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Glutathione S Transferase M1 Polymorphism in Extrahepatic Biliary Atresia

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Abstract

Background: Extrahepatic biliary atresia (EHBA) is a chronic progressive obstructive cholangiopathy of infancy of unknown aetiology. Pathology of bile duct damage involves unanimously neutrophil elastase, variable degrees of fibrosis, and variable CD14+ monocytes intensity staining in the presence of defective p53 and glutathione S transferases Pi class (GST Pi). GST is a super family responsible for detoxification of an array of substances that affect cellular replication and DNA fidelity, of them cytosolic GST Mu is a member.

Aim of Work: Is to study GSTM1 gene polymorphism in EHBA.

Material and Methods: Genotyping of GSTM1 from peripheral blood of 41 infants with EHBA, and from peripheral blood of their mothers was performed. Study commenced by July, 2001 and ended by July, 2004, in New Children Hospital, Cairo University.

Results: All 41 enrolled infants had a null GSTM1 mutation concordant with homozygous deficiency, and all mothers expressed a pattern concordant with affection of only one allele.

Conclusion: All infants suffering from EHBA had null GSTM1 genotype. Phenotypic loss of function of GSTM1 renders subjects with EHBA susceptible to a wide array of substances that affect cellular replication and DNA fidelity. Susceptibility to EHBA is genetic and transmitted in an autosomal recessive fashion from mothers with single gene allele. This work supports that EHBA is a developmental defect.

Key Words: *Extrahepatic biliary atresia – EHBA – Glutathione S transeferase – GSTM1 polymorphism – GST Pi.*

Introduction

EXTRAHEPATIC biliary atresia is the end result of a destructive idiopathic inflammatory process that affects intrahepatic and extrahepatic bile ducts leading to fibrosis and obliteration of the biliary tract with the eventual development of biliary cirrhosis **[1]**. Timely intervention does not halt the march of cirrhosis **[2]**.

The lack of HLA predominance and HLA shared epitopes **[3]**, lack of consistent evidence of viral **[4]** or "auto" immune attack upon the liver **[5]** renders the "auto" immune model an implausible possibility. It is important to recognize that evidence supports immune mediated processes involvement in EHBA. Immune evidence in EHBA includes infiltration by CD4+ helper T lymphocytes, CD8+ suppressor T lymphocytes, CD68+ macrophages, CD14+ **[1,6-8]**, presence of anticytoplasmic antineutrophil antibodies **[9]**, unanimous neutrophil elastase bile duct damage **[10]**, and evidence for endotoxin circulation and up regulation of lipopolysaccharide endotoxin receptor CD 14+ monocytes in EHBA **[10,11]**. Disruption of p53 and glutathione S transferase (GST) Pi stand as evidence against "auto" immune pathogensis **[5]**. Fidelity at resolution and ontogeny respected regeneration is a direct function of p53 **[12]** and indirect function of family of GST **[13]**. GST are responsible for detoxification **[14]** of a wide array of substances that affect cellular replication and DNA fidelity **[13]** that include drugs, pesticides, herbicides, epoxides and carcinogens **[15]**. GST Pi in EHBA is disrupted **[5]**. GST Mu is a class of cytosolic transferases that are also responsible for detoxification of electrophilic compounds, including carcinogens, therapeutic drugs, environmental toxins and products of oxidative stress. The gene encoding the mu class of enzymes are organized in a gene cluster on chromosome 1p13.3 **[16]**. GST Mu class are dimeric, allowing an additional high-affinity site for non-substrate xenobiotics, of phase II detoxification, to be followed by phase III and excretion through bile and/or urine **[17]**.

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This work aimed to study other GST family member involvement in EHBA, namely; genetic GSTM1 polymorphism in EHBA and its inheritance pattern.

Subjects and Methods

Infants known to have EHBA, who were attendants of Hepatology Clinic, New Children Hospital, Cairo University, were enrolled in this study. Their mothers were also included in the study. Mothers consented to the trial. It commenced by July, 2001 and ended by July, 2004.

