Altered Resistin Concentrations in Mid-trimester Amniotic Fluid of Fetuses With Trisomies 18 and 13: A Window onto the Pathophysiology of Trisomies 18 and 13

Abstract. Background/Aim: The study aimed to examine whether resistin is present in second trimester amniotic fluid from pregnancies with trisomy 18 and 13 and evaluate its concentration in comparison with euploid pregnancies. Patients and Methods: The study included 37 women who underwent amniocentesis. Eleven fetuses had trisomy 18, 3 had trisomy 13, while 23 had a normal karyotype. Results: Resistin was detected in all cases. The mean level of resistin in trisomy 18 was statistically significantly lower compared to euploid controls. Resistin levels in all abnormal cases were below its median concentration in euploid controls. ROC analysis showed very good prognostic value for both trisomies. Conclusion: Resistin is a constituent of mid-trimester amniotic fluid of pregnancies with trisomies 13 and 18, exhibiting lower levels than those in euploid fetuses. The reduced levels of resistin in amniotic fluid may be associated with early changes in metabolic pathways and immunoinflammatory responses.

Trisomy 18 and trisomy 13 occur in approximately one out of every 2,000 and 5,000 pregnancies, respectively (1, 2). Trisomy 18 or Edwards syndrome and trisomy 13, also known as Patau syndrome, are the second and third most frequent human autosomal aneuploidies after Down syndrome, respectively, and their incidence increases with maternal age (3). Both syndromes are associated with serious multisystem malformations and severe intellectual disability (4) and have a very high mortality rate with less than 1 in 5 infants surviving their first birthday (5). There is great interest in establishing prenatal diagnosis of these syndromes, while recent studies have focused on explaining the phenotypic features of these aneuploidies through molecular pathways.

Resistin is a 12.5 kDa polypeptide that belongs to the resistin-like molecule family that is secreted by adipocytes, inducing insulin resistance, mainly hepatic and, has thus been suggested as a possible link between obesity and type 2 diabetes (6). In humans, it is encoded by a gene located on chromosome 19 (7). Increased resistin levels have been found in cases of chronic inflammation, such as chronic hepatitis (8), rheumatoid arthritis (9) and chronic kidney disease (10). These data indicate a possible role of resistin in inflammatory response. Resistin concentration is significantly elevated in
pregnant, compared to non-pregnant women and is further increased in cases of pregnancy-induced insulin resistance (11, 12). In addition, there is evidence of involvement of resistin in fetal growth regulation (13).

Maternal plasma resistin in pregnancy has been investigated previously, however, research studies examining the concentration of resistin in amniotic fluid, especially in pathological pregnancies, are scarce. The aim of this study was to investigate resistin levels in amniotic fluid in second trimester of pregnancies with trisomies 18 and 13.

**Patients and Methods**

The study was performed at the Aretaieio University Hospital in cooperation with a private genetic diagnostic center in Athens, Greece. The institutional ethics committee approved the study’s protocol and all women provided written informed consent before the amniocentesis.

Thirty-seven women with singleton pregnancies were included in the study. In all cases gestational age was determined with an early ultrasound examination in the first trimester and amniocentesis was performed in the second trimester. The indications were advanced maternal age, abnormal first trimester combined biochemical and sonographic findings or family history of congenital anomalies.

Ultrasound evaluation and amniocentesis were performed transabdominally by certified practitioners. Multiple pregnancies and pregnancies with fetuses exhibiting anatomical anomalies were excluded.

After amniocentesis, amniotic fluid was centrifuged and supernatants were stored in polypropylene tubes at –80°C, until the time of resistin determination. The presence of extra chromosomes 13 or 18 were ascertained by QF-PCR (quantitative fluorescent polymerase chain reaction) analysis and fetal karyotype was confirmed via conventional cytogenetic cultures.

