Accessed on: http://jkb.ub.ac.id/index.php/jkb/article/view/2628
Jurnal Kedokteran Brawijaya Vol. 31, No. 1, Februari 2020, pp. 58-65
Article History: Received 17 September 2019, Accepted 13 November 2019

Research Article

Iron Status of Newborns and Umbilical Cord Blood Hepcidin Levels in Gender Differences

Status Besi Bayi Baru Lahir dan Kadar Hepsidin Tali Pusat Berdasarkan Jenis Kelamin

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ABSTRACT

Fetal gender might affect the iron status of newborns. Hepcidin has an important role in the process of maternofetal iron transport. This study aims to compare the newborn iron status and the umbilical cord hepcidin levels between male and female gender. *A cross-sectional study was conducted with subjects of 84 clinically healthy newborns. Written informed consent and ethical approval were carried out. Newborn iron status observed included (i) hematologic markers (RBC count, Hb, hematocrit, mean corpuscular volume (MCV) and red cell distribution width), and (ii) biochemical markers (serum iron (SI), serum ferritin (SF), soluble transferrin receptor (sTfR) and cord blood hepcidin). Hematologic markers were checked using Sysmex, XN-1000, while Hepcidin and sTfR were using ELISA. Serum iron was checked using IRON Flex®. Statistical analysis was tested with the independent t-test and the Mann-Whitney. All newborns and their mothers were in normal condition. The mean sTfR levels of newborns were significantly higher in the male group than females (38.3±9.06 vs. 34.3±8.16* nmol/L*) with p=0.033. High sTfR levels reflect a low iron status. In conclusion, fetal gender differences influence the iron status of newborns, and male newborns have a potentially higher iron deficiency.*

Keywords: *Fetal gender, hepcidin, iron status, newborn, sTfR*

ABSTRAK

Jenis kelamin janin mungkin mempengaruhi status besi bayi baru lahir. Hepsidin memiliki peran penting dalam proses transportasi besi maternofetal. Penelitian ini bertujuan untuk membandingkan status besi neonatus dan kadar hepsidin tali pusat antara laki-laki dan perempuan. Studi cross-sectional dilakukan dengan subyek 84 bayi baru lahir normal secara klinis. Persetujuan tertulis dan persetujuan etis telah dilakukan. Status besi yang diperiksa *meliputi* (i) *marker* hematologi (jumlah eritrosit, Hb, hematokrit, *mean corpuscular volume (MCV), red cell distribution width), dan marker biokimia (serum iron (SI), serum ferritin (SF), soluble transferrin receptor (sTfR)* dan hepsidin darah tali pusat). Marker hematologis diperiksa menggunakan Sysmex, XN-1000, sedangkan Hepsidin dan sTfR menggunakan ELISA. SI diperiksa menggunakan IRON Flex®. Analisis statistik diuji dengan t-test independen dan Mann-Whitney. Semua bayi baru lahir dan ibunya dalam kondisi normal. Rerata sTfR bayi baru lahir lebih tinggi secara bermakana pada kelompok laki-laki dibanding perempuan (38,3±9,06 vs 34,3±8,16) dengan p=0,033. Kadar sTfR yang tinggi menggambarkan status besi yang rendah. Kesimpulan, perbedaan jenis kelamin janin mempengaruhi status besi bayi baru lahir, dan bayi laki-laki berpotensi lebih besar untuk kekurangan zat besi.

Kata Kunci: Bayi baru lahir, hepsidin, jenis kelamin fetus, status besi, sTfR

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DOI: http://dx.doi.org/10.21776/ub.jkb.2020.031.01.12

INTRODUCTION

Iron is an essential element, especially during the growth of the brain in the third trimester of pregnancy and at the beginning of human life (1). The balance of iron is essential for all of our cell life (2). Iron deficiency in late pregnancy and early life potentially causes neurological disorder (1), which may be irreversible (3), mainly if it occurs at a critical age, the first two years of life (4,5).

Many factors influencing the iron status of newborns have been identified, including gender. Fetal gender is one of the determinants that can affect iron metabolism during pregnancy. Fetal gender is very likely to affect the transport of iron from the mother to the fetus (maternofetal) and will further determine the iron status of the newborn (6-8).

