Case-controlled study on indirect hyperbilirubinemia in exclusively breast fed neonates and mutations of the bilirubin Uridine Diphosphate-Glucuronyl transferase gene 1A1

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HIGHLIGHTS

- This study aims to investigate the association of neonatal indirect hyperbilirubinemia in breast-fed infants with UGT1A1 polymorphism.
- There was a statistically high significant decrease in weight at sample collection and high significant increase in indirect bilirubin level in patients group compared to control group.
- There was statistically significant difference as regard to genotype frequency [G/G, G/A, A/A], and allele frequency (A,G) between patients and control group. There was statistically significant increase in indirect bilirubin level in G/A – A/A genotypes. By comparing subgroup (A) and subgroup (B).
- There was statistically significant increase in total bilirubin level in subgroup (B). There was statistically high significant difference regarding genotype frequency (G/G, G/A, A/A) and allele frequency (G, A) between subgroup A and B.
- Multiple stepwise regression analysis was done using hyperbilirubinemia as a dependent factor and body weight loss, genotype (G/A) and allele (A) as independent factors. Body weight loss, genotype (G/A) and allele (A) was found to be significant independent predictors for hyperbilirubinemia.

ABSTRACT

Objective: This study aims to investigate the association of neonatal indirect hyperbilirubinemia in exclusively breast-fed infants with UGT1A1 (Uridine Diphosphate-Glucuronyl transferase 1A1) polymorphism.

Methods: 50 neonates were classified into 2 groups: 1) 30 full term neonates with indirect hyperbilirubinemia (gestational age (GA) 39.5 ± 1.2 weeks); 2) 20 apparently healthy full-term neonates. Group 1 was further subdivided based on percentage of body weight lost: (A) less than 10%; (B) 10% or more.

Results: There was a statistically significant decrease in weight at sample collection and significant increase in indirect bilirubin level in patients group compared to control group, there was statistically significant difference as regard to genotype frequency [G/G, G/A, A/A], and allele frequency (A,G) between patients and control group. There was statistically significant increase in indirect bilirubin level in G/A – A/A genotypes. By comparing subgroup (A) and subgroup (B), there was statistically significant increase in total bilirubin level in subgroup (B). There was statistically high significant difference regarding genotype frequency (G/G, G/A, A/A) and allele frequency (G, A) between subgroup A and B. Multiple stepwise regression analysis was done using hyperbilirubinemia as a dependent factor and body weight loss, genotype (G/A) and allele (A) as independent factors. Body weight loss, genotype (G/A) and allele (A) was found to be significant independent predictors for hyperbilirubinemia.

Conclusion: The results of the present study revealed that UGT1A1 polymorphism can be used as a novel predictor for neonatal hyperbilirubinemia in breast fed full term neonates.

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1. Introduction

Neonatal Jaundice is one of the most common neonatal problems. Although up to 60% of term newborns have clinical jaundice in the first week of life, few have significant underlying disease. However hyperbilirubinemia in the neonatal period can be associated with severe illness such as hemolytic disease, metabolic and endocrine disorders, anatomic abnormalities in the liver, and infections [1].

As red blood cells are lysed, they release hemoglobin. Heme molecules (from hemoglobin) are converted to bilirubin. Bili-

rubin (unconjugated or indirect) is bound to serum albumin and transferred to the liver where it is conjugated to glucuronate by glucuron transferase. Conjugated (direct) bilirubin is excreted into bile. A fraction of bilirubin from the stool is reabsorbed into the blood via the portal circulation (enterohepatic circulation) [1].

Neonatal hyperbilirubinemia is “the occurrence of elevated bilirubin levels in blood in neonates”. It may be physiological or pathological if the concentration of unconjugated bilirubin in the blood is too high, it reaches the blood brain barrier and encephalopathy occurs with serious consequences for the child [2]. Neonatal hyperbilirubinemia is a common transitional phenomenon during the first week of life, is caused by an imbalance between bilirubin production and its elimination [3].

Early neonatal jaundice (Onset less than 24 h): Haemolytic disease: eg, haemolytic disease of the newborn (rhesus), ABO in-

compatibility, glucose-6-phosphate dehydrogenase deficiency, spherocytosis. Infection: congenital (eg, toxoplasmosis, rubella, cytomegalovirus (CMV), herpes simplex, syphilis) or postnatal weight loss.

2. Subjects & methods

2.1. Subjects

This case control study was conducted on 30 hyperbilirubinemic full term neonates during the period from June 2015 till February 2016. The cases were chosen from patients attending the pediatric department and outpatient clinic of Benha University Hospital. This study was approved by Ethical committee of Benha University.

