Original Article

Smokeless Tobacco Exposure and Fetal Iron Status: An Analytical Study

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Abstract

Objectives: To compare the cord serum ferritin and fetal iron status in newborns with and without maternal occupational smokeless tobacco exposure and determine the influencing factors. **Methods:** This cross-sectional study included mother–infant dyads with occupational tobacco exposure (exposed) and an unexposed group. Umbilical cord serum ferritin was compared in both groups. Fetal nicotine absorption was established by cord cotinine. **Results:** A total of 140 newborns each were analyzed in each group. There was no significant mean difference (MD) ($P = 0.900$) between the cord serum ferritin in the tobacco exposed and unexposed group. Fetal nicotine absorption was seen in 43.6% of the exposed group. Cord serum ferritin was 14.1 µg/L (95% confidence interval [(95% CI:-43.1, 14.9); P=0.338] lower in this group compared with the group without fetal nicotine absorption. A higher adjusted MD for ferritin was present for maternal hypertension (12.5 [95% CI: −75.5, 100.5]; $P = 0.777$) and gestational diabetes mellitus (21.4 [95% CI: −54.0, 96.9]; $P = 0.571$) in the group with fetal nicotine absorption. Fetal nicotine absorption exaggerated fetal iron depletion in maternal anemia [aOR 4.8 (95%CI: 1.2, 19.0); P=0.025]. **Conclusion:** Cord serum ferritin and fetal iron status were comparable in tobacco exposed and unexposed groups. In those with fetal nicotine absorption, cord ferritin levels reflect the fetal inflammatory state.

Keywords: Bidi, cotinine, ferritin, nicotine, pregnancy

Introduction

Fetal iron plays critical role in erythropoiesis and neurodevelopment. The foetus acquires 1.6–2.0 mg/kg of iron per day from the maternal circulation during the third trimester of pregnancy.[1] Cord ferritin reflects the fetal iron stores and iron deficiency.[2] The fetal ferritin increases from 63 µg/L at 23 weeks of gestational age (GA) to 171 µg/L by term.[3] Newborn infants with iron depletion at birth, as indicated by cord ferritin <76 µg/L, demonstrate neurodevelopmental impairments in long-term follow-up.[3]

Maternal and placental ferritin do not cross into the fetal circulation and alter fetal ferritin levels. Maternal anemia, hypertension, gestational diabetes mellitus (GDM), multiple gestations, and preterm delivery are associated with lower cord ferritin levels.[3] Infants of mothers who smoked during pregnancy also have lower cord ferritin levels.[2] Tobacco smoke contains nicotine and products of combustion such as carbon monoxide, thiocyanate, and cadmium. Fetal iron deficiency in smokers results from disruption of placental iron transport and augmented erythropoiesis due to fetal hypoxia. Carboxy-hemoglobin shifts the oxygen dissociation curve to the left. Thiocyanate interferes with the placental iron transfer. Cadmium gets concentrated in the placenta and disturbs the placental transfer of microelements.[4]

Nicotine is the only component in smokeless exposure. Bidi is a hand-rolled Indian cigarette made up of shredded and sun-dried tobacco dust, rolled in a *tendu* (Diospyros melanoxylon) leaf. Each bidi has approximately 180 mg of tobacco, containing 5 mg of nicotine. A worker rolls an average of 500–600 bidis a day with nicotine exposure equivalent to 2.5–3.0 g.[5] The

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effect of smokeless tobacco on cord ferritin level is not known. We hypothesized that cord ferritin levels would be lower in newborn infants born to mothers with occupational smokeless tobacco exposure.

Methods

Study design, setting, and selection of participants

This cross-sectional analytical study was conducted in a teaching hospital over 18 months (October 2018–February 2020). The study (exposed to tobacco) and control (unexposed to tobacco) groups consisted of mother and infant dyads with and without occupational tobacco exposure (OTE) through bidi rolling. We considered bidi rolling for a minimum of 1 year before and continuing into pregnancy until 16 weeks of gestation as OTE. We excluded all other smoking and nonsmoking tobacco exposures, including chewing, snuff, and environmental smoke. Women in the study and the control group were enrolled when they were admitted just before the delivery.

