RESEARCH PAPER

Maternal Occupational Tobacco Exposure and Newborn Umbilical Cord Serum Leptin Concentration

SWATHI S RAO,[1] A PREETHIKA,[2] DENYA MARY YELDHO,[1] Y SUNIL KUMAR[1] AND RATHIKA D SHENOY[1]

From Department of [1]Pediatrics and [2]Central Research Laboratory, KS Hegde Medical Academy, Nitte (deemed to be University), Mangalore, India.

Correspondence to: Dr Rathika D Shenoy, Professor and Head, Department of Pediatrics, KS Hegde Medical Academy, Nitte (deemed to be University), Mangalore, India.
rathika.shenoy@nitte.edu.in
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Objective: To assess the effect of maternal occupational tobacco handling (*bidi* rolling) on cord serum leptin levels. **Methods:** We enrolled 64 neonates born to women who were *bidi*-rollers, and 64 small for gestational age (SGA) neonates and 57 term appropriate for gestational age (AGA) neonates born to mothers with no tobacco exposure. Cord blood leptin levels between the groups were compared. Adjusted mean difference in leptin was calculated using regression model. **Results:** Cord leptin showed moderate correlation with birthweight (r=0.16; P=0.027) across the groups. Mean (SD) cord serum leptin levels (ng/mL) of study group was 19.79 (13.32), in comparison to 21.4 (13.4) of SGA (P=0.497), and 27.70 (13.96) of term AGA (P=0.002). Maternal occupational tobacco exposure contributed to significant decrease in cord leptin (adjusted mean difference (95%CI): -4.5 ng/mL (-8.82, -0.19); P=0.041). **Conclusion:** Maternal occupational tobacco exposure causes significant reduction in fetal leptin levels.

Keywords: *Barker hypothesis, Bidi-rolling, Cotinine, Nicotine, Small for gestational age*.

Fetal growth is determined by the integrity of the utero-placental unit and fetal adipokine axis. The Barker theory on the role of leptin in initiating the fetal-programming cascade in small for gestational age (SGA) neonates has been of interest for decades [1]. Leptin secreted by the placenta and fetal adipose tissue is important in maintaining energy homeostasis [2]. Leptin has positive correlation with birth weight independent of other maternal factors [3,4]. Smoking during pregnancy causes placental insufficiency and fetal neuro-endocrine dysfunction resulting in SGA neonates [5]. Studies show normal [6-10] to decreased [3,4] cord serum leptin in term and preterm infants born to mothers who smoke, independent of birthweight.

Bidi-rolling is another form of tobacco exposure. Coastal Karnataka is home to *bidi* industry and women constitute the major labor pool involved in rolling and packaging [11]. In a cohort study, we established that occupational tobacco exposure through *bidi* rolling resulted in increased relative risk for SGA and a lower adjusted birthweight [12]. We hypothesized that similar to maternal smoking, tobacco handling during pregnancy may have an effect on the newborn umbilical cord serum leptin levels independent of birthweight.

METHODS

The study was conducted over two years (October, 2017-September, 2019) after institutional ethics committee clearance. The group of interest were 64 neonates born to *bidi* rollers by occupation (Group I). Controls were 64 SGA (Group II) and 57 term appropriate for gestational age (AGA) newborns (Group III) with no maternal occupational tobacco exposure or history of smoking. Group II and Group III newborns were included subsequent to enrolment of each Group I newborn. Infants born to mothers exposed to any other form of tobacco exposure like snuff, chewing, passive and active smoking were excluded in all. Multiple gestations, maternal pre-existing systemic illnesses, early preterm (<32 weeks), very low birthweight, and newborns with major congenital anomalies were also excluded.

Mothers were interviewed for *bidi* rolling practices. Co-variates included pre-pregnancy body mass index (BMI), weight gain, anemia, gestational hypertension (GH), prematurity and neonatal anthropometry. Standard definitions and measurements were used [13]. AGA was defined as birthweight between the 10th and 90th centile and SGA as less than 10th centile in the Lubchenco charts [14].

Cord serum leptin assay was done for all participants; maternal and cord serum cotinine assays were performed only in the study group. Both assays were done by commercial ELISA kits and expressed as ng/mL. A serum

cotinine value ≥2 ng/mL was considered indicative of nicotine absorption [15]. Sera were separated and stored at –80°C until analysis. The tests were repeated twice to minimize errors.

Primary outcome was to assess the effect of maternal tobacco handling on cord serum leptin independent of birthweight and being SGA. Secondary outcome was to look into specific maternal tobacco handling practices that influenced the leptin level.

Sample size calculated was 57 in each group using online software OpenEpiv3 for 90% confidence level, 20% allowable error, 1:2 ratio of study to control groups and mean difference in cord serum leptin of 1.04 ng/mL [4]. Informed written consents were obtained from the participating women.

Statistical analyses: These were performed using SPSS v20.0. For categorical data, frequencies (*n*) and percentages (%) were calculated and Chi square or Fisher exact was applied for significance. For continuous data, either mean (SD) or median (IQR) was calculated based on normality distribution. Intergroup comparisons were performed using independent sample *t* test or ANOVA. Correlation was done by Pearson correlation or Spearman correlation test. Multiple linear regression model was used to determine adjusted mean difference (aMD) of cord leptin for maternal tobacco exposure. A *P* value less than 0.05 was considered significant.

RESULTS

Of the 64 mothers with occupational tobacco exposure, 16 (25%) were SGA. Other maternal and newborn characteristics that influenced the birth weight and/or the cord serum leptin levels are given in ***Table* I**. Cord serum leptin showed moderate correlation with birthweight (r=0.16; P=0.027) across the groups, with no difference between females (n=92) 23.25 (12.78) ng/mL and males (n=93) 22.10 (14.90) ng/mL (P=0.58).