Previous studies on the effect of gender on iron status more often use baby subjects in the first year period, rarely in neonatal subjects. Two old studies, beginning research on infants with fetal growth restriction by Tamura T *et al.,* (1999), reported that the mean serum ferritin levels in the umbilical cord were significantly higher in female than male infants (9), and the second research, Domellöf M et al. in 2002 reported that infants at ages 4, 6, and 9 months, boys had significantly lower Hb, MCV, and ferritin and higher zinc protoporphyrin and transferrin receptors than girls (10). In 2012, Antunes H *et al.,* conducted a study of the effect of gender on iron status of 9 month old infants and concluded that iron deficiency (ID) was significantly more frequent in male infants (had an increased risk of 3.3 times of having ID), independent of rapid growth or longer breastfeeding duration (11).

Currently, hepcidin is believed to be the main regulator of iron metabolism and hemostasis in the human body and plays a role in the process of maternal to fetal iron transport (12). In humans, gender is likely to influence the expression of hepcidin, but research on the role of gender in shaping hepcidin related to iron metabolism in humans has not been satisfactorily explained (6). During pregnancy, hepcidin may play a role in the gender-based differences in iron metabolism (6).

Although many researchers have explored the underlying mechanism, there is no single theory can explain comprehensively the influence of gender on fetal/neonate iron status (6-8). We understand that the male fetuses/infants have a faster growth rate than females; of course, this is an essential factor in explaining the problem of gender influence on infant iron status. From this point of view, logically thinking normally, the fetuses/infant male will need more macronutrients and micronutrients, including Fe, to be able to grow normally. Several studies have recognized male gender as a risk factor both during pregnancy and birth, and various theories discuss the influence of hormonal, physiological, or genetic factors, but the biological mechanism of this gender-specific difference is still unclear (13-15). A better understanding of the mechanisms associated with gender-related differences in iron metabolism during pregnancy can contribute ideas or concepts in preventing and treating infant iron deficiency.

The determinants of iron status in newborns need to be controlled to prevent and adequately manage iron deficiency (ID) in infants. Because the gender differences might affect on the iron status of newborns, our results have an impact on the management of ID in infants. The impact results from the point of view of several aspects, namely: (i) the diagnosis of ID can be made earlier (newborn period) and with considering fetal gender (pregnancy period), (ii) the preventive and therapeutic ID aspects, we can provide input to change the iron supplementation protocol (not only in infants but also in pregnant women), and (iii) from the perceptive prognosis, the earlier the diagnosis and the sooner solution of ID in infants, the higher the baby's expectations for optimal growth and development. The clinical implications of this study not only can affect diagnosis but also therapy and prognosis in the management of iron deficiency in neonates. This study aims to compare the newborn iron status and the umbilical cord hepcidin levels between male and female fetal gender.

METHODS

A cross-sectional study was conducted in three hospitals (one government and two private hospitals) in Purbalingga Regency, Central Java, Indonesia, from September to November 2015, with a total subject of 84 newborns. This study was part of a comprehensive study that assessed various factors associated with the iron status of neonates. To calculate the sample size, we used a sample size formula for mean comparison with independent (non-paired) subjects (16,17). Four independent variables were considered, which included hepcidin, IL-6, sTfR pregnant a term women, and umbilical cord clamping time. We used the largest sample size resulted from the calculation using those four variables. This research protocol received ethical approval from the Health and Medical Research Ethics Commission of the Faculty of Medicine, Diponegoro University/Dr. Kariadi Hospital Semarang, No.48/EC/FK-RSDK/2015. Written informed consent was signed by the father or mother of the newborn subject.

The newborns were eligible in this study (inclusion criteria) if they were born spontaneously, from single and term pregnancy, with an Apgar score ≥7 in the first minute, normal birth weight (≥2.500 to <4.000 grams), and not suffer from major congenital abnormalities. We excluded infant subjects (exclusion criteria) if they were suffering from severe illness and hematologic-oncological disease, and the mother had postpartum hemorrhage.