This group was classified into two subgroups as following:

• Sub group I (A):Neonates had <10% body weight loss during the neonatal period.

• Sub group I (B):Neonates had ≥10% body weight loss during the neonatal period.

2.1.1. Inclusion criteria

1. All subjects exclusive breast fed neonates with gestational age (GA)39.5 ± 1.5 weeks& birth weight 3080 ± 325 g.

2. The measured serum bilirubin level exceeds the following values:10 mg/dl at day1; 14 mg/dl at day 2; 16 mg/dl at day 3; 17 mg/dl at day 4; 18 mg/dl at day5 and 20 mg/dl at day 6 ac-

2.1.2. Exclusion criteria

Neonates with risk factors that affect the level of serum bilirubin were excluded, such as: Hemolytic anemia, infection, intestinal malformation, maternal diabetes, hypertension, neonatal asphyxia.

Group (II): This group included 20 apparently healthy full term neonates with matched age and sex as control group, they were subdivided according to weight into 2subgroups:

• Sub group II (A):12 neonates had normal body weight

• Sub group II (B): 8 neonates had ≥ 10% body weight loss during the neonatal period.

Informed consent was obtained from parents of each participant.

2.2. Methods

Individuals of the studied 2 groups were subjected to the following:

1 Full history taking: maternal history, mode of delivery and family history.

2 Thorough clinical examination: measurement, vital signs and systemic examination.
3 The laboratory investigations included:

2.2.1. Sampling

6 mL of venous blood were withdrawn under aseptic precautions after and distributed as follows:

a. Three ml of venous blood were withdrawn on EDTA, (1.2 mg/ml) for detection of hemoglobin and reticulocyte-count and the remaining stored at ~20 °C for subsequent DNA extraction.

b. Three ml blood were withdrawn for serum separation, after clotting, the sample was centrifuged and the resultant serum was used for measurement of bilirubin level and liver function tests.

2.2.2. Laboratory investigations

A) Routine Laboratory Investigations:

- CBC was done for all samples using a fully automated cell counter, Mythic 18 (Orphee) from Switzerland.

- Transcutaneous bilirubin twice daily (at 8 a.m. and 8 p.m.) by using MJ20 transcutaneous jaundice detector [M & B electronic instruments jaundice detector, version: U 3.0 China] during first week of life. And when the peak transcutaneous bilirubin exceed the level, we so serum bilirubin.

- Serum bilirubin was measured photometrically and the analysis was done using (Photometer BioSystems BTS-310, Spain).

B) Specific Laboratory Investigations:

- Determination of UGT1A1 genotype:

All subjects were genotyped for UGT1A1 polymorphism by real time PCR TaqMan drug metabolism assay by the following steps:

1. DNA was isolated by pure link genomic DNA mini kits (50 preps), (invitrogen by life technologies Cat no (K1820-01) (Lot No. 1607712). Principle:

The PureLinkR Genomic DNA Kits are based on the selective binding of DNA to silica-based membrane in the presence of chaotropic salts. The lysate is prepared from a variety of starting materials such as blood. The cells are digested with Proteinase K at 55 °C using an optimized digestion buffer formulation that aids in protein denaturation and enhances Proteinase K activity. Any residual RNA is removed by digestion with RNase A prior to binding samples to the silica membrane.

The lysate is mixed with (ethanol and PureLinkR Genomic Binding Buffer) that allows high DNA binding PureLinkR Spin Column (Mini Kit). The DNA binds to the silica-based membrane in the column and impurities are removed by thorough washing with Wash Buffers. The genomic DNA is then eluted in low salt Elution Buffer.

2. Amplification by Real-Time PCR: The isolated genomic DNA was amplified using sets of primers & taqman probes designed to detect the target polymorphism. TaqMan Universal Master mix including No UNG (Applied Biosystems, AB) (Lot No:1504030). https://tools.thermofisher.com/content/sfs/manuals/PureLink_Genomic_DNA_Kits.pdf

- VIC dye is linked to the 5' end of the allele 1 probe.
- FAM dye is linked to the 5' end of the allele 2 probe.

