Outcomes following the detection of fetal edema in early pregnancy prior to non-invasive prenatal testing

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**Contribution:**

*What is already known about this topic?*

The significance of fetal edema on ultrasound before 11 weeks of gestation is uncertain.

*What does this study add?*

- This study shows a high association between edema and chromosomal/structural abnormalities in fetuses with a CRL between 28 mm to 44 mm.
- The presence of fetal edema may be an important early marker for adverse pregnancy outcomes.
- Clinical management should include:
  - Individualized counselling regarding options of genetic testing.
  - Detailed ultrasound examination to exclude associated structural abnormalities at 11-13+6 weeks gestation.

**Keywords:** Chromosomal anomalies, Non-invasive prenatal testing (NIPT), cell-free DNA screening, First trimester, Fetal anomalies.

**Data Availability Statement:**

The data supporting the findings of this study are not publicly available due to patient privacy. For enquiries regarding data, please contact the corresponding author, JR.

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ABSTRACT

Objective: To investigate the incidence of structural and chromosomal abnormalities in cases of fetal edema on early ultrasound prior to non-invasive prenatal testing (NIPT).

Methods: A retrospective study of women undergoing pre-NIPT ultrasound with fetal crown-rump length (CRL) of 28-44mm was conducted at a tertiary obstetric ultrasound clinic in Melbourne, Australia. Cases of reported fetal edema were included, and subclassified as isolated nuchal edema (>2.2mm) or generalized edema/hydrops by two operators blinded to outcomes.

Results: We identified 104 cases of fetal edema. Nuchal edema and generalized edema were present in 40 (38.5%) and 64 (61.5%) cases, respectively. Relevant chromosomal anomalies were identified in 19.2% (20/104), occurring in 10.0% (4/40) of the nuchal edema and 25.0% (16/64) of the generalized edema/hydrops cases. Structural anomalies with normal karyotype occurred in four (3.8%) additional cases. Miscarriage occurred in four cases (3.8%) and termination of pregnancy in 18 cases (17.3%). Among cases that reached the 11-13+6 weeks ultrasound, the edema resolved 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 edema in early pregnancy is associated with a high incidence of structural and/or chromosomal abnormalities; 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 \(\leq 44\) mm is unclear. Fetal edema 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 \textit{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 edema on early ultrasound conducted before NIPT.

\textbf{METHODS}

\textit{Study population}

This was a single center descriptive study of a series of cases collected 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 gynecological ultrasound practice and center 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. The NIPT platform used screened for Trisomies 21, 18, 13 and sex chromosome aneuploidy. Pre-test counselling was provided, and informed consent was obtained. All women 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 all patients with fetal edema 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 edema was classified into two groups (Figure 1):

1. Nuchal edema (Figure 1B) – increased edema in the region of fetal neck > 2.2 mm (95th percentile for nuchal translucency measurement at 10 weeks of gestation).7

2. Generalized edema (Figure 1C) – generalized subcutaneous edema (not confined to the nuchal region and measured in its widest diameter) or fetal hydrops (subcutaneous edema with at least one of pleural effusion, pericardial effusion or ascites).

De-identified images and videos of cases with fetal edema 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 edema 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 continuous 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 edema was assessed with analysis of intraclass correlation coefficient (ICC).

The primary outcome was the presence of a clinically relevant chromosomal abnormality, a major structural abnormality in the fetus, or the occurrence of a miscarriage with no genetic testing. Pregnancy outcomes, karyotype and microarray abnormalities and ultrasound findings associated with early fetal edema are described as proportions within each group.

Finally, the association between edema 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 99th 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 26.0® (IBM Corp. Released 2017. IBM SPSS Statistics for Macintosh, Version 26.0. 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 edema with a CRL between 28 mm and 44 mm were identified. Nuchal edema was present in 40 (38.5%) and generalized edema in 64 (61.5%) cases. Fetal hydrops was present in six of the generalized edema 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, edema thickness measurement, nuchal translucency at 11 to 13+6 weeks or cell-free DNA test results between the subgroups of fetal edema.

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

Pregnancy outcomes are summarized in Figure 2. Overall, 85 women (90.4%) chose to have cell-free DNA testing and in seven cases this test yielded a high-risk result; all were confirmed by invasive testing. Twenty-seven women (26.0%) underwent invasive testing following abnormalities on later ultrasound examinations or abnormal cell-free DNA test results. Relevant chromosomal anomalies were identified in 19.2% (20/104), occurring in 10.0% (4/40) of the nuchal edema and 25.0% (16/64) of the generalized edema or hydrops cases. Of
the six cases of fetal hydrops, three 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 (3.8%) cases.

Termination of pregnancy occurred in 18 cases (17.3%) and a spontaneous miscarriage in four cases (3.8%, one of which did not have genetic testing). There were no cases of termination of pregnancy based solely on the pre-NIPT scan. Patients who opted for termination of pregnancy did so following the results of invasive diagnostic testing or after a review at 11-13+6 weeks identified major fetal structural abnormalities. An adverse pregnancy outcome (at least one of clinically significant chromosomal abnormality, major structural defect or miscarriage without genetic testing) occurred in 24% of all cases (25/104), see Table 2 for further details.

