

Aflatoxins in Infants with Extrahepatic Biliary Atresia

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Abstract

Background: Aflatoxin B 1 induced hepatitis was reported to be associated with characteristic features of centrilobular scarring, hepatic venous occlusion, ductular proliferation and cholestasis, focal syncytial giant cell transformation of hepatocytes, and pericellular fibrosis which is congruent to biopsy findings in extra hepatic biliary atresia (EHBA).

Aim of Work: Is to study aflatoxins B1, B2, M1 and M2 in infants with EHBA.

Material and Methods: Aflatoxins B1, B2, M1 and M2 were analyzed in sera and post-Kasai portoenterostomy sacrificed portahepatis liver tissue of 24 neonates and infants with EHBA, sera and breast milk of their mothers. Levels were compared to 17 infants with idiopathic neonatal hepatitis and their mothers' sera and milk. Two-dimensional thin layer chromatography and high performance liquid chromatography were employed for assessment of aflatoxins. Study commenced by July, 2001 and ended July, 2004, in New Children Hospital, Cairo University.

Results: All infants with EHBA were exclusively breast fed. All post-portoenterostomy cores were loaded with aflatoxins B1, (mean \pm SD=2.88 \pm 0.88 ppb), and only 2 had B2 (mean \pm SD=2.58 \pm 0.63 ppb). Their serum contained higher levels of B 1 (mean \pm SD=3.8 \pm 1.73 ppb) ($p=0.02$). All their mothers had aflatoxin B 1 in their sera (mean \pm SD=5.6 \pm 6.9 ppb) and aflatoxin M1 in their expressed breast milk (mean \pm SD=1.6 \pm 1.02 ppb), and their aspartate and alanine transaminases, serum bilirubin levels, albumin and prothrombin time were within normal range. None of the idiopathic neonatal hepatitis group had any detectable aflatoxins B1, B2, or M1 in their sera or in their mothers' sera or milk. Aflatoxin M2 was not detected in any of studied infants' sera, or their mothers or their milk. Infants with aflatoxin B2 had worse outcome.

Conclusion: Porta hepatis adjacent liver tissue was heavily loaded with aflatoxin B 1 in all infants with EHBA. Though EHBA infants and their mothers had very high blood levels of aflatoxin B1, mothers did not have any symptoms. Only two infants had elevated aflatoxin B2 levels. Cytotoxic aflatoxin M1 was transmitted to infants through breast milk.

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Key Words: Extrahepatic biliary atresia – Glutathione S transferase – GSTM1 polymorphism – Aflatoxin B1 – Aflatoxin B2 – Aflatoxin M1 – Aflatoxin M2 – Cholangiopathy.

Introduction

AFLATOXINS are a group of highly substituted coumarins that are acute coagulants [1]. Aflatoxins are abundant in nature, of them aflatoxin B 1 and B2 contaminate food of humans, animals and poultry. Aflatoxin M1 and M2 are the cytopathic metabolites of aflatoxin B 1 and of aflatoxin B2 that are expressed in milk [2]. Epidemic of toxic hepatitis in India in 1978 of aflatoxin B 1 was associated with characteristic features of centrilobular scarring, hepatic venous occlusion, ductular proliferation and cholestasis, focal syncytial giant cell transformation of hepatocytes, and pericellular fibrosis [3]. The same pathology findings are present in livers of infants with extrahepatic biliary atresia (EHBA) [4]. EHBA is characterized by destructive 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 [5]. The causative agent is unknown, but the inflammatory cascade is immune mediated [4,6-9].

This work aimed to study aflatoxins B1, B2, M 1 and M2 in EHBA.

Subjects and Methods

Twenty-four 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.

Aflatoxins B 1, B2, M 1 and M2 were analyzed in sera and in the sacrificed portahepatis liver tissue at Kasai portoenterostomy of infants with EHBA.

Sera and breast milk of their mothers were studied as well. Levels were compared to 17 infants with idiopathic neonatal hepatitis and their mothers' sera and milk.

Diagnosis of EHBA relied upon clinical picture, specific percutaneous liver biopsy findings [4], and operative findings, and diagnosis of idiopathic neonatal hepatitis relied upon exclusion of causes of neonatal hepatitis [10]. Outcome grading was according to Grosfeld et al., (1989) [11].

Analysis for aflatoxins:

Each liver tissue specimen was divided in two and each half was tested twice to verify and reproduce the results. The tissue was homogenized with sodium chloride solution, then centrifuged and conditioned. Aflatoxins extraction and clean up was performed for liver tissue, sera [12] and milk [1]. Two-dimensional thin layer chromatography was used to detect and determine aflatoxins [13]. Confirmation by formation of aflatoxin hemiacetal with sulfuric acid [14,15] was followed by high performance liquid chromatography quantitative determination using water: Methanol: Acetonitrile (67: 20: 13, v/v) solvent system [16]. Levels were expressed as part per billion (ppb).

