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Relation of Uridine Diphosphate Glucuronosyltransferase 1A1 Promoter Gene Polymorphism (211G>A) With Risk of Hyperbilirubinemia in Neonates

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ABSTRACT

Background: The gene 211G>A variants, that underlie complex disorders, are characteristically common in the neonatal hyperbilirubinemia. However, it is the contribution of multiple different co-expressed susceptibility genes that individually confer a small increase in risk coupled with environmental factors that generate complex disorder phenotypes.

Objective: This study aimed to understand the relation of 211G>A promoter polymorphism in UGT1A1 gene and the risk of hyperbilirubinemia in newborns.

Patients and Methods: The study included 50 newborns with hyperbilirubinemia with gestation age of ≥ 37weeks and postnatal age of ≤ 2 weeks with normal birth weight. 34 were males and 16 were females. They were 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. Variation status of UGT1A1 genes in our study was determined by direct sequencing or genotype assays.

Results: This study showed that UGT1A1 promoter gene polymorphism 211G>A genotype can be used as a novel method to detect susceptibility to indirect hyperbilirubinemia in neonates.

Conclusion: Our findings added to the understanding of the significance of UGT1A1 in association with neonatal hyperbilirubinemia in East Delta of Egyptian population. Additionally we are in need for other studies to investigate the protective mechanisms.

Keywords: Neonatal hyperbilirubinemia, UGT1A1 gene. 211G>A promoter polymorphism.

INTRODUCTION

Neonatal hyperbilirubinemia is caused by abnormal metabolism of bilirubin, and is characterized by a syndrome of skin, mucous membrane, and sclera jaundice **(1).** While most cases are physiological, when serum bilirubin concentrations are higher than 12.9 mg/dl in full-term infants and for a prolonged period of time, jaundice is no longer considered physiologic **(2)**.In pathological unconjugated hyperbilirubinemia, increased production of bilirubin, deficiency in hepatic uptake, impaired conjugation of bilirubin, and/or increased enterohepatic circulation of bilirubin are observed **(3)**. However, there is no identifiable factor in almost half of cases. It has been suggested that genetic variation could enhance the risk of neonatal hyperbilirubinemia when coexpressed with other icterogenic conditions **(4)**. Among these, uridine diphosphate glucuronosyltransferase 1A1 (UGT1A1) was identified to be associated with neonatal hyperbilirubinemia **(5)**. It is the key rate-limiting enzyme in the liver for bilirubin glucuronidation, which is a clearance mechanism for numerous dietary and environmental chemicals, including bilirubin **(6)**.

The polymorphisms of the UGT1A1 coding region or the promoter may produce structural or functional enzymatic deficiencies, leading to intermittent elevation of unconjugated serum bilirubin, resulting in hyperbilirubinemia known as Gilbert's syndrome (GS) and Crigler-Najjar syndrome (CNS). Numerous polymorphisms of UGT1A1 have been reported in patients with GS and CNS, including GlycineArginine and 211G>A promoter **(7).**

The Gly71Arg (G71R) of the UGT1A1 gene has been reported as a genetic risk factor for GS, which might reduce the activity of the enzyme, and then cause mild unconjugated hyperbilirubinemia **(8).**

In Japanese population, the allele frequency of 211G>A in infants with neonatal jaundice was significantly higher than in control infants **(9).** The interaction among genetic polymorphisms and clinical risk factors may modulate the hyperbilirubinemia risk. Better knowledge on gene–environment interaction may advance our understanding of this complex disorder **(4).**

Previous data have also shown that the effects of variants in UGT1A1 gene appear to be variable across populations **(10).**

The study aimed to study the contribution of 211G>**A** promoter polymorphism in **UGT1A1** gene and the risk of hyperbilirubinemia in newborns.

PATIENTS AND METHODS

This study was carried out in Neonatal Intensive Care Unit, Pediatrics Department, and

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Biochemistry Department, Zagazig University Hospitals over one-year duration from April 2017 to April 2018.

Ethical approval:

This study was done after being approved from Ethical Committee of Faculty of Medicine, Zagazig University (IRB). Consent of parents of the participants was obtained.