Additional eligibility criteria included maternal age between 20 and 35 years, singleton pregnancy, parity ≤3, and term (37–42 weeks) gestation. We excluded mothers with obesity (body mass index ≥30 kg/m^2), severe anemia (hemoglobin <70.0 g/L), or delivering large infants (birth weight >90th centile for GA). Maternal chorioamnionitis, cord serum C-reactive protein (CRP) >6 mg/L or cord ferritin >370 µg/L were considered evidence of peripartum infection and excluded.[3] The study was initiated with the institutional ethical committee approval; we obtained informed consent from the mothers.

Data collection and estimations

Demographic data included maternal details, newborn anthropometry (weight, length, and head circumference) and classification (appropriate or small). We interviewed the study group for bidi rolling practices, such as the age at which they started bidi rolling, the number of bidis rolled in a day and the duration of rolling during pregnancy.

From the placental end, 3 ml of cord blood was collected at delivery. Serum ferritin was automated (Roche Elecsys, Germany; Cobas e411, Hitachi, Japan). Cotinine concentration was determined by ELISA (Calbiotech, USA) and semi-quantitative CRP by latex agglutination. The tests were run in duplicate for confirmation.

Definitions

Cord ferritin <76 µg/L and <35 µg/L indicated fetal iron-depleted and fetal iron-deficient statuses.[3,4] A cord serum cotinine ≥2 ng/mL was considered significant for fetal nicotine absorption and nondisclosure of tobacco exposure in the unexposed.[6] Maternal anemia, GDM and hypertension were defined according to standard definitions.[7-9]

Outcomes

The primary outcome was cord ferritin and fetal iron status of infants with and without maternal smokeless tobacco exposure. The secondary outcome was to compare determinants of cord ferritin and fetal iron status in those with and without evidence of nicotine absorption.

Statistical analysis

Statistical analysis was performed using software package IBM SPSS version 20 (IBM, SPSS Inc, Chicago, Illinois, USA). Central tendencies were expressed as mean (standard deviation) or median (interquartile range [IQR]) based on normality of distribution (Kolmogorov–Smirnov test). Comparison of the mean cord ferritin between groups was assessed using independent sample *t*-test (exposed and unexposed) and ANOVA (group IA – exposed with fetal nicotine absorption; IB – exposed without fetal nicotine absorption; II – unexposed). The fetal iron status was compared by Chi-square (Fisher exact if count <5). The results were summarized as mean difference (MD) and odds ratio (OR), respectively, with 95% confidence interval (CI). We used the Spearman test for correlation. Regression statistics were used to determine the influence of maternal and fetal demographic factors on cord ferritin (adjusted MD (aMD) by linear) and fetal iron-depletion status (adjusted OR [aOR] by binary logistic) by labeling the three groups. A $P < 0.05$ was considered significant.

Results

Of the 150 mother and infant dyads recruited under each of the study arms (Group I-exposed and Group II-unexposed to tobacco), 140 pairs in either group were included in the final analysis [Supplementary Figure 1]. The tobacco exposed group was further divided into those with fetal nicotine absorption (Group IA; $n = 61$) and without (Group IB; $n = 79$).

Primigravida constituted 37.1% ($n = 45$) in either group. The mean age of the women in the study group was 28.6 ± 4.0 years and majority (72.2%) was multigravida. Furthermore, majority of the women in group I belonged to the lower middle class (52.1%) when compared to group II (30.2%). The women in the study group began bidi rolling at a median age of 18 years (IQR 5). The median duration of exposure before present pregnancy was 9 years (IQR 7). They rolled an approximate 500 bidis per day during pregnancy for a median duration of 26 weeks (IQR 8). They had increased risk for anemia (OR 1.8 [1.0, 3.2]; $P = 0.044$) and hypertension (OR 3.5 [1.1, 10.9]; $P = 0.024$). Their newborn infants had lower birth weight (MD − 156.8 g [−249.0, −64.6]; $P = 0.001$) and a higher proportion of SGA (OR 2.2 [1.2, 4.2]; $P = 0.013$).

