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Research Article

An Assessment of the Relationship between Maternal Iron Supplementation and hyperbilirubinemia in Neonates: A Nested Case-Control Study

Mohamad-Ali Moghimi[1], Janmohamad Malekzadeh[2*], and Masoud Moghimi[3]

[1]Students Research Committee, Yasuj University of Medical Sciences, Yasuj, Iran. email: moghimi_saeed@yahoo.com
[2]SocialDeterminants of Health Research Center, Yasuj University of Medical Sciences, Yasuj, Iran. Email:malekjmd@yahoo.com
[3]Nursingand midwifery school, Yasuj University of Medical Sciences, Yasuj, Iran. Email: moghimimasod@yahoo.com
[*]**Corresponding author:**Janmohamad Malekzadeh, email: malekjmd@yahoo.com, malekzadehj@yums.ac.ir

ABSTRACT

Backgrounds: Jaundice due to increased bilirubin concentration develops during the first week of life in 60% of term neonates. Bilirubin precipitation in the brain may results in kernicterus, and may even result in death. In addition to mother-child blood type incompatibility, G6PD, hypothyroidism, and low breast-feeding, iron might be also effective in jaundice incidence due to its effects on enzymes activities. Iron supplementation, an important source of iron for pregnant mothers and their neonates, may results in extra iron intake. However, we could not find any research investigating the relationship between maternal iron supplementation and neonatal jaundice incidence. Therefore, we evaluated the relationship between iron supplementation in pregnant females with the incidence of neonatal jaundice.

Methods: We compared the iron status and supplementation of mothers in two groups of neonates: those who were hyperbilirubinemic in the first week of their lives (cases; n=60) and healthy normobilirubinemic neonates (controls; n=60). At first, we measured pregnant women's hematologic indices before laboring and took the history of anemia and iron supplementation during their pregnancy. In the second stage, we measured and compared serum iron indices for both groups of mothers.

Results: The results showed that the percentage of mothers who had high levels of iron supplementation was significantly higher in the case group than the control group (27.6% vs. 8.5%; p< 0.01, respectively; OR=3.69; CI=1.23-11.05). Study findings also showed a non-significant difference in serum iron (114.2±38.4 vs. 107± 34.9 mg/dl), TIBC (208 ± 82 vs. 224±182 mg/dl) and serum ferritin (26.6±18.12 vs. 25.06±17.0) of the case group and the control, respectively.

Conclusion: According to this study, we conclude that increased iron intake in pregnant mothers can increase the incidence of neonatal physiologic jaundice.

Keywords: physiologic jaundice, hyperbilirubinemia, hemoglobin, neonates, hyperbilirubinemia, iron status

INTRODUCTION

Jaundice is a common neonatal disorder affecting 60% of term and 80% of preterm neonates in the first week of life, although most of the cases are physiologic (Najib, Saki, Hemmati, & Inaloo, 2013).Bilirubin accumulation in the blood is responsible for jaundice and is produced through heme catabolism by the action of hemeoxygenase and biliverdin reductase(Kapitulnik & Maines, 2012).High levels ofbilirubin is neurotoxic and may cause neonatal kernicterus,auditory neuropathyor death (Greco et al., 2016; Shapiro, 2003; Vert & Grojean, 2002). Affected neonates may become mentally retarded and get into disorders such as seizure, hear loss, and

psychomotor disabilities(Shapiro, 2003). The common causes of pathologic hyperbilirubinemia are Rh immunization, G6PD deficiency, and prematurity (Davutoglu, 2010).Also, high levels of iron, manganese, and iodine in total parenteral nutrition solution was associated with greater severity of conjugated hyperbilirubinemia(Klein, Revenis, Kusenda, & Scavo, 2010). Iron is not only an essential element in the function of heme oxygenase-1 and heme synthesis, but the elevated iron intake also results in the generation of reactive oxygen and nitrogen species (RONS), lipid peroxidation, oxidative stress (Fisher & Naughton, 2004) andliver inflammation (Halliwell & Gutteridge, 1999; Shapiro, 2003). Excessive levels ofiron alters the activity of uroporphyrinogen decarboxylase (URO-D) and aminolevolinat synthase, damages liver tissues, and parenchymal cells and, as a results, may alterporphyrinogen metabolism(Bygum & Brandrup, 2000; Koskenkorva-Frank, Weiss, Koppenol, & Burckhardt, 2013).

Iron deficiency during pregnancy isassociated with serious risksfor mother and her child, including a higher risk of hemorrhage in the mother, an increased risk of premature birth, smaller infants, and somedevelopmental problems. Therefore, to prevent this consequences, pregnant women's dietis supplemented with ironin some countries(Gambling, Lang, & McArdle, 2011). The World Health Organization(WHO) recommends 60 mg iron/d for 6 monthsduring pregnancy, which is a very high amount (Gambling, et al., 2011).Also, many physicians may prescribe higher doses for treating asevere anemia duringpregnancy.

