Increased Hyaluronic Acid Level in Mid-Trimester Amniotic Fluid of Trisomy 21 Fetuses with Hydrops

YL Chang (✉ j12054@cgmh.org.tw)  
Chang Gung Memorial Hospital Linkou Branch  
https://orcid.org/0000-0003-2025-5492

An-shine Chao  
Chang Gung Memorial Hospital

Chang shuenn-dyh  
Chang Gung Memorial Hospital

Shu-Han You  
Chang Gung Memorial Hospital

Po-Jen Cheng  
Chang Gung Memorial Hospital

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Abstract

Background: Trisomy 21 is the most prevalent chromosomal anomaly among new-borns with mental disability. The phenotypes with trisomy 21 may involve short stature, mental retardation, shortness of the extremities, congenital heart disease and hydrops.

Material and methods: To evaluate the hyaluronic acid concentrations in amniotic fluid of trisomy 21 fetuses with and without hydrops.

Materials and Methods: Totally 56 amniotic fluid supernatant samples were collected from pregnant women who underwent genetic amniocentesis for this study: group 1 were euploid fetuses without hydrops (n=30), group 2 were trisomy 21 fetuses without hydrops (n=16), group 3 were euploid fetuses with hydrops (n=6) and group 4 were trisomy 21 fetuses with hydrops (n=4). Hyaluronic acid concentrations in AF supernatants were evaluated by ELISA method.

Results: There are no significant differences of hyaluronic acid concentrations between euploid and trisomy pregnancy in AF obtained from genetic amniocentesis. AF in the gestational age of amniocentesis among the four groups of fetuses; maternal age at amniotic fluid collected is oldest in group 2 fetuses. The amniotic fluid hyaluronic acid concentration is highest in group 4 fetuses, and is not significant difference among the other three groups. (P= 0.014, one way ANOVA test).

Conclusion: Elevated hyaluronic acid concentration in amniotic fluid may associate with the pathophysiology of fetal hydrops in trisomy 21 fetuses.

Background

Trisomy 21 is the most prevalent chromosomal anomaly among new-borns with mental disability characterized by a triplication of the whole or distal part of human chromosome 21. The prevalence rate of trisomy 21 is estimated at 1 per 1000 new-borns(1) or approximately 1 per 500 recognized pregnancies(2). The phenotypes with trisomy 21 may involve short stature, mental retardation, shortness of the extremities, congenital heart disease and hydrops. (3)

Studies had shown that the nuchal skin of trisomy 21 fetuses was related to an increased amount of hyaluronic acid (HA)(4), and HA concentration was also found elevated in the umbilical cord of trisomy 21 fetuses(4–7). Authors had ever generated amniotic fluid mesenchymal stem cells (AFMSCs) from a pair of discordant trisomy 21 monozygotic twins in which the trisomy 21 fetus presented as hydrops. (8) Hyaluronic synthase 2 (HAS2) gene expression had been revealed more up-regulated in the AFMSCs of trisomy 21 fetuses with hydropic change than the AFMSCs of euploid co-twin. Since the antenatal diagnosis of trisomy 21 usually is made by chromosome analysis from amniocentesis at mid-trimester, authors are interested to know whether the HA concentration in amniotic fluids is different between trisomy 21 and euploid fetuses at the gestational age when genetic amniocentesis is performed.
The aim of this study was therefore to evaluate the HA content in the amniotic fluid of euploid and trisomy 21 fetuses upon receiving genetic amniocentesis.

**Materials And Methods:**

From the year 2011 December to 2014 December, we prospectively stored AFs of pregnant women who underwent genetic amniocentesis at Chang Gung Memorial Hospital, Lin-Kou Medical Center, Taoyuan, Taiwan, a tertiary referral center, accommodating over 3650 beds, due to advanced maternal age, abnormal Down syndrome screening results, fetal ultrasound anomalies, etc.

Before amniocentesis, written informed consent was obtained from all participants. In this study, genetic amniocenteses were performed between 16 and 21 gestational weeks using a transabdominal approach; about 20 ml of AF was aspirated from each patient. After amniocytes were collected via centrifugation for culture and cytogenetic studies, we consecutively stored 4 ml of fresh supernatant in an 80°C refrigerator. This study was approved by the Ethical Committee of Chang Gung Memorial Hospital (institutional review board no. 93-6366 and 102-2979A3).

The diagnosis of trisomy 21 was based on the cytogenetic study. All cases of trisomy 21 were reviewed, if the written informed consent was obtained then the supernatant was obtained from the stored laboratory. AF supernatants from euploid fetuses during the same study period were obtained for the purpose of the comparative group.

HA concentrations were determined by a commercial ELISA kit (R & D Systems, Minneapolis, MN, USA) by means of a sandwich fluorescent ELISA-like assay. Values were converted to µg/ml with reference to a standard curve generated with each assay.

