Outcomes following the detection of fetal oedema in early pregnancy prior to non-invasive prenatal testing: a retrospective cohort study

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Abstract

Objective: To investigate the incidence of structural and chromosomal abnormalities in cases of fetal oedema on early ultrasound prior to non-invasive prenatal testing (NIPT). Design: Retrospective cohort study. Setting: Tertiary obstetric ultrasound clinic in Melbourne, Australia. Population: Women undergoing pre-NIPT ultrasound examination from January 2013-November 2018 with fetal crown-rump length (CRL) of 28-43 mm. Methods: Cases of reported fetal oedema or increased nuchal thickness were included. Clinical information was collected from the clinic's patient management database. Oedema was subclassified as isolated nuchal oedema (>2.2 mm) or generalised oedema/hydrops by two operators blinded to pregnancy outcomes. Main Outcome Measures: Incidence of chromosomal or structural defects following the detection of fetal oedema. Results: We identified 104 cases of reported fetal oedema with a CRL between 28-44 mm. Nuchal oedema and generalised oedema were present in 40 (38.5%) and 64 (61.5%) cases respectively. Outcomes were available in 93 cases (89.4%). Relevant chromosomal anomalies were identified in 21.5% (20/93), occurring in 12.1% (4/33) of the nuchal oedema and 26.7% (16/60) of the generalised oedema/hydrops cases. Structural anomalies with normal karyotype were found in an additional four (4.3%) cases. Miscarriage occurred in four (4.3%) cases and termination of pregnancy in 18 cases (19.4%). Oedema resolved by 11-13+6 weeks in 81.9% and these cases had less adverse outcomes than those with NT[?]3.5 mm (10.9% vs 76.5%, p<0.001). Conclusions: Fetal oedema in early pregnancy is associated with a high incidence of structural or chromosomal abnormalities, and these rates increase with progressive severity.

INTRODUCTION

Since the clinical implementation of non-invasive prenatal testing (NIPT) by cell-free DNA analysis of maternal blood, the test has been incorporated in prenatal care as a safe and accurate method for the detection of common chromosomal abnormalities. While there are many advantages to NIPT, the most significant is its high accuracy in screening for trisomies 21, 18 and 13 with a detection rate of 99% for T21, and low false-positive rate of less than 0.1%.[1]

In its most recent guidelines on NIPT, the International Society of Ultrasound in Obstetrics and Gynecology stated that all pregnant women should be offered a first trimester ultrasound regardless of their intention to undergo cell-free DNA screening.[2]However, there are no established guidelines or pathways around incorporating an early pre-test ultrasound in current clinical practice. In a recent study of 2,337 women who underwent an ultrasound between 10 and 14 weeks of gestation, 16.1% had an unexpected finding detected on the pre-NIPT scan. These findings altered the clinical management for the patients involved.[3]

As a tertiary ultrasound unit our current model of care for NIPT is to offer a pre-test scan between 9 and 10^{+6} weeks of gestation and post-test genetic counselling. This scan provides an opportunity to confirm

viability, establish gestational age and identify miscarriage, multiple pregnancy or early twin demise, all of which can affect the choice of the first trimester aneuploidy screening method. This creates an opportunity for early assessment of the fetus.

While the implications of increased nuchal translucency measurement (NT) at the 11 to 13^{+6} weeks scan (crown rump length [CRL] between 45 mm-84 mm) and its association with aneuploidy and structural defects is well established[4], the implications of increased nuchal translucency measurement at CRL [?] 44 mm is unclear.

Fetal oedema at the pre-NIPT scan is a potential early marker for both structural and chromosomal anomalies. However, in the absence of any evidence-based guidelines this finding presents challenges in both the counselling and management of pregnant women seeking aneuploidy screening. In a prospective cohort study, Grande *et al* . constructed a reference range for NT in fetuses with CRL between 28-44 mm and concluded that an increased NT at this early gestation appeared to be an effective marker for the common aneuploidies.[5] The objective of this study is to investigate the incidence of structural and chromosomal abnormalities in cases of reported fetal oedema on early ultrasound conducted before NIPT.

METHODS

Study population

This was a single centre retrospective cohort study conducted between January 2013 and November 2018. The cases were obtained from the ultrasound database at Monash Ultrasound for Women, Melbourne, Australia, which is a dedicated tertiary obstetric and gynaecological ultrasound practice and centre for fetal diagnosis, offering first and second trimester screening for chromosomal and structural anomalies during pregnancy.