Samples from the three pregnancies with trisomy 13 and from 11 cases with trisomy 18, confirmed by karyotyping (study groups), were compared with 23 samples derived from uncomplicated singleton pregnancies with a normal karyotype (control group). For each pregnancy, maternal age (in completed years), gestational age (in weeks) at amniocentesis and fetal sex were recorded. Levels of resistin in the amniotic fluid were measured by a commercially available enzyme-linked immunosorbent assay (ELISA) kit (ALPCO Diagnostics, Salem, NH, USA). The detection limit of the kit was 100 pg/ml and the intra-assay and inter-assay coefficients of variation were 2.9% and 7.2%, respectively. Analysts were blinded to the clinical information.

Quantitative variables were expressed as mean±standard deviation (SD) or median (IQR, interquartile range: 25th-75th percentiles), depending on whether they followed the normal distribution or not, respectively. Student’s t-test was applied to detect differences between groups when continuous variables were normally distributed. Otherwise, the Mann–Whitney U-test was applied. Normality was statistically examined using the Kolmogorov-Smirnov test. The correlation between continuous variables was studied using Pearson’s r coefficient for normal distributions as well as linear regression models. The overall comparison of resistin levels among the study and control groups was performed with the non-parametric Kruskal–Wallis test and post-hoc analysis, was performed with Conover’s test adjusted for multiple testing with the Benjamini–Hochberg method. Qualitative variables were expressed as numbers and percentages. Proportions were compared with Chi-square or Fisher’s exact tests as appropriate. Receiver operator curve (ROC) analysis was performed to examine the diagnostic value of resistin concentration in mid-trimester amniotic fluid for trisomies 13 and 18. All statistical analyses were performed using MedCalc Statistical Software version 12.7.7 (2013 MedCalc Software bvba, Ostend, Belgium). P<0.05 was considered statistically significant.

**Results**

A total number of 37 singleton pregnancies were examined, 3 pregnancies with trisomy 13, 11 cases with trisomy 18 and 23 euploid pregnancies serving as controls. Gestational age at amniocentesis ranged from 15 weeks plus 3 days to 22 weeks plus 5 days in all participants. The fetal sex ratio (males/females) was 1/2 and 5/6 among cases of trisomy 13 and 18, respectively, and 11/12 in the control group. Characteristics of women and resistin concentration in amniotic fluid according to the fetuses’ status are presented in Table I.

| Characteristic                              | Cases with Trisomy 13 (n=3) | Normal pregnancies (n=23) |
|--------------------------------------------|----------------------------|--------------------------|
| Maternal age (years) (mean, SD)            | 36.7 (6.7)                 | 35.5 (4.3)               |
| Gestational age (weeks) (mean, SD)         | 18.7 (2.1)                 | 18.4 (1.6)               |
| Resistin (ng/ml) (median, IQR)             | 2.4 (2.4-2.8)              | 3.3 (2.9-3.7)            |

| Characteristic                              | Cases with Trisomy 18 (n=11) | Normal pregnancies (n=23) |
|--------------------------------------------|-----------------------------|--------------------------|
| Maternal age (years) (mean, SD)            | 39.7 (4.2)                  | 35.5 (4.3)               |
| Gestational age (weeks) (mean, SD)         | 18.8 (2.2)                  | 18.4 (1.6)               |
| Resistin (ng/ml) (median, IQR)             | 2.5 (0.4)                   | 3.3 (0.8)                |

Table I. Characteristics of women and resistin concentration in amniotic fluid of pregnancies with trisomies 13 and 18 and normal controls.
Maternal age, gestational age and resistin levels in cases with trisomy 18 and controls did not significantly deviate from the normal distribution (trisomy 18: maternal age: \(p=0.368\), gestational age: \(p=0.855\), resistin: \(p=0.487\), euploid group: maternal age: \(p=0.104\), gestational age: \(p=0.312\), resistin: \(p=0.962\)), although the abovementioned variables could not be considered to follow a normal distribution in trisomy 13 due to the limited number of cases.

