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# Phenotypic Study of Egyptian Pediatric Patients with Criglar Najjar Syndrome

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## Abstract

*Background:* Crigler-Najjar syndrome (CNS) is an uncommon autosomal recessive hereditary condition characterized by severe unconjugated nonhemolytic hyperbilirubinemia from infancy. It is caused by a defect of bilirubin uridine diphosphate glucuronosyltransferase (UGT) 1A.

*Aim of Study:* We aimed to identify mutations in exons (2-4) in the UGT1A1 gene in Egyptian CNS patients.

*Material and Method:* The UGT1A1 gene's exons (2-4) were then molecularly examined using PCR amplification and singe sequencing of the coding regions after ten Egyptian CNS patients had been clinically identified.

*Results:* The patients were between the ages of 3 and 18, with an average age of 8.3±4.74 years. All patients (100%) had positive consanguinity, and 50% of cases had positive family history. Forty percent of the patients had delayed verbal and/or social or motor milestones, whereas the remaining sixty percent of patients had normal developmental milestones (6/10). The aggressive behavior exacerbates the illness. CNS type I affected 10 patients (100%) while type II affected 0 instances (0%). No deleterious mutations were found in exons (2-4) of UGT1A1 gene.

*Conclusion:* This study found no mutations in the three studied exons (exons 2-4) of the UGT1A1 gene. This highlights the importance of studying the rest of the exons of the UGT1A1 gene.

*Key Words: Crigler-Najjar syndrome – Indirect hyperbilirubenemia, Jaundice – Uridine diphosphate glucuronosyltransferase.*

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## Introduction

**NEWBORNS** with Crigler-Najjar syndrome have hyperbilirubinemia due to this uncommon autosomal recessive condition. The hallmark of Crigler-Najjar syndrome is the lack or reduced activity of UDP-glucuronosyltransferase (UGT), an enzyme necessary for the hepatic glucuronidation of unconjugated bilirubin. One of the main causes of congenital nonhemolytic jaundice is this deficit **[1].** The degree of UGT activity determines the classification of the syndrome into two types: Type 1: People with Crigler-Najjar syndrome type 1 (CN1) have severe symptoms brought on by low or absent enzyme activity. Their extreme jaundice can be fatal, and phototherapy is a necessary part of their treatment. Individuals suffering from this type of illness have an extremely significant chance of experiencing neurological aftereffects, which could result in irreversible harm like kernicterus. For CN1 syndrome, liver transplantation is the sole treatment **[2].** Type 2: The milder symptoms of this kind of Crigler-Najjar syndrome are caused by a decrease in enzyme activity. People who have type 2 Crigler-Najjar syndrome (CN2) often take conservative approaches to treating their ailment, which is characterized by sporadic jaundice that is induced by stress. In CN2, there is a low incidence of permanent neurological impairment and liver transplantation **[3].**

An absence or reduced level of the UGT enzyme as a result of a genetic deficiency in the bilirubin UDP-glucuronosyltransferase family 1, polypeptide A1 (UGT1A1) gene causes Crigler-Najjar syndrome. Common mutations in CN1 include stop codon creation, exon skipping, missense mutations, deletions, changes in intron splice donor and receptor locations, and insertions. These mutations result in total failure of the UGT enzyme. On the

other hand, CN2 is caused by a point mutation in the UGT1A1 gene, which lowers the amount of UGT enzyme produced **[1]**. The incidence rate of Crigler-Najjar syndrome is between 0.6 to 1 in 1 million babies worldwide, making it an extremely rare condition **[4]**.

Serum unconjugated bilirubin levels should be tested in patients suspected of having Crigler-Najjar syndrome; CN1 patients have much greater levels than CN2 patients. Unconjugated bilirubin in CN1 patients ranges from 2 to 25mg/dL, with severe instances potentially reaching 50mg/dL. In most cases, CN2 levels are less than 20mg/dL. However, genetic testing techniques are the mainstay of current diagnosis **[3]**. Most CN2 patients had their serum bilirubin concentration drop after receiving phenobarbital for two weeks **[3]**. Notably, phenobarbital therapy has no effect on CN1.