Fetal gender was determined based on physical examination when the baby was born. The blood samplings from the umbilical cord and newborns vena were performed immediately after birth. Newborn iron status parameters in this study included RBC count, Hb, Ht, MCV, RDW, SI, SF, sTfR, and the cord blood hepcidin. The hematologic status (RBC count, Hb, Ht, MCV, and RDW) of newborns was checked using a hematology analyzer (Sysmex, XN-1000 hematology analyzer), while hepcidin and sTfR were using the ELISA method. SI was tested using IRON Flex® reagent cartridge, Cat. No. DF85. The statistical analysis to compare newborn iron status and umbilical cord hepcidin levels between male and female sexes was tested with the independent t-test and the Mann-Whitney test. The statistical test used 95% confidence intervals, with a limit of significance at $p<0.05$.

RESULTS

This research is the first study, which aims to compare the newborn iron status and the umbilical cord hepcidin levels between male and female gender in Indonesia. Our research was conducted on 84 newborns at Purbalingga Regency,

Central Java, Indonesia. In the initial process, as many as 108 pregnant women/parents of prospective subjects interviewed, seven pregnant women refused to participate in the study, mostly because of fear or worry about the process of taking blood. There were two infants with clinical features of Down syndrome, four babies were born with respiratory distress, and 11 babies had failed blood sampling or laboratory techniques, could not continue the research process, so we fulfilled a total of 84 baby subjects.

Table 1 shows the characteristics of the newborns subjects and their mothers based on fetal gender. The mothers and their babies in both male and female fetal gender groups are in good clinical condition. Several maternal characteristics that might influence the iron status of the newborns have been identified.

All mothers are from Javanese ethnicity and have performed antenatal care (ANC) ≥4 times following minimum recommendations. Maternal education in males compared to female groups, the percentage of mothers with higher education than senior high school (university) was 11(28.9%) vs. 27(71.1%) and senior high school or below was 27(71.1%) vs. 37(80.4%), and these results were not significantly different, with p=0.46.

Between male and female gender groups, maternal age (27.63±5.98 vs. 27.26±4.89 years), and maternal systolic and diastolic blood pressure (120±9.81 vs. 119.72±8.76 and 72.84±6.43 vs. 73.74±6.55 mmHg) did not differ significantly (p>0.05). The other characteristics of mothers were presented in Table 1 and were not found to be statistically significant differences between the two fetal gender groups, with p>0.05.

All newborns were born from healthy mothers (not suffering from hypertension, pre-eclampsia/eclampsia, and antepartum or postpartum hemorrhage). The newborn subjects in both fetal gender groups were born spontaneously, from a single pregnancy, and did not suffer from major congenital abnormalities. All newborns subjects in male and female gender groups were born in normal birth weight (3256.32±303.42 vs. 3135.33±303.16 gr, p=0.07), term pregnancy (39.00±1.18 vs. 39.41±0.99 weeks, p=0.09), with the same median Apgar score in the fifth minute [9 (8-10)], and normal other conditions. All these newborns' characteristics between the two fetal gender groups (in Table 1) were not significantly different (p>0.05).

Table 1. Characteristics of the mother and newborn subjects based on fetal gender

Characteristics	Group based on fetal gender		p
	Males (n=38)	Females (n=46)	
A. Characteristics of mother			
Maternal age, year ($\bar{x}$±SD)	27.63±5.98	27.26±4.89	0.91[a]
[median (range)]	27(15-40)	26(20-38)	
Maternal education			
- > Senior High School, n (%)	11(28.9)	9(19.6)	0.46[c]
- ≤ Senior High School, n (%)	27(71.1)	37(80.4)	
Systolic, mmHg ($\bar{x}$±SD)	120±9.81	119.72±8.76	0.89[a]
Diastolic, mmHg ($\bar{x}$±SD)	72.84±6.429	73.74±6.55	0.55[b]
Active smoking			
- Yes, n (%)	1(2.6)	0(0.0)	
- No, n (%)	37(97.4)	46(100.0)	0.92[c]
Passive smoking			
- Yes, n (%)	18(47.4)	19(41.3)	
- No, n (%)	20(52.6)	27(58.7)	0.74[c]

