Analysis of common mutation for GALT gene in newborns with galacatosemia Nineveh governorate

تحديد الطفرات الوراثية التي تصيب ألجين GALT لدى الأطفال المصابين الكلاكتوسيميا في محافظة نينوى

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Abstract

Iraq contains many diseases that have never been counted or examined, including diseases related to food, which has deteriorated in recent years, and has rapid and direct impact especially on the children category, one of these diseases is galactosemia. Classical galactosemia, deficiency of galac tose-1-phosphate uridyltransferase GALT, is characterized by acute symptoms of hepatomegaly, jaundice, sepsis, cataract, vomiting, and diarrhea and growth retardation. Our previous molecular study showed that the most common mutation of the GALT gene is a missense mutation of Q188R (replacement of glutamine-188 by arginine in exon 6 and N314D mutation replacement of aspargen-314 by aspartic acid) in exon 10. The aim of this study was to determine the possibility of diagnosing galacatosemia, and to search for galactosemia mutation Q188R and N314D in Iraqi population. Blood samples were collected from babies admitted to the children's hospitals in Mosul City depending on the clinical symptoms of disease and then serum was taken. Measuring the Galactose-1-Phosphate uridylytransferase GALT enzyme activity and galactose -1- phosphate in serum by ELISA technique was done. DNA samples were analyzed by the polymerase chain reaction followed by digestion with restriction endonuclease *HpaII* and *AvaII* for Q188R and N314D mutation. The results showed a significant decrease in the level of the GALT enzyme in children with galactosemia 21.7 ± 0.45 and among non-diagnosed children 79.93 ± 1.44 compared with control group 160.33 ± 0.93 as well as a significant decrease in the level of the enzyme among mothers 20.5 ± 1.92 was observed. Gal-1-P level was significantly higher in the cases than that of the control group, while that of the not diagnosed children and mothers groups showed inconsistent difference. Also the result showed absence allele frequency for Q188R mutation and present allele frequency for N314D mutation in Iraqi population. In conclusions It is possible to depend on measurement of Galactose-1-Phosphate as indicator in the diagnosis of Galactosemia in newborn, the main mutation in GALT gene causes galactosemia is N314D in Iraqi population.

Key words: galactosemia, *GALT* gene mutation, PCR.

المستخلص

حسب الادلة المشار اليها فان العراق يحوي العديد من الامراض غير المشخصة والتي لم يتم احصائها ومن هذه الامراض ماهو مرتبط بالغذاء وخاصة ما هو في فنة الاطفال ومن هذه الامراض هو الكلاكتوسيميا ، تهدف الدراسة الى امكانية تشخيص الاصابة بالكلاكتوسيميا سريريا والتحري عن وجود الطفرات الاكثر شيوعا Q188R & N314D المسببة للمرض في المجتمع العراقي . تم جمع عينات الدم من الاطفال الراقدين في مستشفيات الاطفال في مدينة الموصل بالاعتماد على العلامات السريرية للمرض وتم فصل مصل الدم ومن ثم قياس مستوى الا نزيم galactose-1-phosphate uridyltransferase وقياس تركيز galactose-1-phosphate باستخدام تقنية الـ ELISA وتم تحليل الـ DNA للعينات باستخدام تقنية الـ PCR ومن ثم الهضم باستخدام الانزيمات القاطعة *Hpa11 & Ava11* للطفرات N314D & Q188R على التوالي . اظهرت نتائج الدراسة انخفاض في مستوى الانزيم GALT لدى الاطفال المصابين بالكلاكتوسيميا 21.74 ± 0.49 pg/ml وكذلك في الاطفال غير المشخصين 79.93 ± 1.44 pg/ml مقارنة مع مجموعة السيطرة 160.33 ± 0.93 pg/ml كذلك اظهرت الدراسة ارتفاعا في مستوى Gal-1-p لدى المرضى 130.3 ± 12.7 pg/ml مقارنة مع الاصحاء 66.0 ± 10.0 pg/ml. فضلا عن ذلك غياب الطفرة Q188R لدى المرضى ووجود الطفرة N314D في الاطفال المصابين بالكلاكتوسيميا . نستنتج من هذه الدراسة امكانية الاعتماد على مستوى Gal-1-p كمؤشر للاصابة بالكلاكوسيميا لدى الاطفال . وان الطفرة الرئيسية في الجين GALT المسببة للاصابة بالكلاكتوسيميا في المجتمع العراقي هي N314D .