The study included 50 newborns with hyperbilirubinemia with gestation age ≥ 37weeks and postnatal age ≤ 2 weeks with normal birth weight. They were with 34 males and 16 females 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:

Newborns with hyperbilirubinemia enrolled in the study were:

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

Control group:

Control newborns enrolled in the study were:

- Neonates of ≥ 37 weeks of gestation age.
- Neonates of ≤ 2 weeks of postnatal age.
- There was mild clinical jaundice or the peak of total serum bilirubin (TSB) was <12 mg/dl.

Exclusion Criteria:

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

1) Hemolytic anemia
   - Elevated reticulocyte count.
   - Fall in Hb.
   - Peripheral smear showing spherocytes nucleated RBCs or anisopoikilocytosis.
   - Fetomaternal blood group incompatibility (e.g. Rh and ABO).
   - Positive direct Coombs' test.
2) Infection (CRP +ve).
3) Major congenital malformations such as intestinal malformation.
4) Maternal diabetes, hypertension, neonatal asphyxia.
5) Liver disease with 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) (using Coulter Counter T660, Coultronics; France) including Hb, Hct, platelet count, differential leucocytic count and blood indices and direct Comb's test.

Genetic analysis:

Detection of number of 211G>A repeats of promoter area of the Uridine Diphosphate glucuronosyltransferase 1A1 (UGT1A1) gene by PCR-based restriction fragment length polymorphism (RFLP) according to **Kumar *et al.*** [(11)].

Collection of samples:

Total of 2 mL of blood sample was collected in sterile EDTA containing tubes from every participant under complete aseptic condition for DNA extraction. Then, the extracted DNA was kept at -20°C for detection of number of promoter area of the UGT1A1 gene according to **Kumar *et al.* [(11)].**

DNA quantitation:

For determination of DNA concentration and evaluation of DNA purity, 20 µL of each extracted DNA sample was added to 1 mL of deionized water in quartz cuvette and absorbance was measured at 260 and 280 nm wavelengths using Milton Roy Spectronic 3000 Array. DNA has a maximum absorbance at 260 nm as the resonance structures of pyrimidine and purine bases are responsible for the absorbance. An absorbance of 1.0 at 260 nm gives DNA concentration 50 µg/ml. Proteins absorb maximally at 280 nm due to the presence of tyrosine, phenylalanine and tryptophan and absorption at this wavelength is used for detection of protein in DNA samples. This was done by determination of the A260/ A280 ratio. This ratio for pure double-stranded DNA was customarily taken to be between 1.8 and 1.9 [(12)].

Principle:

The number of 211G>A was determined by amplification of the promoter area of the UGT1A1 gene by PCR followed by SSCP analysis.

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. 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 Level for all above mentioned statistical tests done. The threshold of significance is fixed at 5% level (P-value).

RESULTS

By 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) (Table 1).

Table (1): Demographic characteristics if neonates

Variable	Patients (N=30)	Control (N=20)	*t*	*P* value
Age (days) Mean ± SD	5.1 ± 0.9	5.0 ± 1.3	0.72	0.057 (NS)
Gestational age (wks) Mean ± SD	37.2 ± 0.74	37.4 ± 0.84	0.12	0.71 (NS)
Birth Weight (kg) Mean ± SD	3.21 ± 0.187	3.187 ± 0.29	1.07	0.25 (NS)
Weight at sample collection (kg) Mean ± SD	2.87 ± 0.177	3.19 ± 0.286	3.37	0.001 (HS)
	No/(%)	**No/(%)**	χ^2	***P* value**
Sex (No. & %) Male Female	21 (70%) 9 (30%)	12 (60%) 8 (40%)	0.71	0.41 (NS)
Mode of delivery NVD CS	22 (73.3%) 8 (26.7%)	15 (75%) 5 (25%)	0.71	0.41 (NS)

Data are presented as *n* (%), **mean ± SD:** mean ± standard deviation
CS: Cesarean **NVD**: Normal vaginal delivery **NS**: no significance, **HS**: Highly significance

Table (2) showed highly statistically significant increase in reticulocytic count, TSB and direct bilirubin in the hyperbilirubinemia group than in the control group (p < 0.01). However, no statistically significant difference was observed between hyperbilirubinemia group and control group as regards Hb, WBCs, and platelets count (p > 0.05).

