Hepatocellular carcinoma and impact of aflatoxin difuranocoumarin derivative system – A case report

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SUMMARY

Introduction Hepatocellular carcinoma (HCC) is the most frequent type of liver malignancy. As a carcinogen, aflatoxin B1 (AFB1) causes HCC by inducing deoxyribonucleic acid adducts that lead to genetic changes in liver cells and may be the cause of HCC in up to 30% of cases. The incidence of HCC has been on the rise and is an issue in the countries of the Western Balkans.

Case Outline This paper presents a case of a 37-year-old woman who was diagnosed with HCC, without hepatitis B, hepatitis C, or liver cirrhosis. The patient consumed milk and dairy products in quantities of over two liters per day over the course of 20 years, which indicates the impact of aflatoxin in milk on HCC. A positive signal for the presence of AFB1 was detected by ELISA (enzyme-linked immunosorbent assay) in-house using immunoperoxidase screening test.

Conclusion As carcinogenic difuranocoumarin derivative, aflatoxin B1 is the most likely cause of malignant transformation of hepatocytes, which resulted in hepatocellular carcinoma in this patient.

Keywords: aflatoxin molecule; hepatocarcinogenic; hepatocellular carcinoma; Vojvodina

INTRODUCTION

Hepatocellular cancer (HCC) is the most frequent liver cancer, causing the mortality in men and women with ≈500,000 new cases per year and >600,000 deaths annually [1]. Hepatitis B virus and hepatitis C virus infection, aflatoxin B1 exposure, iron, and arsenic are the most frequent of all registered chemical stressors. Regions of developing countries that have increased exposure to aflatoxin correspond with regions where HCC incidence is most evident. There are four major aflatoxins, namely B1, B2, G1, and G2, and two additional monohydroxilated metabolic products, M1 and M2, which are found in milk and dairy products. AFB1 is the emerging mycotoxin produced by *Aspergillus flavus* and *Aspergillus parasiticus*. The fungal mycotoxin, AFB1, can be found in agricultural products such as maize, cereals, rice, and dried fruits that are stored in hot and humid conditions [2, 3, 4]. Reports from epidemiological studies have demonstrated that AFB1 is the most hepatocarcinogenic mycotoxin especially for HCC [5].

The aim of this paper is to present a patient with HCC and positive anamnesis of a milk lover, absence of viral etiological factors for HCC, and evidence of AFB1 as the most significant factor in etiopathogenesis of hepatocellular carcinoma in Vojvodina, an agricultural region where the presence of AFB1 has been proven in both animal feed and human bodily fluids.

CASE REPORT

A Caucasian woman, age 37, graphic designer, married, with two children, with no previous surgery, had a traffic trauma, which is why she was referred for upper abdomen ultrasound. The ultrasound registered a tumour mass in the right liver lobe, which had over 10 cm in diameter. After that, computed tomography scan and magnetic resonance (MR) imaging gave the diagnosis of primary liver tumour (Figure 1), which was compressing the right and middle hepatic veins. Other laboratory parameters showed slightly elevated hepatic enzymes with normal bilirubin levels. There were no hepatitis antibodies or elevated alpha-fetoprotein (AFP). The patient was in good physical condition, bicycle driver and hiker. The only suggestion she gave in medical interview before the operation was that she consumed over two liters of milk and other dairy products (yoghurt and white cheese) per day over the course of 20 years. Simplified methods of ELISA in-house testing using immunoperoxidase screening test detected a positive signal for the presence of AFB1 in the patient's serum in 12 repetitions. In addition, the same simplified screening methodology of ELISA did not show a positive signal for the viral hepatitis B and C. The patient was operated on in January 2012, when right hepatectomy was performed on non-cirrhotic liver. Pathology revealed a hepatocellular carcinoma and immunohistochemistry confirmed with CD34 that liver sinusoids in the tumour mass

