ORIGINAL ARTICLE

ASSOCIATION OF CORD HEPCIDIN AND IRON PARAMETERS WITH MATERNAL HEPCIDIN, IRON STATUS MARKERS AND NEONATAL MORPHOMETRICS

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Background: Hepcidin is the only iron regulating hormone in human body. The foetus begins to synthesize Hepcidin from the 1st trimester of gestation to control unidirectional iron transfer from mother to foetus. The objective of the present study was to determine the correlation of cord Hepcidin with iron status markers of mothers, neonates and neonatal morphometrics. **Method:** Twenty-five healthy pregnant women and their neonates were included in the study. Haemoglobin, Iron status markers and Hepcidin were analyzed in maternal and cord blood. Neonatal anthropometric variables were measured. Pearson/Spearman correlation was used for association of different variables of mother-neonate pairs. **Results:** Maternal Hepcidin showed positive correlation with maternal Iron, Ferritin and Transferrin Saturation (TS) (r=0.455 and p=0.022, r=0.511 and p=0.009, r=0.440 and p=0.025 respectively). Cord Hepcidin was positively correlated with cord iron, Ferritin and Transferrin saturation (r=0.593 and p=0.002, r=0.792 and p=0.000, r=0.546 and p=0.005 respectively). Neonatal morphometric variables were neither correlated to cord Hepcidin nor with any other Cord blood variable. Maternal Ferritin showed positive correlation with cord Haemoglobin (r=0.456, p=0.022) and cord Ferritin (r=0.460, p=0.021). **Conclusion:** There was no correlation between cord Hepcidin and neonatal morphometrics. Maternal and neonatal Hepcidin are independent of each other in iron status regulation.

Keywords: Neonatal morphometrics, Hepcidin, Ferritin, Transferrin saturation

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INTRODUCTION

Hepcidin is a peptide hormone secreted from liver, and mainly regulates iron level in body. It performs this task by regulating Ferroportin transporter which is the only iron transporter in the body. This transporter is mainly present across the basolateral membrane of enterocytes of duodenum, macrophages, hepatocytes and placental syncytiotrophoblasts.[1] Hepcidin binds with Ferroportin and causes the internalization and degradation of this receptor, and thus decreases absorption of iron across basolateral membrane.[2] The production of Hepcidin is enhanced during inflammation and elevated iron levels whereas Hypoxia and anaemia decrease its production.[3]

Maternal Hepcidin level decreases during normal pregnancy in 2nd to 3rd trimester in response to iron deficiency.[4] Decreased or undetectable Hepcidin during pregnancy make sure unimpeded unidirectional iron transfer from mother to foetus. Mothers with higher Hepcidin deliver lesser iron to foetus.[5] The maternal iron bound with transferrin is taken to the placenta where this transferrin cannot cross it. Iron is then bound with foetal transferrin which delivers iron to foetal circulation.[6]

Foetus synthesizes and secretes its own Hepcidin from the 1st trimester which regulates foetal iron level independently.[7] Cord blood contains higher Hepcidin concentration compare to maternal blood which prevents iron-overload in neonates.[4,8] Low Hepcidin level in infants reflects increased iron demand which may otherwise lead to iron deficiency anaemia. Higher Hepcidin concentration in infants is linked with lower birth weight and anemia.[9] The objective of the present study was to determine the correlation of cord Hepcidin with iron status markers of mothers and neonates, and neonatal morphometrics.

MATERIAL AND METHODS

Twenty-five (25) apparently healthy pregnant women at the time of delivery were recruited for this study from the Department of Obstetrics and Gynaecology, Fatima Hospital, Karachi. Informed written/verbal consent was obtained from each patient. The data were collected from Sep 2015 to Mar 2016. Twenty-five (25) healthy neonates delivered to the recruited mothers were also included for their cord blood samples. Only women with singleton and term pregnancies, without any complications were included.

The APGAR score was recorded immediately after birth, at 1st and 5th minutes of delivery. Birth weight was measured using digital weighing scale. Birth length of infants was recorded in centimetres from crown to heels. Head circumference was measured at the largest area of head usually from frontal prominence to occipital protuberance using a measuring tape.

Five ml of venous blood was withdrawn from mother, and also from umbilical vein of the foetus. Two ml blood was transferred to EDTA containing test

tube for Haemoglobin (Hb) estimation, and 3 ml blood was transferred to plain test tube and left standing for 30 minutes for clotting. Later, it was centrifuged at 4,000 rmp for 15 minutes until clear serum was obtained.