All patients were referred by their treating physician for cell-free DNA screening and blood samples were collected onsite. Pre-test counselling was provided, and informed consent was obtained. Women who opted to use NIPT were offered an ultrasound prior to blood sampling to confirm fetal number, viability and gestational age. They were subsequently advised to return for fetal structural assessment between 12 and 13^{+6} weeks of gestational age.

Further genetic counselling was provided for those patients with fetal oedema on the early scan and the option of either continuing with the planned NIPT or undergoing invasive testing was made after review by an obstetric sonologist in consultation with the referring physician and wishes of the patient. If invasive testing was undertaken, fetal chromosome analysis was performed by fluorescent in-situ hybridization (FISH) plus conventional karyotyping (in cases of abnormal FISH) or microarray analysis (in cases of normal FISH).

Procedures

The ultrasound database was searched for the terms “oedema”, “edema” and for increased nuchal translucency measurements in the reports of all pre-NIPT ultrasound examinations. Cases of singleton pregnancy with a CRL between 28 mm and 44 mm were then selected for analysis. Early fetal oedema was classified into two groups (see figure 1):

1. Nuchal oedema (figure 1B) – increased oedema in the region of fetal neck $>$ 2.2 mm (95^{th} percentile for nuchal translucency measurement at 10 weeks of gestation[7]).
2. Generalised oedema (figure 1C) – generalised subcutaneous oedema or fetal hydrops (subcutaneous oedema with at least one of pleural effusion, pericardial effusion or ascites).

De-identified images and videos of cases with fetal oedema were reviewed and classified independently by two operators with extensive experience in obstetric ultrasound, both blinded to the outcomes. In case of discordance, a third operator’s opinion was requested.

Cases of multiple pregnancy, missed miscarriages, CRL below 28 mm or above 45 mm or nuchal thickness less than 2.2 mm and without other signs of fetal oedema were excluded. Ultrasound examinations were

performed using Voluson E10 (GE Healthcare Ultrasound, Zipf, Austria) machines, equipped with a 3D 4-8 MHz probe for transabdominal and a 5-9 MHz probe for transvaginal examinations.

Statistical analysis

Categorical variables were expressed as absolute numbers and percentages, and continues variables were expressed in medians and interquartile ranges (IQR). Differences in characteristics between the study groups were examined with chi-square or Fisher's exact test in case of categorical variables, and t test or Mann-Whitney U test for continuous variables depending on the distribution. The agreement between the two operators regarding classification of the type of oedema was assessed with analysis of intraclass correlation coefficient (ICC).

The primary outcome of the study was the occurrence of chromosomal abnormalities. Pregnancy outcomes, karyotype and microarray abnormalities and ultrasound findings associated with early fetal oedema are described as proportions within each group.

Finally, the association between oedema thickness with CRL 28 - 44 mm and the nuchal translucency at 11 to 13^{+6} weeks was assessed through linear regression analysis, and rates and outcomes of persistently increased nuchal translucency above the 99^{th} percentile (3.5 mm) were reported. For this purpose, adverse outcomes were defined as at least one of miscarriage, termination of pregnancy, relevant chromosomal abnormality or major structural defects.

A two-tailed 0.05 significance level was adopted, and statistical analyses were performed in SPSS version 25.0® (IBM Corp. Released 2017. IBM SPSS Statistics for Macintosh, Version 25.0. Armonk, NY: IBM Corp.).

RESULTS

During the study period, 10,478 pre-NIPT were performed at any gestational age and 104 cases of reported fetal oedema with a CRL between 28 mm and 44 mm were identified. Nuchal oedema was present in 40 (38.5%) and generalised oedema in 64 (61.5%) cases. Fetal hydrops was present in six of the generalised oedema cases (9.4%).

The characteristics of the study population and pre-NIPT ultrasound are shown in Table 1. The median maternal age was 33.6 years (interquartile range (IQR) 30.3-37.6) and there were no significant differences in the maternal characteristics, gestational age, oedema thickness measurement, nuchal translucency at 11 to 13^{+6} weeks or cell-free DNA test results between the subgroups of fetal oedema.

The agreement between the two operators in subclassifying the cases into nuchal oedema, generalised subcutaneous oedema or fetal hydrops was high (ICC 0.91, 95%CI 0.87 – 0.94, $p<0.001$).