Table 1. Characteristics of the mother and newborn subjects based on fetal gender (Continued)

Characteristics	Group based on fetal gender		p
	Males (n=38)	Females (n=46)	
A. Characteristics of mother			
Ante-natal care			
- ≥ 4 times, n (%)	38(100.0)	46(100.)	-
- < 4 times, n (%)	0(0.0)	0(0.0)	
Fe tablet supplementation,			
- Yes, n (%)	38(100.0)	46(100.0)	-
- No, n (%)	0(0.0)	0(0.0)	
B. Characteristics of newborns			
Umbilical cord clamping time,			
- 20 seconds, n (%)	23(60.5)	19(41.3)	0.13[c]
- 3 minutes, n (%)	15(39.5)	27(58.7)	
Birth weight, gram ($\bar{x}$±SD)	3256.32±303.42	3135.33±303.16	0.07[a]
Gestation, week ($\bar{x}$±SD)	39.00±1.18	39.41±0.99	0.09[a]
Heart rate, beats/min [median (range)]	130.61±7.08	129.28±9.75	0.49[a]
Apgar Score 5 min, [median (range)]	9.00(8-10)	9.00(8-10)	0.08[b]

Remarks: Statistical test using 95% confidence intervals; a, Independent t-test; b, Mann-Whitney Test; c, Chi-Square

Table 2 shows the comparison (based on the gender) between hematological and biochemical markers of the iron status of newborns. Hematologic markers in the male group compared with the female group, the mean of RBC count (4.88 ± 0.45 vs. 4.82 ± 0.57), Hb levels (17.39 ± 1.44 vs. 17.47 ± 1.94), and Ht levels (49.45 ± 4.57 vs. 49.48 ± 5.37) did not differ significantly, p = 0.580, p = 0.835, and p = 0.979, respectively. Other hematologic markers were not significantly different between the two gender groups, with p >0.05.

The results of biochemical markers showed that the mean sTfR levels of newborns were higher in the male compared to females group (38.3±9.06 vs. 34.3±8.16nmol/L) with p=0.033, which indicated fetal gender influences newborn sTfR levels. Other biochemical parameters (serum iron and serum ferritin) did not differ significantly in the two sex groups, p>0.05. The levels (median) of the hepcidin umbilical cord were higher in the male than in the female group [4.10 (1.66-6.63) vs. 3.85 (1.58-6.90) ng/ml], but were not statistically significant, with p=0.364 (Table 2).

Table 2. Comparative analysis of the iron status of newborns and umbilical cord hepcidin levels based on fetal gender

Iron status parameter	Group of the newborns by gender		p
	Males (n=38)	Females (n=46)	
A. Hematologic Markers			
RBC count, 10^6/mm^3 ($\bar{x}$±SD)	4.88 ± 0.45	4.82 ± 0.57	0.58[a]
Hb, g/dL ($\bar{x}$±SD)	17.39 ± 1.44	17.47 ± 1.94	0.84[a]
Ht, % ($\bar{x}$±SD)	49.45 ± 4.57	49.48 ± 5.37	0.98[a]
MCV, fL ($\bar{x}$±SD)	100.20 ± 4.22	102.90 ± 5.89	0.15[a]
RDW, % [median (range)]	16.55 (15.30 - 19.50)	16.80 (15.20 - 19.90)	0.62[b]
B. Biochemical Markers			
Serum iron (SI), µg/dL ($\bar{x}$±SD)	105.58 ± 49.57	119.20 ± 54.73	0.24[a]
Serum ferritin, ng/ml ($\bar{x}$±SD)	405.5 (102.40 - 822.00)	360.9 (38.02 - 918.60)	0.76[b]
sTfR, nmol/L ($\bar{x}$±SD)	38.3 ± 9.06	34.3 ± 8.16	0.03[a]
Hepcidin (umbilical cord), ng/ml [median (range)]	4.10 (1.66 - 6.63)	3.85 (1.58 - 6.90)	0.36[b]

Remarks: Statistical test using 95% confidence intervals; a, Independent t-test; b, Mann-Whitney Test

