Organ weights and ratios for postmortem identification of fetal growth restriction: utility and confounding factors

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+A: Abstract

Objectives The postmortem fetal brain:liver weight ratio is commonly used as a marker of nutrition for diagnosis of fetal growth restriction (FGR), but this is based on limited data. Additionally, there are limited data regarding the effects of intrauterine retention, fetal maceration and postmortem interval on organ weights and their ratios at autopsy. Our aims were to examine the relationships between gestational-age-adjusted and sex-adjusted fetal organ weights at autopsy, maternal factors, cause of intrauterine death and effects of intrauterine retention, and to determine whether brain:liver weight ratio is a reliable marker of FGR in fetal deaths.

Methods As part of a larger study examining autopsy findings in intrauterine death, data from two specialist centers in London was collated in a specially designed database. Autopsy and clinical information for > 1000 intrauterine deaths between 2005 and 2013 were extracted. Adjusted (delta) organ weights were calculated by plotting against gestational age, female and male brain, liver, thymus, heart, combined kidney, combined lung, spleen and combined adrenal gland weights. Polynomial regression was used to determine best fit and to calculate expected (50th centile) organ weights and deviations from expected. We compared adjusted organ weights and body:organ weight ratios in fetuses which were small-for-gestational-age (SGA) at autopsy (birthweight <10th centile for normal livebirths) versus those which were not, and in macerated vs non-macerated fetuses.

Results The majority of fetal organs (brain, liver, heart, thymus, lungs, kidneys and thyroid) in SGA fetuses were significantly lighter than those of non-SGA fetuses. Body:organ weight ratios for thymus, liver and spleen were significantly greater in SGA fetuses, indicating these organs being disproportionately small. The majority of organs

were significantly lighter in macerated compared with non-macerated fetuses and body:organ weight ratios for most organs (liver, thymus, lung, pancreas, adrenal gland, kidney, heart) were significantly greater in macerated compared with non-macerated fetuses. When SGA cases with demonstrable placental histological abnormalities werecompared to other SGA cases, there was a significant difference in the brain:liver weight ratio (median, 6 *vs* 3).

Conclusion Changes after intrauterine death lead to loss of fetal weight, with preferential weight loss of visceral organs such as liver. Maceration therefore affects brain:liver weight ratio and such changes should be adjusted for during interpretation of such ratios. Fetal organ weights may be affected significantly by maternal factors, mechanism of death and postmortem changes. The fetal brain:liver weight ratio may provide useful information regarding intrauterine growth status at time of death, providing that adjustment is made for effects of intrauterine retention and that appropriate cut-off values are used.

+A: Introduction

The guidelines of the Royal College of Pathologists regarding autopsy in stillborn infants recommend the weighing of all organs[1]. There are limited published data regarding the effects of maternal factors on fetal organ weights, which is somewhat surprising since factors such as body mass index and ethnicity are associated with stillbirth[2–5]. Intrauterine fetal growth restriction (FGR) has been suggested as a possible important contributor to stillbirth[3,6–8], but there are difficulties in establishing this diagnosis after death[9], since small-for-gestational age (SGA) with weight below a given centile of the normal range is not analogous to FGR[10].

There are few published studies examining the effects of cause of death in stillbirth on organ weights and hence the potential usefulness of organ weight metrics for determining cause or mode of death. Postmortem brain:liver weight ratio is commonly used as a marker of nutrition in fetuses for diagnosis of FGR, but this is based on limited, historical data with littleinformation regarding effects of intrauterine retention, fetal maceration and postmortem interval on brain:liver weight ratio[11–13].

The aims of this study were, therefore, to examine whether there are significant relationships between gestational-age-adjusted and sex-adjusted fetal organ weights at autopsy, and maternal factors, cause of intrauterine death and changes due to intrauterine retention, and to determine whether brain:liver weight ratio is a reliable marker of FGR as the cause of fetal death.

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+A: Methods

As part of a larger study examining several aspects of autopsy findings in intrauterine death, detailed data from > 1000 autopsies for investigation of intrauterine fetal death

(IUFD) from two specialist centers in London (Great Ormond Street Hospital and St George's Hospital) were collated and entered into a specially designed Microsoft Access Autopsy Database (Microsoft Corp., Redmond, WA, USA). More than 400 data fields were included for each case, with criteria defined objectively prior to commencement of the study to ensure data consistency.