3. Statistical analysis

The collected data were tabulated and analyzed using SPSS version 16 soft ware (Spss Inc, Chicago, ILL Company). Categorical data were presented as number and percentages while quantitative data were expressed as mean ± standard deviation, and range. Chi square test (X2), or Fisher’s exact test (FET), odds ratios (ORs) and the corresponding 95% CI were calculated when applicable. Quantitative data were tested for normality using student t-test if normally distributed, or Man Whitney U test, and Spearman’s correlation coefficient (rho) if not normally distributed. Binary logistic regression analysis with the adjusted Odds Ratios was used to detect the significant predictors of hyperbilirubinemia. The accepted level of significance in this work was stated at 0.05 (P < 0.05 was considered significant).

Mean: Is the sum of the values in a set of data divided by the number of the values in the set. It is denoted by the sign X (called X bar).

Standard deviation (SD): is the positive square root of the variance.

Chi square test: compares between 2 or more categorical groups (tables 2 × 2 or more).

Fisher’s exact test is used when you have two nominal variables. Fisher’s exact test is more accurate than the chi-squared test when the expected numbers are small.

OR (Odds Ratio) is a risk index – odds in exposed/odds in non exposed, it quantifies the risk among study group compared with the control group. 95% CI of OR = is the interval within which the
investigator is 95% confident that the true population OR lies. "Z" test = is a test of significance that compares between 2 proportions.

Student "t" test compares between 2 means of 2 independent groups: t-value is the ratio of the difference between the two means/calculated SD of this difference.

rho → Spearman's correlation coefficient: evaluates the linear association between 2 quantitative variables (one is the independent var. X, and the other is the dependent var. Y).

Mann Whitney U test: non parametric test used to compare 2 non parametric quantitative variables.

4. Results

By comparison between patient and control groups, it was found that, there were no-significant difference between both groups regarding, gestational age, age, sex, mode of delivery and birth weight (P > 0.05). While, there was a statistically high significant decrease in total bilirubin level in patient group (17.9 ± 3.23 vs 14.1 ± 3.98) mg/dl (Table 2).

There was a statistically high significant increase in indirect serum bilirubin level in subgroup I (B) (17.9 ± 3.23 vs 14.1 ± 3.98) mg/dl (Table 2).

By comparing patient and control groups, it was found that, there was a statistically significant difference as regard to genotype frequency [G/G, G/A, A/A] (P < 0.05), and allele frequency (A/G) P = 0.011 (Table 3) and (Fig. 1).

By comparing genotype groups (G/G, G/A, A/A), it was found that there was no-significant difference as regard to gestational age, age, sex, birth weight and mode of delivery (P > 0.05), there was a statistically significant increase in total bilirubin level in G/A - A/A genotypes (P < 0.05) (Table 4). There was a statistically significant decrease in weight at sample collection in G/A, A/A genotypes (P < 0.05). Also there was a statistically high significant increase in weight loss percent in G/A (P ≤ 0.001) (Table 4).

There was a statistically high significant difference regarding genotype frequency (G/G, G/A, A/A); (P < 0.001) and allele frequency (G, A); (P < 0.005) (Table 5). By comparing control subgroups, there was no-significant difference regarding genotype frequency (Table 6). A multiple stepwise regression analysis was done using hyperbilirubinemia as a dependent factor and body weight loss, genotype (G/A) and allele as independent factors. Body weight loss, genotype (G/A) and allele (A) was found to be a significant independent predictors for hyperbilirubinemia. (Table 7).

5. Discussion

Due to the disparity of results among different neonates we aimed at studying such association in Egyptian neonates having indirect hyperbilirubinemia. The study was designed to clarify the association of UGT1A1 polymorphism and neonatal hyperbilirubinemia in breast fed Egyptian neonates. All neonates were subjected to full history taking and clinical examination. Total & indirect serum bilirubin was assessed in all neonates, and then all samples were genotyped for the UGT1A1 polymorphism using taqMan genotyping technique.

In the present study, the groups were well matched for various demographic features. there, these results were in agreement with Hiroko et al. [9], who reported that there was no statistically

Table 1

| Parameter                  | Polymerase activation | PCR (40 cycles) |
|----------------------------|-----------------------|-----------------|
| Temperature (°C)           | 95 °C                 | 60 °C           |
| Time (min:sec)             | 02:00                 | 01:00           |

