Occurrence of Aflatoxin M_1 in Human Milk Investigated in Karachi, Pakistan

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ABSTRACT

Aflatoxins are strong toxic and cancer producing substance which can be excreted in lactating humans in the form of aflatoxin M_1 (AFM_1), exposed to food contaminated with mycotoxins. In the current study, breast milk samples of 62 lactating mothers from two well renowned hospitals at Karachi, were evaluated for AFM_1 by High Performance Liquid Chromatography (HPLC). AFM_1 was detected in 10 out of 27 samples collected through Civil Hospital (CH) and 11 out of 35 from Jinnah Postgraduate Medical Centre (JPMC). In human breast milk sample collected from Civil Hospital, AFM_1 was detected in a range of 28pgmL^{-1} to 71pgmL^{-1}, and in human breast sample from JPMC level of AFM_1 was ranging from 27pgmL-1to 970pgmL^{-1}. The levels of AFM_1 in human breast milk detected in the period of studies was found to be frequent (Correlation coefficient (r^2= 0.97), but at were limited to a lower level. The levels were found to be lesser than that of FDA limit of 0.05mgkg^{-1}. Development and improvement of the newborn child is quick and hence it is conceivable that exposure of AFM_1 through human breast milk contains a noteworthy health impacts. Current study helps to carry out further study to determine the possible sources of exposure of aflatoxin in the women in future.

Keywords: Aflatoxin M_1, High Performance Liquid Chromatography (HPLC), Human Breast Milk, Toxicity.

INTRODUCTION

Human breast milk is believed to be the perfect nourishing food for the growth of newborn [1]. However, alongwith healthful and immunologically advantageous components, some carcinogenic substances such as Aflatoxin M1 (AFM1) has also been observed in human milk [2-3]. Aflatoxins M_1 has also been detected in cow and Buffalo milk and reported earlier [4]. Several countries have regulated AFM_1 level in milk and milk products whereas the limit 0.05 mgkg^{-1} set by European Union is considered to be the lowest in the world [5]. Various studies to identify the pre-natal and post-natal health effect on human by this carcinogenic substance have earlier been reported [6-7]. Infants have a slower rate of metabolizing carcinogens than that in adults; causing long circulation period of the substance [1].

Recent reports have already indicated the presence of AFM_1 in dairy milk and human milk in different countries [2, 4, 7-11]. AFM_1 has been observed in the breast milk of lactating females of various parts of the world and it has become very important to understand that lactating mothers can be a potent source of AFM1 exposure to the infant [6-7, 12-13]. In the earlier studies it is observed that growth pattern of fetus to infant influence the risk to health in future life [14-18].

Keeping in view significant health hazards of aflatoxins, the aim and purpose of current study was to estimate the level of aflatoxin in human breast milk sample collected through two renowned hospitals of Karachi. The expected results of this study will highlight the occurrence of aflatoxin in human breast milk and danger of its presence for mother and child both. The data of the current study helps to carry out further study to indicate the possible sources of exposure of aflatoxin in the Pakistani women.

MATERIALS AND METHOD

Chemicals and Reagents

HPLC grade Methanol and acetonitrile (99.9%) were used for analysis. Standards of Aflatoxin M_1 (analytical grade) were stored at 4oC prior to use.

Data Collection

Before donating breast milk sample, every volunteer mother signed up a consent letter for providing her breast milk for analysis which includes; permission to take part in the research project, and to the use the volunteer's information. Volunteers filled up a set of printed questions, devised for the purposes of the statistical study of food intake including dried fruits and peanuts, fish, grain, meat, milk or milk products, legumes and vegetable oil. Employment status, total income, education, area of residence, and other data including age, health status and medication were also recorded. Mothers' and infants' height and mass (at the time of delivery and at the time of current study) were also obtained from the hospitals. In both of the hospitals patients appear from poor vicinity i.e. Lower Class (LC) and Lower Middle Class (LMC) of Karachi or various parts of interior Sindh. Most of the lactating women included in the study were born and residing in Karachi, Pakistan.

Sample Collection

A total of 62 milk samples were collected from two different hospitals (located at the central area of Karachi) 27 from Civil Hospital (CH) and 35 from Jinnah Post Graduate Medical Centre (JPMC). The samples were collected by self-expression of volunteer mothers approached through the nurses and paramedical staff working in the hospital. The samples were collected for a period of three months, i.e. three months after the start of nursing.

Sterile plastic containers were used to collect breast milk before feeding the infants. The samples were maintained at 4oC before extraction. 10mL of each breast milk sample was heated to 37oC with constant shaking and then it was centrifuged with a speed 3000 rpm for 15 min at 5oC. The samples were diluted with 20mL hot (80oC) demineralized water before charging in to C18 cartridge. Before passage of diluted milk sample it was rinsed with 10mL acetonitrile and then 10mL water. Washing of cartridge was carried out with 10mL water then 10mL ammonia:acetonitrile:water (1:10:89, v/v/v) and 10mL acetic acid:acetonitrile:water (1:10:89, v/v/v).

HPLC Analysis

Reverse-phase HPLC (model LC-10ADvp solvent delivery system; auto injection, Shimadzu, Japan) C_{18} Brownlee reverse phase column (220x4.6mm, particle size 5 m) with C_{18} guard column (Perkin Elmer) was used with fluorescence detection set at 440nm emission and 360nm excitation. The mobile phase was water:acetonitrile:methanol (66:17:17, v/v/v). The oven temperature was maintained to 40oC with a flow rate of 1mL/min and injection volume for standard and sample extracts was kept 30μL. The calibration solution of AFM_1 ranging from 0.04-10 $ngmL^{-1}$ was prepared in 1 mL 2:3 vol/vol mixture of methanol and water and then it was filtered through PVDF membrane having pore size 0.45 μm. Since aflatoxins are possible carcinogen, care has always been practiced to avoid exposure and 10% sodium hypochlorite was used for decontamination. The limit of detection (LOD) of Aflatoxin M1 for the human breast milk samples was obtained as $200pg/mL^{-1}$.

Statistical Analysis

Standard deviation was estimated by using one way analysis of variance ANOVA. Calibration curves and linear regression curve showed r2 values above 0.97 indicating good linearity.

CONCLUSIONS

Aflatoxin exposure to the residents of Karachi has been estimated earlier in buffalo milk [4] and human breast milk in current study. The current study provides important evidence of contamination of aflatoxin in human breast milk, but at a very limited number of samples and in the period of study. In view of the results of current study, a detailed study is required to be carried out to reduce the hazard of aflatoxin to newborn baby through human breast milk in Karachi. It is also recommended to analyse individual exposure to aflatoxin in lactating women residing in various provinces of Pakistan. A longitudinal study to indicate the possible sources of aflatoxin exposure in the Pakistani diet may also be conducted.

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