For the purposes of this study, we extracted information including autopsy and antenatal details from all IUFDs (excluding terminations) between 2005 and 2013 inclusive. Fetal body weight and organ weights were identified and data were analyzed through queries and statistical tests run using Microsoft Access and Microsoft Excel (Microsoft Corp.), GraphPad Prism (GraphPad Software Inc., San Diego, CA, USA) and Stats Direct (StatsDirect Ltd., Altrincham, UK). For calculation of adjusted ('delta') organ weights, female and male fetal organ weights were plotted separately against gestational age, and polynomial regression calculations were used to determine best fit and to calculate expected (50th centile) organ weights for sex and gestation. The regression equation was interpolated to determine the 2.5th centile for gestation and the SD in grams for each gestational age was calculated as: (50th centile value – 2.5th centile value)/1.96. Delta values for each organ were calculated as: (observed organ weight – expected organ weight)/SD, allowing comparison of organ weights between different clinical groups, adjusting for the effects of gestational age and fetal sex. Raw values were used for ratio calculations.Body:organ weight ratios were calculated for all major organs and brain:liver and brain:thymus ratios calculated since these have been previously suggested as possible markers of FGR. For consistency with the existing literature, SGA was defined as birth weight < 10th centile of the expected normal range for fetal sex and

gestational age (using World Health Organization reference range > 23 weeks of gestation). Cause of death was assigned according to predefined objective criteria.

+A: Results

There were 1064 autopsies for investigation of IUFDdelivered across the second and third trimesters. Data on organ weights and gestational age at delivery were available for 911 brains, 957 livers, 930 thymuses, 967 hearts, 913 spleens, 952 adrenals (combined weight), 313 lungs (combined weight), 406 kidneys (combined weight), 745 pancreas' and 154 thyroids. The relationships between organ weights and gestational age for the major organs are shown in Figure 1. Based on weights adjusted for gestational age and fetal sex (delta organ weight), the majority of organs (brain, liver, heart, thymus, lungs, kidneys and thyroid) in SGA fetuses were significantly lighter than those in non-SGA fetuses ($P < 0.0001$, $P < 0.0001$, $P < 0.0001$, $P < 0.0001$, $P < 0.0001$, $P < 0.0001$, $P = 0.03$ respectively). The liver and thymus were significantly lighter in cases with a placental cause of death (defined here as abruption, antenatally diagnosed FGR, pre-eclampsia and definite placental parenchymal abnormalities) compared with cases of unexplained death ($P < 0.0001$ and $P = 0.01$, respectively).

Body:organ weight ratios for the thymus, liver and spleen were significantly greater in cases of SGA compared with non-SGA fetuses ($P < 0.001$ for all) indicating that the thymus, liver and spleen were disproportionately small. The brain:thymus weight ratio was significantly greater in SGA fetuses ($P < 0.0001$) indicating that the thymus was also disproportionately lighter than the brain.

The majority of organs (brain, liver, thymus, heart, lungs, adrenals, and thyroid) were significantly lighter in macerated compared with non-macerated fetuses ($P < 0.01$ for all). Furthermore, body:organ weight ratios for most organs (liver, thymus, lung,

pancreas, adrenal gland, kidney, heart) were significantly greater in macerated compared with non-macerated fetuses ($P < 0.01$ for all), demonstrating that macerated fetuses had disproportionately lighter organs compared with body weight, i.e. that internal organs lose weight more than do the musculoskeletal elements of the body during the period of intrauterine retention following intrauterine death. Similarly, the brain:liver weight ratio was significantly greater in macerated compared with non-macerated fetuses ($P < 0.0001$; median (range), 3.94 (0.09–9.97) *vs* 2.78 (1.17–9.95); median difference, 1.08 (95% CI, 0.90–1.27)), demonstrating preferential loss of liver compared to brain weight after intrauterine death with retention. The brain:liver weight ratio remained significantly greater even when only unexplained deaths were evaluated ($P < 0.0001$; median (range), 3.84 (0.09–9.88) *vs* 3.05 (1.47–9.95); median difference, 0.77 (95% CI, 0.52–1.04)). In retained (macerated) IUFDs the brain:liver weight ratio should therefore be adjusted by 0.8–1 to take into account secondary effects of maceration (Figure 2).