Diagnosis of EHBA relied upon clinical picture, specific percutaneous liver biopsy findings [2], and operative findings.

Methods:

Polymerase chain reaction (PCR) genotyping of GSTM1 polymorphism was performed from peripheral blood of 41 infants with EHBA, and from peripheral blood of their mothers. Study commenced by July, 2001 and ended July, 2004, in New Children Hospital, Cairo University.

GSTM1 Polymorphism:

DNA was isolated from whole peripheral blood using Qiagen. The polymorphic detection of GSTM1 gene was typed using the multiplex PCR [17]. The PCR primers used were as follows: P1: 5'CGCCATCTTGTGCTACATTGCCCG, P2: '5ATCTTCTCCTCTTCTGTCTC and P3: '5TTCT GGATTGTAGCAGATCA. P1 and P3 amplify a 230 bp product that is specific to GSTM1, whereas P1 and P2 aneal to GSTM1 and GSTM4 genes, yielding a 157 fragment that serves as an internal control. PCR was performed in 20ml containing 20ng of genomic DNA, 0.5 [illegible]mol/L of primer, 200 [illegible]mol of each dNTPs, 10mmol/L Tris Hcl (pH 8.3), 50mmol/Kcl, 1.5mmol/L Mg cl_2 and 0.5U of amphitaq DNA polymerase (promega). After denaturation for 4min at 94ºC. The PCR was performed for 35 cycles of 30 seconds at 94ºC, 1min at 58ºC and 1min at 72ºC. The last elongation step to 7min. The presence of one or both GSTM1 allele identified by a 230bp, or its complete deletion (null type) was analyzed by electrophoresis on 1.2% agarose gel. The absence of amplifiable GSTM1 (in the presence of the GST4 amplified control) indicated a null genotype.

Statistical analysis:

Statistical analysis in this study was conducted using the Statistical Package for Social Sciences version 15 (SPSS, Chicago, IL, USA). Simple frequency, descriptive analysis, cross-tabulation, tests of significance (*t*-test for parametric data, and X^2 tests for non parametric data) were employed.

Results

The enrolled 41 infants suffering from EHBA had undergone Kasai portoenterostomy at a mean age of (83±21 days), all had non-correctable type of EHBA. 21 (51.2%) were products of consanguineous marriages. None had history of similar affection in a family member. At presentation their mean total and direct bilirubin levels were 15.8 ±6-1 and 8.1±4.2mg%. Mean alanine amino transferase (ALT) level was 2.3±1.03 folds of upper level of normal, aspartate amino transferase (AST) was 2.3±1.03 folds of upper level of normal (4±2.9), alkaline phosphatase was 2.1 ±1.04 folds of upper level of normal for age, gamma gluatmyl transpeptidase (GGT) was 15±3.7 folds of upper normal for age, with mean prothrombin concentration of 70±49.6 seconds.

All enrolled mothers had normal levels of ALT, AST, alkaline phosphatase, GGT, total and direst bilirubin, and normal prothrombin time and concentration.

GSTM1 Polymorphism:

All neonates and infants had null GSTM1 mutation concordant with homozygous allele deficiency and all mothers had only one allele affection, concordant with heterozygous expression of GSTM1.

No correlations were computed because of constant genetic null GSTM1 in all enrolled subjects and heterozygous expression in their respective mothers.

Discussion

All enrolled neonates and infants with EHBA demonstrated null type GSTM1 gene mutation, which is different from the reported 54% percent null GSTM1 expression in children with EHBA who received liver transplantation reported by Carcillo and coworkers from University of Pittsburgh [18]. It might be due to a heterogeneity of EHBA aetiology i.e., different etiologies ending in bile duct damage and biliary atresia. The involvement pattern in our studied infants and the consistent constant heterozygous expression in mothers, suggest a susceptibility to oxidative stress in infants with EHBA. The findings suggest that

EHBA is a developmental defect, and that heterozygous GSTM1 mutation was not associated with hepatic affection in mothers. The recent published work demonstrated that also GST Pi and p53 are disrupted in EHBA **[5]**. Evidence points to a defect of regeneration in EHBA, where infants get immune mediated bile duct damage followed by regeneration with defective structure with lack of ontogeny respected regeneration **[5,10]**.