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 χ^2 tests for non parametric data) were employed.

Results

The enrolled 24 infants suffering from EHBA had undergone Kasai portoenterostomy at a mean age \pm standard deviation (SD) of 82 ± 21 days, (range 47-132 days), all had non-correctable type of EHBA. 12 (50%) were products of consanguineous marriages. None had history of similar family member affection. They were all exclusively breast fed. Apart from statistically significant higher gamma glutamyl transpeptidase ($p=0.000$), there was no statistical difference among studied infants as regards their serum bilirubin, transaminases, and prothrombin concentration (Table 1).

Aflatoxin levels:

All post-portoenterostomy cores were loaded with aflatoxins B1, (mean \pm SD= 2.88 ± 0.88 ppb), and only 2 had B2 (mean \pm SD= 2.58 ± 0.63 ppb). Their serum contained higher levels of B 1 (mean \pm SD= 3.8 ± 1.73 ppb) ($p=0.02$). Their mothers had

aflatoxin B 1 in their sera (mean \pm SD= 5.6 ± 6.9 ppb) and aflatoxin M1 in their expressed breast milk (mean \pm SD= 1.6 ± 1.02 ppb), and their aspartate and alanine transaminases, serum bilirubin levels, albumin and prothrombin time were within normal range. None of the idiopathic neonatal hepatitis group had any detectable aflatoxins B1, B2, M1 or M2 in their sera or in their mothers' sera or milk. Aflatoxin M2 was not detected in any of studied infants' sera, or their mothers or their milk.

Outcome:

Among the EHBA group after 18 months of follow-up, none had cleared their jaundice and had good bile flow (successful outcome), 8 (33.3%) had persistent jaundice, moderate bile flow and stable disease (improved outcome), 4 (16.7%) had no bile flow and progressive disease (failed outcome), and 12 (50%) died within 18 months of operative intervention (mean \pm SD= 3.49 ± 6.4 months, range=0.3-18 months). Outcome severity did not correlate with level of aflatoxins in blood or liver tissue ($p=0.54$). The 2 infants with aflatoxin B2 suffered from death within 18 months of operative intervention. Among those with idiopathic neonatal hepatitis, cholestasis resolved in 12 (70.5%) completely, while 5 (29.4%) did not, where 2 had improved outcome and 3 suffered failed outcome with progressive course.

Table (1): Serum bilirubin, transaminases, gamma glutamyl transpeptidase and prothrombin concentration in studied infants.

	EHBA 24 infants	INH 17 infants	<i>p</i> value
Total bilirubin (mg%)	13.3 \pm 3.4	11.6 \pm 2.9	0.15
Direct bilirubin (mg%)	9.6 \pm 2.9	8.5 \pm 1.9	0.089
ALT folds of upper range of normal	3.1 \pm 0.72	3.25 \pm 0.92	0.63
AST folds of upper range of normal	4 \pm 1.22	4.12 \pm 1.2	0.70
GGT folds of upper range of normal	14.33 \pm 3.6	5.9 \pm 2.8	0.000*
Prothrombin concentration in %	73.2 \pm 9.2	63.8 \pm 16.5	0.067

EHBA = Extrahepatic biliary atresia.
INH = Idiopathic neonatal hepatitis.
ALT = Alanine aminotransferase.
AST = Aspartate aminotransferase.
GGT = Gamma glutamyl transpeptidase.

Discussion

All infants with EHBA had elevated levels of aflatoxin B 1 in liver tissue and sera, two infants had elevated levels of aflatoxin B2, and all their mothers had elevated aflatoxin B 1 in sera, and aflatoxin M1 in their milk. None of the idiopathic

neonatal hepatitis infants or their mothers had any detectable levels of aflatoxins in their sera or breast milk. Aflatoxin M2 was not detected in any of studied infants' sera, or their mothers or their milk.

Given that aflatoxins B 1 are only transmitted from eating food contaminated by aflatoxins [1-3], and all infants with EHBA enrolled in this study were exclusively breast fed, the aflatoxins must have been transmitted through the placenta from the mothers to their fetuses [17,18]. If aflatoxins were responsible for the damaged livers why were livers of the mothers spared? And why do infants with EHBA develop cholestasis late and not at birth or immediately after birth? If aflatoxins were responsible for extra damage, why were both groups matched as regards the serum bilirubin and transaminases? Are aflatoxins responsible for raised gamma glutamyle transpeptidase in EHBA? If so why were mothers spared?