As compared to group III (term AGA with no maternal tobacco exposure), cord serum leptin levels were significantly lower in group I (maternal tobacco exposure) [Mean difference (95% CI)= –7.91 (–12.92,–2.90); P=0.002] and group II (SGA with no maternal tobacco exposure) [MD (95% CI) = –6.30 (–11.33, –1.28); P=0.014]; even term AGA newborns of group I had significantly lower levels than term AGA newborns of group III [MD (95% CI) = –8.5 (–13.89, –3.11); P=0.002]. No significant difference was found between the levels in group I and group II (P=0.49).

Mothers in the study group started *bidi* rolling at median (IQR) age of 20 (18,23) years. Their median (IQR) tobacco exposure was 6.75 (4,10.75) years. They rolled a median (IQR) of 500 (500,600) *bidis* a day and majority (84.4%) stopped rolling by median (IQR) 22 (20.5,29.5) weeks of gestation. Evidence of nicotine absorption was found in 24 (37.5%) of maternal and 22 (34.4%) of cord blood. Median (IQR) maternal cotinine was 3.35 (0,15.15) ng/mL; and median (IQR) cord serum cotinine 4.0 (0, 17.25) ng/mL (range 0-30.45). Cord leptin had significant negative correlation with longer years of occupational tobacco handling (r = –0.34; P=0.001) and longer tobacco exposure (gestational week) during pregnancy (r = –0.33; P=0.007). There was no correlation between cord leptin, maternal cotinine and cord cotinine.

Maternal occupational tobacco exposure contributed to significant decrease in cord leptin by 4.50 ng/mL [95%CI: –8.82, –0.19; P=0.041] when adjusted for maternal gestational hypertension, prematurity and birthweight. *Bidi* rolling practices associated with decrease in cord leptin value included longer years of occupational exposure [aMD (95%CI): –1.31 (–2.22, –0.41); P=0.005] and longer weeks of exposure into pregnancy [aMD (95%CI): –0.72 (–1.35, –0.09); P=0.025] when adjusted for number of *bidis* rolled in a day, quantity of tobacco stored at home and engagement of other family members in the same occupation.

DISCUSSION

In our study, the cord serum leptin levels of the newborns born to mothers who were *bidi* rollers were significantly

Table I Comparison of Maternal and Neonatal Variables Among the Study Groups

Variable	*Group I (n=64)*	*Group II (n=64)*	*Group III (n=57)*
Maternal			
Age, y	28.3 (4.03)	27.3 (4.5)	26.9 (4.01)
BMI, kg/m^2	21.7 (3.6)	21.8 (2.8)	22.7 (1.9)
Weight gain, kg$^{\$}$	9.96 (2.71)	8.23 (1.80)	9.5 (2.30)
Hemoglobin, g/dL	11.6 (1.2)	11.6 (1.2)	11.9 (0.9)
Newborn			
Gestational age, wk	38.2 (1.3)	37.9 (1.2)	38.4 (0.9)
Birthweight, g*	2829.4 (374.3)	2355.9 (182.9)	3213.9 (300.2)
Length, cm*	48.6 (1.88)	47.2 (1.44)	49.3 (1.99)
HC,cm*	33.7 (1.13)	32.5 (1.06)	33.8 (0.8)
Leptin, ng/mL$^{\wedge\#}$	19.79 (13.31)	21.4 (13.40)	27.7 (13.96)

All values in mean (SD); Group I: Maternal Tobacco Exposure, Group II: Small for gestational age without tobacco exposure, Group III: Term Appropriate for gestational age without tobacco exposure; HC-head circumference; BMI-body mass index; $^{\$}$Pregnancy weight gain; $^{\#}P=0.005$. $^{*}P<0.001$;^cord serum leptin.

WHAT THIS STUDY ADDS?

- *Bidi* rolling during pregnancy reduces the cord blood leptin levels independent of birthweight and being born small for gestational age.

lower when compared to those born to the reference group. Mantzoros, *et al*. [4] documented that the decrease in mean cord leptin in pregnant smokers was more pronounced in preterm neonates. A significant negative correlation between cord leptin and number of cigarettes smoked has also been reported [4,8]; though, Kayemba-Kay, *et al.* [3] showed a positive correlation. Fang, *et al.* [7] noted that the median cord leptin concentration in smokers was less than that of the non-smokers.

Nicotine influences cord leptin through decreased secretion due to uteroplacental insufficiency, decreased birthweight and catecholamine-mediated decreased fetal adiposity [4]. In the present study, longer the years of tobacco exposure and longer the mother continued with her occupation into pregnancy, lower was the cord leptin. The lower age in most women in this study substantiates that they begin this occupation in late adolescence, unwittingly helping their mothers [11]. The cord blood leptin level of the tobacco exposed newborns was comparable to the unexposed SGA babies. This indicated that these newborns with *in utero* tobacco exposure had an adiposity similar to that of an SGA newborn in spite of being born AGA. It also suggested a common pathophysiology compromising the circulation of the growing fetus in both. Maternal malnutrition may be an additional factor common to both the groups [2].

This was a single centre study with a small sample size. Nicotine absorption was demonstrable only in about one-third, probably related to altered metabolism kinetics during pregnancy [12,15]. There is wide variation in reported cord leptin values with several maternal, labour and newborn factors influencing the same [7,16]. We included two control population of newborns and statistically adjusted various covariates that could influence cord leptin levels. Future considerations include a longitudinal study with other fetal hormones in newborns with maternal occupational tobacco exposure.

In conclusion, maternal occupational exposure to tobacco *via bidi* rolling decreases cord serum leptin independent of birthweight and being SGA. Maternal *bidi* rolling is a demographic risk factor for altered neuroendocrine function of the fetus.

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