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硕　士　学　位　论　文

初探唾液酸在母婴血液和组织中的表达水平对正常和辅助生殖妊娠结局的影响

Effect of sialic acid expression in maternal-fetal blood and tissues of natural and assisted reproductive technology's pregnancy outcomes

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Content

摘 要

研究背景：唾液酸（Sialic acid, Sia）是一类以九碳糖神经氨酸为基本结构的衍生物的总称，在自然界广泛存在，并发挥重要的生物功能。N-乙酰神经氨酸（Neu5Ac），N-羟乙酰神经胺酸（Neu5Gc）和脱氨神经氨酸（KDN）是唾液酸三种主要亚型，其中，前两者是哺乳动物中最常见的存在形式，但在人体中很难检测到 Neu5Gc。目前关于唾液酸的研究多集中于感染、肿瘤、冠心病、神经发育迟缓等疾病以及神经系统发育这方面的健康问题，它因广泛存在于人类的组织和体液包括血液、唾液、胃液、脑脊液，并随健康与疾病的变化而变化，而被作为生物标记物来辅助诊断多种疾病，具有重要的研究意义。由于唾液酸和人类生殖过程密切相关，唾液酸水平随着妊娠过程而逐渐变化，如唾液、血清、羊水中的唾液酸含量在健康女性孕期持续增加，以供胎儿的正常发育需求。唾液酸也是母乳、脑神经节苷脂中的重要成分，婴幼儿通过母乳摄入唾液酸对其早期生长发育尤其是神经系统的发育起到重要的促进和保护作用。因此，我们推测唾液酸在妊娠过程的变化，与孕妇代谢、胎儿发育密切相关，可能用于胎儿的发育状态等方面进行检测诊断。

辅助生殖技术（assisted reproductive technology，ART）是指用医疗手段辅助自然受孕过程的某一或全部环节来完成生育的方法，涉及诸多非自然受孕过程，与自然妊娠存在较大差异。随着中国二孩政策的开放，新生儿出生率升高，高龄孕妇逐渐增多，ART 经过近四十年的快速发展，给许多不孕不育患者带来福音，人群规模也将继续庞大。但是，同时随之而来的孕产期风险和不良妊娠结局已成为 ART 的重要问题，给母婴健康带来威胁。如何提高 ART 妊娠的母婴结局，必然引起全社会的高度重视。唾液酸是人类生殖过程的重要参与者，但目前尚未有关于唾液酸和辅助生殖妊娠的研究。本课题将辅助生殖妊娠的母婴与自然妊娠进行对比，首次分析唾液酸在 ART 母婴血液、脐带和胎盘中表达的变化，探讨了唾液酸含量与辅助生殖妊娠结局的关系，为评估母婴健康、减少妊娠不良结局、预测和辅助诊断等提供研究基础。

研究目的：本研究通过调查辅助生殖技术的妊娠结局，测量并比较母婴血液

和组织包括血清、血浆、红细胞膜、脐带和胎盘，不同唾液酸的含量、存在形式及分布，探讨唾液酸与辅助生殖妊娠结局之间的关系。

研究方法：分别于 2016 年 2 月~7 月厦门大学附属成功医院，2017 年 1 月~2 月福建省福州市妇幼保健院招募辅助生殖技术妊娠和自然妊娠孕妇各 60 例、59 例，其中 21 例妊娠糖尿病的孕妇，总共 119 例，以一般资料调查法调查母婴的基本情况；同时采用液相色谱-质谱联用法来测量血液和脐带、胎盘中唾液酸的含量。

研究结果：ART 孕妇（1）年龄、生活条件、不良妊娠结局发生率如妊娠期并发症发生率、早产率、低出生体重儿率、多胎率均显著高于自然妊娠组孕妇（P＜0.01），新生儿身长明显低于自然妊娠组新生儿（P＜0.01）；（2）脐带血血浆中结合态 KDN 含量显著低于自然妊娠组（P＜0.05），外周血血清、脐带血血清中的总唾液酸含量分别为 34.33±2.05μmol/g protein，24.07±2.76μmol/g protein，均显著高于对照组（P＜0.05）；（3）孕妇、脐带血、新生儿的红细胞膜以及脐带、胎盘组织中的总唾液酸含量分别为 574.63±32.93μmol/g protein，302.20±30.10μmol/g protein，44.83±3.41μmol/g protein，33.12±10.07μmol/g protein，229.99±19.83μmol/g protein。其中新生儿红细胞膜上的总唾液酸显著高于自然妊娠组（P＜0.01），脐带、胎盘中的总唾液酸显著低于自然妊娠组（P＜0.01）；（4）不同血样及组织中都没有检测到 Neu5Gc，且唾液酸的主要存在形式为结合态 Neu5Ac，游离态 KDN 占总 KDN 的构成比均高于自然妊娠组。