Pregnancy outcomes were available in 93 (89.4%) cases. Overall, in seven cases cell-free fetal DNA testing yielded high-risk results, and they were all true positive findings (Table 2). Relevant chromosomal anomalies were identified in 21.5% (20/93), occurring in 12.1% (4/33) of the nuchal oedema and 26.7% (16/60) of the generalised oedema or hydrops cases. Of the six cases of fetal hydrops, three (50%) were affected by trisomy 18 and three resulted in a phenotypically normal infant at birth after low risk cfDNA testing and normal NT at 11 to 13^{+6} weeks.

Other structural anomalies with normal karyotype were identified in another four (4.3%) cases. Seventy-one infants (76.3%) were liveborn (66 with normal ultrasound follow up examinations and phenotypically normal infants at birth, one with monosomy X, two with major fetal anomalies and normal chromosomal microarray, and two with variants of unknown significance on the chromosomal microarray). Miscarriage occurred in four cases (4.3%), of which three had confirmed aneuploidy on products of conception, and termination of pregnancy was performed in 18 cases (19.4%, 16 with chromosomal abnormalities and two with normal karyotype but major structural anomalies at 11 to 13^{+6}weeks, Table 2).

There was a significant association of the oedema thickness at early gestational age and increased nuchal

translucency at 11 to 13^{+6} weeks ($p<0.001$, $R^2=0.187$, Figure 1). Nevertheless, the oedema resolved (NT below 3.5 mm) by 11 to 13^{+6} weeks in 81.9% of the cases, and these cases had a significantly lower adverse outcome rate than those with NT [?] 3.5 mm (10.9% versus 76.5%, $p<0.001$).

DISCUSSION

Main Findings

This study showed a high incidence of chromosomal anomalies in cases of fetal oedema diagnosed in early pregnancy, with increasing rates seen in progressively more generalised cases of oedema. The incidence of chromosomal anomalies increased with severity of the oedema and the presence of hydrops (12.1% of the nuchal oedema and 26.7% of the generalised oedema cases). Fetuses with reported increased nuchal translucency had a measurement over 2.2 mm which is the 95^{th} percentile for gestation, based on previously published charts for NT in fetuses with CRL < 45 mm.[5] Clinically relevant chromosomal anomalies in our series were present in more than one fifth of the cases which is higher than expected. While fetal oedema can be seen as the presentation of various disorders other than chromosomal anomalies, namely fetal infection, fetal anemia secondary to conditions like thalassemia and twin-to-twin transfusion syndrome,[6] the aim of this study was to assess the outcomes of fetal oedema with a focus on chromosomal and structural anomalies.

Strengths and Limitations

To our knowledge, this is the first study of outcomes following detection of oedema in fetuses with CRL between 28 mm and 44 mm in women requesting cell-free DNA testing for aneuploidy screening.

The main limitations of this study are its retrospective design and the fact that the database search relied on reported fetal oedema, potentially underestimating the incidence of this finding by not including cases where oedema was present but not mentioned in the report due to poorly defined terms and the assumption by some reporting physicians that mild oedema may be a normal transient finding in early gestation. Nevertheless, it is likely that the cases with significant oedema were captured and the incidence seems reasonable. Additionally, fetal oedema in early pregnancy is rare and the number of cases with fetal hydrops was small, but the higher incidence of poor outcomes in the more severe cases is biologically plausible.

The lack of standardized definition for early fetal oedema and differentiation between cystic hygroma and increased nuchal translucency has been highlighted in various studies resulting in inconsistent reporting of outcomes.[6] The authors refrained from using the term cystic hygroma to describe nuchal oedema as frequently with the high resolution of the ultrasound probes fine septations are seen in cases with increased nuchal translucency. Additionally, the presence of fine septations as an independent risk factor for chromosomal anomalies is not well established.[7]

Interpretation

The direct relationship of chromosomal anomalies with increasing nuchal translucency measurements is well established in fetuses between 45 mm and 84 mm in several studies.[8, 9] However, there is little known about this relationship in fetuses under 45 mm. A prospective cohort study of 672 fetuses concluded that a nuchal translucency measurement above the 95^{th} percentile in fetuses at 9 to 10 weeks could be used clinically as a marker for aneuploidy.[5] The authors reported that NT was above 95^{th} percentile in 64% of the fetuses with trisomy 21, 71% of those with trisomy 13 or trisomy 18 and in all cases of monosomy X.[5] Scholl and Chasen[10]have compared the outcomes of fetuses with cystic hygroma both with CRL < 45 mm and CRL [?] 45 mm and concluded that the rate of chromosomal abnormalities and birth outcomes were lower in the CRL < 45 mm fetuses with cystic hygroma as compared to the ones with CRL [?] 45 mm. The cases in the mentioned study represent the extreme end of the nuchal oedema group (median nuchal measurement 5.5 mm),[10] while our cases included nuchal measurement over 2.2 mm and had a median nuchal thickness of 3.1 mm. The results of this study reinforce the value of a pre-NIPT ultrasound and suggest an increased risk of chromosomal and structural anomalies in fetuses with early subcutaneous oedema.