Transfer of iron to the fetus is regulated in response to maternal iron status (O'Brien, Zavaleta, Abrams, & Caulfield, 2003), andmaternal iron overload may lead to excessive iron accumulation in the fetus(Rao & Georgieff, 2007).Excessive iron is proinflammatory and toxic for cells and induces inflammatory cytokine release from liver koppfer cells. It altersfunctionsof heme metabolizing enzymes and, as a results, may alterblood bilirubin concentration, especially in neonates(Otogawa et al., 2007). In the present nested case-control study, we evaluated the relation between mothers' iron status and supplementation with the incidence of neonatal hyperbilirubinemia. Our findings show the necessity of re-evaluating iron supplementation in pregnancy.

MATERIALS AND METHODS:

This nested case-control study was carried out as a two-stage process. Firstly, we registered all the pregnant mothers with a full term pregnancy (36-40 weeks of gestational age, n=440) who were referred to the delivery room for delivery. Data on age, education level, employment status, height, weight, number of parities, number of deliveries, number of abortions, history of jaundice in previous neonates, history of iron deficiency anemia, and history of iron supplementation in the recent pregnancy were recorded for all the pregnant mothers. The address and the phone number of those pregnant mothers who consented to be contacted for follow up were recorded. 5-milliliters of venous blood wassampled from each mother. The hematologic indices including hemoglobin, hematocrit, mean corpuscular volume and red blood cell numbers were measured using autoanalyzerand recorded in respective data sheets. One milliliter of their blood serum was stored in -70° C freezer in two half-milliliteraliquots in order to measure their iron indices if they were selected as the study subjects.

In the second stage, namely after delivery, the neonates were followed for hyperbilirubinemia incidence for a week. Neonateswithin a serum bilirubin range of 6-12 mg/dl were regarded as cases. Any pathologic hyperbilirubinemia with known pathologic causes was excluded from the study.Seventy healthy neonates whose serum bilirubin was lower than 6 mg per deciliters and who did not show icterus signs were selected as controls. Controls were matched with cases in gestational age, gender, and birth weight. Children with low birth weight and prematuritywere excluded from the study. Neonates'mothers (cases and controls) were compared for anemia history,

iron status, hematologic indices, and iron supplementation history during their recent pregnancies.Maternal anemia was defined based on WHO classifications for hemoglobincorrected for altitude (WHO, 2011). A serum ferritin cut-off point of 20mg/l was used for diagnosing maternal iron deficiency(McMahon, 2010).

Sample size: Considering a 95 percent confidence interval($Z_{1-\alpha/2=1.96}$), 90 percent test power ($z_{1-\beta=0.86}$) and assuming that iron overload may cause a 20 percent increase in hyperbilirubinemia incidence(i.e., from 65 to 85 percent), using the EPI info software for calculating the required sample size, (Dean, Dean, Burton, & Dicker, 1991),sample size was estimatedto be 60 cases in each group.

Statistical analysis:Data were controlled for distribution. All data were normally distributed. Quantitative data were compared using an independent samples t test, qualitative data were compared using a chi-square test. Odds ratio (OR)and its 95 percent confidence interval were calculated for bivariate variables related to hyperbilirubinemia. A conditional forward logistic regression analysis was run to determine the factors most strongly related to hyperbilirubinemia. SPSS version 19 was used for statistical analysis.

FINDINGS:

Table 1 shows findings on demographic and medical history of pregnant mothers who participated in the study (cases and controls). Findings did not show any significant differences between mothers' characteristics, including education, employee status, history of icterus among previous children, presence of anemia, and children's gender.

Table 1: Demographic Variables among Pregnant Mothers of Neonates in Case and Control Groups

Groups / Factors		Cases Number(percent)	Controls Number(percent)	
Mother Education levels	Illiterate	7(16.3)	10(25)	Chi^2=0.98 P=0.6
	Primary-secondary	28(65.1)	23(57.5)	
	Highschool and higher	8(18.6)	7(17.5)	
Mother Employee status	Unemployed	56(93.3)	51(86.4)	Chi^2=1.56 P=0.21 .85(.41-1.74)
	Employed	4(6.7)	8(13.6)	
Mother Delivery type	Normal	31(51.7)	28(47.5)	Chi^2=0.21 P=0.64 .85(.41-1.74)
	Cesarean	29(48.3)	31(52.5)	
Icterus history	Yes	21(35)	14(23.7)	Chi^2=1.8 P=0.18 1.73 (.78-3.86)
	NO	39(65)	45(76.3)	
Neonate gender	Female	33(45)	32(45.8)	Chi^2=0.007 P=0.9 1.03(.50-2.12)
	Male	27(55)	27(54.2)	
Anemia in mother	Yes	19(31.7)	12(20.3)	Chi^2=1.98 P=0.16 1.81 (.79-4.2)
	No	41(68.3)	47(79.7)	

Table 2: Mean and Standard Deviation of Gestational Age and Mothers' AnthropometricIndices in BothCases and Controls (data are Mean±standard deviation)