Statistical analysis was conducted with the SPSS software (version 11.0 for Window; SPSS Inc, Chicago, IL). Continuous variables were first tested for normal distribution using the Kolmogorov-Smirnov test. Two-sample Student t test or Mann-Whitney U test was used to compare the values of two groups, and one-way ANOVA test was applied for data more than three groups, and post hoc tests were generated by Bonferroni test. The correlation of gestational age at amniocentesis and AF HA concentrations was evaluated by the Pearson correlation test. A probability value of less than 0.05 was considered to be statistically significant.

**Results**

During the study period, there were 3520 genetic amniocentesis procedures performed, and among them, 28 cases were karyotyped as trisomy 21. In 20 (71.4%) cases, the patient signed the consent and their supernatant was subject to analysis. Four (20.0%) of the 20 trisomy 21 fetuses presented as fetal hydrops at amniocentesis. Then we further collected 35 AF samples from the euploid fetuses that matched gestational age at amniocentesis and with a similar sex ratio. Of the 35 samples, there were six fetuses with hydrops fetalis at amniocentesis. After comparing the AF HA concentrations between
euploid fetuses and trisomy 21 fetuses, we found the AF HA concentration is not significantly different between the two groups of pregnancy and the maternal age and gestational age at amniocentesis were older and higher in mothers with trisomy 21 fetuses. (Table 1) We further compare the AF HA concentration in trisomy 21 fetuses with and without hydrops during amniocentesis, we found the AF HA are higher in trisomy 21 fetuses with hydrops than in trisomy 21 fetuses without hydrops. (Table 2)

**Table 1**

| Characteristic of cases between euploid and trisomy 21 |
|------------------------------------------------------|
| **Euploid fetuses (n = 36)** | **Trisomy 21 fetuses (n = 20)** | **P value** |
| Gestational age at amniocentesis (weeks) | 17.2 ± 1.1 | 17.9 ± 0.9 | 0.021※ |
| Maternal age at amniocentesis (year-old) | 33.9 ± 4.7 | 36.1 ± 4.7 | 0.08※ |
| Amniotic fluid HA concentration (µg/ml) | 22.2 ± 8.8 | 25.3 ± 11.9 | 0.30※ |
| Sex ratio (male/female) | 21/15 | 9/11 | 0.40# |

※: Two-sample Student t test
#: Fisher’s Exact Test

HA: hyaluronic acid

**Table 2**

| Characteristic of cases between trisomy 21 with and without hydrops |
|--------------------------------------------------------------------|
| **Trisomy 21 fetuses without hydrops (n = 16)** | **Trisomy 21 fetuses with hydrops (n = 4)** | **P value** |
| Gestational age at amniocentesis (weeks) | 17.9 (17.4 ~ 18.5) | 17.7 (16.5 ~ 18.8) | 0.36※ |
| Maternal age at amniocentesis (year-old) | 36.9 (35.3 ~ 38.8) | 31.5 (19.8 ~ 41.3) | 0.61※ |
| Amniotic fluid HA concentration (µg/ml) | 20.5 (18.2 ~ 25.5) | 29.1 (7.3 ~ 69.1) | 0.022※ |
| Sex ratio (male/female) | 8/8 | 1/3 | 0.59# |

※: Mann-Whitney Test
#: Fisher’s Exact Test

HA: hyaluronic acid
Then the total 56 AF cases were divided into four groups: group 1 was euploid fetus without hydrops (n = 30), group 2 was trisomy 21 without hydrops (n = 16), group 3 was euploid fetuses with hydrops (n = 6) and group 4 was trisomy 21 fetus with hydrops (n = 4). The 56 sample AF HA were checked again, and the author found the AF HA concentration is significantly different among the four groups of pregnancy (one-way ANOVA test, \( p = 0.014 \)). (Fig. 1) After post-Hoc examinations, the AF HA was found as highest in group 4 pregnancy and were not significant different among groups 1, 2 and 3 pregnancy. (Fig. 1)

The correlation of the gestational age of amniocentesis and AF HA concentration, and the correlation of maternal age and AF HA concentration were both not significant. (Pearson correlation test, \( p \) value = 0.468 and 0.141, respectively) All trisomy 21 fetuses (groups 2 and 4) ended up with termination of pregnancy. The outcomes of the five euploid fetuses with hydrops (group 3) were: one fetal demise at a gestational age of 20 weeks without obvious anomaly, one with marked ascites suspected caused by meconium peritonitis and then dying in utero at a gestational age of 20 weeks, one having the pregnancy terminated at 24 weeks due to maternal severe preeclampsia, and the other two delivered at 36 and 37 weeks without apparent fetal anomaly and with hydropic change resolved.

**Discussion**

By this study, authors found the HA concentration is higher in AF of trisomy 21 fetuses presenting as hydrops fetalis. In trisomy 21 fetus without hydrops, the HA concentration in AF was not significantly different from euploid fetuses whether with or without hydrops.

