸. Many of these mechanisms have been drawn from clinical and pathological correlation and are reflected in the results from our study with DVM occurring in diabetics and in the setting of chromosomal abnormalities.

The terminology used in describing the findings of DVM varies, with “placental maturation defect” ⁷ and more recently “distal villous immaturity” ¹⁸ being used. Our use of the term “delayed” arose from a desire to assist clinicians in making a conceptual separation between this abnormality of maturation and the more familiar pathology reflected by the term “accelerated maturation”. In the context of the terms “accelerated” and “delayed”, we agree with Redline’s recent observation that “affected placentas are really poor phenocopies of normal premature and term placentas” ¹⁸.

In conclusion, vasculo-syncytial membranes are reduced in cases of DVM compared to controls and may be implicated in the etiology of perinatal mortality. DVM is a placental abnormality that may be present to varying degrees of severity. With an estimated recurrence risk of the order of 5%, an increased awareness of this entity will contribute to the understanding of perinatal mortality.

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Figure Legends:

Figure 1. High power view (40X) of normal terminal villi with multiple vasculo-syncytial membranes.

Figure 2: High power view (40X) of terminal villi of placenta with delayed villous maturation.

Figure 3: Quantitative and Qualitative Comparison and Analysis.

Boxplot highlighting significantly higher median levels among controls compared to all other groups.