Table 2

| Variable                  | Patients (N = 30) | Controls (N = 20) | t    | P    | Subgroup I A (N = 22) | Subgroup I B (N = 8) | t    | P    |
|---------------------------|-------------------|-------------------|------|------|-----------------------|----------------------|------|------|
| Gestational age (wk)      | 39.1 ± 0.64       | 39.2 ± 0.82       | 0.11 | 0.91 | NS                    | NS                   |      |      |
| Mean ± SD                 | 31.1 ± 1.4        | 3.0 ± 1.5         | 0.52 | 0.60 | NS                    | NS                   |      |      |
| Age (days)                | 3.1 ± 1.4         | 3.0 ± 1.5         | 0.52 | 0.60 | NS                    | NS                   |      |      |
| Mean ± SD                 | 3.2 ± 1.5         | 3.1 ± 1.3         | 0.05*| 0.96 | NS                    | NS                   |      |      |
| No.(%)                    | Male              | Female            |      |      |                      |                      |      |      |
| Sex                       | 20 (66.7%)        | 11 (55.0%)        | 0.69 | 0.41 | NS                    | NS                   | 15 (68.2%) | 5 (62.5%) | 3 (37.5%) | 1.0 | NS |
| Mode of delivery          |                   |                   |      |      |                      |                      |      |      |
|                         | NVD               | CS                |      |      |                      |                      |      |      |
| Birth weight (gm)         | 3251.6 ± 184.9    | 3179.5 ± 290.5    | 1.08 | 0.28 | NS                    | NS                   | 3267.7 ± 202.5 | 3207.5 ± 125.2 | 0.71 | 0.48 | NS |
| Mean ± SD                 | 3197.5 ± 284.4    | 3.48              | 0.001 |      | NS                    | NS                   | 3021.4 ± 173.7 | 2831.7 ± 88.97 | 3.03 | 0.002 | S  |
| Weight loss (%)           | 7.46              | 11.60             | 4.13 | <0.001 | NS                    | NS                   | 7.46 ± 1.18    | 11.60 ± 0.81 | 4.13 | <0.001 | HS |
| T. bilirubin (mg/dl) Mean | 17.6 ± 4.33       | 21.1 ± 1.21       |      |      | NS                    | NS                   | 17.6 ± 4.33   | 21.1 ± 1.21  |      |      |
| Indirect. bilirubin (mg/dl) Mean | 14.1 ± 3.98 | 17.9 ± 3.32 | 2.14 | 0.032 | NS | NS | 14.1 ± 3.98 | 17.9 ± 3.32 | 2.14 | 0.032 | NS |

NVD: Normal vaginal delivery, CS: Caseran section.
NS: Non-significant, X² = Chi-square, SD = standard deviation, t = student-t-test, P > 0.05 – non-significant, P < 0.001 – very highly significant.
significant difference between the studied groups regarding body weight and age at time of sample collection. Also these results were in accordance with a study done by Hung et al. [10].

While Hui et al. [11], reported that the age at time of sample collection and body weight at birth showed a statistically significant difference between the studied groups this could be due to difference in number of studied populations.

In the present study there were no statistically significant difference between the studied groups regarding gender (sex) and gestational age, while Hiroko et al. [9], reported that neonates with shorter gestational age were risk predictors of development of neonatal hyperbilirubinemia. These results were in agreement with Hung et al. [10] and Melih et al. [12] reported that there was no significant difference between the studied groups regarding gender and gestational age.

The present study showed that maximal body weight loss was significantly higher in subgroup (B) cases especially in cases with the G/A and A/A genotype compared to those carrying the G/G genotype, so it is considered a significant risk factor and a significant independent predictor for the development of hyperbilirubinemia.

**Table 3**

| Genotype | Groups | Count | % within groups | Total | FET & P |
|----------|--------|-------|-----------------|-------|---------|
|          | Patients |       |                 | Controls |        |
| G/G      | 19      | 19    | 63.3%           | 38     | 6.65 & 0.025 (S) |
| G/A      | 10      | 1     | 33.3%           | 11     | 0.042 (S) |
| A/A      | 1       | 0     | 3.3%            | 1      | 0.012 (S) |
| Allele   |         |       |                 |        |         |
| G        | 48      | 39    | 80.0%           | 87     | OR      |
| A        | 12      | 1     | 20.0%           | 13     | 9.7     |

FET: fisher exact test, OR: odds ratio, CI: confidence interval.