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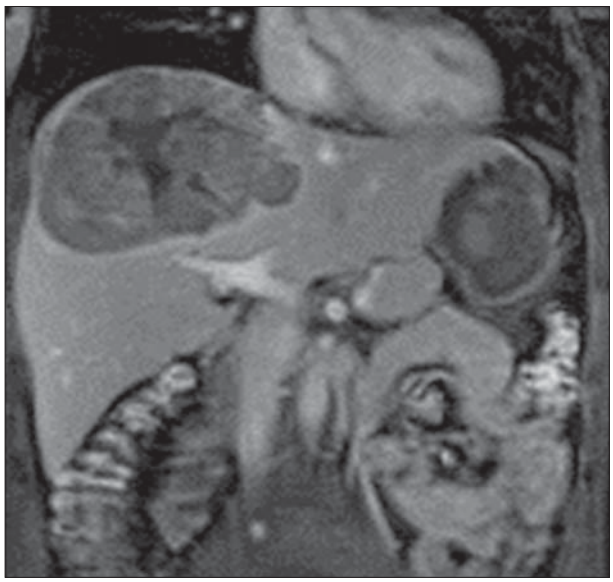

Figure 1. Initial MR imaging, performed on a 3T machine: coronal post-contrast T1W image shows a hypovascular mass that is dominantly hypointense with low signal intensity in the central portion of the tumour, which correlates to necrosis

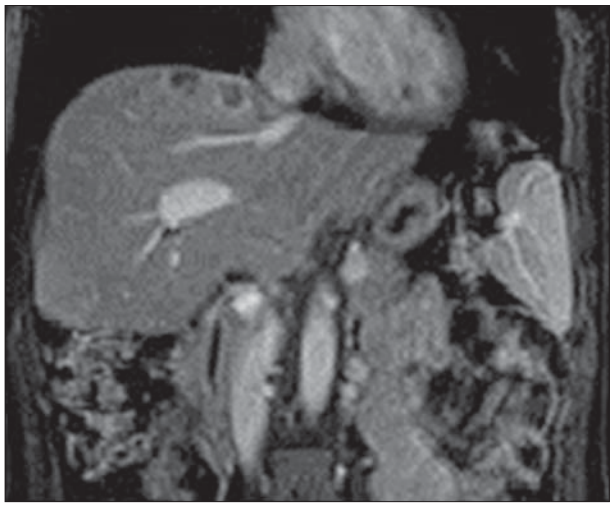

Figure 2. Liver MR imaging performed 13 months after the surgery (on the same 3T scanner): coronal post-contrast T1W image demonstrates multifocal tumour recurrence, two hypointense nodes with rim enhancement

were positive, whereas in the non-tumorous liver tissue (on the resection margin) they were negative. Nine months after the operation there were no new HCC in the rest of the liver. Thirteen months later, there were two new liver nodules on MR imaging (Figure 2), and the second operation was done with resection of the HCC metastases, as well as one diaphragm metastasis. In the second year after this operation, a new tumour mass developed in the rest of the liver, and radiofrequency ablation was performed. The patient died two years after the second operation.

DISCUSSION

The International Agency for Research on Cancer (IARC) classifies AFB1 as a carcinogenic substance within the first group of carcinogens for HCC. In April 2004, Lewis et al. [6] studied contamination of maize on the market and found that 55% of the maize was contaminated by AFB1. The association between aflatoxin levels in the maize found on the market and an aflatoxicosis outbreak was confirmed. The study focused on detecting AFM1 in cow, goat, and donkey milk, different dairy products, complete feed mixtures for dairy cattle, as well as meat and eggs [7].

Depending on the induction of cancer, mutagens are classified as genotoxic and non-genotoxic molecules. Genotoxic molecules are microcomponents of nutrition, such as aflatoxins, which cause genetic alterations. AFB1 may induce cancer, and the target organ that metabolizes food contaminated with aflatoxins is the liver. Aflatoxins, especially AFB1, may be metabolized by cytochrome-P450 enzymes to a highly nucleophilic-reactive genotoxic intermediate (AFBO) or hydroxylated to two isomers (AFM1 and AFQ1) and a demethylated (AFP1) metabolite. Unstable reactive intermediate AFBO forms adduct to DNA with strong covalent bonds, resulting in AFB1-guanine molecular system or in forming of AFB1-albumin and other protein adducts. AFB1-guanine adducts induce the p53 gene mutation, which is responsible for carcinogenic effects. Another way for causing the aflatoxicosis is binding the AFBO to amino acids [8].

