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Uridine Diphosphate Glucuronosy ltransferase 1A1 Promoter Gene Polymorphism (211G>A) inneonatal Hyperbilirubinemiaat Zagazig University hospital

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Abstract

Background:*Neonatal jaundice is usually benign, but in some cases it can progress to severe hyperbilirubinemia, acute bilirubin encephalopathy (ABE) and kernicterus/chronic bilirubin encephalopathy (CBE). In Egypt, severe neonatal hyperbilirubinemia accounted for 33% of total admissions to (NICU). UGT1A1 is the key enzyme for bilirubin conjugation, and mutations of UGT1A1 cause unconjugated hyperbilirubinemia syndromes known as Crigler-Najjar syndrome and Gilbert's syndrome. The aim was to study the relation of UGT1A1, 211 G to A promoter gene polymorphism with the risk of hyperbilirubinemia in neonates.****Methods****: The study included 50 neonates; case group consisting of 30 neonates with the peak of total serum bilirubin ≥ 16 mg /dl and control group consisting of 20 neonates with the peak total serum bilirubin) levels <12 mg/dl. UGT1A1 genes was determined.* ***Results****: This study results that UGT1A1 promoter gene polymorphism 211G>A genotype can be used as a novel method to detect susceptibility to indirecthyperbilirubinemia in neonates.* ***Conclusion:*** *Detection of 211G>A variant of UGT1A1 promoter polymorphism gene was comparable between neonatal hyperbilirubinemia and control group. Heterozygous (G/A) and homozygous (G/G, A/A) genotype variants of the promoter region in UGT1A1 polymorphism in healthy neonates and idiopathic hyperbilirubinemia should be considered.*

Key Words: *Neonatal hyperbilirubinemia, UGT1A1 gene. 211G>A promoter polymorphism.*

I. Introduction:

Over 60% of all newborns develop neonatal jaundice (NNJ), a physiologic condition characterized by yellowish discoloration of the skin and conjunctiva as a consequence of increased levels of serum bilirubin during the first week of life **(1)**.

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Neonatal jaundice is usually benign, but in some cases it can progress to severe hyperbilirubinemia, acute bilirubin encephalopathy (ABE) and kernicterus/chronic bilirubin encephalopathy (CBE) [(2)]. ABE and CBE are largely preventable if severe hyperbilirubinemia is identified early and treated promptly with effective phototherapy or, for hazardous cases, exchange transfusion. Guidelines for managing jaundice have been proposed by the

American Association of Pediatrics (AAP), the UK National Institute for Health and Care Excellence (NICE) and others [(3)].

Blirubin is eliminated from the body by conjugation with glucuronic acid in the liver by the enzyme uridine 5′-diphosphate glucuronosyltransferase 1A1 (*UGT1A1*) [(4)].

Egypt, severe neonatal hyperbilirubinemia accounted for 33% of total admissions to the outborn neonatal ICU (NICU) with about 10 cases of kernicterus occurring each year [(5)].

Genetic association studies have linked a number of single nucleotide polymorphisms (SNPs) with unconjugated hyperbilirubinemia[(6)].

UGT1A1 is the key enzyme for bilirubin conjugation, and mutations of *UGT1A1* cause unconjugated hyperbilirubinemia syndromes known as Crigler-Najjar syndrome and Gilbert's syndrome [(7)].

Genetic variants involving red blood cell (RBC) enzyme glucose-6-phosphate dehydrogenase (EC 1.1.1.49; G6PD) and bilirubin conjugating enzyme uridinediphosphoglucuronate-glucuronosyltransferase 1A1 (EC 2.4.1.17; *UGT1A1*) have been commonly associated with neonatal hyperbilirubinemia[(8)].

The goal of the present study was to study the relation of *UGT1A1*, 211 G to A promoter gene polymorphism with the risk of hyperbilirubinemia in neonates.

II. Patients and Methods

This study was carried out in Neonatal Intensive Care Unit, Pediatrics Department, and Biochemistry Department at Zagazig University Hospitals over one-year duration from April 2017 to April 2018.