**Table 4**

| Group | n. | Mean ± SD | Range | t  | P    |
|-------|----|-----------|-------|----|------|
| G/G   | 19 | 3272.1 ± 206.30 | 2980–3950 | 0.79 | 0.43 (NS) |
| G/A-A/A | 11 | 3216.3 ± 142.98 | 3050–3400 | 2.52 | 0.018 (S) |
| G/G   | 19 | 3028.0 ± 180.88 | 2730–3600 | 2.52 | 0.018 (S) |
| G/A-A/A | 11 | 2873.6 ± 118.59 | 2740–3100 | 2.52 | 0.018 (S) |
| G/G   | 19 | 7.4 ± 1.35 | 5.4–10.5 | 5.59 <0.001 (HS) |
| G/A-A/A | 11 | 10.6 ± 1.74 | 7.7–12.6 | 5.59 <0.001 (HS) |

**Table 5**

| Subgroups | Group A | Group B | P value |
|-----------|---------|---------|---------|
| Genotype  | G/G Count | 18 | 1 | P < 0.001 (HS) |
| % within subgroups | 81.8% | (12.5%) |
| G/A Count | 3 | 7 |
| % within subgroups | 13.6% | (87.5%) |
| A/A Count | 14.5% | 0 (0.0%) |
| % within subgroups | 4.5% | 0 (0.0%) |

**Table 6**

| Group | n. | Mean ± SD | Range | t  | P    |
|-------|----|-----------|-------|----|------|
| G/G   | 19 | 17.2 ± 4.5 | 9.1–19.8 | 2.27 | 0.03 (S) |
| G/A-A/A | 11 | 20.7 ± 1.136 | 19–22 | 2.27 | 0.03 (S) |

Man Whitney U (MWU) test was used.

**Fig. 1.** Example of the allelic discrimination of the UGT1A1*28 variant by TaqMan PCR.
These results were in agreement with Chang et al. [13] and Hiroko et al. [9], who reported that maximal body weight loss was an independent risk factor for the development of neonatal indirect hyperbilirubinemia. It has been suggested that body weight loss due to inadequate feeding of breast fed neonates which may increase intestinal absorption impair hepatic conjugation and/or excretion of bilirubin because of energy deficiency and causes hyperbilirubinemia.

The present study showed that neonates in group (B) had a significantly higher peak transcutaneous bilirubin levels especially those with G/A, A/A genotypes. These results were in accordance with a study done by Youyou et al. [14], who reported that there was association between 211 G/A genotype, which is a risk factor for neonatal hyperbilirubinemia and higher serum indirect bilirubin level.

Also these results were in agreement with Hiroko et al. [9], who reported that peak bilirubin levels were significantly higher in cases who carrying G/A genotype compared to others carrying G/G or A/A genotype.

The present study showed that there was statistically significant differences between the studied groups regarding genotype frequency (G/G, G/A, A/A) these results were in agreement with Hiroko et al. [9] and Zibi et al. [15] who reported that there were statistically significant difference in the genotype frequencies of UGT1A1 between patient groups.

The present study showed that there was statistically significant difference between the studied groups regarding allele frequency (G/A). These were in agreement with Zibi et al. [15] who reported that there were significant differences between G allele and A allele.

The present study showed that incidence of hyperbilirubinemia was statistically significant in subgroup (B) is especially with those who carrying GA, A/A genotypes than carrying G/G genotypes these results were in agreement with Hiroko et al. [9] who reported that the incidence of hyperbilirubinemia was significantly higher in group (B) neonates with heterozygous UGT1A1-211G > A genotype, this discrepancy may be due to different technique used.

In the present study, multiple stepwise regression analysis was done by using hyperbilirubinemia as dependent factors and body weight loss, genotype (G/A) and allele (A) as independent factor and body weight loss, genotype (G/A), allele (A) was found to be significant independent predictors for hyperbilirubinemia.

These results were in agreement with Hiroko et al. [9] who reported that maximal body weight loss is the only independent risk factor for the development of hyperbilirubinemia and UGT1A1 polymorphism become risk factor in neonates showing 10% or more body weight loss during the neonatal period (only in infants with inadequate feeding).

Also these results were in accordance with Zibi et al. [15] who reported that the A allele was found to be associated with a risk of hyperbilirubinemia. Based on these results, it could be reasonable to speculate that neonates with body weight loss 10% or more, A-allele compared to neonate with body weight loss less than 10%, G-allele, may have an increased risk for developing hyperbilirubinemia.

Inadequate feeding may increase the bilirubin burden and cause apparent hyperbilirubinemia in neonates, who have a polymorphic change in the genes involved in the transport of bilirubin.

The results presented here need to be confirmed by using larger groups and other related genes, also only long follow up study would be necessary to confirm the association between this polymorphism and hyperbilirubinemia.

### 6. Conclusion

This study suggests that UGT 1A1 polymorphism can be used as a novel method to detect susceptibility to indirect hyperbilirubinemia in Egyptians exclusively breast fed neonates with weight loss >10% or more and can help for early manage newborns with hyperbilirubinemia.