The brain:liver weight ratio was significantly greater in SGA fetuses compared with non-SGA fetuses ($P < 0.0001$; median (range), 4.84 (0.51–9.88) *vs* 3.42 (1.32–8.92); median difference, 1.38 (95% CI, 1.03–1.72; Figure 3)), indicating that SGA fetuses have a disproportionally lighter liver in relation to brain weight suggesting that the liver disproportionately fails to gain weight more than the brain in SGA. However, when SGA cases were separated into cases FGR with placentalFGR-associated placental pathology (abruption, pre-eclampsia, maternal vascular malperfusion), and compared with those without such placental changes, there was a significant difference in their brain:liver weight ratio ($P < 0.0001$; median (range), 6.03 (0.51–10.00) *vs* 4.27 (1.17–9.88); median difference, 1.55 (95% CI, 0.86–2.21)), indicating that

'pathological' SGA due to FGR with placental pathology is associated with disproportionally lighter livers in relation to brain weight compared with SGA cases without placental abnormalities, distinguishing pathological FGR from 'normal, small' SGA cases.

A brain:liver weight ratio of 6 differentiated placental FGR from SGA without significant placental pathology, withsensitivity of 53% and specificity of 80% (Figure 4a), and would identify SGA with placental disease versus all other causes of death with sensitivity of 55% and specificity of 92% (Figure 4b). However, tehre was no brain:liver weight ratio that absolutely distinguished these groups; some cases of 'placental FGR' had a brain:liver ratio of 2–3, whilst some cases with no evidence of placental disease or other pathology had ratios > 6. However, cases with birth weight $> 10^{th}$ centile without placental pathological lesions only rarely had brain:liver weight ratio above 6 and the brain:liver weight ratio remained significantly greater in cases of placental cause of death compared with other causes, regardless of bodyweight centile ($P < 0.0001$; median (range), 6.10 (0.51–10.00) vs 3.59 (1.17–9.88); median difference, 2.38 (95% CI, 1.83–2.94)).

+A: Discussion

The findings of this large study highlight several issues regarding the use of organ weight assessment at autopsy for diagnosis of FGR in IUFD. First, changes with intrauterine retention after death, such as maceration, lead to loss of fetal weight, with preferential weight loss of visceral organs, such as the liver, compared with the body. Second, maceration affects organ weights differentially and hence affects their ratios, for example the brain:liver weight ratio. Such changes should be adjusted for during

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interpretation.. Third, the brain:liver weight ratio in non-SGA fetuses has a median normal value of around 2.5–3 but the normal range extends to 5. Finally, the brain:liver weight ratio can be used to distinguish non-FGR-SGA from FGR, and stillbirths with placental pathologies from other causes of death, with a ratio of 6 providing optimal sensitivity and specificity. However, there is no ratio value that identifies FGR unequivocally.

Most organs were significantly lighter in SGA compared with non-SGA fetuses and even after adjusting for body weight organs such as the liver and thymus were disproportionately lighter, with preferential visceral organ weight loss with maceration. Furthermore, the liver and thymus were disproportionately lighter in cases with a placental cause of death even after accounting for the reduction in fetal body weight. One previous study has reported reduction in weights of visceral organs with increasing maceration, but did not provide data on organ weight ratios or mechanisms of death[15].

Previous data based on smaller, less-defined datasets have suggested a relationship between SGA, FGR and fetal brain:liver weight ratio.[9-11] Despite these studies themselves reporting that the feature is crude and performs poorly[9], the use of a brain:liver weight ratio of above 3–3.5 being indicative of FGR has entered the pediatric pathology literature[9,11,13]. However, these studies included heterogeneous populations, did not strictly separate pathological FGR versus SGA and did not account for effects of intrauterine retention, all of which we are addressed here.. Previous fetal brain:liver weight ratio data is from a first study of 95 mixed stillbirths and neonatal deaths from more than 40 years ago[13]. The average ratio in those with a birth weight of 1SD above the mean was three in gestations > 27 weeks, a finding similar to the present data, with SGA cases having an increased ratio. The second study was from 2001, in which the

brain:liver weight ratio was examined in 182 stillbirths[11]. Growth restriction was based on body weight $< 10^{th}$ centile and it was reported that SGA fetuses had significantly greater ratios compared with controls[11]. It was also stated that there was marked overlap between the SGA and control groups.