The study was conducted to include 50 newborns with hyperbilirubinemia with gestation age (≥ 37weeks) and postnatal age (≤ 2 weeks) with normal birth weight with 34 male and 16 female divided into two groups; case group consisting of 30 neonates with the peak of total serum bilirubin (TSB) levels ≥ 16 mg /dl and control group consisting of 20 neonates with the peak total serum bilirubin (TSB) levels <12 mg/dl.

Inclusion Criteria:

Cases:

Newborns with hyperbilirubinemia enrolled in the study are:

- Neonates (≥ 37 weeks) of gestation age,
- Neonates (≤ 2 weeks) of postnatal age,
- The peak of total serum bilirubin (TSB) levels ≥ 16 mg /dl

Control group:

- Control newborns enrolled in the study are:
- Neonates (≥ 37 weeks) of gestation age,
- Neonates (≤ 2 weeks) of postnatal age,
- There was mild clinical jaundice or the peak of total serum bilirubin (TSB) <12 mg/dl.

Exclusion Criteria:

Neonates with risk factors that affect the level of serum bilirubin were excluded, such as:

1) Hemolytic anemia
2) Infection (CRP +ve)
3) Major congenital malformations such as intestinal malformation
4) Maternal diabetes, hypertension, neonatal asphyxia
5) Liver disease ,Conjugated bilirubin > 20% of the STB
6) Hypothyroidism
7) Polycythemia
8) Cephalohematoma

Methods:

All neonates enrolled in the study were subjected to the following:

Full history.Laboratory investigations:(Serum total and direct bilirubin level. Blood group of baby and mother. Complete blood count (CBC).Direct Comb's test.

Genetic analysis

Detection of number of 211G>A repeats of promoter area of the (UGT1A1) UridineDiphosphateglucuronosyltransferase 1A1 gene by PCR-based restriction fragment length polymorphism (RFLP) according to **Kumar et al.**[9].

STATISTICAL ANALYSIS

The collected data were computerized and statistically analyzed using SPSS program (Statistical Package for Social Science) version **25.0**. Qualitative data were represented as frequencies and relative percentages.Chi square (χ^2) test was used. **Odds Ratio.** Mann Whitney test was used to calculate difference between quantitative variables in not normally distributed data in two groups.ANOVA *F*-test test was used to calculate difference between quantitative variables in more than two groups in normally distributed data.Kruskal Wallis test was used to calculate difference between quantitative variables in more than two groups in not normally distributed data.The significance Levelfor all above mentioned statistical tests done. The threshold of significance is fixed at 5% level (P-value)

III. Results:

Figures (1, 2, 3, 4, 5, 6) show thatby comparison between neonatal hyperbilirubinemia group and control group, it was found that, there were no significant differences between both groups regarding age, sex, gestational age (GA), birth weight, and mode of delivery ($P > 0.05$); while there was a statistically high significant decrease in weight at sample collection in patient group ($p < 0.001$)

This table shows a highly statistically significant increase in reticulocytic count, TSB and direct bilirubin in the hyperbilirubinemia group than control group ($p < 0.01$); but no statistically significant difference observed between hyperbilirubinemia group and control group as regards Hb, WBCs, and platelets count ($p > 0.05$)**(Table 1).**