	Age(years)	Height(cm)	Weight(kg)	BMI(kg/m^2)	Neonate birth weight(gr)	Gestational age(weeks)
cases	28.2±4.8	162±6	73.8±13.2	29.3±4.3	3258±427	38.6±1.6
controls	28.2±4.8	156±7.6	70.2±14.3	29.8±6.4	3327±394	38.8±1.2
P value	0.09	0.027	0.19	0.8	0.3	0.4

Table 3:A Comparison of Hematologic and Iron Indices of Neonates' Mothers in Cases and Controls(data are Mean±standard deviation)

Indices	Hgb(g/dl)	Hct(%)	RBC(10^6 cells/mm)	MCV(fl)	Serum iron(mg/dl)	TIBC(µg/dl)	Ferritin(ng/ml)
Cases	12.2±1.1	37.3±3.2	4.3±0.4	85.2±7	114.2±38.8	207.6±82.4	26.6±18.12
Controls	12.6±1.16	37.3±3.3	4.3±0.4	87.3±7	107.9±34.2	223.8±183.6	25.06±17.0
P value	0.17	0.54	0.51	0.1	0.35	0.53	0.65

Table 4: frequency table for anemia and iron status among mothers in cases and controls neonates

Group / indices		Cases		Controls		Chi^2 , P value OR (CI for 95%)
		Number	Percent	Number	Percent	
*Hgb (g/dl)	<12.3	29	48.3	28	48.3	Chi^2=0.001, p=0.9 .99(.49-2.05)
	≥ 12.3	31	51.7	30	51.7	
	total	60	100	58	100	
Serum ferritin(ng/ml)	≤20	23	39.7	27	47.4	Chi^2=1.7 p=0.19 1.37(.65-2.87)
	>20	35	60.3	30	52.6	
	Total	58	100	57	100	
**Iron supplement (tablet/day)	1	42	72.4	51	91.1	Chi^2=6.6 p=0.01 3.89(1.31-11.49)
	2	16	27.6	5	8.9	
	total	58	100	56	100	
MCV (*fl*)	<80	11	19	5	8.8	Chi^2=2.5 p=0.11 .41(.13-1.27)
	≥80	47	81	52	91.2	
	total	58	100	57	100	

*Hemoglobin corrected for altitude (1839 meters from the sea level)

** Ferrous sulfate tablet containing 60 mg elemental iron

The forward stepwise conditional logistic regression analysis showed that the higher iron supplementation (two tablet of ferrous sulfate containing 60 mg elemental iron versus one tablet) is most strongly related tohyperbilirubinemia incidence among mothers' neonates (Table 5).The odds ratio for hyperbilirubinemia incidence in cases receiving two tablet of ferrous sulfate was 3.69 times of those receiving one tablet per day.

Table 5: Forward Stepwise Logistic Regression Showing the Iron Supplementation Odds Ratio (Two Tablets against One Tablet Ferrous Sulfate) for Physiologic Jaundice Incidence

Variables in the Equation									
		B	S.E.	Wald	df	Sig.	Exp(B)	95% C.I.for EXP(B)	
								Lower	Upper
Step 1[a]	fesupplem(1)	1.306	.560	5.449	1	.020	3.692	1.233	11.057
	Constant	-.208	.216	.928	1	.335	.813		
a. Variable(s) entered on step 1: fesupplem.									

DISCUSSION:

Our study suggested that high iron supplementation in pregnant womenmay be associated with greater incidence rate of physiologic jaundice in their neonates. Study findings also showed that iron status indices in the icterus neonates were higher than in the non-icterus group. Excessive iron supplementation in mothers may increase iron stores in their fetus(Rao & Georgieff, 2007), and increased iron stores may be poisoning for liver enzymes involved in heme metabolism(McDonald, Middleton, Dowswell, & Morris, 2014; Pena & Kiselyov, 2015). The mothers' serum ferritin and serum iron in the icterus group were insignificantly higher than the control group, while iron binding capacity was higher in mothers of the control group. These findings confirm the hypothesis that iron overload may alter heme metabolism in the neonate's liver. This finding is

supported by Pena et al.'s review on transitional metals(Pena & Kiselyov, 2015). Pena and his colleague reported that iron, as a transitional metal, may increase transcription factor EB (TFEB), resulting in increasedheme oxygenase-1 expression. Hem oxygenase-1 is responsible for heme conversion to biliverdin.Biliverdin in turn is converted to bilirubin and may increase the icterus incidence probability(Maines, 2001). McDonald et al. reported that delayed umbilical cord clamping is associated with increased iron transfer to newborns and also increased jaundice incidence at the same time(McDonald, et al., 2014).In conclusion, our study findings show that the extra iron supplementation in pregnant mothers may be a risk factor for hyperbilirubinemia and jaundice among their neonates. To be more precise, we suggest cohort studies with greater sample sizes or conducting experimental studies.

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