The mean cord ferritin of group I was 138.5 ± 85.9 µg/L and of group II was 139.8 ± 83.8 µg/L with no significant difference in mean (−1.3 [95% CI: −21.2, 18.7]; $P = 0.900$). Fetal iron depletion was present in 25.0% ($n = 35$) of group I and 28.6% ($n = 40$) of group II, and was comparable [OR 0.8 (95%CI: 0.5,1.4; p=0.500]. Fetal iron deficiency status likewise was comparable between group I (10.0%; $n = 14$) and Group II (7.9%; $n = 11$) with OR of 1.3 (95% CI: 0.6, 3.0; $P = 0.530$).

Nicotine absorption (Group IA) was evident in 43.6% ($n = 61$). The median cord serum cotinine was 18 ng/mL (IQR 10.0). In the exposed group, the cord ferritin of infants in group IA was 14.1 µg/L lower [(95%CI: - 43.1, 14.9); P=0.338)] when compared to Group IB. Figure 1 shows the subgroup analysis of mean cord ferritin levels in pregnancy-related conditions in the three groups. The mean ferritin level in group IA infants was higher in those born to mothers with hypertension and GDM unlike the observations in maternal anemia and SGA.

Table 1 gives the aMD for cord ferritin for various determinants in the three groups. Group IA infants born to mothers with hypertension and GDM had higher aMD. Table 2 gives the aOR for fetal iron-depletion for various determinants in the three groups. Maternal age ($P = 0.013$) and anemia ($P = 0.025$) were significant determinants in group IA infants.

Discussion

We studied the fetal iron status by determining cord ferritin in newborn infants born to mothers occupationally exposed to (smokeless) tobacco. We also established fetal nicotine absorption in newborn infants born to mothers exposed to tobacco using cotinine assay. We compared the determinants of cord ferritin with a tobacco unexposed group. The maternal and newborn demographic factors differed significantly among the tobacco exposed and unexposed groups.

Contrary to our hypothesis, smokeless tobacco exposure did not lower cord ferritin when compared to those who were unexposed. This finding is unlike the effect which is seen in smoking, where the cord ferritin levels are lower in newborns of mothers who smoked.[2] Pateva *et al*.[10] showed a significant negative correlation of cord ferritin with the number of packs smoked. Conversely, Morton *et al*.[11] did not find smoking a determinant factor for fetal iron status.

Siddappa *et al*.,[3] showed lower intercepts of cord ferritin in infants of diabetic mothers than the controls. A similar trend was seen in pregnancies complicated by hypertension and GDM in our study, but only in the group without fetal nicotine absorption or tobacco exposure. The higher serum ferritin we observed in infants with nicotine absorption born to mothers with hypertension and GDM is not reported in the literature. Maternal exposure to smokeless tobacco like bidi rolling[5] and Swedish snuff[12] increases the risk for hypertension. In contrast, smoking reduces the incidence of preeclampsia mediated by CO.[13]

Serum ferritin is an acute-phase reactant and mirrors the degree of inflammation. We postulate that among bidi rollers, the fetal nicotine absorption resulted in higher ferritin levels through its proinflammatory effect in pregnancies complicated by hypertension and GDM. A similar effect is demonstrated in other smokeless tobacco use like electronic-cigarette vapors (EV) that contain nicotine, glycerine, and propylene glycol. In *in-vivo* animal models, the EV components altered epithelial function in various organs and led to leakage of inflammatory proteins into the systemic circulation. These