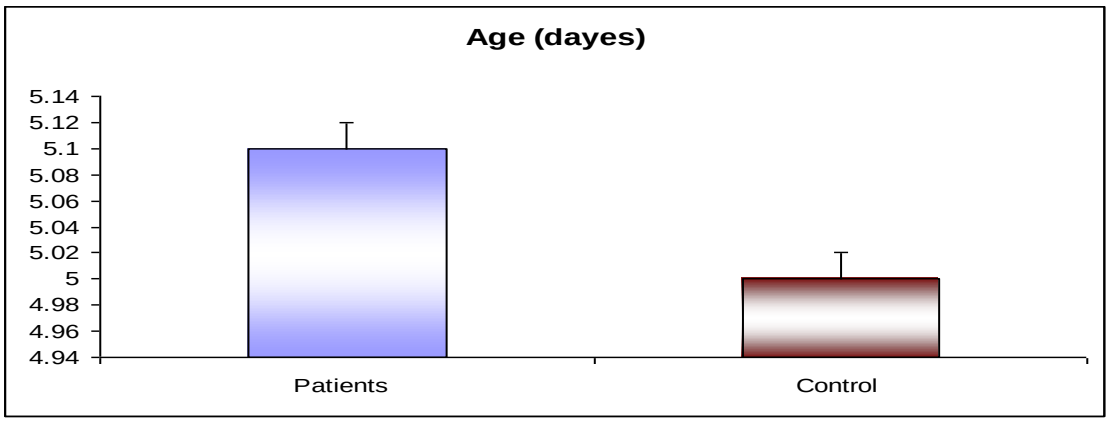

Figure (1): Bar chart for comparison of age between patients and controls

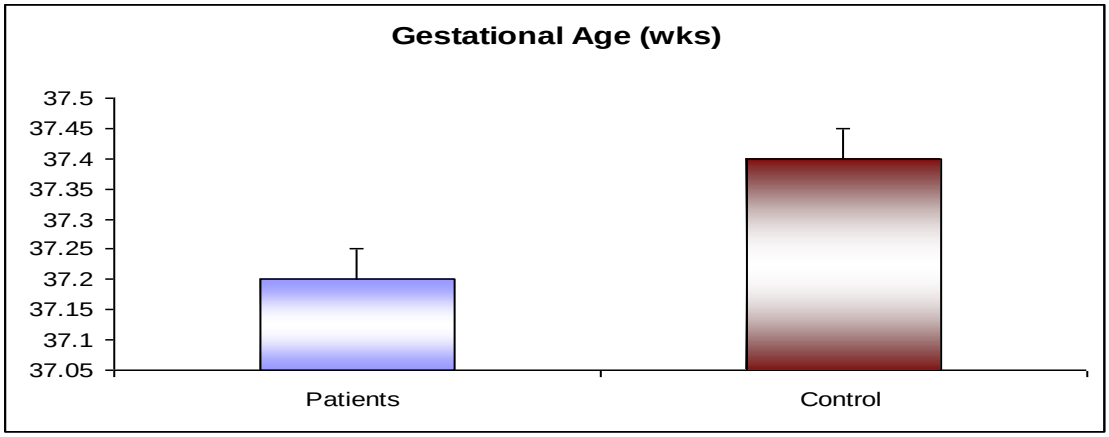

Figure (2): Bar chart for comparison of gestational age between patients and controls