Nevertheless, role of oxidative stress and over expression of CD14+ highlight role of bacteria lipopolysaccaride in mediating bile duct injury **[10]**. This highlights a possible role of oxidant stress induced bile duct damage, in the susceptible infants with GSTM1 null mutation.

The null GSTM1 mutation in our studied EHBA population provides explanation to toxic effects of ursodeoxycholic acid in children with EHBA who fail to process phase II detoxification **[19]**.

Conclusion:

This work provides evidence to support that EHBA is a multiple hit developmental defect associated with regeneration that ignores respecting ontogeny. The susceptibility to EHBA seems to be conferred through a null GSTM1 mutation concordant with homozygous allele deficiency transmitted in an autosomal recessive fashion from mothers expressing a single affected allele.

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الملخص العربى

الخلفية العلمية: مرض انسداد القنوات المرارية خارج الكبد مرض مزمن متصاعد فى الرضع غير معلوم السبب. تلف القنوات المرارية فى هذا المرض دائما ما يكون ما ينطوى علي انزيم الإلاستيز الصادر من خلايا النيوتروفيل، ودرجات متفاوتة من التليف، وخلايا الوحيدات البيضاء الحاملة لمجموعة التمايز ١٤+ ذات درجات متفاوتة في قبول ضبغة الانسجة، و بروتينن "ب" ٥٣ معيب، وينطوى كذلك على انزيم جلوتاثيون الناقل للكبريت من فصيل "بى" معيب. عائلة انزيمات جلوتاثيون الناقل للكبريت عائلة كبيرة ومسئولة عن إزالة مجموعة كبيرة من السموم التى تؤثر على تكاثر الخلية، وتؤثر علي سلامة الحمض النووى، وتشمل عائلة انزيمات جلوتاثيون الناقل للكبريت على انزيم من فصيل "ميو١".

الهدف من البحث: دراسة تعدد اشكال الجين المسئول عن الانزيم جلوتاثيون الناقل للكبريت من فصيل "ميو" فى الرضع مرضى انسداد القنوات المرارية خارج الكبد.

المواد والأساليب: قمنا بالتنميط الجيبنى للانزيم جلوتاثيون الناقل للكبريت من فصيل "ميو١" فى عينات من ٤١ رضيع من مرضى إنسداد القنوات المرارية خارج الكبد وامهاتهم. الدراسة بدأت فى يوليو ٢٠٠١ و انتهت فى يوليو ٢٠٠٤ بمستشفى الاطفال الجامعى - كلية الطب - جامعة القاهرة.

النتائج: التنميط الجيبنى للانزيم جلوتاثيون الناقل للكبريت من فصيل "ميو١" في جميع عينات الرضع كانت سلبية لتعدد الأشكال للجينن بينما وجدنا جميع عينات أمهات هؤلاء الرضع متخالفة التنميط الجينى للانزيم جلوتاثيون الناقل للكبريت من فصيل "ميو".

الاستنتاج: كل الرضع مرضى انسداد القنوات المرارية خارج الكبد يعانون من تنميط جيبنى سالب للانزيم جلوتاثيون الناقل للكبريت من فصيل "ميو١"، مما يجعل الرضع عرضة لمجموعة كبيرة من السموم التى تؤثر علي تكاثر الخلية، و تؤثر على سلامة الحمض النووى. قابلية التأثر فى الرضع مرضى إنسداد القنوات المرارية خارج الكبد محددة جينيا، و تنتقل بصورة جسمية متنحية من الامهات احادية الجين للانزيم جلوتاثيون الناقل للكبريت من فصيل "ميو١". هذا العمل يؤيد ان مرض انسداد القنوات المرارية خارج الكبد للرضع يمثل عيب نشوء.