DISCUSSION

During the last period of pregnancy and the beginning of human life, the need for iron increases because the nervous system rapidly develops (1). Long-term neurocognitive problems have a strong association with iron deficiency at critical times during brain development (18,19). We should control all risk factors that can cause iron deficiency in newborns. In this study, we determined the difference of sTfR levels of the newborns based on the fetal gender differences. Our research found that the mean sTfR levels of newborns were significantly higher in the male compared to the female group. An increase in sTfR levels can indicate the presence of iron-deficient erythropoiesis (the stage of iron deficiency without anemia) or the presence of iron deficiency anemia. So that we proved that fetal gender differences influence the iron status of newborns, and male newborns have a potentially higher iron deficiency.

Before discussing the results further, first, we looked at other factors, besides gender, that might affect the iron status of the newborn, and we would ensure that gender differences influenced our results. Our study limited the criteria for inclusion of newborns with a normal range of birth weight (≥2.500 to <4.000 grams) and at term pregnancy (37 - ≤41 weeks). All newborns were born in normal clinical conditions.

Some things that affect infant iron storage, in addition to low birth weight and shorter pregnancy duration, are maternal iron status (20). Iron supplementation during pregnancy influence the maternal iron status (21). In developed countries, iron supplementation (IS) is currently a controversial issue related to the benefits and risks for mothers and their babies. However, IS during pregnancy is still strongly recommended in developing countries (22), such as Indonesia. Iron supplementation in pregnant women can affect the iron status of the newborns (23). In this research, all mothers received supplementation of Fe tablets, so the Fe tablet supplementation factor did not affect our results and was not further analyzed.

Ethnicity might affect someone in choosing the preferred food menu. A study in Singapore reported that Malay and Indian pregnant women were more likely to have iron deficiency than ethnic Chinese. Differences in dietary practice, where ethnic Chinese eat more meat (source of haem-iron), Malay women consumed fewer fruits and vegetables and have a lower vitamin C intake, which can reduce iron absorption, while Indian women are tended to be vegetarian (24). All subjects were born from healthy Javanese mothers, so ethnicity did not affect the results of this study.

Many studies showed positive effects of antenatal care (ANC) visits on perinatal outcomes (25). The results of our research, in both groups of fetal gender, all mothers performed ANC of ≥4 times, following the recommendation of the World Health Organization (WHO) for all low-risk pregnant women at least to have four consecutive ANC visits, so that the ANC factor does not affect the results of this study.

Some of the maternal characteristics below might influence the status of iron newborns. Parity, especially grand multiparity (parity ≥5), remains a risk in pregnancy, including iron deficiency in mothers and is associated with an increased prevalence of maternal and neonatal complications (26). In our study, none of the mothers had grand multiparity. Maternal education was linked to several neonatal outcomes, and compared with low-level educated mothers, those with high education had reduced odds of preterm and small for gestational age (SGA) birth. All health professionals agree that babies with premature birth and SGA can affect their iron status (27,28). Table 1 showed that maternal education in the two study groups of gender was not different. Hypertensive disorder of pregnancy is a serious problem because it is associated with an increased risk of adverse fetal and neonatal outcomes (29). Our result, both systolic and diastolic blood pressure, were not significantly different between the two fetal gender groups. Smoking in pregnant women, either active or passive smoking during pregnancy has been known to harm the well-being of a developing fetus and cause iron deficiency in infants (30). In our study (Table 1), between the two study groups, the number of either active or passive smoking was not statistically different, so it did not affect the results of the study.

Our results showed that all the factors above included parity, maternal education, blood pressure, and other characteristics did not differ significantly between the two groups of fetal gender, so we did not analyze these factors further.

The effect of fetal gender on pregnancy outcome, especially neonatal outcome, has long been studied (9). Evidence suggests that females have an advantage over males with a better clinical outcome in the perinatal period. Literature review on fetal gender showed that the incidence of preterm delivery (8,31) and cesarean section were higher for male fetuses (8). The pregnancies of male fetuses are also associated with higher rates of labor dystocia, cord problems, fetal distress, low Apgar scores, and perinatal mortality. So, the male gender is an independent risk factor for adverse pregnancy outcomes (7). Although, in general concept, the male fetuses have lower clinical performance than females, it does not apply to the occurrence of intrauterine growth restriction (32). The incidence of intrauterine growth retardation occurred more often in female than male fetuses (7,8). But overall, male fetuses have lower clinical performance than females (7).