妊娠糖尿病的孕妇（1）脐带血血浆中总唾液酸含量（41.42±19.05μmol/g protein）明显高于无妊娠糖尿病的孕妇（P＜0.05）；（2）不同血样及组织中都没有检测到 Neu5Gc，且唾液酸的主要存在形式为结合态 Neu5Ac，游离态 KDN 占总 KDN 的构成比均高于无妊娠糖尿病的孕妇；（3）血清唾液酸水平与新生儿血糖成正相关性（P＜0.05）。

所有孕妇（1）外周血清、红细胞膜、新生儿红细胞膜上的总唾液酸含量与有无除妊娠糖尿病以外的妊娠期并发症有正相关性（P＜0.05），脐带、胎盘中的总唾液酸含量与其有负相关性（P＜0.01）；（2）外周血血清、脐带血血清、新生儿红细胞膜上的总唾液酸含量越高，婴儿不良妊娠结局的风险就越高（P＜0.05）；（3）新生儿体重与孕妇外周血血清唾液酸含量成负相关（P＜0.01），

与胎盘组织中的唾液酸含量成正相关（P＜0.05）；（4）筛选出的健康母婴，外周血血清、红细胞膜、脐带血血清、血浆、红细胞膜、脐带、胎盘以及新生儿红细胞膜中的总唾液酸水平范围分别为 23.60±16.97μmol/g protein，404.64±220.24μmol/g protein，14.22±2.72μmol/g protein，0.04±0.02μmol/g protein，237.77±58.14 μmol/g protein，137.27±112.77μmol/g protein，459.69±297.90μmol/g protein，27.73±9.65μmol/g protein，且没有检测到 Neu5Gc。

结论：ART 孕妇的血清、脐带血清、新生儿红细胞膜的唾液酸水平显著高于自然妊娠孕妇，脐带、胎盘组织的唾液酸水平显著低于自然妊娠孕妇；妊娠糖尿病的孕妇脐带血血浆中总唾液酸含量显著高于无妊娠糖尿病孕妇；孕妇外周血血清唾液酸水平越高，新生儿在正常范围内血糖越高，体重越低；孕妇胎盘组织中唾液酸水平越高，新生儿体重越高；孕妇血清、新生儿红细胞膜的总唾液酸水平越高，母婴不良妊娠结局的风险越高；脐带、胎盘组织的总唾液酸水平越高，孕妇患妊娠期并发症的风险就越高。

关键词：唾液酸；辅助生殖技术；液相色谱-质谱联用；妊娠结局

Abstract

Background: Sialic acids (Sia) are a diverse family of nine-carbon sugar neuraminic acid as the basic structure of derivatives, widely distributing in nature and playing important roles in lots of vital physiological processes. N- acetylneuraminic acid (Neu5Ac), N-glycolylneuraminic acid (Neu5Gc) and 2-keto-3-deoxy-D-glycero-D-galactonononic acid (KDN) are sialic acid's main subtypes, the first two are the most common form in mammals, but Neu5Gc is devoid in the human. Biomedical research of Sia is focus on infection diseases, tumor，coronary disease, development of nervous system and neurodevelopmental delay disease. Sia is expressed in all body tissues and body fluids including blood, saliva, gastric juice, brain spinal fluid in human. As Sia expression levels vary with health and disease state of indivadual, therefore Sia can be used as a biomarker for diseases diagnosis in the clinic. In human productive medcine, Sias expression levels are gradually change during pregnancy. For instance, Sia concentration in saliva, serum and amniotic fluid of healthy pregnant women increases constantly for fetal development during pregnancy. Since Sia is an enssential nutrient in human milk and brain gangliosides and sialylated glyproteins , ectogenic Sia plays an building block role for early infants' growth and development. Our hypothesis is that the changes of different form Sia levels in blood and tissues during pregnancy and labor are closely associated with maternal-fetal's Sia metabolism, brain Sia accumulation of fetus, complications of pregnant women and pregnant outcomes.