الكلمات المفتاحية : الكلاكتوسيميا ، طفرة الجين *GALT* ، PCR

Introduction

Galactosemia is an autosomal recessive disorder caused by a deficiency of one of the enzymes involved in the utilization of dietary galactose; galactokinase EC 2.7.1.6, galactose-1-phosphate uridyl transferase EC 2.7.7.12, or uridinediphosphate galactose-4-epimerase EC 5.1.3.2 (18). The most common form of galactosemia is due to a deficiency of galactose-1-phosphate uridyltransferase GALT [1], which catalyzes the reaction between galactose-1-phosphate and UDP-glucose to form glucose-1-phosphate and UDP-galactose. The deficiency of GALT leads to the accumulation of galactose-1-phosphate and to the oxidation and reduction of galactose to galactonate and galactitol, respectively. The main clinical features of galactosemia are a failure to thrive, diarrhea and dehydration, vomiting, jaundice, hepatomegaly, hypoglycemia, and cataracts [2,3]. Treatment with a galactose-free diet prevents these acute severe symptoms, but there is increasing evidence that the long term prognosis for these patients is not as good as was first hoped. Long term complications include below-average IQ, ovarian dysfunction, speech difficulties, delayed growth, and impaired motor function and balance, suggesting persistent metabolic defects in the brain and ovarian tissues. The biochemical basis of both the acute and chronic symptoms is not well understood, and there is no correlation between outcome and genotype, residual GALT activity or the red blood cell level of galactose-1-phosphate, which is used to monitor treatment [1,4].

The GALT gene was cloned and sequenced originally from *Escherichia coli* and yeast [5]. By using islands of amino acid sequence identity among these species, a human GALT cDNA was cloned and correctly sequenced [4]. More recently, the human GALT gene was cloned and fully sequenced [5]. With these normal sequences in hand, several groups began to use direct sequencing of PCR-amplified cDNA or genomic DNA to identify candidate mutations in the GALT genes of galactosemic patients. For example, the Q188R mutation, which substitutes an arginine for glutamine at codon 188 in exon 6, has a prevalence of 70% in Caucasians with galactosemia [1,2,5]. The Q188R allele is associated with essentially no activity in human erythrocytes or lymphoblasts or in yeast engineered to express the human alleles [3,6].

Another common mutant allele of the GALT genome is an A-to-G transition in bp 2744 of exon 10, which results in the substitution of an acidic aspartate (D), for an asparagines (N) at codon 314 (N314D) [7]. The N314D variation had extraordinary concordance with the biochemical phenotype of the Duarte allele [5,8]. The N314D mutation is frequent and has an estimated population prevalence of 5.9%, which conforms with previous estimates of the population frequency of the Duarte allele's biochemical phenotype [9,10]. Thus, this study was to determine the possibility of diagnosing galacatosemia and to search for galactosemia mutation Q188R and N314D in Iraqi population.

Materials and Methods

Blood samples were collected from babies admitted to the children's hospitals in Mosul city depending on the clinical symptoms of galactosemia disease. Enzyme and molecular analyses were performed on 1cc of EDTA blood. Measuring the GALT enzyme activity with galactose -1- phosphate using ABO Swetzirland co. kit by ELISA technique. Patients and families gave informed consent to participate, and the research project was approved by the institutional review board at Emory University.

Two sets of PCR primers were designed that flanked exons 6:

1481-F 5'-GGGTCGACGTCGGATGTAACGCTGCCACTCA-3'

Int7-R 5'-GGGGACACAGGGCTFGGCTCTCTCCCA-3';

and Primer sets for intron G (5'CGCGAATTCCCTTGCCTATTTGCTGACCAC and intron J (3' GGGGTCGACGCCTGCACATACTGCATGTGA

Amplify a 949-bp segment of DNA includingexons 8-10 of the human GALT gene.