Approximately more than four billion of the world's population is exposed to environment contaminated by aflatoxins through the food chain, particularly in developing countries [1]. Kew [9] has published data on the daily intake of maize (g/person/day) in Eastern Europe. In Vojvodina, there is no systematic or regular monitoring of aflatoxin in agricultural products, milk, and dairy products. There is also no biomonitoring of human milk, serum, and liver tissue. This region has no data on the impact of AFB1 and AFM1 on HCC. This paper presents a case report of a possible carcinogenic effect of AFB1 on the development of HCC in the agricultural region of Vojvodina. The patient was a woman, age 37, who suffered from HCC, without hepatitis B, hepatitis C, or liver cirrhosis, but who consumed milk and dairy products in quantities of over two liters per day over the course of 20 years.

The results obtained by Spirić et al. [3] show a weak contamination of 54 analyzed cheese samples from the market. In 13% of all analyzed samples, the level of AFM1 contamination was above the adopted limit of 0.25 mg/kg [4]. Tomašević et al. [8] detected AFM1 in milk and dairy products from the same market and found that in more than 30% of analyzed samples, the levels exceeded the EU's maximum residue limit. In order to assess carcinogenic effects of AFB1, the authors suggest routine analyses of aflatoxin metabolites, DNA and protein adducts in the blood, tissues, and urine in patients with HCC [9]. There are two methods for detecting only AFB1 with sophisticated equipment (ultra-high pressure liquid chromatography tandem mass spectrometry – UHPLC-MS/MS) or common detection by an in-house ELISA and in-house immunoperoxidase test. AFB1 detection in serum specimens was performed using an in-house ELISA, developed

doi: 10.2298/SARH1612661I

as per standard procedures with slight modifications, in which the peroxidase-conjugated anti-AFB1 was used at a concentration of 1:1,000 [10].

The impact of aflatoxin alone on HCC needs to be examined in patients who have neither hepatitis B, hepatitis C, nor liver cirrhosis, with the aim of finding new antagonists for preventing and minimizing the genotoxic effect of aflatoxin in liver tissue.

The described case of a patient with diagnosed and surgically treated HCC, as well as the outcome characteristic for this illness where, aside from the presence of AFB1 in the blood, no viral etiological factors had been proven, indicates that in rural regions where the exposure to carcinogens is increased, all patients with HCC need to be tested for AFB1 in bodily fluids. Legislation must follow clinical observations.

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Хепатоцелуларни карцином и утицај дифуранокумаринског система афлатоксина – приказ случаја

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КРАТАК САДРЖАЈ

Увод Хепатоцелуларни карцином (ХЦК) најчешћи је тип малигнитета јетре. Афлатоксин (АФБ1), као канцерогено једињење, изазива ХЦК индуковањем дезоксирибонуклеинских продуката, који доводе до генетских промена у ћелијама јетре, и може изазвати до 30% случаја ХЦК. Учесталост хепатоцелуларног карцинома расте и представља проблем у земљама Западног Балкана.

Приказ болесника У овом раду је приказан случај младе жене, старости 37 година, која је оболела од ХЦК, без присуства хепатитиса Б, хепатитиса Ц и без цирозе јетре. Пацијенткиња је конзумирала млеко и млечне производе у количинама преко два литра дневно током 20 година, што указује на утицај афлатоксина у млеку на ХЦК. Позитиван сигнал на присуство АФБ1 је детектован помоћу *ELISA* (имуноензимски тест високе осетљивости) применом имунопероксидаза „скрининг" процедуре.

Закључак Афлатоксин, као канцерогени дифуранокумаринski дериват, највероватније је проузроковао малигну трансформацију хепатоцита и довео до настанка хепатоцелуларног карцинома јетре код ове пацијенткиње.

Кључне речи: молекул афлатоксина; хепатокарциногени; хепатоцелуларни карцином; Војводина

Примљен • Received: **Ревизија • Revision:** **Прихваћен • Accepted:**