Exposure to Microplastics in Neonates Receiving Parenteral Nutrition: A Prospective Cohort Study in Neonatal Intensive Care Units (Protocol)

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1. Background

Microplastics are defined as plastic particles smaller than 5 millimeters in diameter. This definition, established by the National Oceanic and Atmospheric Administration (NOAA), remains the most widely accepted standard. These particles exhibit diverse morphologies, ranging from fragments and fibers to films, granules, and even foam. Due to their minuscule size, they are often referred to as "PM2.5 of the ocean." Microplastics are ubiquitous, present in water bodies, soil, air, and the food chain, with detection reported in seawater, seafood, drinking water, and even honey. Primary microplastics (MPs) originate directly from personal care products, synthetic textiles, and industrial production processes, while secondary microplastics result from the weathering and mechanical degradation of larger plastic products. Recent advancements in analytical techniques have led to the detection of microplastics in human blood, lungs, placentas, and even breast milk, confirming their systemic distribution and potential for accumulation in the human body. These findings have sparked significant public and scientific concern regarding their long-term health impacts, particularly in the most vulnerable population—neonatal infants.

Newborns, particularly those requiring hospitalization due to preterm birth, low birth weight, or severe intestinal dysfunction, are exposed to scenarios markedly different from the general population. In neonatal intensive care units (NICUs), life support and nutritional supply are highly dependent on plastic medical devices such as intravenous infusion bags, tubing, infusion pumps, and ventilator circuits. These devices may contain intentionally added microplastics during production or release secondary microplastics through physical friction or chemical degradation during use. Of particular significance is that for critically ill neonates who cannot be fed orally, parenteral nutrition (PN) serves as their sole source of survival. However, the entire "plastic tubing" system constituting the parenteral nutrition delivery pathway represents a potential, persistent source of microplastic exposure. Studies have demonstrated that microplastic particles can be detected in infusion solutions after passing through plastic equipment[]. This implies that neonates receiving intravenous nutrition may be subjected to a unique exposure pattern of "iatrogenic, persistent microplastic infusion."

However, clinical studies on neonatal exposure to AP remain limited. Moreover, most neonatal biomonitoring studies are based on in vitro experiments and random urine samples collected at hospital admission or discharge, without considering the time-dependent nature of exposure. Additionally, there is a lack of research on neonatal microplastic load, particularly quantitative data in blood. Therefore, this study aims to assess the current status of neonatal microplastic exposure during NICU hospitalization. Through a meticulously designed gradient exposure cohort, this study seeks to systematically evaluate and compare the microplastic load in the blood of neonates receiving long-term parenteral nutrition for the first time.

2、Materials and Methods

2.1. Study Population and Stratification

This study was a prospective observational study. In 2 02 5,12 neonates were enrolled in the NICU of the Second Affiliated Hospital of Guangdong Province, Jinan University. All

subjects mothers had no underlying conditions such as heart disease, chronic kidney disease, mental illness, hypertension, diabetes, gestational hypertension, or gestational diabetes. Researchers conducted face-to-face interviews to record demographic information. All participants signed informed consent forms and completed questionnaires. A total of 12 neonatal blood samples, 12 breast milk samples, and 12 bottle milk samples were collected. To accurately assess the dose-response relationship between the duration of parenteral nutrition and microplastic load, neonatal blood samples were divided into the following three groups: (1) Long-term exposure group (n=4): Preterm infants requiring total parenteral nutrition support for>14 days due to severe feeding difficulties, necrotizing enterocolitis (NEC), short bowel syndrome, or other serious intestinal diseases. (2) Short-term exposure group (n=4): Preterm infants receiving total parenteral nutrition support for 3 to 7 days due to early adaptation issues (e.g., respiratory distress, transient feeding intolerance) and having successfully transitioned to total enteral nutrition for at least 48 hours prior to blood sample collection. This group represented common, transient, routine iatrogenic exposure in NICUs. Control group (n=4):The cohort consisted of healthy full-term neonates with gestational age ≥37 weeks. These infants received no intravenous nutritional support or planned fluid therapy, serving as baseline microplastic levels in the absence of relevant iatrogenic exposure. The study strictly adhered to the ethical guidelines of the Declaration of Helsinki, and the protocol was approved by the Medical Ethics Committee of the Second Affiliated Hospital of Guangdong Province, Jinan University.