PCR was carried out in a final volume of 20 µl, with pri mex from promega, The following cycling conditions were used: cycle 1: 95for 5 min, 65° for 1 min, and 72 for 1 min; cycles 2-34: 95 for 45 s, 65° for 1 min, and 720 for 1 min; cycle 35: 95 for 45 min 65 for 1 min, and 72 for 8 min [6,11]

The PCR products were then digested with restriction endonucleases *HpaII* and *SinI*, as recommended by the manufacturer (Promega) for 3 hours and were analyzed by electrophoresis through a 2% agarose gel [6,11]

Uncut amplification products were 607 bp for Q188R mutation and 949 bp for N314D mutation. Restriction endonuclease digestion, these fragments were cut into a collection of smaller fragments-some constitutive and others diagnostic for either the presence or absence of each of the two mutations, Q188R and N314D.

Statistical analysis

Results were analyzed based on T-test P≤0.05

Results

Table (1) showed a significant decrease in the level of the GALT enzyme in children with galactosemia 21.7 ± 0.45 compared with healthy children who were considered as a control group 160.33 ± 0.93 as well as a significant decrease in the level of the enzyme among mothers 20.5 ± 1.92 was observed. Similar finding is shown among non-diagnosed children 79.93 ± 1.44 compared with the control group. The percentage of the effectiveness of enzyme was 41.7%. The level of g-1-p in babies with galacatosemia a highly significant way 114.4 ± 8.4 and non-diagnosed children 52.8 ± 6.8 compared with the control group 25 ± 2.76, while no significant difference in the level of Gal-1-p among mothers 25 ± 1.3.

Table (1): GALT enzyme concentration and Gal-1-phosphate (p.g \ ml) among study groups

Groups	No.	GALT enzyme concentration	Galactose-1-phosphate
Controls	**40**	**160.33±0.93**	**25 ± 2.76**
Non-diagnosed	**13**	**79.8 ± 1.44**	**52.8 ± 6.8**
Cases	**17**	**21.7 ± 0.45**	**114.4 ± 8.4**
Mothers	**21**	**20.5±1.92**	**25 ± 1.3**

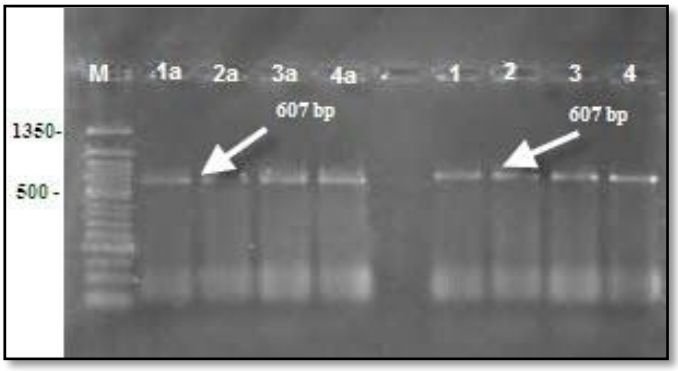
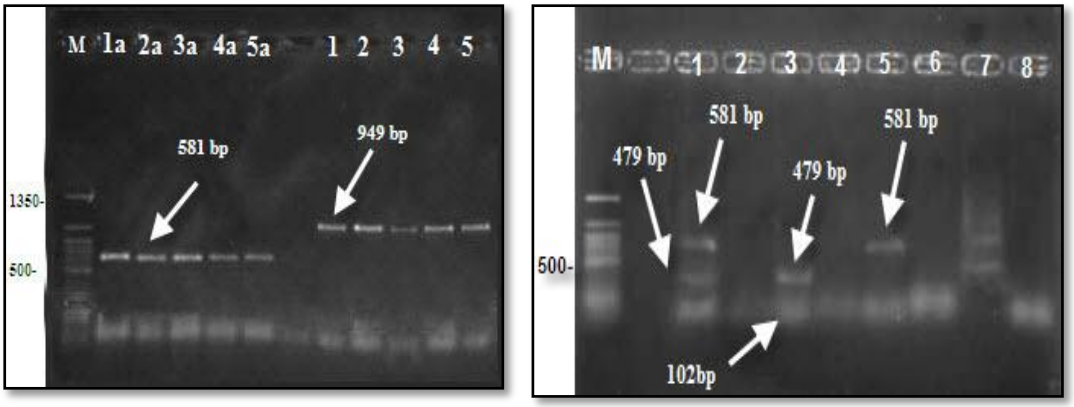