The main treatment strategy for CN1 is to lower unconjugated bilirubin levels through plasmapheresis and phototherapy. While most patients make it through puberty without suffering significant brain damage, they are at risk of developing kernicterus later in life. Currently, liver transplantation is the only option for treating CN1 **[5,6]**. Phototherapy is the main treatment approach, and it is often used to treat neonatal hyperbilirubinemia. Because older children and adults have thicker skin, more pigmentation on their skin, and smaller bodies relative to their bulk, phototherapy is less effective in these populations **[5,6]**. For CN1, liver transplantation is the only effective and long-term therapy option. Since kernicterus may not be curable once it manifests, preventive liver transplantation is strongly advised to avoid the disease **[7]**. Phenobarbital has no effect on CN1, but in patients with CN2, it can lower serum bilirubin levels by 25% **[8]**.

## Material and Methods

Study participants were ten children diagnosed with Crigler-Najjar syndrome based on clinical and laboratory findings. The study was carried out at the Pediatric Department of Menoufia University's National Liver Institute from November 2020 to May 2021. The study was approved by the Research Ethical Committee of Menoufia University's National Liver Institute. Every method employed was compliant with the 2008 Helsinki Declaration amendment and the ethical standards established by the committee in charge of supervising human experimentation. Every patient who desired to take part in the trial gave their informed consent through their parents or guardians.

1- Patients' medical records were retrieved. Personal history, clinical presentation, family history, clinical examination and laboratory investigations of the 10 patients were recorded. Blood samples for liver function analysis were obtained. Liver function tests.

- Alanine transaminase (ALT): The normal value is up to 44 U/L.
- Aspartate Transaminase (AST): The normal value is up to 34 U/L.
- Total Serum Bilirubin: The normal range is up to 1.1mg/dL.
- Direct Bilirubin: The normal range is up to 0.2mg/dL.

On a Cobas-600 autoanalyzer (Japan), all parameters of the liver function tests were completed.

## Results

Ten putative male Crigler-Najjar syndrome patients were included in this study: The patients were between the ages of 3 and 18, with an average age of 8.3±4.74 years. All patients (100%) had positive consanguinity, and 50% of cases had positive family history. Three patients (30%) were in the governorates of Gharbia, one patient (10%) was in Dakahlia, and six patients (60%) were in Buhaira.

Forty percent of the patients had delayed verbal and/or social or motor milestones, whereas the remaining sixty percent of patients had normal developmental milestones (6/10). The aggressive behavior exacerbates the illness. CNS type I affected 10 patients (100%) while type II affected 0 instances (0%).

Following their admission to the newborn intensive care unit (NICU), every patient in the study received phototherapy 10/10 (100%). Two patients (20%) out of the ten required an exchange transfusion (2/10). After examining the various forms of patient care, it was found that 100% of the patients were on phenobarbitone, 30% of patients were on levetiracetam, and 20% of patients had had liver transplants. Every patient (100%), according to their liver profiles upon presentation, had indirect hyperbilirubinemia. The average levels of total and direct bilirubin were 26.98±5.52 and 0.735±0.3mg/dl, respectively. Aspartate aminotransferase had a mean of 38.6±19.73u/l while alanine aminotransferase had a mean of 30.8±16.3u/l (Table 1).

Table (1): Represent liver enzymes (AALLT, AST), total bilirubin and direct serum bilirubin.

| Patients | AST IU/L | ALT IU/L | TSB mg/dL | DSB mg/dL |
|---|---|---|---|---|
| Pt1 | 36 | 59 | 26.3 | 0.9 |
| Pt2 | 27 | 29 | 22 | 0.69 |
| Pt3 | 40 | 43 | 31 | 0.96 |
| Pt4 | 92 | 21 | 40 | 0.1 |
| Pt5 | 92 | | | |
| Pt6 | 44 | 52 | 20.6 | 0.78 |
| Pt7 | 31 | 16 | 25 | 0.9 |
| Pt8 | 24 | 36 | 22.2 | 0.3 |
| Pt9 | 32 | 26 | 31.6 | 1.2 |
| Pt10 | 26 | 11 | 25 | 0.86 |

### *Discussion*

Biallelic mutations in UGT1A1, which encodes uridine 5'-diphosphate glucuronyltransferase (UGT1A1), cause Crigler–Najjar syndrome (CNS; MIM 218800), a rare autosomal recessive disorder. UGT1A1 mediates the glucuronidation of native Z, Z-bilirubin into mostly diglucuronides with some monoglucuronide. Bilirubin becomes hydrophilic through serial conjugation with glucuronides, which is necessary for the hepatobiliary system to excrete it *[4]*.