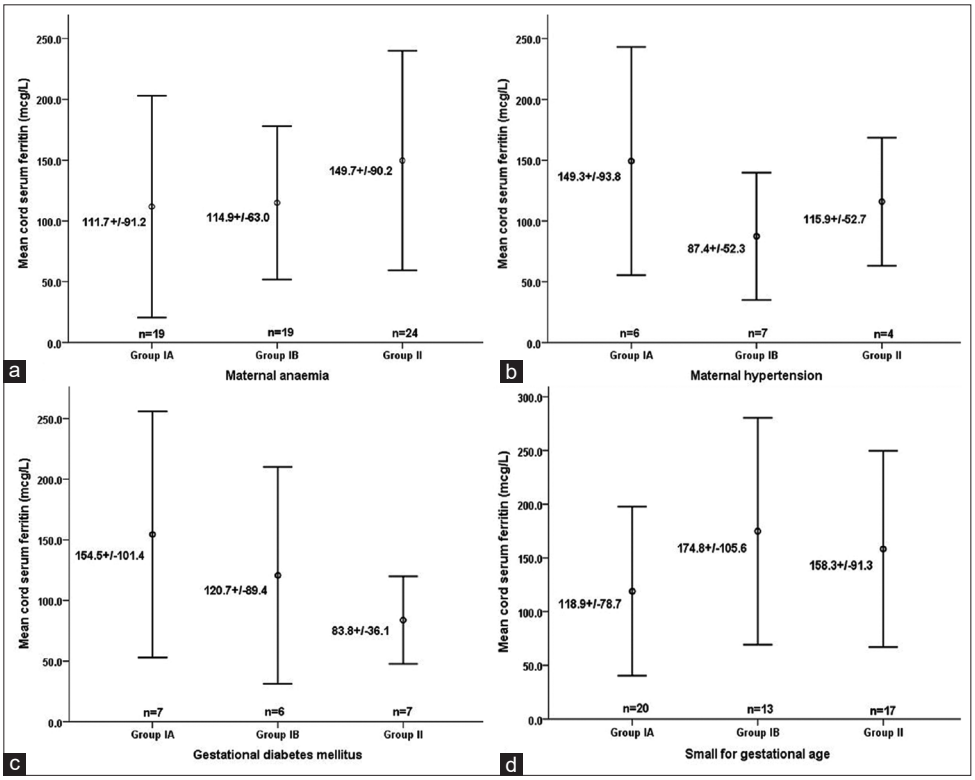

Figure 1: Comparison of mean cord serum ferritin stratified for tobacco exposure and nicotine absorption in pregnancies complicated by (a) maternal anemia, (b) maternal hypertension, (c) gestational diabetes mellitus, and (d) small for gestational age

Table 1: Determinants of mean cord serum ferritin in new-borns of study group with nicotine absorption (Group IA) and without nicotine absorption (Group IB) and comparison with unexposed (Group II)

Variable	Cord serum ferritin (μg/L), adjusted mean difference (95% CI)*		
	Group IA (n_1=61)	Group IB (n_2=79)	Group II (n_3=140)
Maternal age**	−1.1 (−6.4-4.2)	−2.6 (−8.4-3.3)	−2.0 (−5.6-1.6)
Haemoglobin	21.3 (−2.2-44.8)	9.5 (−4.7-23.7)	5.2 (−7.9-18.3)
Hypertension (n_1=6; n_2=7; n_3=4)	12.5 (−75.5-100.5)	−61.9 (−125.7-1.9)	−17.5 (−105.2-70.3)
Diabetes mellitus (n_1=7; n_2=6; n_3=7)	21.4 (−54.0-96.9)	−25.6 (−94.8-43.5)	−52.1 (−118.9-14.6)
Small for gestational age (n_1=20; n_2=13; n_3=17)	−1.2 (−56.4-53.9)	34.8 (−14.2-83.9)	0.5 (−15.1-16.1)
Constant	−84.8 (−399.9-230.2)	107.6 (−103.5-318.6)	115.9 (−517.4-749.3)

*P≥0.05, **Significantly correlates with duration of bidi rolling in tobacco exposed. Group IA: Tobacco exposed with foetal nicotine absorption, Group IB: Tobacco exposed without foetal nicotine absorption, Group II: Tobacco unexposed, CI: Confidence interval

Table 2: Determinants of foetal iron depletion in new-borns of study group with nicotine absorption (Group IA) and without nicotine absorption (Group IB) and comparison with unexposed (Group II)