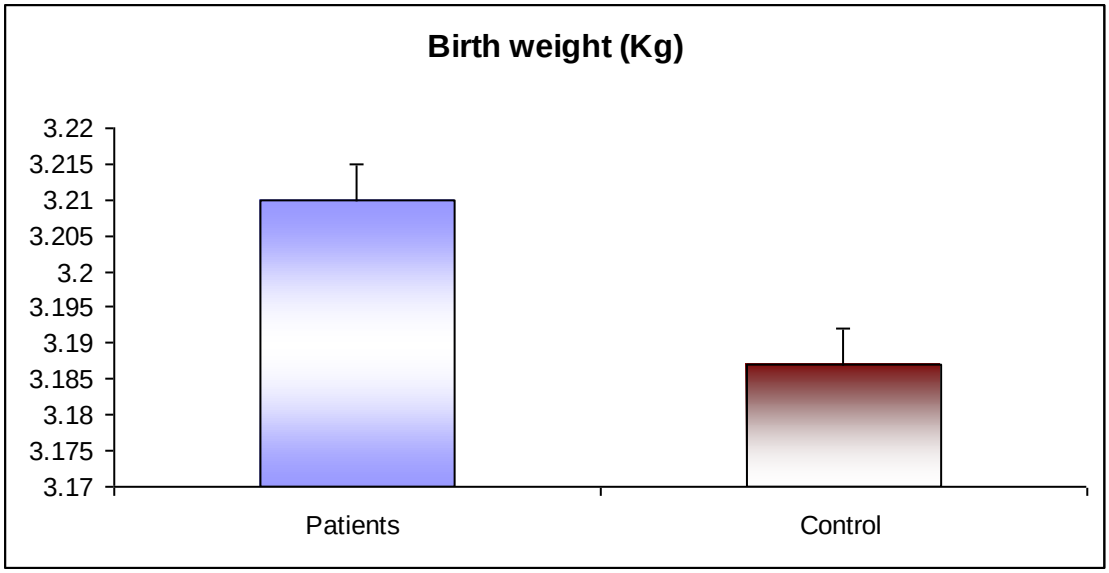

Figure (3): Bar chart for comparison of weight between patients and controls

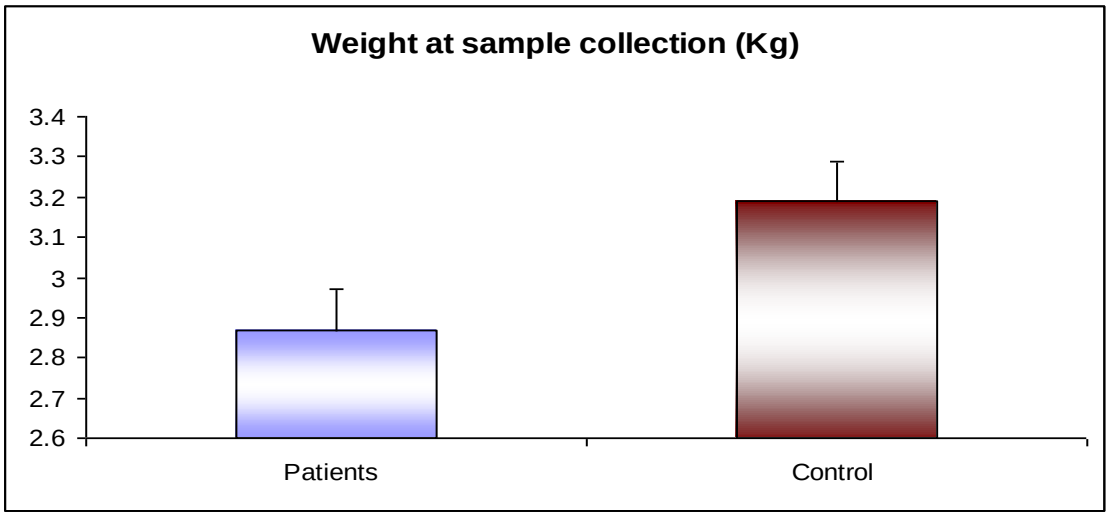

Figure (4): Bar chart for comparison of weight at sample collection between patients and controls