Assisted reproductive technology (ART) is a method to complete reproductive process by using medical auxiliary means in the process of one or all links, involving lots of non natural conception process. There is a big difference with natural pregnancy. ART has been used in clinic nearly forty years, it benefits to patients with infertility, but its adverse outcomes for mother and newborns are increased, since the use of ART has increased substantially in China. Sia is an important molecule

involving in the process of human reproductive process, but the role of Sia in ART women has not been studied at present. We compared pregnancy outcomes between ART and natural pregnancy, analyzed the level of Sia in different blood samples and tissues to explore the underlying mechamisim of the relationship of Sia content and ART pregnancy outcome, these studies provide unique opportunities to improve perinatal outcome of ART women and natural pregnancy women.

Objective: We tested the hypothesis that changes of different form Sia levels in serum, plasma, erythrocyte membrane, umbilical cords and placentas during pregnancy and labor are closely associated with maternal-fetal's Sia metabolism, brain Sia accumulation of fetus, complications of pregnant women and pregnant outcome.

Methods: One hundred and nineteen pregnancy women including ART and natural pregnancy in Chenggong Hospital affiliated to Xiamen University and Fuzhou Maternity and Child Health Care Hospital were involved in this study. There were 60 ART and 59 natural pregnant women respectively，including 21 pregnant women of gestational diabetes mellitus. Sia levels of blood samples，umbilical cords and placentas were quantified by sensitive LC-MS/MS.

Results: Pregnancy women of ART(1) Age, living conditions and the incidence of adverse pregnancy outcomes such as pregnancy complications, premature birth, low birth weight, multiple pregnancy rate were significantly higher than control group($P<0.01$), neonatal length was significantly shorter than control group($P<0.01$); (2) The conjugated KDN of cord blood plasma was significantly lower than that of control group($P<0.05$), total Sia content of serum(34.33±2.05μmol/g protein) and in cord blood serum(24.07±2.76μmol/g protein) were significantly higher than that of control group($P<0.05$); (3) The total Sia content of erythrocyte membrane in pregnant women, cord blood and infants, umbilical cords, placentas were 574.63±32.93μmol/g protein，302.20±30.10μmol/g protein，44.83±3.41μmol/g protein，33.12±10.07μmol/g protein，and 229.99±19.83μmol/g protein respectively. Those infants' Sia in erythrocyte membrane was significantly higher than control group($P<0.01$), while umbilical cords and placentas' Sia were significantly lower than control

group(P＜0.01); (4) Neu5Gc was not detected in any samples, and the main form of Sia is conjugated Neu5Ac. The proportion of free KDN in total KDN was higher than control group.

Pregnant women of gestational diabetes mellitus(GDM) (1) Total Sia content of cord blood plasma (41.42±19.05μmol/g protein) was significantly higher than pregnancy women who did not have GDM(P＜0.05); (2) Neu5Gc was not detected in any samples, and the main form of Sia is conjugated Neu5Ac. The proportion of free KDN in total KDN was higher in GDM women than without GDM pregnant women; (3) There was positive correlation between total Sia content of serum in GDM women and the neonates blood glucose(P＜0.05).

All pregnant women including ART, GDM and others (1) There were positive correlation between total Sia content of serum, erythrocyte membrane, infants' erythrocyte membrane and pregnancy complications except GDM(P＜0.05), negative correlation between total Sia content in umbilical cords, placentas and pregnancy complications except GDM(P＜0.01); (2) The higher total Sia content of serum, cord blood serum and infants' erythrocyte membrane was, the higher incidence of adverse fetal outcomes was(P＜0.05); (3) There was negative correlation between total Sia content of serum and new born weight(P＜0.01), positive correlation between total Sia content in placentas and new born weight(P＜0.05); (4) Total Sia content of serum, erythrocyte membrane, cord blood serum, plasma, erythrocyte membrane and umbilical cords, placentas, infants' erythrocyte membrane among screened healthy mothers and infants were 23.60±16.97μmol/g protein，404.64±220.24μmol/g protein，14.22±2.72μmol/g protein，0.04±0.02μmol/g protein，237.77±58.14 μmol/g protein，137.27±112.77μmol/g protein，459.69±297.90μmol/g protein，27.73±9.65μmol/g protein respectively, and Neu5Gc was not detected in any samples.

Conclusion: Total Sia concentration of ART pregnancy women was significantly higher than that of natural pregnancy in serum, cord blood serum, infants' erythrocyte membrane, and lower than that of natural pregnancy in tissues of umbilical cords and placentas respectively. Total plasmatic Sia level of GDM pregnancy women was significantly higher than that of others. The higher total Sia content of maternal serum