The high incidence of chromosomal and structural abnormalities in cases of fetal oedema in early pregnancy

found in this study also suggests that in these cases and particularly in those with marked oedema or hydrops, cell-free DNA testing may not be the ideal screening modality, despite its high accuracy in detecting the most prevalent trisomies. Women facing abnormal findings before 11 weeks of gestation should receive appropriate genetic counselling and may opt for invasive testing for genetic analysis. Regardless of which investigation method is chosen, a detailed specialized anatomical assessment of the fetus at 11 to 13^{+6} weeks is needed, given that a significant proportion of these cases, with or without chromosomal abnormalities, will present with structural defects.

The rate of resolution of nuchal oedema at the 11 to 13^{+6} weeks ultrasound in our series was 81.9%. Although resolution of increased nuchal thickness before 14 weeks has been previously described in only one fifth of the fetuses with early nuchal enlargement, persistence of an increased nuchal translucency has been consistently associated with adverse outcomes.[11]

CONCLUSION

Fetal oedema in early pregnancy is associated with a high incidence of structural and chromosomal abnormalities, and these rates increase with progressive severity of fetal oedema. Identification of fetal oedema on the pre-NIPT scan should be followed by a detailed 11 to 13^{+6} weeks fetal anatomy assessment and individualized counselling regarding the different options for aneuploidy screening and diagnosis should be offered in these cases.

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Disclosure of interests

The authors report no conflicts of interest.

Contribution to Authorship

JR, KH, CT, MM, MJM were involved in the acquisition of data, analysis and interpretation of data and drafting and critical revision of the manuscript. SM, JR, DLR, MM and FC led the conception and design of the study and revised article. DLR was involved in data analysis and interpretation, construction of tables and figures, and article revision. All authors approve the final article.

Details of ethics approval

The study was conducted in accordance with the policies outlined by the National Health and Medical Research Council and was approved by the Monash Health Human Research Ethics Committee (HREC Ref No. RES-19-0000-021L) on 13 March 2019.

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REFERENCES

1. Gil MM, Accurti V, Santacruz B, Plana MN, Nicolaides KH. Analysis of Cell-Free DNA in Maternal Blood in Screening For Aneuploidies: Updated Meta-Analysis. Ultrasound Obstet Gynecol. 2017.

2. Salomon LJ, Alfirevic Z, Audibert F, Kagan KO, Paladini D, Yeo G, et al. ISUOG updated consensus statement on the impact of cfDNA aneuploidy testing on screening policies and prenatal ultrasound practice. Ultrasound Obstet Gynecol. 2017;49(6):815-6.

3. Vora NL, Robinson S, Hardisty EE, Stamilio DM. Utility of ultrasound examination at 10-14 weeks prior to cell-free DNA screening for fetal aneuploidy. Ultrasound Obstet Gynecol. 2017;49(4):465-9.

4. Kagan KO, Sonek J, Wagner P, Hoopmann M. Principles of first trimester screening in the age of non-invasive prenatal diagnosis: screening for chromosomal abnormalities. Arch Gynecol Obstet. 2017;296(4):645-51.

5. Grande M, Solernou R, Ferrer L, Borobio V, Jimenez JM, Bennasar M, et al. Is nuchal translucency a useful aneuploidy marker in fetuses with crown-rump length of 28-44 mm? Ultrasound Obstet Gynecol. 2014;43(5):520-4.

6. Beke A, Joo JG, Csaba A, Lazar L, Ban Z, Papp C, et al. Incidence of chromosomal abnormalities in the presence of fetal subcutaneous oedema, such as nuchal oedema, cystic hygroma and non-immune hydrops. Fetal Diagn Ther. 2009;25(1):83-92.

7. Mack LM, Lee W, Mastrobattista JM, Belfort MA, Van den Veyver IB, Shamshirsaz AA, et al. Are First Trimester Nuchal Septations Independent Risk Factors for Chromosomal Anomalies? Journal of ultrasound in medicine : official journal of the American Institute of Ultrasound in Medicine. 2017;36(1):155-61.