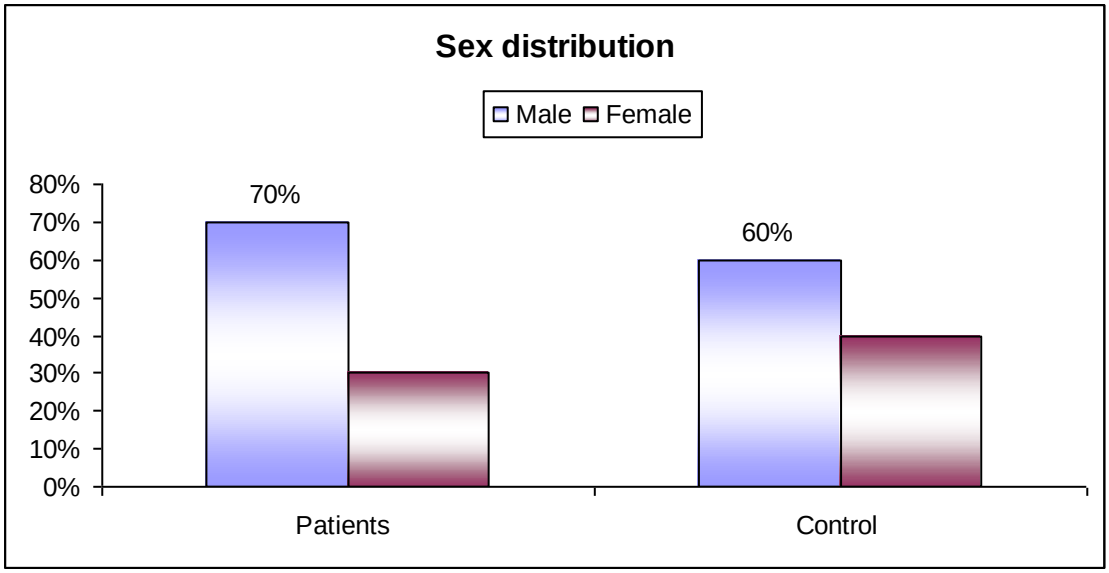

Figure (5): Bar chart for comparison of Sex distribution between patients and controls

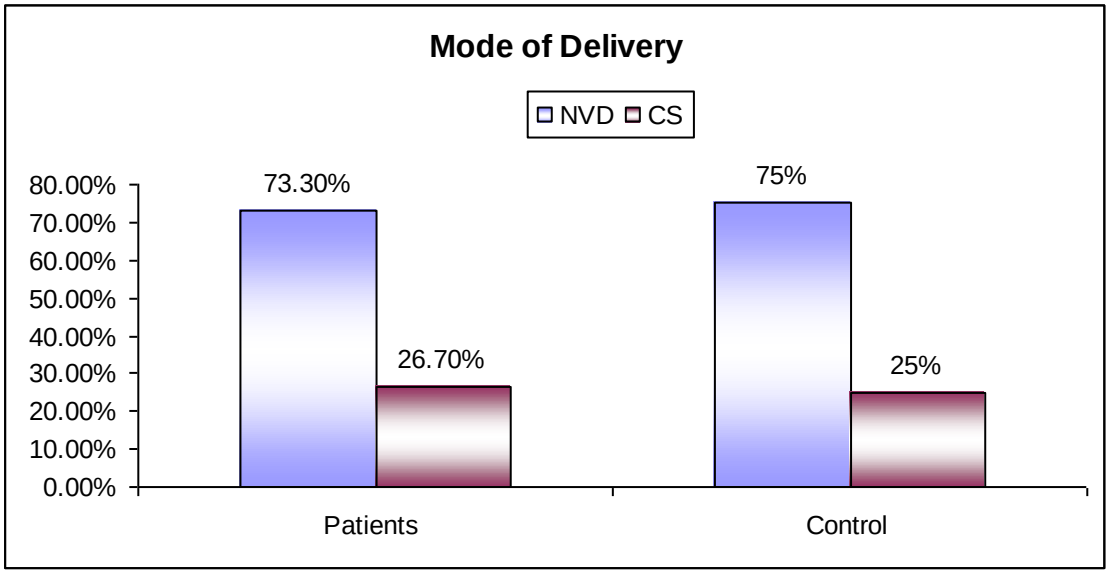

Figure (6): Bar chart for comparison of mode of delivery between patients and controls

Table (1): Comparison of laboratory data among studied groups

Variable	Patients (N=30)	Control (N=20)	*Test*	*P* value
Hb(gm/dl) Mean ±SD	16.54± 1.5	15.6± 2.01	t=1.80	0.08 (NS)
WBCs ($\times 10^3$/L)			t=0.05	0.96

Mean ±SD	10.99±3.18	11.03±2.4	(NS)
Platelets Mean ±SD	311.3± 79.06	327.8±75.1	t=0.72 0.47 (NS)
Retieculocytic count (%) Mean ±SD	2.0± 0.08	1.53±0.12	t=2.74 0.009 (HS)
TSB (mg/dL) Mean ±SD	19.69±1.96	10.44±1.0	t=19.31 <0.001 (HS)
Direct bilirubin (mg/dL) Mean ±SD	1.09±0.61	0.565±0.28	MW=3.39 0.001 (HS)
ABO setting* (%)	0.0%	0.0%	0 1.0

Hb: Hemoglobin WBC: White blood cell TSB: Total serum bilirubin

*Mother blood group and baby blood group A or B NS: Non significant HS: Highly significant**.**

Table (2): Genotype frequency of 211 G>A variant of UGT1A1 promoter in patients versus control groups