The most common sonographic finding in trisomy 21 fetuses was cystic hygroma or nuchal fold thickness which together was found in 33% of cases, and the hydrops as the second common finding was 9.6%. (9) There are two possibilities authors suspected as the etiologies of the higher HA concentration in AF of trisomy 21 fetuses with hydrops: the first suspicion is that the amniocytes would secret more HA in trisomy 21 fetus with hydrops; another thought is that for trisomy 21 fetus with hydrops, the HA may more easily leak through the fetal parts like skin or umbilical cord into the amniotic fluid. Authors had ever obtained amniotic fluid mesenchymal stem cells (AFMSCs) from a pair of discordant trisomy 21 monozygotic twins in which the trisomy 21 fetus presented as hydrops. (8) Hyaluronic synthase 2 (HAS2) gene expression had been found as significantly up-regulated in the AFMSCs of trisomy 21 fetus with hydropic change as compared with the AFMSCs of euploid co-twin by human transcriptome array. (10) In the pair of discordant trisomy 21 monozygotic twin, the HA concentration in this trisomy 21 affected twin with hydrops was 32.63 µg/ml and it was 9.31 µg/ml in the euploid twin. HAS2 was the most highly expressed HA synthetic enzyme (5, 11). So this finding that hydropic trisomy 21 AFMSCs with up-regulated HAS2 gene expression may lend support to the finding that amniocytes in trisomy 21 fetus with hydrops would secret more HA than the euploid fetus. Also, there were reports saying that the HA concentrations in the umbilical cord (4–7) and fetal nuchal area (12) were higher in trisomy 21 fetuses and the possibility that the high HA in AF of hydropic trisomy 21 fetuses may come from the fetal nuchal area or umbilical cord cannot be ruled out. Owing to the two
aforementioned mechanisms, the finding that the high HA concentration in the amniotic fluid of trisomy 21 fetuses with hydrops can be explained away.

The gestational age of amniocentesis was found as higher and maternal age was older in trisomy 21 pregnancy than in euploid pregnancy. But from the correlation test, the AF HA concentration is not significantly correlated to the gestational age and the maternal age at amniocentesis.

Besides being implicated in its association with hydropic change in trisomy 21 fetuses, HA and HAS2 expression are of great importance during embryonic development. Downregulation of HA had been reported as essential for fetal limb morphogenesis and misexpression of HAS2 from the onset of limb development in vivo resulted in limb malformation (13). HA also had been reported as important in many other morphogenetic processes such as craniofacial development(14). Those findings may indicate the possibility of a link between higher HA concentration and fetal anomalies in trisomy 21 fetuses. HAS2 is regulated by the induction of COL6A2 (15). Hypothetically, inhibition of the HAS2 or COL6A2 gene expressions in trisomy 21 fetuses in early gestational age can decrease the overexpression of HA, which may open a window for fetal therapy for trisomy 21 fetus.

There are limitations of this study, including a small case number in the affected groups, and selection bias since we do not test the AF HA concentration in all euploid pregnancies. The strength of this study is we set the comparative group to test the AF HA concentration in the euploid fetuses with hydrops change at amniocentesis to elucidate the effect of hydropic change to the AF HA concentration; the authors found the fetal hydrops in euploid pregnancy did not significantly influence the AF HA level.

**Conclusion**

HA concentration is found elevated in AF of trisomy 21 fetuses with hydropic change at genetic amniocentesis. Because HA play important roles in the brain and skeletal development in fetal life, hence increased HA concentration in AF with trisomy 21 fetus with hydrops may adversely affect the pathophysiology of Down syndrome. To inhibit the HAS2 or COL6A2 gene expressions in trisomy 21 fetuses in early gestational age may pose as a potential candidate to prevent the anomalies induced by high HA concentration.

**Abbreviations**

HA  
hyaluronic acid  
AF  
amniotic fluid  
AFMSCs  
amniotic fluid mesenchymal stem cells  
HAS2
Hyaluronic synthase 2 gene

**Declarations**

**Ethics approval and consent to participate**

With an approval by the Institute Review Board of Chang Gung Memorial Hospital (IRB #93-6366 and 102-2979A3). All the included cases signed the consent.

**Consent for publication**

Not applicable.

**Availability of data and materials**

The datasets obtained and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Competing interests**

The authors declare that they have no competing interests.

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**Authors’ contributions**

YLC, ASC and SHY design the study. All authors have participated to collect the data. All authors have contributed to drafting or revising the manuscript, approved this final version of the manuscript to be published, and are willing to take public responsibility for the accuracy and integrity of its content.

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Figures

The amniotic fluid hyaluronic acid (HA) concentrations in the four groups of fetuses: the HA concentration is highest in group 4 fetuses (one-way ANOVA test, p= 0.014) and are not significant difference among group 1, 2 and 3 fetuses.

P value among groups are generated by Bonferroni test

Group 1: euploid fetuses without hydrops (n=30),

Group 2: trisomy 21 fetuses without hydrops (n= 16),

Group 3: euploid fetuses with hydrops (n=6)

Group 4: trisomy 21 fetuses with hydrops (n=4).

Figure 1

The amniotic fluid hyaluronic acid (HA) concentrations in the four groups of fetuses: the HA concentration is highest in group 4 fetuses (one-way ANOVA test, p= 0.014) and are not significant difference among group 1, 2 and 3 fetuses.