8. Kagan KO, Avgidou K, Molina FS, Gajewska K, Nicolaides KH. Relation between increased fetal nuchal translucency thickness and chromosomal defects. Obstet Gynecol. 2006;107(1):6-10.

9. Molina FS, Avgidou K, Kagan KO, Poggi S, Nicolaides KH. Cystic hygromas, nuchal edema, and nuchal translucency at 11-14 weeks of gestation. Obstet Gynecol. 2006;107(3):678-83.

10. Scholl J, Chasen ST. First trimester cystic hygroma: does early detection matter? Prenat Diagn. 2016;36(5):432-6.

11. Muller MA, Pajkrt E, Bleker OP, Bonsel GJ, Bilardo CM. Disappearance of enlarged nuchal translucency before 14 weeks' gestation: relationship with chromosomal abnormalities and pregnancy outcome. Ultrasound Obstet Gynecol. 2004;24(2):169-74.

FIGURES

Figure 1: Oedema classification

TABLES

Table 1. Characteristics of the study population.

	Nuchal oedema n = 40 (38.5%)	**Subcutaneous oedema or hydrops n = 64 (61.5%)**	**Total N = 104**
Maternal age (years)	32.6 (30.1-37.5)	33.7 (30.6-37.8)	33.6 (30.3-37.6)
Weight (Kg)	61.0 (53.9-72.3)	64.0 (58.0-70.0)	64.0 (55.3-70.0)
Height (cm)	160.5 (158.0-166.5)	163.0 (158.0-169.0)	162.0 (158.0-168.0)
Body mass index (kg/m^2)	23.0 (20.0-27.8)	23.9 (21.1-26.3)	23.4 (20.8-26.9)
Parity			
Nulliparous	19 (47.5)	33 (51.6)	52 (50.0)
Parous	21 (52.5)	31 (48.4)	52 (50.0)
Previous chromosomal abnormality	2 (5.0)	4 (7.8)	7 (6.7)
Gestational age at pre-NIPT ultrasound (weeks)	10.5 (10.4-10.7)	10.6 (10.4-10.9)	10.6 (10.4-10.9)
Conception			

	Nuchal oedema n = 40 (38.5%)	Subcutaneous oedema or hydrops n = 64 (61.5%)	Total N = 104
Spontaneous	36 (90.0)	56 (87.5)	92 (88.5)
IVF	4 (10.0)	8 (12.5)	12 (11.5)
Crown-rump length (mm)	39.4 (35.8-40.7)	39.4 (36.0-41.6)	39.4 (26.0-41.3)
Oedema thickness [?] 10+6 weeks (mm)	3.2 (2.6-3.8)	3.1 (2.5-4.1)	3.1 (2.5-4.1)
Nuchal translucency 11-13+6 weeks (mm)	2.5 (2.2-3.0)	2.4 (1.9-3.2)	2.5 (2.0-3.1)
High risk cell-free DNA test	2/34 (5.9)	5/51 (9.8)	7/85 (8.2)

Categorical variables are given in absolute number and percentages, continuous variables are given in medians and interquartile ranges.

Table 2. Associations with chromosomal and structural abnormalities in each fetal oedema subgroup.

	Chromosomal abnormalities	Structural abnormalities with normal CMA	Pregnancy outcomes
Nuchal oedema (n =33)*	Trisomy 18, one Trisomy 21, one Monosomy X, one Mosaic Monosomy X / 46, X,idicY(q11.2), one	Right atrial isomerism, Bilateral thumb abnormalities, Duodenal stenosis, one Transposition of the great arteries, one	Miscarriages, two TOP, three Live births with fetal abnormalities, two Live births of phenotypically normal infants, 26 (two with VOUS)
Generalised oedema (n = 60)*	Trisomy 18, six Trisomy 21, three Trisomy 22, one Monosomy X, two Trisomy 18 and trisomy X, one 2.48 Mb deletion 4p16.3, one Duplication 8p23.3p11.21 and deletion 9p24.3p22.3, one Heterozygous deletion 15q11.2 and CNG 17q12, one	Hypoplastic left heart syndrome, one Single umbilical artery and cord cyst, one Short femur and FGR, one Duplex left kidney, one	Miscarriages, two TOP, 15 Live birth with monosomy X, one Live births of phenotypically normal infants, 42

* Including cases with known pregnancy outcome. CMA: chromosomal microarray; Mb: mega-bases; CNG: copy number gain; TOP: Termination of pregnancy; VOUS: variant of unknown significance on chromosomal microarray