### Conflicts of interest

No conflict of interest.

### Sources of funding

No fund.

### Ethical approval

Ethical approval was taken by Benha university.

### Author contribution

**Amal E. Mohammed**: study design.

**Eman G. Behiry**: data collections, data analysis, writing.

**Akram E. El-Sadek**: data collections, data analysis, writing.

**Waleed E. Abdulghany**: data collections, data analysis.

**Dalia M. Mahmoud**: data collections, data analysis.

**Abdelfattah A. Elkholy**: data collections.

### Guarantor

Eman G. Behiry.

### References

[1] B.J. Stoll, R.M. Kliegman, Jaundice and hyperbilirubinemia, in: R.E. Behrman, R.M. Kliegman, H.B. Jason (Eds.), Nelson Textbook of Pediatrics, seventeenth ed., Saunders, Philadephia, 2004, pp. 592–606.

[2] I. Mesic, V. Milas, M. Medimurec, Z. Rimar, Unconjugated pathological jaundice in new borns, Coll. Antropol. 38 (1) (2014) 173–178.

[3] D.K. Stevenson, H.J. Vreman, R.J. Wong, Bilirubin production and the risk of bilirubin neurotoxicity, Semin. Perinatol. 35 (3) (2011) 121–126.

[4] K.K.I. Hahn, J.J. Wolff, J.M. Koilesar, Pharmacogenetics and irinotecan therapy, Am. J. Health Syst. Pharm. 63 (22) (2006) 2211–2217.

[5] P.J. Bosma, Inherited disorders of bilirubin metabolism, J. Hepatology. 38 (2003) 107–117.

[6] A. Kadakol, S.S. Ghosh, B.S. Sappal, G. Sharma, J.R. Chowdhury, N.R. Chowdhury, Genetic lesions of bilirubin uridine-diphosphoglucuronate...
glucuronosyltransferase (UGT1A1) causing Crigler-Najjar and Gilbert syndromes: correlation of genotype to phenotype, Hum. Mutat. 16 (2000) 297–306.

[7] J. Prithviraj, H. Vincent, N. Jothikumar, Design of FET TagMan probes for multiplex real-time PCR using an internal positive control, BioTechniques 46 (2009) 519–524.

[8] V.K. Bhutani, L.A. Johnson, A proposal to prevent severe neonatal hyperbilirubinemia and kernicterus, J. Perinatol. 29 (2009) 561–567.

[9] Hiroko Sato, Toshihiko Uchida, Kentaro Toyota, Tomohiro Naka Mura, Gen Tamiya, Miyako Kanno, et al., Association of neonatal hyperbilirubinemia in breast-fed infants with UGT1A1 or SLCO1B1 polymorphisms, J. Hum. Genet. 60 (2015) 35–40.

[10] C.C. Hung, H.C. Mei, I.Y. Hwai, N.S. Yi, et al., 211 G to A Variation of UDP-Glucuronosyl transferase 1A1 gene and neonatal breastfeeding jaundice, Pediatr. Res. 69 (2011) 170–174.

[11] Y. Hui, W. Qian, Z. Lei, B.Z. Xiang, et al., Clinical significance of UGT1A1 genetic analysis in Chinese neonates with severe hyperbilirubinemia, Pediatr. Neonatol. (2015) 1–8.

[12] O. Melih, Sule Y. Babaoglu, A. Sukru, et al., Neonatal jaundice and bilirubin UDP-glucuronosyl transferase 1A1 gene polymorphism in Turkish patients, Basic Clin. Pharmacol. Toxicol. 98 (2006) 377–380.

[13] P.F. Chang, Y.C. Lin, K. Liu, S.J. Yeh, Y.H. Ni, Risk of Hyperbilirubinemia in breast fed infants, J. Pediatr. 159 (2011) 561–565.

[14] Z. Youyou, W. San-nan, L.i. Hong, Z. Weifeng, Zha Weifeng, Wang Xuli, Liu Yuanyuan, et al., Association of UGT1A1 variants and hyperbilirubinemia in breast-fed full-term Chinese infants, PLoS One 9 (2014) 8.

[15] Y. Zibi, Z. Kaichang, W. Li, L. Ying, Sun Jianmei, Association of neonatal hyperbilirubinemia with UGT1A1 gene polymorphisms: a meta-analysis, Med. Sci. Monit. 21 (2015) 3104–3114.