Table (2): 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 ($\times 10^3/mm^3$) Mean ± SD	10.99 ± 3.18	11.03 ± 2.4	t=0.05	0.96 (NS)
Platelets (x $10^3/mm^3$) 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 ± 0.31	0.565 ± 0.18	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 (3) showed that, the homozygous G/G was found in 10 patients (33.3%) versus 14 (70%) from control group. However, the heterozygous G/A was found in 14 patients (46.7%) versus 5 (25%) from control group. As regards the homozygous A/A, it 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 regarding genotype frequency of 211 G>A variants [G/G, G/A, and A/A] in UGT1A1 promoter region (**Table 3**). 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 (3): 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 G>A	**G/G**	Count % within groups	10 (33.3%)	14 (70%)	24 (48%)	R	----
	G/A	Count % within groups	14 (46.7%)	5 (25%)	19 (38%)	3.92 (1.06-14.44)	0.04 (S)
	A/A	Count % within groups	6 (20%)	1 (5%)	7 (14%)	8.4 (1.02-18.10)	0.04 (s)

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

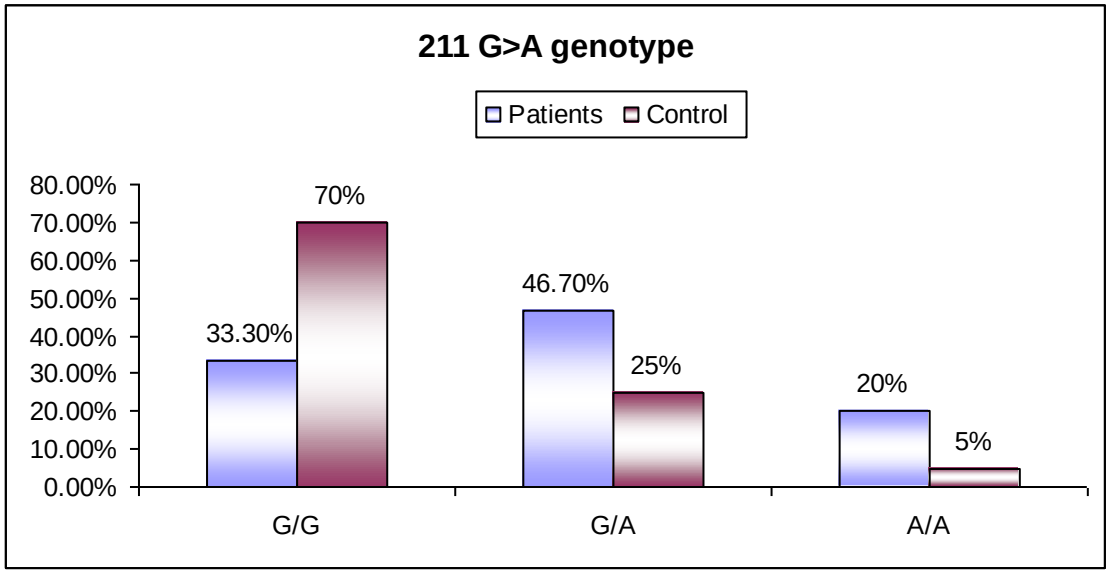

Figure (1): Bar chart for comparison of 211 G>A genotype between patients and controls

By comparing hyperbilirubinemia group and control group, it was found that, there was a statistically significant difference as regards allele frequency (G, A) in UGT1A1 promoter region (P = 0.007) (Table 4).