Hb was measured on Haematology analyzer (Sysmex KX-21, Sysmex Corporation, Japan). Serum Ferritin (Pontine Scientific Inc. Canton, Michigan, USA), and Serum Hepcidin (Sunlong Biotech Co. Ltd., Zhejiang, China) were measured with ELISA (Chem plate reader, Shenzan Electronics, China). Serum Iron and TIBC were analyzed calorimetrically on Clinical Chemistry analyzers (BioSciences Pte Ltd, Singapore; and Microlab 300, EliTech-Merck, Neitherlands). Transferrin saturation was calculated using formula:

Serum iron/TIBC×100

SPSS-21 was used for statistical analyses. Distribution of data was checked using Shapiro Wilk test. Normally distributed data were presented as Mean±SD whereas non-normally distributed data were presented as median [IQR]. Pearson/Spearman correlation was used to check the association between different variables and $p \leq 0.05$ was considered statistically significant.

RESULTS

General characteristics of mothers and neonates are presented in Table-1. The hepcidin concentration of mothers and neonates in the present study was 6.971±2.432 and 72.596±13.357 ηg/ml respectively. The correlation of maternal and neonatal hepcidin with their respective Hb and iron parameters is tabulated as Table-2. The levels of all haematological parameters including Hb, iron status markers and hepcidin were comparatively lower in maternal blood than foetal cord blood. Both maternal and neonatal hepcidin showed positive correlation with their serum iron (r=0.455 and r=0.593, p=0.022 and p=0.002 respectively), ferritin (r=0.511 and r=0.792, p=0.009 and p=0.000 respectively) and Transferrin saturation (TS) (r=0.440 and r=0.546, p=0.025 and p=0.005 respectively).

Birth weight, body length and head circumference showed no association with cord hepcidin or any other haematological variable of the neonate (Table-3). APGAR scores at 1st and 5th minute did not correlate with any variable of neonates. Table-4 presents the relationship between maternal and neonatal Hb, iron status and hepcidin with each other. Maternal ferritin showed positive correlation with neonatal Hb (r=0.456, p=0.022) and ferritin (r=0.460, p=0.021) whereas neonatal hepcidin was found to be positively associated with maternal total iron binding capacity (TIBC) (r=0.440, p=0.002).

Cord hepcidin concentration was found to be non-significantly lower in neonates of overweight mother as compare to obese and normal mothers (67.38±12.83, 76.60±13.35, and 78.79±11.65 respectively). Neonates at term with different gestational age on One-way ANOVA showed no significance difference in serum hepcidin concentration (p=0.839).

Table-1: Baseline characteristics of mothers and neonates

Parameter	Values
Mother	
Age (Years)	27.84±6.256
BMI (Kg/m^2)	25.89±3.54
Parity	2 [4]
Miscarriages	0 [1]
Neonates	
APGAR score at 1st minute	8 [2]
APGAR score at 5th minute	9 [1]
Gestational age (weeks)	37.76±1.011
Length (Cm)	49.52±2.61
Weight (Kg)	2.99±0.55
Head circumference (Cm)	34.78±1.22

Mean±SD for Normally distributed variables, Median [IQR] for Non-normally distributed variables

Table-2: Correlation of maternal and neonatal hepcidin with haemoglobin and iron status markers at delivery (n=25)

Variables	Maternal Hepcidin			Neonatal Hepcidin		
	Mean±SD	*r*	*p*	Mean±SD	*r*	*p*
Haemoglobin (g/dl)	11.408±0.923	0.052	0.789	13.814±1.172	0.268	0.196
Iron (µg/dl)	112.52±25.239	0.455	0.022*	127.84±16.449	0.593	0.002*
Ferritin (ηg/ml)	11.704±7.643	0.511	0.009*	64.96±21.275	0.792	0.000*
TIBC (µg/dl)	490.84±35.725	-0.142	0.497	243.92±21.762	-0.115	0.584
TS (%)	23.251±5.967	0.440	0.025*	52.789±8.155	0.546	0.005*

*Significant

Table-3: Correlation of neonatal morphometrics with studied haematological parameters

Haematological Parameters	Birth weight		Body Length		Head Circumference	
	r	*p*	*R*	*p*	*r*	*p*
Haemoglobin	-0.164	0.433	-0.239	0.251	-0.072	0.731
Iron	-0.050	0.813	0.005	0.981	-0.151	0.472
Ferritin	-0.094	0.654	-0.286	0.165	-0.220	0.290
TIBC	-0.124	0.555	-0.073	0.728	0.272	0.188
TS	0.049	0.817	0.058	0.781	-0.271	0.190
Hepcidin	0.003	0.988	-0.254	0.220	0.387	0.268