Resistin was detected in all samples obtained from aneuploid pregnancies. A positive correlation between resistin concentrations and gestational age was found in normal pregnancies (\(r=0.68, 95\%\text{CI}=0.38-0.85, p<0.001\), linear regression model: \(b=0.35, 95\%\text{CI}=0.18-0.52, p<0.001\)). This translates to an average increase of amniotic fluid resistin concentration by 0.35 ng/ml for every advancing gestational week in mid-trimester pregnancies (Figure 1). In contrast, levels of resistin were not significantly correlated with gestational age in pregnancies with trisomy 18 (\(r=–0.33, 95\%\text{CI}=–0.87-0.56, p=0.473\)). Furthermore, no significant association was found between resistin concentration and maternal age in trisomy 18 cases (\(r=0.53, 95\%\text{CI}=–0.21-0.88, p=0.528\)) or controls (\(r=0.31, 95\%\text{CI}=–0.12-0.64, p=0.311\)). Finally, resistin amniotic fluid levels did not differ by fetal sex in either group (trisomy 18: \(p=0.702\), controls: \(p=0.113\)).

Mothers who carried a fetus with trisomy 18 were older than normal controls (mean age: 39.7±4.2 vs. 35.5±4.3 years, respectively, \(p=0.019\), mean difference: 4.2 years, 95\%CI=0.7-7.6). On the other hand, no difference in maternal age was observed between cases of trisomy 13 and controls (\(p=0.675\)). Gestational age was similar in both pathological cohorts and in controls (trisomy 13 vs. controls: \(p=0.789\), trisomy 18 vs. controls: \(p=0.617\)).

The mean second trimester level of resistin in cases with trisomy 18 was 2.5 (±0.4) ng/ml, statistically significantly lower as compared with 3.3 (±0.8) ng/ml in euploid controls (\(p=0.004\)) (mean difference: 0.8 ng/ml, 95\%CI=0.3-1.4). Resistin was on average 25% lower (95\%CI=8-41) in the amniotic fluid from pregnancies with trisomy 18 compared with the normal group. Nine out of 11 cases with trisomy 18 (81.8%) had low resistin concentrations (≤2.7 ng/ml), compared with 4 out of 23 healthy pregnancies (17.4%) (OR=21.4, 95\%CI=3.3-139.2, \(p=0.001\)). Additionally, median resistin amniotic fluid levels in pregnancies with trisomy 13

![Figure 1. Positive association of amniotic fluid resistin concentration with advancing gestational age in mid-pregnancy (Linear regression model: \(Y=0.35X-3.07, b=0.35, 95\%\text{CI}=0.18-0.52, p<0.001\)).](image1)

![Figure 2. Scatter-plot diagram of resistin concentration (ng/ml) in fetuses with trisomies 13 and 18 and normal fetuses.](image2)

![Figure 3. Boxplot diagram of the distribution of resistin levels (ng/ml) according to the presence of trisomy 13 or 18 or euploidy.](image3)
were lower than in controls, although the difference marginally failed to reach statistical significance (2.4 ng/ml vs. 3.3 ng/ml, \( p=0.071 \)). In a dichotomous evaluation, all trisomy 13 cases (3/3, 100%) had resistin concentrations of <2.9 ng/ml whereas in 17 out of 23 controls (73.9%) resistin levels were ≥2.9 ng/ml, this difference also being of marginal statistical significance (OR=18.8, 95%CI=0.9-417.1, \( p=0.063 \)). A statistically significant difference among aneuploid fetuses and controls was established after ranking the resistin levels for the whole sample (Kruskal-Wallis test: \( p=0.004 \)). While post-hoc analysis, adjusted for multiple testing, provided \( p=0.004 \) for the comparison trisomy 18 vs. controls and a marginal significance of \( p=0.052 \) for trisomy 13 vs. controls (Figures 2 and 3).

ROC analysis for trisomy 13 showed an AUC (area under the curve) at 82.6% (95%CI=62.8-94.5, \( p<0.001 \)). At a cut-off limit of 2.9 ng/ml, determination of resistin mid-trimester amniotic fluid concentration classified correctly all trisomy 13 cases (sensitivity 100%) with specificity 73.9%. Furthermore, ROC analysis for trisomy 18 provided AUC=82.6% (95%CI=65.8-93.4 \( p<0.001 \)) performing best at a cut-off resistin level of 2.7 ng/ml (sensitivity 81.8%, specificity 82.6%).