A range of irreversible neurological sequelae *[11]* can accompany elevated levels of unconjugated bilirubin, which can cause a devastating encephalopathy known as kernicterus *[12]* adapted from the German kern ("nucleus") and icterus ("jaundice") which refers to the deposition of bilirubin pigment in the cranial nerve and subthalamic nuclei, basal ganglia (especially the globus pallidus), and hippocampus *[12]*. Historically, residual UGT1A1 activity and its inducibility upon phenobarbital exposure have been use to approximately characterize the severity of CNS. This, however incomplete, enables categorization into clinical variations characterized as Gilbert syndrome (~20% UGT1A1 enzymatic activity), type 2 (<10% UGT1A1 enzymatic activity; CNSII), and type 1 (lack of UGT1A1 enzymatic activity; CNS-I) (MIM PS237450) *[12]*.

According to the National Organization for Rare Disorders, the incidence of CNS is one in 750 000–1,000 000 live births, making it a "ultra-rare orphan disease". Because of our limited knowledge of the pathophysiology, clinical symptoms, and disease progression of rare disorders, as well as the lack of corporate and academic backing for the development of effective treatments, rare disorders typically have a high unmet demand. The burden is also increased by delayed diagnosis and the social isolation that patients and their families go through *[13]*.

Crigler Najjar type 1 was reported in mutations in exon 2: missense mutation A291V (French origin) and G308E (Portugal origin), in exon 3: missense mutation Q357R (Tunisian origin), and non-sense mutations: W335X and Q357X (French origin). Mutations in exon 4 include missense mutations (A368T, A401P, and K426E) of French origin, S381R (Italian origin), and frame shift mutation 1223insG (French origin) *[14]*.

In our study, molecular analysis of (2-4) exons in the UGT1A1 gene revealed no mutations. This may be because the mutations in our cases belong to exons 1 or 5, which were not included in our study. Sixty percent of the patients had normal developmental milestones, while forty percent of the patients had delayed verbal, social, or physical milestones (6/10). The sickness is made worse by the hostile behavior. Ten individuals (100%) had CNS type I, whilst zero cases (0%), had type II.

Every patient in the trial received phototherapy 10/10 (100%) after being admitted to the newborn intensive care unit (NICU). Out of the ten patients, two (20%) needed an exchange transfusion (2/10). Upon reviewing the different patient care plans, it was discovered that 20% of patients had received liver transplants, 30% of patients were taking levetiracetam, and 100% of patients were on phenobarbitone. Based on their liver tests at the time of presentation, all patients (100%) exhibited indirect hyperbilirubinemia. With a mean ± SD of 25.92±7.45mg/dL (16.6 times ULN), we discovered that TSB ranged in patients from 10.60 to 40mg/dL. Patients' direct serum bilirubin levels (mg/dL) varied between 0.10 and 2.40, with a mean ± SD of 0.71±0.5.

About aminotransferases, our analysis revealed that ALT varied between 11 and 63IU/L in patients, with a mean ± SD of 35.68±16.71IU/L, which was ~1.2 times ULN. With a mean ± SD of 39.29±17.88IU/L, the AST ranged in patients from 16 to 92IU/L, indicating that it was 1.6 times ULN.

*Conclusion:*

This study found forty percent of the patients had delayed milestones.

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## دراسة نمطية للمرضى المصريين المصابين بمتلازمة كريجلار نجار

ان الاطفال المصابين بمتلازمة كريجلار نجار يعانون من فرط البيليروبين بسبب هذه الحالة المتنحية الغير شائعة. والسمة المميزة لمتلازمة كريجلار نجار هى نقص او انخفا ض الانزيم الناقل يوريدين ثنائى فوسفات جلوكيورونوسيل وهو انزيم ضرورى لعملية الجلوكورونيدات الكبدية للبيليروبين غير المقترن.

أحد الاسباب الرئيسية لليرقان الخلقى غير الانحلالى هو هذا النقص. تحدد درجة نشاط الانزيم تصنيف المتلازمة الى نوعين :

النوع ١: يعانى الاشخاص المصابون بمتلازمة كريجلار نجار من النوع ١ من أعراض حادة ناجمة عن غياب الانزيم يمكن ان يكون اليرقان الشديد قاتلا، ويعتبر العلاج الضوئى جزء ضروريا من علاجهم. الافراد الذين يعانون من هذا النوع يكونون أكثر عرضة للآثار العصبية لاحقا، والتى يمكن أن تؤدى الى ضرر لا رجعة فيه مثل اليرقان النووى. ويعتبر زراعة الكبد هى العلاج الوحيد لهذا النوع.