Table-4: Correlation between maternal and neonatal haematological variables

Haematological Parameters of Mothers		Haematological Parameters of Neonates					
		Haemoglobin	Iron	Ferritin	TIBC	TS	Hepcidin
Haemoglobin	***r***	0.068	0.018	-0.103	-0.189	0.101	-0.012
	p	0.747	0.931	0.624	0.367	0.630	0.956
Iron	***r***	0.024	0.095	0.261	-0.238	0.219	0.007
	p	0.909	0.651	0.208	0.251	0.293	0.972
Ferritin	***r***	0.456	0.341	0.460	0.061	0.225	0.364
	p	0.022*	0.095	0.021*	0.743	0.279	0.073
TIBC	***r***	0.203	0.208	-0.160	-0.100	0.256	0.440
	p	0.331	0.319	0.445	0.635	0.217	0.002*
TS	***r***	-0.026	0.007	0.156	-0.188	0.106	-0.131
	p	0.904	0.973	0.457	0.368	0.613	0.532
Hepcidin	***r***	0.143	-0.092	0.131	0.276	-0.230	-0.026
	p	0.494	0.643	0.533	0.181	0.268	0.900

*Significant

DISCUSSION

For the present study, healthy non-anaemic[10] women having Hb>11.0 g/dL were recruited with their neonates and cord blood. Cord hepcidin was measured and correlated with the neonatal anthropometric variables, APGAR score and iron parameters of both mother and neonates. All the neonates were full term (37.76±1.011) and having APGAR score >7 at 1st and 5th minutes of life. The average birth length of the neonates in our study was in accordance of term neonates of other countries[11] but less than the studies conducted in Poland.[12,13] The average birth length of term newborn in present study was lower than the healthy term neonates in other countries[12–14]. Our finding are consistent with birth weight of full term Indian neonates[11]. The mean head circumference of neonates included in present study was consistent with Villar[11], Chelchowska[12], and Rechberger[13].

The hepcidin concentration in mother was in consistent to results of Rehu[8] for the non-anaemic pregnant women but having insufficient iron stores. The pregnant women in our study were also non-anaemic (Hb>11.0 g/dL) but about 2/3rd of them had insufficient iron stores (SF<15 ηg/ml). We and others[8,15,16] found maternal serum hepcidin concentration several folds lower than the cord hepcidin concentration. The decreased iron stores and hepcidin level in mothers depicts the need of iron transfer to foetus over maternal need as the foetus largely grows in late pregnancy.

Basu *et al*[17] found positive association of hepcidin with the Hb and iron parameters, both in maternal and cord blood, whereas, Simavli[18] has found no correlation of hepcidin with Hb and other iron parameters. Albandery[19] observed positive correlation of hepcidin with ferritin but negative association with Hb in anaemic pregnant women having gestational diabetes. The cord hepcidin concentration in our study is lower than other studies[17,20] which might be due to lower iron stores in cord[21] as this would allow the maximal iron transfer from mother to foetus.

No correlation was observed between cord hepcidin and neonatal anthropometrics or with the APGAR score. Chelchowska *et al*[12] reported that effect of maternal hepcidin and correlated it with neonatal birth weight and length in smoking and non-smoking mothers. They observed that maternal hepcidin was directly associated with neonatal birth weight and birth length in both smoking and non-smoking mothers. They inferred decreased birth weight, head circumference, and body length in infants of smoker mothers but there was no difference in APGAR score in the two groups.[12]

In consistency to our results, Kumar *et al*[22] also found positive correlation of maternal ferritin with cord ferritin and Hb, but Sunmin Lee *et al*[10] and Rechberger *et al*[13] found results contrary to our findings. The newborns born to these non-anaemic mothers had Hb 13.814±1.172 g/dL and their ferritin concentration was 64.965±21.276 ηg/ml which represents no anaemia but quite low iron stores (cut-off for low iron stores in cord blood=SF<75 ηg/ml).[23] It was reported that maternal serum ferritin has relation to cord ferritin if maternal SF<13.6 µg/L and above this value, no association existed.[23] We identified positive correlation between maternal and cord blood SF. Low iron store in cord shows concern as cord serum ferritin is a strong predictor of iron status in infants up to 2 years of age. The neonatal iron insufficiency is the causation of impaired myelination in CNS and has its effects on neonates' auditory and visual transmission that persist even in childhood.[24] As reported by WHO, iron deficient infants might have affected cognitive, neurologic and psychomotor functions in childhood.[25]

Cord hepcidin was positively correlated with cord iron, ferritin and transferrin saturation but not with any anthropometric variable and APGAR score. Young *et al*[16] reported positive association of cord ferritin and iron with hepcidin. We also noted positive correlation with cord iron, ferritin and transferrin saturation.

CONCLUSION

Maternal and cord hepcidin are independent of each other. Relatively decreased hepcidin concentration can be due to depleted iron stores in cord that facilitates iron transfer. Cord hepcidin was affected with stored iron of

cord and mothers but was not associated to neonatal anthropometric variables. Maternal hepcidin was not influenced with any variable of cord.

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