Overall, in the total number (14/14, 100%) of affected pregnancies (trisomy 13 or 18), resistin concentration was ≤3.2 ng/ml while in the majority of euploid pregnancies (12/23, 52.2%) resistin level was >3.2 ng/ml (OR=31.5, 95%CI=1.7-590.8, \( p=0.021 \)). ROC analysis resulted in AUC=82.6% (95%CI=66.6-93.1 \( p<0.001 \)) and by selecting a resistin concentration cut-off of 2.7 ng/ml, 11 out of 14 aneuploid fetuses and 19 out of 23 normal fetuses were correctly diagnosed (sensitivity 78.6%, specificity 82.6%) (Figure 4).

**Discussion**

Amniotic fluid is produced daily by the fetal urinary system and lungs and until the keratinization of fetal skin, at the end of the second trimester, the composition of amniotic fluid is similar to that of fetal plasma. Consequently, the concentration of fetal proteins in second trimester amniotic fluid is correlated with fetal serum concentration (14).

In the present study, resistin concentrations in the amniotic fluid of mid-trimester fetuses with trisomy 13 or 18 were found to be significantly decreased compared with euploid pregnancies.

To our knowledge this is the first study analyzing resistin levels in the amniotic fluid of trisomy 18 and 13 pregnancies. Decreased concentrations of amniotic fluid resistin have previously been reported in pregnancies with trisomy 21 (15). Our results are in agreement with those of a previous study which investigated the levels of cytokines in amniotic fluid (16). In this report, the researchers measured the amniotic fluid levels of interleukin-6, interleukin-8 and tumor necrosis factor-\( \alpha \).
factor-beta in a group of 15 pregnancies with fetal chromosomal abnormalities, which included only 3 cases of trisomy 18 and 1 fetus with trisomy 13. Resistin has been detected in the umbilical venous blood (13). Furthermore, there are also reports that resistin is expressed in the placenta (17, 18) and that this expression is increased in full-term pregnancies. These data imply that the placenta is a possible source of resistin production and that placenta pathology can lead to reduced resistin levels. Kusanovic et al. (19) detected resistin in amniotic fluid for the first time and reported that its levels increase in the presence of an intrauterine infection and intra-amniotic inflammation. Additionally, there is evidence that the median amniotic fluid resistin concentration more than doubled in full-term pregnancies, compared to its levels in mid-trimester (14-18 weeks). In accordance with the above, in the present study, that resistin levels were found to be significantly increased in the amniotic fluid of normal pregnancies with advancing gestational age during the second trimester (15-22 weeks). Placental cytokines are involved in the molecular pathways leading to preterm birth (20), and elevated levels of amniotic fluid resistin were reported in cases of preterm labor (19, 21). However, in a cohort of 96 twin pregnancies, mid-trimester amniotic fluid resistin was not shown to predict early preterm birth (22). In another study of 70 singleton pregnant women undergoing amniocentesis at 15-20 weeks of gestation, Bugatto et al. (23) found a moderate significant positive correlation between amniotic fluid resistin and body mass index (r=0.353, p=0.003). In the abovementioned study, amniotic fluid resistin levels did not differ between male and female fetuses and this finding was confirmed in the present cohort.

Although the primary objective of the present study was not prenatal diagnosis, ROC analysis revealed that amniotic fluid resistin provides very good prognostic value for trisomies 13 and 18. The AUC was equal (82.6%) for trisomy 13 and trisomy 18 (as well as for either of the two), although with a slightly wider confidence interval for trisomy 13 due to the limited number of cases. However, due to the fact that determination of resistin concentration in amniotic fluid requires amniocentesis, which carries a minimal risk of miscarriage and also permits karyotyping testing, it could not serve as a screening test for these aneuploidies. Finally, as expected, given the increase in the incidence of trisomy 18 with maternal age (3), in our sample, mothers carrying fetuses with a trisomy were older than controls. However, this had no effect on the results, because resistin concentration was found to be independent of maternal age in both groups.