النوع ٢: الأعراض الخفيفة لهذا النوع من متلازمة كريجلار نجار تنتج عن انخفاض نشاط الانزيم. يتبع الأشخاص المصابون بهذا النوع أساليب محافظة فى علاج مرضهم، والذى يتميز باليرقان المتقطع الناتج عن الاجهاد. وفى هذا النوع هناك انخفاض فى معدل الاصابة بالضعف العصبى الدائم وزراعة الكبد. غياب او انخفاض مستوى الانزيم الناقل هو نتيجة لنقص وتراثى فى عائلة البيليروبين جين متعدد الببتيد والذى يسبب متلازمة كريجلار نجار.

تشمل الطفرات الشائعة فى كريجلار نجار انشاء كودون التوقف، وتخطى الاكسون، والطفرات الخاطئة، والحذف، وتغيرات فى المواقع الجهات المانحة والمستقبلات لوصلة الانترون والادراج. تؤدى هذه الطفرات الى الفشل التام للانزيم الناقل.

من ناحية أخرى ان سبب النوع الثانى هو طفرة نقطية فى الجين مما يقل كمية الانزيم الناتج. يتراوح معدل الاصابة بمتلازمة كريجلار نجار بين ٠,٦ الى ١ من كل ١ مليون طفل فى كل أنحاء العالم مما يجعلها حالة شديدة الندرة.

يجب قياس مستوى البيليروبين غير المقترن فى الدم لدى المرضى المشتبه فى اصابتهم بمتلازمة كريجلار نجار، حيث أن مرضى النوع الأول يعانون من ارتفاع أكبر بكثير فى مستوى البيليروبين غير المقترن من مرضى النوع الثانى والذى قد يصل الى ٢٥ مجم/ديسيلتر، مع احتمال وصول الحالات الشديدة الى ٥٠ مجم/ديسيلتر.

ومع ذلك تعتبر تقنيات الاختبارات الجينية هى الدعامة الأساسية للتشخيص الحالى.

ان عقار الفينوباربيتال يعمل على انخفاض نسبة البيليروبين فى الدم فى مرضى النوع الثانى بينما ليس له تأثير على مرضى النوع الاول.

تتمثل استراتيجية العلاج الرئيسية فى خفض مستوى البيليروبين غير المقترن من خلال فصادة البلازما والعلاج الضوئى. فى حين أن معظم المرضى يصلون الى مرحلة البلوغ دون التعرض لتلف كبير فى الدماغ، الا انهم معرضون لخطر الاصابة باليرقان النووى فى وقت لاحق من حياتهم.

ان العلاج الضوئى هو العلاج الرئيسى و غالبا ما يستخدم لعلاج فرط البيليروبين عند حديثى الولادة. نظرا لأن الاطفال الاكبر سنا يكون جلدهم أكثر سمكاً وتصبغاً للبشرة، لهذا يكون العلاج الضوئى أقل فعالية.

تعتبر زراعة الكبد هى الخيار العلاجى الوحيد الفعال وطويل الأمد. وبما أن اليرقان النووى قد لا يكون قابلا للشفاء، ينصح بشدة باجراء زراعة كبد وقائية. ان الفينوباربيتال ليس له تأثير على مرضى النوع الاول، لكنه يقلل مستوى البيليروبين فى مرضى النوع الثانى بنسبة ٢٥٪.

المرضى والأساليب: شملت هذه الدراسة عشر اطفال من أولئك الذين ترددوا على قسم أمراض الكبد والجهاز الهضمى. والتغذية للاطفال بالمعهد القومى للكبد، جامعة المنوفية خلال الفترة من نوفمبر ٢٠٢٠ حتى مايو ٢٠٢١ تم التوقيع على موافقة خطية مستنيرة توضح الهدف من الدراسة قبل بدء الدراسة، بعد موافقة لجنة الأخلاقيات فى المعهد القومى للكبد، جامعة المنوفية.

جميع المرضى للتحاليل التالية:

أ- وظائف الكبد (البيليروبين الكلي والمباش ر، ، ألانين أمينو ترانسفيراز، ترانساميناز).

الخلاصة: توصلت هذه الدراسة الى أنه ٤٠٪ من مرضى متلازمة كريجلار نجار يعانون من تاخر فى النمو.