The mechanism of fetal gender influencing the status of iron newborns is still a matter of discussion. Previous (old) research in 1999, Tamura T, et al. examined the effect of gender differences on neonatal outcomes, and found the mean ferritin concentration in females was significantly higher than in male infants, but detailed explanation the mechanism of the gender difference was unknown (9). Currently, we don't find a single theory that explains the effect of gender on the iron status of the newborn. Several studies have recognized the male gender as a risk factor during pregnancy and childbirth. However, the biological mechanism of this gender-specific difference is still unknown, even though various theories discuss the influence of hormonal, physiological, or genetic factors (13-15).

Antunes H *et al.,* in 2012 reported a study of iron deficiency (ID) risk factors in infancy and concluded that ID was significantly more frequent in male infants, independent of rapid growth or longer breastfeeding duration (11), so male newborns potentially have a higher birth-weight than female. It means that the growth of the male fetus is faster

than females.

As a consequenced above, during pregnancy, the male fetuses need more nutrients for growth, including iron. More iron necessity for male fetal growth is one of the risks of iron deficiency. Thus, male fetuses might have lower iron status than females if the mother did not meet the iron necessities for fetal growth during pregnancy. We must anticipate this condition to prevent an iron deficiency in newborns. Our study showed that the mean sTfR levels of newborns were significantly higher in the male compared to the female group, but the other iron status parameters of newborns (including hematologic markers, other biochemical markers, and umbilical cord hepcidin levels) did not differ. We will discuss these results more, including explaining why the sTfR parameter was affected by gender differences.

Our study (Table 2) showed that the red blood cell (RBC) count, Hb, and Ht levels in male compared with female newborns did not differ significantly. Compared to other studies, our results (RBC count, Hb, and Ht) were consistent with a previous study in 2015 by Glasser L *et al.,* which stated that iron status parameters rarely showed differences based on gender, and they found that RBC count, Hb, and Ht levels were not significantly different in both genders (33). In contrast to our study, research in 2011 in Taiwan by Chang Y *et al.,* showed that gender had a significant effect on cord blood RBC count, Hb, and Ht levels, and they drew a conclusion that the female neonates had significantly lower RBC count, Hb, and Ht levels (34). The differences between the studies suggest that other determinants could affect the iron status of newborns.

Serum iron (SI) may be affected by gestation, gender, maternal iron status, maternal-fetal nutrient exchange, hypoxemia condition, decreased uteroplacental perfusion, and inflammation during pregnancy (35). SI can identify iron deficiency with low sensitivity. The amount of iron intake and duration of time may influence SI status (35). In this research, the mean of SI was higher in female newborns compared with that in male (119.20±54.73 vs. 105.58±49.57), but not statistically significantly different. These results indicate that the effect of gender on SI levels in newborns is inconsistent.

In our results (Table 2), the median of newborn serum ferritin (SF) levels were higher in male than female gender newborns [405.5 (102.40-822.00) vs. 360.9 (38.02-918.60) ng/ml], but the difference was not significant. SF can reflect the body's iron storage (36). In contrast to previous (old) research, in infants with fetal growth restriction, the mean serum ferritin levels in the umbilical cord were significantly higher in female than male infants (9). High or increased serum ferritin levels indicate filled iron stores and even overload iron conditions (37). Low levels of SF are specific for iron deficiency, and however, ferritin is an acute-phase protein that can increase during inflammation or infection (38), so it can cause a “masking effect” on low iron storage conditions. Based on the data and discussion above, we need other iron status parameters that are not affected by pregnancy (as inflammation). We need a more stable and also sensitive parameter of the iron status of newborns.