Resistin has been strongly associated with chronic and acute infection, while it has additionally been reported that resistin mRNA expression increases in blood mononuclear cells that are exposed to interleukins and tumor necrosis factor-alpha, implying a proinflammatory role of resistin in inflammatory response (24). Since, resistin levels were found to be significantly lower in fetuses with trisomies 18 and 13, this protein is possibly involved in the speculated inflammatory cascade of these syndromes.

Pregnancy is characterized by maternal hyperglycemia, hyperinsulinemia and insulin resistance accompanied by increased transfer of crucial nutrients and energy to the fetus, essential for its normal development. It has been suggested that resistin may play a major role in serum glucose concentration, thereby increasing insulin resistance. In normal pregnancies resistin has been found in high concentrations in maternal serum, as well in fetal umbilical blood (25). Furthermore, in preterm neonates’ umbilical cord resistin concentration was significantly lower than in full-term neonates (26) Resistin was also found to be lower in neonates from mothers with diabetes who received insulin during pregnancy compared to healthy mothers (27). Resistin has also been shown to induce the production of vascular endothelial growth factor and the normal proliferation of the placental unit (28). These data indicate a possible role for adipocytokines in normal intrauterine development. The results of our study, showing decreased resistin amniotic fluid levels in pregnancies with a trisomy 18 or 13, supports this hypothesis. The levels of resistin may reflect metabolic disorders or inadequate placentation. However, further research is necessary for a deeper understanding of the pathway that leads from a pathologic karyotype to low levels of resistin.

Our results showed that in the amniotic fluid of trisomy 18 and 13 fetuses there were reduced concentrations of resistin, the final product of a gene that is not directly related to chromosomes 18 or 13. However, the exact mechanism via which the genes located in these chromosomes affect the expression of resistin remains unclear.

**Conclusion**

The results of the present pilot study revealed that the levels of resistin, in second trimester amniotic fluid were lower in pregnancies with trisomy 18 and 13, compared to those in euploid pregnancies. Our data, although limited, support the hypothesis that certain features of these aneuploidies may be associated with altered concentrations of resistin in second trimester amniotic fluid, evidence which could contribute to the clarification of their metabolic and inflammatory pathways. Further research should be encouraged in order to clarify the observed differences as well as the role of resistin and other adipocytokines in the pathophysiology of these karyotype abnormalities.

**Conflicts of Interest**

The Authors have no conflict of interest to disclose regarding this study.
Authors’ Contributions

Nikolaos Vrachnis: Conception and design, acquisition of data, drafting the article and critically revising it, gave final approval of the submitted version. Erminia Dalakli: conception and design, acquisition of data, drafting the article and critically revising it, gave final approval of the submitted version. Dimitrios Zygouris: Acquisition of data, drafting the article, gave final approval of the submitted version. Nikolaos Vlachadis: Analysis and interpretation of data, drafting the article, gave final approval of the submitted version. Nikolaos Salakos: Analysis and interpretation of data, critically revising the article, gave final approval of the submitted version. Dimitrios Botsis: Analysis and interpretation of data, critically revising the article, gave final approval of the submitted version. Sophia Kalantaridou: Analysis and interpretation of data, critically revising the article, gave final approval of the submitted version. Dimitrios Botsis: Analysis and interpretation of data, critically revising the article, gave final approval of the submitted version. Nikolaos Drakoulis: Acquisition of data, drafting the article, gave final approval of the submitted version. George Mastorakos: Analysis and interpretation of data, critically revising the article, gave final approval of the submitted version. Efthymios Deligeoroglou: Analysis and interpretation of data, critically revising the article, gave final approval of the submitted version. Zoe Iliodromiti: Conception and design, drafting the article and critically revising it, gave final approval of the submitted version.

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