			Groups		**Total**	**Adjusted OR (95% CI)**	***P* value**
			Patients (n=30)	**Controls (n=20)**			
Genotype 211 G>A	**G/G**	Count % within groups	10 (33.3%)	14 (70%)	24 (48%)	R 3.92 (1.06-14.44) 8.4 (1.02-18.10)	---- 0.04 (S) 0.04 (s)
	G/A	Count % within groups	14 (46.7%)	5 (25%)	19 (38%)		
	A/A	Count % within groups	6 (20%)	1 (5%)	7 (14%)		

OR: Odds Ratio **CI**: Confidence interval

Table (3): Comparison of studied groups regarding allele frequency

			Groups		Total	Adjusted OR (95% CI)	P value
			Patients	Controls			
Allele	**G**	Count % within groups	34 (56.7%)	33 (82.5%)	67 (67%)	3.61 (1.38-9.44)	0.007 (HS)
	A	Count % within groups	26 (43.3%)	7 (17.5%)	33 (33%)		

OR: Odds Ratio **CI**: Confidence interval

Table (4): Comparison of the weight of the studied patients according to genotype

Group	No.	Mean ±SD	Range	*t*	*P* value
Birth weight (kg)					
G/G	10	3.27±0.20	2.900-3.600	0.83	0.32 (NS)
G/A-A/A	20	3.21±0.14	3.050-3.300		
Weight at sample collection (kg)					
G/G	10	3.280±0.180	2.800-3.600	2.57	0.019 (S)
G/A-A/A	20	2.872±0.118	2.700-3.200		
Weight loss (%)					
G/G	10	7.8±1.28	5.5-10.3	5.43	<0.001 (HS)
G/A-A/A	20	11.2±1.63	7.5-12.5		

Table (5): Comparison of the laboratory data among studied patients according to genotype

Group	No.	Mean ±SD/ Range	*Test*	*P* value
Hb (gm/dl)				
G/G	10	13.5±1.9	F=1.50	0.11 (NS)

		1-14		
G/A	14	12.6±1.4 1-13		
A/A	6	13.3±1.7 1-15		
WBCs (×10^3/L)				
G/G	10	9.63±3.2 3-11	K=2.28	0.32 (NS)
G/A	14	10.08±3.7 3-9.5		
A/A	6	10.82±4.06 3-10		
Platelets count (%)				
G/G	10	298±73 70-400	F=11.167	0.20 (NS)
G/A	14	300±76 70-320		
A/A	6	300±80 70-320		
Reticulocytic count (%)				
G/G	10	1.7±0.04 0.02-2.5	F=0.782	0.028 (S)
G/A	14	2.3±0.07 0.05-2.5		
A/A	6	3.7±0.08 0.08-4		

TSB (mg/dL)				
G/G	10	19.82±1.57 16.3-20.9	F=5.77	0.01 (S)
G/A	14	21.2±3.4 18-22.4		
A/A	6	24.6±3.6 20-28		
Direct bilirubin (mg/dL)				
G/G	10	0.8±0.1 0.2-1	KW=3.087	0.005 (S)
G/A	14	1.0±0.11 0.2-2		
A/A	6	2.4±1.35 0.3-3.2		

This table showed that, the homozygous G/G was found in 10 (33.3%) patients, versus 14 (70%) from control group; but the heterozygous G/A was found in 14 (46.7%) patients, versus 5 (25%) from control group. As regard, the homozygous A/A was found in 6 (20%) patients versus 1 (5%) of control group. By comparing hyperbilirubinemia group and control group, it was found that, there was a statistically significant difference as regard to genotype frequency of 211 G>A variants [G/G, G/A, and A/A] in UGT1A1 promoter region. As expected, more neonates in the hyperbilirubinemia group were observed to have the 211 G/A (p = 0.04 for G/A & 0.04 for A/A)**(Table 2).**

By comparing hyperbilirubinemia group and control group, it was found that, there was a statistically significant difference as regard to allele frequency (G, A) in UGT1A1 promoter region (P = 0.007) (**Table 3**).