Figure (1): Detection of Q188R mutation by the RFLP-PCR procedure, M ladder, lane 1, 2, 3, 4 undigested PCR product 607 bp and lane (1a, 2a, 3a, 4a) digested with *HpaII* enzyme and no Q188R mutation



(a) **(b)**

Figure (2): Detection of N314D mutation by the RFLP- PCR procedure. (a) M ladder, lane 1, 2, 3, 4, 5 undigested PCR product 949 bp and lane (1a, 2a, 3a, 4a, 5a) digested fragment with AvaII and produce one band 581 bp. (b) M ladder, lane 1,7 N314D parent heterozygote with two bands 581+479 bp, lane (3) N314 mutation with two bands 479+102 bp and lack 581 band and lane 5 control with one band 581bp.

Discussion

In the present study a significant decrease in the level of the enzyme GALT in children with galactosemia compared with healthy as well as a significant decrease in the level of the enzyme in the mothers and non-diagnosed children compared with the control group these results agreed with other studies [7,12], which demonstrated a decrease of GALT enzyme level in patients with galactosemia compared with healthy group. The low level or even absence of this enzyme in such patients may be due to mutations in GALT gene and thus an imbalance in gene expression. Besides that, these mutations may lead to the disappearance of an enzyme such as Q188R and N314D and there are a number of mutations [6,13], that lead to a decline in the proportion of gene expression and thus decrease the level of the enzyme as is the cases group, which will be the second part of the study, which involves identifying mutations and genetic variation of the gene GALT. Gal-1-P level was significantly higher in the cases than that of the control group, while that of the not diagnosed children and mothers groups showed inconsistent difference. These findings are agreed with others [3,4] which showed a high GALT in patients with galactosemia, compared with healthy controls and that the main reason for this finding is the absence or decrease GALT enzyme activity in patients with galactosemia. Gal-1-p is the most likely toxin that initiates cell death. It is the commonly measured and is frequently used to monitor efficacy of treatment. In severe classical galactosemia, galactose-restriction results in reduction of Gal-1-p, although levels remain detectably elevated over controls even under under good nutritional control probably due to endogenous galactose production [13,14].

The Q188R mutation is caused by an A-to-G transition, which is located in a highly conserved region of the gene. Most patients reported with classical galactosemia carried this mutation [11]. In patients with two Q188R alleles the clinical outcome is generally poor [15]. We not detected any alleles carrying the Q188R mutation out of the analyzed cases in Iraqi population. These findings differ from the published population studies reporting 62-64% Q188R frequency in Germany [4], 85% in Ireland, including 95.5% in the Irish subpopulation called ”Travelers” [14], 60% in the Austrian [9], 46% in the Czech and Slovak population [7] and 0 % in Japan [16]. The American studies report an average of 62% frequency in the classical galactosemic population, 71% among the Americans with British descent and 13% in the Afro-American patients [5,17]. About 0.6 % of the average American population was found to carry this allele [9]. The difference of our result might be due to different sampling, since our patients were all identified by newborn screening.

The N314D (Duarte) mutation is due to an A-to-G transition in the exon 10, introducing a new Ava II restriction site beside the 5 normally present ones. Enzyme assays have shown that this mutation causes about 80 percent decrease in the enzyme activity for each carried alleles. The N314D mutation is usually associated with the Duarte phenotype, the outcome of these patients is better [5,8,11].

The Elsas group found the incidence of the mutation for 58.8% among galactosemic patients with Duarte biochemical phenotype, and they reported a 5.9 % frequency for the Duarte allele in heterogeneous population of non-galactosemic Americans [15]. We found Duarte mutation in 10 alleles out of 45 galactosemic patients. Other mutations such as K285N are commonly reported in Central European countries [18,19].

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