There was a significant association of the edema thickness at early gestational age and increased nuchal translucency at 11 to 13+6 weeks (p<0.001, R²=0.187, Figure 3). Among cases that reached the 11-13+6 weeks ultrasound, the edema resolved (NT below 3.5 mm) in 81.9% (77 of the 104 cases in the entire cohort, 74.0%), 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 edema diagnosed in early pregnancy, with increasing rates seen in progressively more generalized cases of edema. The incidence of chromosomal anomalies increased with severity of the edema and the presence of hydrops (10% of the nuchal edema and 25% of the generalized edema cases). Fetuses with reported increased nuchal translucency had a measurement over 2.2 mm which is the 95th percentile for gestation, based on previously published charts for NT in fetuses with CRL < 45 mm. Clinical relevant chromosomal anomalies in our series were present in almost one fifth of the cases which is higher than expected. While fetal edema 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, the aim of this study was to assess the outcomes of fetal edema with a focus on chromosomal and structural anomalies.

**Strengths and Limitations**

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

The main limitation of this study is its retrospective descriptive design with lack of a control group. Descriptive studies such as this case series are hypotheses generating by nature and require confirmation by analytical studies. We report a
high rate of adverse outcomes, and although direct estimates of effect cannot be currently obtained, the rate of adverse pregnancy outcomes in this group are significantly higher than those expected in our population. We are in the process of collecting pregnancy outcomes of a large cohort of women with normal pre-NIPT scans in early pregnancy to allow comparison of the groups. Another limitation is the fact that the database search relied on reported fetal edema, potentially underestimating the true incidence of this finding by not including cases where edema was present but not mentioned in the report due to poorly defined terms and the assumption by some reporting physicians that mild edema may be a normal transient finding in early gestation. Nevertheless, it is likely that the cases with significant edema were captured and the incidence seems reasonable. Additionally, fetal edema 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 edema 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 edema 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 \(\geq\) 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 \(\geq\) 45 mm. The cases in the mentioned study represent the extreme end of the nuchal edema 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 edema.

The high incidence of chromosomal and structural abnormalities in cases of fetal edema in early pregnancy found in this study also suggests that in these cases
and particularly in those with marked edema 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 edema at the 11 to 13⁺⁶ weeks ultrasound in our series was 81.9%. This rate, however, clearly overestimates the resolution rates because a proportion of these pregnancies miscarry or proceed straight to invasive testing following an abnormal NIPT result. 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 edema in early pregnancy is associated with a high incidence of structural and chromosomal abnormalities, and these rates increase with progressive severity of fetal edema. Identification of fetal edema 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.

**Details of ethical 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).
REFERENCES


FIGURES

Figure 1: Edema classification. A) Sagittal image of a normal nuchal measurement (2.0 mm) at 10 weeks and 2 days of gestational age. B) 2D and 3D images of nuchal edema (4.0 mm) at 10 weeks and 3 days of gestational age. C) Nuchal edema, subcutaneous edema and bilateral pleural effusion at 10 weeks and 4 days of gestational age.

Figure 2: Flow chart demonstrating management and pregnancy outcomes within each group of fetal edema.

Figure 3: Scatter plot of the association between fetal edema prior to 11 weeks and the nuchal translucency at 11-13+6 weeks of gestational age (*n* = 94, *slope* = 1.018, *R*² = 0.194).
Table 1. Characteristics of the study population.