Table (4): 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

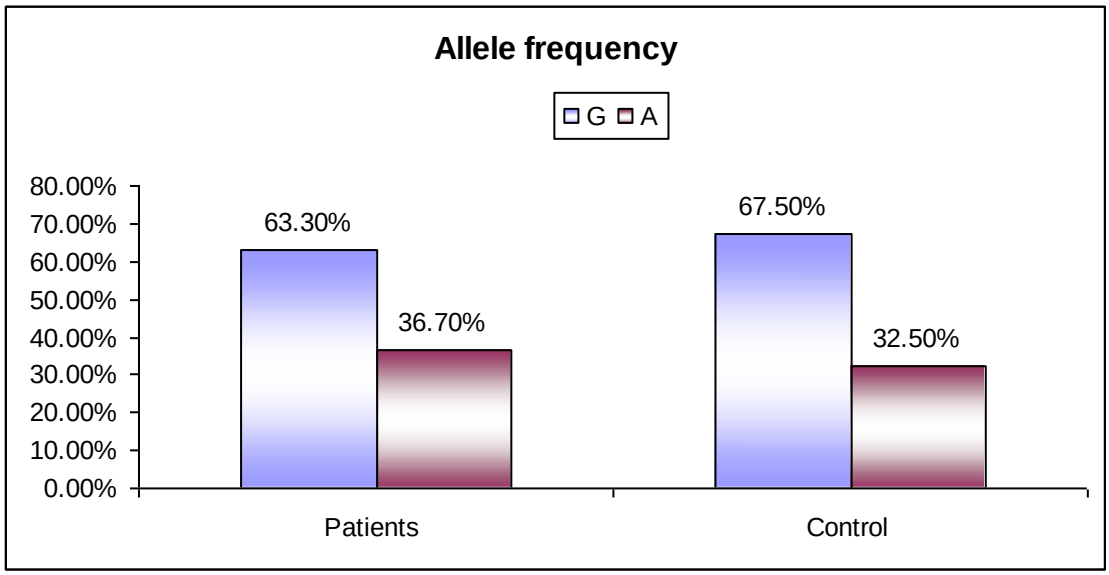

Figure (2): Bar chart for comparison of allele frequency between patients and controls

DISCUSSION

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 are in agreement with **Hui *et al.*** [13] who 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.*** [14], reported that neonates with shorter gestational age were risk predictors of development of neonatal hyperbilirubinemia. These results are in agreement with **Melih *et al.*** [15] reported that there was no significant difference between the studied groups regarding gender and gestational age.

The present study showed a statistically significant increase in reticulocytic count, TSB and direct bilirubin in the hyperbilirubinemia group than in the control group ($p < 0.05$). However, no statistically significant difference was observed between hyperbilirubinemia group and control group regarding Hb, WHCs, and platelets count ($p > 0.05$). These results are in agreement with **Chang *et al.*** [16]**.**

In the present study, by comparing hyperbilirubinemia group and control groups, it was found that, there was a statistically significant difference as regards 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 are in agreement with **Mohammed *et al.*** [17] who reported there was statistically significant differences between the studied groups regarding genotype frequency (G/G, G/A, A/A). In addition, these results are in agreement with **Hiroko *et al.*** [14] 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.*** [17] who reported that, there was statistically significant difference between the studied groups regarding allele frequency (G/A). In addition, these are 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 that 211G>A was detected infrequently; the estimated allele frequency was 0.002 in control newborns and 0.002 in hyperbilirubinemia group. In contrast, a much higher allele frequency has been documented in East Asian populations, such as Chinese (0.23) **Long *et al.*** [1], Koreans (0.23) and Japanese (0.13) accounting for higher prevalence of neonatal hyperbilirubinemia in these groups [21]. Our findings also differed from a previous study conducted in the North-Western part of India where this variant was not detected **Agrawal *et al.*** [4]. 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 regarding 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). There was a statistically high significant increase in weight loss percent in G/A, A/A genotype (P < 0.001). These results are in agreement with **Mohammed *et al.* (17)** 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) were found to be significant independent predictors for hyperbilirubinemia. These results also are in agreement with **Chang *et al.* (16)** and **Hiroko *et al.* (14)** who reported that maximal body weight loss was an independent risk factor for the development of neonatal indirect hyperbilirubinemia.

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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