Aflatoxins B1 and B2 have a relatively short half-life [19], and when metabolized by cytochrome P 1 A2, they are converted to M1 and M2 which are water soluble and expressed in urine and milk [20]. Aflatoxin M 1 is evanescent with a half life of 15min [21]. The reports describing aflatoxin M1 in breast milk, reported that the infants who received this breast milk with aflatoxin M1 were free of liver disease, albeit strong association with stunting and wasting [22].

Moreover, aflatoxin M1 and M2 are cytopathic under specific conditions [21]. Mothers did not seem to be affected with the elevated levels of aflatoxin B 1 in their sera or M 1. Our results support the assumption that aflatoxin M1 in the breast milk that the infants with EHBA feed might be responsible for the repeated attacks of cholangitis, even after timely Kasai, yet the reports of infants receiving breast milk that contains aflatoxin M1 without having liver affection is a strong argument against this assumption as such [22].

In Conclusion: Porta hepatis adjacent liver tissue was found heavily loaded with aflatoxin B1 in all infants with EHBA. Though EHBA infants and their mothers had very high blood levels of aflatoxin B1, mothers did not have any symptoms. Cytotoxic aflatoxin M1 was transmitted to infants through breast milk. We report that neither sera of infants with idiopathic neonatal hepatitis nor their mothers contained aflatoxins B1 or B2.

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الملخص العربي

الخلفية العلمية: سموم افلاتوكسين "ب ١" تؤدي الى التهاب كبدي له صفات منها: وجود ندبات بالمناطق المركزية للفصيص الكبدي، وانسداد الوريد الكبدي، وتكاثر القنوات المرارية، وركور للصفراء، وتحول نسيجي يؤدي في خلايا الكبد الى خلايا كبدية عملاقة، وتليف في محيط الخلايا الكبدية. وهي نفس التغييرات الكبدية النسيجية المصاحبة لمرض انسداد القنوات المرارية خارج الكبد في الرضع.

الهدف من البحث: دراسة سموم الافلاتوكسينات "ب ١" و "ب ٢" و "م ١" و "م ٢" في الرضع مرضى انسداد القنوات المرارية خارج الكبد.

المواد والأساليب: قمنا بقياس سموم الافلاتوكسينات "ب ١" و "ب ٢" و "م ١" و "م ٢" في دماء و العينات الكبدية التي تم استئصالها من بوابة الكبد من ٤٢ من الرضع مرضى انسداد القنوات المرارية خارج الكبد أثناء جراحة شق بوابة الكبد. وفي دماء ولبن الوالدات لذات الرضع، وتم مقارنة النتائج لعينات من رضع يعانون من مرض التهاب كبد الرضع ومن امهاتهم ولبن الوالدات لذات الرضع. وتم استخدام البيان اللوني دقيق الطبقات ثنائي الابعاد والبيان اللوني عالي السيولة لقياس تلك السموم.

الدراسة بدأت في اكتوبر ١٩٩٩ وانتهت في يوليو ٢٠٠٢ بمستشفى الاطفال الجامعي - كلية الطب - جامعة القاهرة.

النتائج: تلقى جميع الاطفال الرضع مرضى انسداد القنوات المرارية خارج الكبد رضاعة طبيعية من الأم حصريا. أثبت التحليل تواجد سموم الافلاتوكسينات "ب ١" في كل عينات الدماء والعيينات الكبدية التي تم استئصالها من بوابة الكبد من ٤٢ من الرضع مرضى انسداد القنوات المرارية خارج الكبد، وتواجد سموم الافلاتوكسينات "ب ٢" في العينات الكبدية التي تم استئصالها من بوابة الكبد من ٢ من الرضع مرضى انسداد القنوات المرارية خارج الكبد. كما تواجدت سموم الافلاتوكسينات "ب ١" في دماء امهات جميع ال ٤٢ رضيع مرضى انسداد القنوات المرارية خارج الكبد وسموم الافلاتوكسينات "م ١" في البانهن. بينما لم توجد أي سموم الافلاتوكسينات "ب ١" و "ب ٢" و "م ١" و "م ٢" في دماء الرضع الذين يعانون من مرض التهاب كبد الرضع أو امهاتهم أو البانهن.

الاستنتاج: العينات الكبدية التي تم استئصالها من بوابة الكبد من الرضع مرضى انسداد القنوات المرارية خارج الكبد أثناء جراحة شق بوابة الكبد كانت محملة بسموم الافلاتوكسينات "ب ١". بالرغم من وجود كميات كبيرة من سموم الافلاتوكسينات "ب ١" في الرضع مرضى انسداد القنوات المرارية خارج الكبد وامهاتهم الا ان الامهات لم يكن يعانين من اي اعراض للمرض. سموم افلاتوكسين "م ١" تعبر الى الرضع عبر الرضاعة الطبيعية من الأم.