This table showed a comparison of weight among hyperbilirubinemia group according to genotype groups (G/G, G/A, A/A), it was found that there was no significant difference as regard to birth weight (P = 0.32), there was a statistically significant decrease in weight at sample collection in G/A, A/A genotypes in 211 G>A variants (P = 0.019). As regard, there was a statistically high significant increase in weight loss percent in G/A, A/A genotype (P < 0.001)**(Table 4).**

This table showed a comparison of clinical laboratory data among hyperbilirubinemia group according to genotype groups (G/G, G/A, A/A), it was found a statistically significant increase in reticulocytic count, TSB, and direct bilirubin at birth age collection with G/A, A/A genotype in 211 G>A variants ($P < 0.05$); but there was no significant difference as regard to Hb, WBCs, and platelets count ($P > 0.05$)**(Table 5).**

IV. Discussion

The study population of the current study consisted of 30 neonates had hyperbilirubinemia during the period study with the peak total serum bilirubin (TSB) level ≥ 16 mg/dL. Total 21 (70%) of neonates were male and 9 (30%) were female; with mean age was 5.1±0.9 days. The mean neonatal birth weight was 3.21± 0.187 Kg; and the mean neonatal born at term were (37.2±0.65 weeks of gestation); and 22 (72.3%) of neonates were normal vaginal delivery (NVD) at mode of delivery. The mean weight at sample collection was 2.87± 0.177 Kg.

There were also 20 apparently healthy full term neonates with matched age and sex as control group with TSB level < 12 mg/dL were enrolled in the study. They were 12 (60%) male and 8 (40%) were female; with mean age was 5.0±1.3 days. The mean neonatal birth weight was 3.187± 0.29 Kg; and the mean neonatal born at term were (37.4±0.84 weeks of gestation); and 15 (75%) of neonates were NVD at mode of delivery. The mean weight at sample collection was 3.19± 0.286 Kg.

In the present study, the studied groups were well matched for various demographic data, it was found that, there were no significant differences between both groups regarding age, sex, gestational age (GA), birth weight, and mode of delivery ($P > 0.05$). While, there was a statistically high significant decrease in weight at sample collection in hyperbilirubinemia group ($p < 0.001$).

These results were in agreement with **Hiroko et al.** [(10)], who reported that there was no statistically significant difference between the studied groups regarding age, birth weight and age at time of sample collection.

Also these results were in accordance with a study done by **Hung et al.** [(11)].

While **Hui et al.** [(12)] 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.

The present study showed no statistically significant difference between the studied groups regarding gender (sex) and gestational age, while **Hiroko et al.** [(10)], reported that neonates with shorter gestational age were risk predictors of development of neonatal hyperbilirubinemia.

These results were in agreement with **Melih et al.** [(13)] and **Hung et al.** [(11)]reported that there was no significant difference between the studied groups regarding gender and gestational age.

The present study shows a statistically significantincrease in reticulocytic count, TSB and direct bilirubin in the hyperbilirubinemia group than control group ($p < 0.05$); but no statistically significant difference observed between hyperbilirubinemia group and control group as regards Hb, WHCs, and platelets count ($p > 0.05$).

These results were in agreement with **Chang et al.** [14]; **Hiroko et al.** [10] and **Mohammed et al.** [15].

In the present study, by comparing hyperbilirubinemia group and control groups, it was found that, there was a statistically significant difference as regard to genotype frequency of 211 G>A variant [G/G, G/A, and A/A] in *UGT1A1* promoter region. As expected, more neonates in the hyperbilirubinemia group were observed to have the 211 G/A (p = 0.002).

These results were in agreement with **Tiwari et al.** [16]; **Zhou et al.** [17]and **Mohammed et al.** [15] who reported there was statistically significant differences between the studied groups regarding genotype frequency (G/G, G/A, A/A).

Also, these results were in agreement with **Hiroko et al.** [10] and **Zibi et al.** [18] who reported that there were statistically significant differences in the genotype frequencies of *UGT1A1* between patient groups.

In the present study, by comparing hyperbilirubinemia group and control groups, it was found that, there was a statistically significant difference as regard to allele frequency (G, A) in *UGT1A1* promoter region (P = 0.014).