The sTfR is a parameter of iron deficiency at the cellular level. The sTfR expression increases when there is an inadequate supply of iron for tissue demand or an increased need for iron associated with the erythropoiesis process (39). TfR is present on the surface of the cell membrane and binds to Tf-Fe entering the cell. The amount of TfR in the cell membrane is proportional to the amount of sTfR in the plasma. In the cellular level of iron deficiency, there will be an increase in TfR synthesis. The increasing levels of sTfR can indicate an iron-deficient erythropoiesis or iron deficiency anemia (IDA). The level of sTfR in blood plasma is an indicator of functional iron availability, and its presence does not depend on iron stores (40,41).

Cellular iron homeostasis depends on coordination between proteins that regulate the absorption, storage, use, and release of cellular iron (42). Cells will respond to iron deficiency by increasing the expression of sTfR to maximize the internalization of iron-transferrin bonds. This condition causes "iron reserve synthesis" to be delayed to ensure the availability of cellular iron (42).

In our study, the results of the biochemical marker (Table 2) showed that the mean sTfR levels of newborns were higher in the male group than females. The increasing levels of sTfR indicate low iron status, so our result shows that male newborns have lower iron status than female newborns. It shows that male sex is negatively related to the iron status of newborns.

Our study also noted that almost all iron status parameters for newborns were not significantly different in the two gender groups, except for sTfR levels. It shows that sTfR is a proper and good parameter, and this may be more sensitive than others. The sTfR measurement is stable, is not affected by diurnal variations, and changes before the protoporphyrin erythrocytes or mean corpuscular volume (MCV) change (35). Not only sensitive, but the sTfR parameter is also specific for iron deficiency. It can distinguish iron deficiency anemia (IDA) from anemia due to chronic disease (41,43). The level of sTfR remains normal in acute or chronic inflammation and liver disease, but sTfR increases in hemolytic anemia, thalassemia, and polycythemia. The sTfR levels will decrease in hypoplastic anemia and kidney failure (35). Serum sTfR levels can be a useful marker for the diagnosis and treatment evaluation of IDA in children (44). Based on the analysis above, the sTfR parameter is more reliable, stable, and more sensitive in determining the iron status of the newborn.

The influence of different gender on the iron status of the newborn is possible through the role of hepcidin. Hepcidin is a protein, and in humans, is encoded by the HAMP gene, which is located in the long arm of chromosome 19 at position 13.1 (6). Gender and genetic background have been shown to modulate hepcidin expression in mice. The role of sex in the regulation of human hepcidin gene expression in the liver is unclear. However, hepcidin can play a role in gender-based differences in iron metabolism and liver disease (6).

Ferroportin that is currently the only cellular iron exporter found in several organs, including on the "fetal side" of the syncytiotrophoblasts placenta, plays an essential role in the release of iron and regulating the maternofetal iron transport. Hepcidin plays a role in controlling this ferroportin by binding directly and internalized into the lysosome, resulting in ferroportin degradation and loss of its function (12,45).

Hepcidin is believed to be the main regulator of iron metabolism and hemostasis in the human body (12).

Hepcidin is negatively related to iron status (45). Hepcidin levels of the umbilical cord blood (after born) can describe fetal hepcidin levels in the late pregnancy period (46). Cord blood is an ideal source for laboratory examinations in just-born neonates (34).

During pregnancy, iron is transferred from the mother to the fetus, and hepcidin regulates maternofetal iron transport across the placenta (6,12). Both maternal and fetal hepcidin might determine the transfer rate of placental iron (47). Fetal iron levels are related to maternal liver TfR and hepcidin expression. The fetus might have a control role in mobilizing maternal iron storage. Hepcidin is produced by fetal liver acts as a negative regulator of iron absorption from the placenta (45). The fetal hepcidin expression has an inverse correlation with TfR expression on placental membranes in animal models (48). Hepcidin derived from the fetus can play a role in ferroportin regulation expressed on the basolateral side of syncytiotrophoblast and determines the levels of iron entry into the fetal circulation (47). All that can affect the expression of hepcidin might influence maternal iron transport to the placenta and fetuses so that it might affect the iron status of newborns.