The current data therefore confirm an association between increased brain:liver weight ratio and SGA, and furthermore demonstrate that this increase is due to those with pathological FGR with placental disease, which affects around half of apparent SGA cases. This dataset also provides the normal range in a large series of intrauterine deaths. The findings indicate that a brain:liver weight ratio ≥ 6 provides a clinically useful marker with which to distinguish pathological FGR from non FGR small fetuses and from other causes of death, the latterwith specificity of 92% and a sensitivity of 55%. Furthermore, it is possible to differentiate pathological FGR with concurrent placental abnormalities and placental cause of death from cases with biometry below the 10th centile but with no placental abnormalities using a brain:liver weight ratio of 6, with specificity 80% and sensitivity 53%. Following intrauterine death with retention and maceration, use of an unadjusted brain:liver weight ratio will erroneously overestimate the frequency of pathological FGR, since the liver loses weight disproportionately to the brain; in the presence of maceration the brain:liver weight ratio should be adjusted by a value of around 1.

Given that the brain:liver weight ratio appears to provide useful information regarding mechanism of death, it remains undetermined whether such information could be obtained non-invasively. Data are available evaluating postmortem magnetic resonance imaging (MRI) measurement of fetal organ volumes compared with standard autopsy organ weights, but with small numbers of cases at present; an MRI study

including 25 perinatal deaths at 16–40 weeks' gestation, in which MRI was performed prior to autopsy, reported a linear relationship between MRI organ volume estimates and autopsy organ weights[16]. These preliminary data suggest that postmortem MRI may have the potential to replace invasive autopsy measurements of brain:liver weight ratio, but further, larger studies are needed.

The strengths of the current study include the use of a large dataset (at least five times larger than that of the previous largest study[11]) and use of predefined criteria for classification and recording of variables. Furthermore, this is the first study to evaluate pathologically determined mechanisms in SGA fetuses rather than using weight $< 10^{th}$ centile as an 'outcome'. Finally, the study design allowed evaluation of the effects of confounding factors, such as maceration and intrauterine retention, on the brain:liver weight ratio. Potential weaknesses include those associated with use of a retrospective dataset derived from routine clinical measurements. For example, there was no evaluation of scale calibration. This is, however minimized by using only cases from Clinical Pathology Accreditation -approved centers, in which appropriate quality assurance structures are in place, working to established standards. Finally, these data demonstrate an association between organ weight ratios, mainly the brain:liver weight ratio, and placental findings, but do not in themselves provide evidence that FGR was the cause of death, merely indicating that it was present.

In conclusion, fetal organ weight ratios, such as the brain:liver weight ratio, may provide important information regarding fetal intrauterine growth status at autopsy, provided that appropriate adjustment is made for the effects of intrauterine retention and maceration, and that appropriate cut-off values are used.

+A: Acknowledgments

+A: References

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Figure 1 Scatterplots with polynomial regression lines for relationship between gestational age at intrauterine death across the second and third trimesters and fetal organ weight at postmortem in a series of intrauterine deaths: (a) brain, (b) liver, (c) thymus, (d) heart, (e) combined kidneys, (f) combined lungs, (g) spleen and (h) combined adrenal glands.

Figure 2 Distribution of brain:liver weight ratio in a series of intrauterine deaths, according to whether there was maceration (------) or no maceration (——) at postmortem. With intrauterine death, retention and maceration, the fetal liver loses weight disproportionately relative to the brain and the curve shifts to the right.

Figure 3 Distribution of brain:liver weight ratio in a series of intrauterine deaths, according to whether they were appropriate-for-gestational age with an unexplained cause of death (——; median value, 3), small-for-gestational age (SGA) with a placental cause of death, i.e. growth-restricted (-----; median value, 6) or SGA with no placental pathology (·······; median value, 3). The three groups had significantly different brain:liver weight ratio distributions ($P<0.0001$).

Figure 4 Receiver–operating characteristic curves of brain:liver weight ratio for cases or 'fetal growth restriction with placental pathology versus other SGA cases (a) and versus all other causes of death (both SGA and non-SGA) (b), based on a brain:liver weight ratio of 6.