<table>
<thead>
<tr>
<th></th>
<th>Nuchal oedema (n = 40 (38.5%))</th>
<th>Subcutaneous oedema or hydrops (n = 64 (61.5%))</th>
<th>Total (N = 104)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>32.6 (30.1-37.5)</td>
<td>33.7 (30.6-37.8)</td>
<td>33.6 (30.3-37.6)</td>
</tr>
<tr>
<td>Weight (Kg)</td>
<td>61.0 (53.9-72.3)</td>
<td>64.0 (58.0-70.0)</td>
<td>64.0 (55.3-70.0)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>160.5 (158.0-166.5)</td>
<td>163.0 (158.0-169.0)</td>
<td>162.0 (158.0-168.0)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>23.0 (20.0-27.8)</td>
<td>23.9 (21.1-26.3)</td>
<td>23.4 (20.8-26.9)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nulliparous</td>
<td>19 (47.5)</td>
<td>33 (51.6)</td>
<td>52 (50.0)</td>
</tr>
<tr>
<td>Parous</td>
<td>21 (52.5)</td>
<td>31 (48.4)</td>
<td>52 (50.0)</td>
</tr>
<tr>
<td>Previous chromosomal abnormality</td>
<td>2 (5.0)</td>
<td>4 (7.8)</td>
<td>7 (6.7)</td>
</tr>
<tr>
<td>Gestational age at pre-NIPT ultrasound (weeks)</td>
<td>10.5 (10.4-10.7)</td>
<td>10.6 (10.4-10.9)</td>
<td>10.6 (10.4-10.9)</td>
</tr>
<tr>
<td>Conception</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spontaneous</td>
<td>36 (90.0)</td>
<td>56 (87.5)</td>
<td>92 (88.5)</td>
</tr>
<tr>
<td>IVF</td>
<td>4 (10.0)</td>
<td>8 (12.5)</td>
<td>12 (11.5)</td>
</tr>
<tr>
<td>Crown-rump length (mm)</td>
<td>39.4 (35.8-40.7)</td>
<td>39.4 (36.0-41.6)</td>
<td>39.4 (26.0-41.3)</td>
</tr>
<tr>
<td></td>
<td>Group 1</td>
<td>Group 2</td>
<td>Group 3</td>
</tr>
<tr>
<td>--------------------------</td>
<td>---------</td>
<td>---------</td>
<td>---------</td>
</tr>
<tr>
<td><strong>Oedema thickness ≤ 10+6 weeks (mm)</strong></td>
<td>3.2 (2.6-3.8)</td>
<td>3.1 (2.5-4.1)</td>
<td>3.1 (2.5-4.1)</td>
</tr>
<tr>
<td><strong>Nuchal translucency 11-13+6 weeks (mm)</strong></td>
<td>2.5 (2.2-3.0)</td>
<td>2.4 (1.9-3.2)</td>
<td>2.5 (2.0-3.1)</td>
</tr>
<tr>
<td><strong>High risk cell-free DNA test</strong></td>
<td>2/34 (5.9)</td>
<td>5/51 (9.8)</td>
<td>7/85 (8.2)</td>
</tr>
</tbody>
</table>

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.

<table>
<thead>
<tr>
<th>Chromosomal abnormalities</th>
<th>Structural abnormalities with normal CMA</th>
<th>Pregnancy outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Nuchal oedema</strong> (n = 40)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Trisomy 18, one</td>
<td>• Right atrial isomerism, Bilateral thumb abnormalities, Duodenal stenosis, one</td>
<td>• Miscarriages, two</td>
</tr>
<tr>
<td>• Trisomy 21, one</td>
<td>• Transposition of the great arteries, one</td>
<td>• TOP, three</td>
</tr>
<tr>
<td>• Monosomy X, one</td>
<td></td>
<td>• Live births with fetal abnormalities, two</td>
</tr>
<tr>
<td>• Mosaic Monosomy X / 46, X, idicY(q11.2), one</td>
<td></td>
<td>• Live births of phenotypically normal infants, 26 (two with VOUS)</td>
</tr>
<tr>
<td><strong>Generalized oedema</strong> (n = 60)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Trisomy 18, six</td>
<td>• Hypoplastic left heart syndrome, one</td>
<td>• Miscarriages, two</td>
</tr>
<tr>
<td>• Trisomy 21, three</td>
<td>• Single umbilical artery and cord cyst, one</td>
<td>• TOP, 15</td>
</tr>
<tr>
<td>• Trisomy 22, one</td>
<td>• Short femur and FGR, one</td>
<td>• Live birth with monosomy X, one</td>
</tr>
<tr>
<td>• Monosomy X, two</td>
<td>• Duplex left kidney, one</td>
<td>• Live births of phenotypically normal infants, 42</td>
</tr>
<tr>
<td>• Trisomy 18 and trisomy X, one</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 2.48 Mb deletion 4p16.3, one</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Duplication 8p23.3p11.21 and deletion 9p24.3p22.3, one</td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Heterozygous deletion 15q11.2 and CNG 17q12, one</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CMA: chromosomal microarray; Mb: mega-bases; CNG: copy number gain; TOP: Termination of pregnancy; VOUS: variant of unknown significance on chromosomal microarray
Normal nuchal measurement
Pre-NIPT Ultrasound CRL 28-44mm (n=10478)

Fetal Edema (n=104)
Group A = 40
Group B = 64

NIPT + 11-13+6 Ultrasound (n=84)
Group A = 34
Group B = 50

Low risk NIPT (n = 76)
Group A = 31
Group B = 45

Failed NIPT (n = 1)
Group A = 1
Group B = 1

Chromosome AB (n = 2)
Group A = 0
Group B = 2
Structural AB & normal CMA (n = 2)
Group A = 1
Group B = 1

Miscarriage (n = 0)
TOP (n = 3)
Group A = 2
Group B = 3

Failed NIPT (n = 7)
Group A = 2
Group B = 5

Chromosome AB (n = 8)
Group A = 3
Group B = 5
Structural AB & normal CMA (n = 0)

Miscarriage (n = 2)
TOP (n = 6)
Group A = 1
Group B = 4

Normal ultrasound (n = 6)
Group A = 2
Group B = 4

Abnormal ultrasound (n = 14)
Group A = 4
Group B = 10

Chromosome AB (n = 10)
Group A = 1
Group B = 9
Structural AB & normal CMA (n = 3)
Group A = 3
Group B = 0

Miscarriage (n = 2)

TOP (n = 0)

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