The research during pregnancy in humans on the role of gender in influencing hepcidin-related to iron metabolism has not been satisfactorily explained. During pregnancy, fetal gender might affect the metabolism and transport of iron maternofetal (6-8). In different mouse strains, different gender has a different hepcidin expression, as well. In humans, fetal gender likely also influences the hepcidin's appearance, and hepcidin might play a role in the gender-based differences in iron metabolism (6).

In this study (Table 2), we found that the levels of umbilical cord hepcidin was higher in male than the female group [4.10(1.66-6.63) vs. 3.85(1.58-6.90) ng/ml], but not significantly different. In conditions where there are differences in cord hepcidin levels, it indicates that the fetus responds to the situation of iron hemostasis in the body during pregnancy. Increased fetal hepcidin levels cause some ferroportin (as the only iron exporter in the fetal side placenta) degraded. Because hepcidin acts as a negative regulator of iron transmission from the placenta to the fetus (45), high levels of hepcidin during late pregnancy will cause a decrease in iron transfer to the fetus. So, it is clear that fetuses with high hepcidin levels tend to have low iron status.

The lower overall iron status in males and the higher CRP concentration in females can mainly explain this gender difference effect in hepcidin concentration (49). Several studies have reported higher rates of anemia and lower iron status in male compared to female infants, which were attributed to the gender-specific growth rates or hormone-mediated differences (sex hormone) in iron metabolism (49,). Sex hormones, especially estrogen, affect the work of the liver. Estrogen can also increase the production of reactive oxygen species that may also regulate the transcription of hepcidin in the liver. It is, therefore, possible that estrogens also play a role in sex-specific regulation of hepcidin expression in the liver (6). Estrogen is involved in hepcidin expression via a GPR30-BMP6-dependent mechanism, providing new insight into the role of estrogen in iron metabolism (50).

In contrast to previous animal studies, hepcidin expression in the liver has been reported to differ by gender, and the liver of female mice (not neonates) express significantly higher hepcidin levels than males (6). The higher hepcidin levels in female mice are associated with increased levels of iron in the liver, but it is unclear whether the cause is an increase in iron levels (6). However, we should not forget that besides iron, hepcidin is also regulated by other stimuli, which may play a vital role in the expression of hepcidin in the liver.

Gender considerations in patient management that have been carried out since the perinatal phase help to optimize the process of general care and health since early life "as an important step in bridging individualized treatment"(15). An understanding of the mechanisms associated with gender-related differences in iron metabolism is vital for overcoming the iron deficiency in infants.

This report has many limitations studies because not all factors that influence neonatal iron status are examined, for example, maternal inflammatory factors (CRP, IL-6 levels), dietary factors during pregnancy, and maternal iron status (ferritin, hepcidin, and sTfR level). Future research should involve these variables. Because the inclusion criteria are clinically healthy neonates, these results cannot be generalized to abnormal conditions, for example, asphyxia, congenital defects, and small for gestational age babies.

The implications of the results of this study are (i) because the male fetus tends to have rapid growth and iron deficiency, it is necessary to think of iron supplementation in pregnant women based on gender-specific that male fetuses need more iron supplementation in pregnant women. Of course, it requires further investigation. (ii) Clinicians, especially pediatricians, need to think of gender-based supplementation, so male babies can get more iron supplementation than female. This gender-based iron supplementation in children requires research related to the dose and effectiveness. (iii) The iron status parameters of sTfR are more sensitive and stable, so it is better to use the sTfR even though we realized that there are technical problems and costs for some regions.

The status of iron newborns (using parameters: RBC count, Hb, Ht, MCV, RDW, SI, and SF) have not been affected by gender differences, but when using the sTfR parameter, it shows that male newborns have higher levels of sTfR than female. High sTfR levels reflect low iron status, thus male newborns potentially more likely to suffer from iron deficiency. Our research also found that male newborns have a higher umbilical cord hepcidin level than females (although not significantly different), so male newborns are potentially iron deficient. Overall, we conclude that fetal gender differences influence the iron status of newborns (using the sTfR parameter), and male newborns have potentially higher for iron deficiency. We also found that the sTfR parameter is more sensitive in measuring the iron status of the newborn.

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