Variable	Foetal iron depletion status (cord serum ferritin <76 μg/L), AOR (95% CI)		
	Group IA (n_1=61)	Group IB (n_2=79)	Group II (n_3=140)
Maternal age**	1.2* (1.0-1.4)	1.0 (0.8-1.2)	1.1 (1.0-1.2)
Anemia (n_1=19; n_2=19; n_3=24)	4.8* (1.2-19.0)	2.3 (0.6-7.9)	1.3 (0.5-3.7)
Hypertension (n_1=6; n_2=7; n_3=4)	0.6 (0.1-6.6)	3.3 (0.6-18.2)	1.4 (0.1-17.3)
Diabetes mellitus (n_1=7; n_2=6; n_3=7)	0.4 (0.1-3.0)	1.0 (0.1-9.8)	1.4 (0.3-7.4)
Small for gestational age (n_1=20; n_2=13; n_3=17)	0.6 (0.1-2.5)	0.3 (0.0-3.1)	0.7 (0.2-2.4)

*P<0.05, **Significantly correlates with duration of bidi rolling in tobacco exposed. Group IA: Tobacco exposed with fetal nicotine absorption, Group IB: Tobacco exposed without fetal nicotine absorption, Group II: Tobacco unexposed, AOR: Adjusted odd's ratio, CI: Confidence interval

mice exposed to EV had elevated plasma cotinine levels than those exposed to air for 60 min.[14]

Maternal anemia lowers fetal iron stores. Moderate maternal anemia is associated with depleted iron stores and severe maternal anemia with deficient iron stores in the newborn.[15] Smokeless forms of tobacco are associated with maternal iron deficiency.[16,17] Mothers with severe anemia were excluded in our study; however, the proportion with fetal iron deficiency was higher among the exposed. Maternal anemia, in association with fetal nicotine absorption, was significant for fetal iron-depleted status. Maternal age, which correlated with the duration of tobacco exposure, was also a significant determinant that exaggerated fetal iron depletion in women with anemia.

Strengths and limitations

Ours was a novel study as there is no literature available on occupational smokeless tobacco exposure (bidi rolling) and newborn iron status. We used cord serum ferritin estimation to quantify fetal iron stores, which is the most reliable marker.[3] Furthermore, we quantified the fetal nicotine absorption by measuring the cord serum cotinine. Cotinine achieves a fetal concentration of 88% and has a half-life of 16 h. This was a single-site study. Maternal tobacco exposure was based on questionnaires. We did not estimate maternal ferritin and cotinine levels. Women with pregnancy-related complications within the individual groups constituted small numbers. We could not establish a direct correlation between cord ferritin and cord serum cotinine.

Conclusion

The cord ferritin and fetal iron status of newborn infants born to mothers with smokeless tobacco exposure were comparable to those born to unexposed mothers. However, in those with fetal nicotine absorption, ferritin levels reflected a potential response to an inflammatory state. Alternate biomarkers of iron deficiency, such as reticulocyte hemoglobin and zinc protoporphyrin/haem ratio may be better than serum ferritin for screening for neonatal iron deficiency in this population.

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Conflicts of interest

There are no conflicts of interest.

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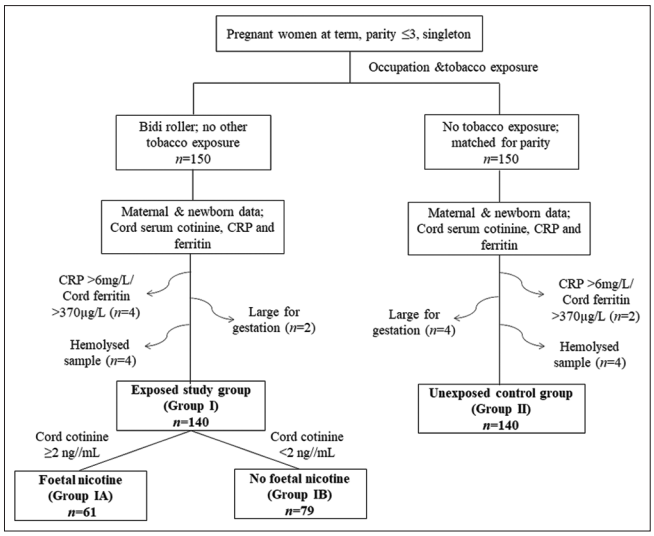

Supplementary Figure 1: Recruitment Flow chart