**Associations of maternal exposure to multiple plasma metals with the risk of fetal congenital heart defects: a prospective nested case-control study**

Z. Nie¹, YANQIU Ou¹

¹Guangdong Cardiovascular Institute, Guangzhou, China

**Funding Acknowledgements:** Type of funding sources: Public Institution(s). Main funding source(s): Key Area R&D Program of Guangdong Province (No.2019B020227005) Guangdong Provincial Clinical Research Center for Cardiovascular disease (2020B1111170011)

**Background:** Congenital heart defects (CHDs) are the most common congenital anomalies with complex etiologies. There is knowledge gap with respect to the association between single and combined exposures to multiple heavy metals during pregnancy and the risk of CHDs in the fetus. Thus, we aimed to examine the association between maternal plasma heavy metal concentrations in mid-pregnancy and the risk of CHDs in the fetus.

**Methods:** A prospective nested case-control study was conducted in a cohort of 11,578 newborns. Exposure odds ratios (ORs) were compared between 164 CHD cases and 164 non-malformed controls delivered at the same hospital, individually matched by maternal age (±5 years) and parity. Concentrations of 21 metals were determined in maternal peripheral blood plasma via an ultra-performance liquid chromatography inductively coupled to mass spectrometry (ICP-MS). Single- and multiple-metal logistic regressions, the adaptive least absolute shrinkage and selection operator (LASSO) penalized regression analysis and restricted cubic spline (RCS) analysis were applied to explore the associations and dose-response relationships of plasma metals with CHD. We also applied Bayesian Kernel Machine Regression (BKMR) model to evaluate the cumulative effect of the exposure metals.

**Results:** Median (interquartile range, IQR) Vanadium (V) [0.3 (0.3-0.4) vs. 0.3 (0.3-0.4) μg/L, P = 0.027] and cadmium (Cd) [0.1 (0.0-0.1) vs. 0.1 (0.0-0.1) μg/L, P = 0.036] levels were different between the CHDs and control groups. Compared with the first quartile, the Directed acyclic graph (DAG) model-adjusted ORs (95% CI) were 3.12 (1.72-5.64) for kalium (K), 2.21 (1.23-3.94) for manganese (Mn), 0.55 (0.33-0.94) for cerium (Ce), 1.96 (1.06-3.64) for lead (Pb) in the second quartile, and ORs (95%CI) were 1.88 (1.08-3.26) for V, 0.53 (0.30-0.93) for Cr, 1.92 (1.10-3.35) for copper (Cu), 2.09 (1.18-3.70) for Cd, 2.14 (1.24-3.70) for stannum (Sn) and 3.05 (1.69-5.49) for Pb in the third quartile. In the multi-metal model, compared to the lowest quartile, the ORs for Sn and Pb in the second quartile were 1.32 (95% CI: 0.69-2.55) and 1.94 (95% CI: 0.98-3.84), respectively, and the ORs for both metals in the third quartiles were 2.15 (95% CI: 1.16-3.98) and 3.32 (OR=0.66, 95% CI: 1.68-6.55), suggesting that Sn and Pb had relatively stable positive results with a linear dose progression. BKMR models with 2 cluster for 9 metals showed that the overall effect of metal mixture was not statistically significant at or below 35th percentile and at or above the 70th percentile, compared to their median levels. Pb was the major contributor to the combined effect.

**Conclusions:** Elevated level of plasma plumbum was associated with increased risk of CHDs. Further investigations in larger perspective cohorts are needed to confirm our findings.
fig 1