2.2 Interview Content

At enrollment, investigators conducted interviews to collect demographic information from newborns and pregnant women, including education level (high school or below, bachelors degree, masters degree or above), per capita annual income (<100,000 yuan, 100,000-200,000 yuan,>200,000 yuan), occupation (government official, professional technician, general clerk, commercial service, other), number of pregnancies (primipara, multipara), mode of pregnancy (natural delivery, assisted delivery), delivery outcome (preterm, full-term), delivery method (vaginal delivery, cesarean section), newborn gender (male, female), and neonatal weight. Details are provided in Table S1.

2.3 Sample Collection

Samples were collected by trained pediatricians and pediatric nurses. After delivery, the newborns blood was drawn using a non-plastic blood collector and then injected into a glass anticoagulation tube (KERONG, 3mL, China Chengdu). Breast milk and milk from bottles were collected by trained nurses or family members and quickly placed into sample boxes. To avoid contamination, all consumables that came into contact with the samples during the sampling process were non-plastic products. All samples were stored in a-80℃ environment and strictly anonymized.

2.3 Microplastic Analysis

The sample was transferred to a glass vial and subjected to digestion for a specified duration to obtain the digestion solution. The sample was then processed through a

membrane and analyzed using a confocal micro-Raman spectrometer. All reagents and instruments (e.g., test tubes, beakers, pipettes) used during the procedure were glassware, with no contact with plastic components. When a monochromatic light beam was incident on the transparent sample, most of the light transmitted while a small portion was scattered in various directions. When photons undergo inelastic collisions with sample molecules, the scattered light exhibits energy differences compared to the incident light, resulting in changes in both frequency and direction. This phenomenon is termed Raman scattering. The mechanism of Raman scattering involves energy exchange between photons and molecules, altering the photons energy. Raman shift spectra are particularly suitable for analyzing molecular structures, especially non-polar bonds (e.g., C-C bonds) and crystalline materials. Combined with confocal microscopy, point-by-point scanning generates high-resolution chemical images, enabling precise localization of microplastic components and their distribution.

2.4 Statistical Analysis

All data analyses were performed using SPSS Statistics 26, with statistical significance set at a two-tailed test ($p < 0.05$). First, normality testing (Shapiro-Wilk test) and homogeneity of variance were conducted for measurement data. Variables conforming to normal distribution were described as mean ± standard deviation, while non-normally distributed variables (primarily microplastic concentration data) were described as median (interquartile range, IQR). Categorical data were presented as frequency (percentage). For baseline characteristic comparisons among the three groups, normal-distributed continuous variables were analyzed using one-way ANOVA, non-normally distributed continuous variables were analyzed using the Kruskal-Wallis H test, and categorical variables were analyzed using Fishers exact test. Some charts were created using GraphPad Prism 9.0 software, and data were rigorously reviewed. Statistical analysis of some data was primarily completed using R software (version 4.4.1). Some plots were generated using ggplot. Correlation coefficients were calculated using the R function cor.test, and hypothesis testing results were calculated using the R function wilcox.test.

Survey Form

Characteristics	Categories
Education	High school and below
	Bachelor’s degree
	Postgraduate and above
Occupation	Governmental official
	Profession and technology
	General office

	Business services
	Others
Annual income per capita (CNY)	<100 thousand
	100 − 200 thousand
	>200 thousand
Parity	Primipara
	Multipara
Mode of conception	Natural conception
	Assisted reproduction
Birth outcome	Preterm
	Full-term
Mode of delivery	Vaginal delivery
	Cesarean delivery
Sex of newborns	Male
	Female
Newborn weight (kg)	-