These results were in agreement with **Mohammed et al.** [15] who reported that, there was statistically significant difference between the studied groups regarding allele frequency (G/A).

Also, these were in agreement with **Zibi et al.** [18]who reported that there were significant differences between G allele and A allele.

However, 211G>A variant was associated with reduced isozyme activity, ranging from 60% in heterozygous state to 14 to 32% of normal levels in homozygous state [19; 20].

The present study showed 211G>A was detected infrequently; the estimated allele frequency was 0.002 in control newborns and 0.002 inhyperbilirubinemia group.

In contrast, a much higher allele frequency has been documented in East Asian populations, such as Chinese (0.23) [21], Koreans (0.23) and Japanese (0.13) [22], accounting for higher prevalence of neonatal hyperbilirubinemia in these groups.

Our findings also differed from a previous study conducted in the North-Western part of India where this variant was not detected [23]. Difference in sample size and genetic heterogeneity of the population could account for this discrepancy.

This study showed a comparison of weight among hyperbilirubinemia group according to genotype groups (G/G, G/A, A/A), it was found that there was no significant difference as regard to birth weight (P = 0.32), there was a statistically significant decrease in weight at sample collection in G/A, A/A genotypes in 211 G>A variants (P = 0.019). As regard, there was a statistically high significant increase in weight loss percent in G/A, A/A genotype (P < 0.001).

These results were in agreement with **Mohammed et al.** [15] who reported that, there was statistically significant increase in indirect bilirubin level in G/A-A/A genotypes. They also concluded that, 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. They demonstrated that body weight loss, genotype (G/A) and allele (A) was found to be significant independent predictors for hyperbilirubinemia.

These results also were in agreement with **Chang et al.** [14]and **Hiroko et al.** [10] who reported that maximal body weight loss was an independent risk factor for the development of neonatal indirect hyperbilirubinemia.

This study showed a comparison of clinical laboratory data among hyperbilirubinemia group according to genotype groups (G/G, G/A, A/A), it was found a statistically significant increase in reticulocytic count, TSB, and direct bilirubin at birth age collection with G/A, A/A genotype in 211 G>A variants ($P < 0.05$); but there was no significant difference as regard to Hb, WBCs, and platelets count ($P > 0.05$).

These results were in agreement with **Zibi et al.** [18]. While, **Hiroko et al.** [10] 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.

Huang et al. [24] suggested that the co-expression of the *UGT1A1* polymorphisms could diminish hepatic bilirubin uptake and decrease hepatic bilirubin-conjugating capacity, which further impairs bilirubin clearance and increases the severity as well as the risk of hyperbilirubinemia.

In **D'Silva et al.** [6]study, they observed that 76.80% of the cases co-expressed three or more variants as compared to 40.33% of the controls and the mean serum bilirubin levels and requirement of phototherapy was also higher in those neonates who co-expressed higher number of variants.

The gene variants that underlie complex disorders are characteristically common in the population; however, it is the contribution of multiple different co-expressed susceptibility genes individually conferring a small increase in risk that is required coupled with environmental factors that generate complex disorder phenotypes. This is evident from **D'Silva et al.** [6]study wherein they found that the serum bilirubin levels were the highest in those cases who co-expressed all the 5 variants (TSB: 381.15 ± 15.73 μmol/L) as compared to the cases who co-expressed only 2 of the variants (TSB: 303.86 ± 12.65 μmol/L)

It is interesting to note that all the hyperbilirubinemic neonates who had more variants also required phototherapy. Hence, **Olusanya et al.** [3]can be concluded that although individual polymorphisms may increase the total serum bilirubin levels, however, the risks of development of neonatal hyperbilirubinemia as well as the severity are much higher when these polymorphisms are co-expressed together.

V. Conclusion:

Detection of 211G>A variant of UGT1A1 promoter polymorphism gene was comparable between neonatal hyperbilirubinemia and control group. Heterozygous (G/A) and homozygous (G/G, A/A) genotype variants of the promoter region in UGT1A1 polymorphism in healthy neonates and idiopathic hyperbilirubinemia should be considered.

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