Aflatoxin, G1, G2 and M1 Prenatal Exposure and its Sero-Dynamics amongst Pregnant Mothers in Adamawa State, North East of Nigeria

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Abstract

Introduction: Of the known mycotoxins, the most important in relation to direct hazard to human health are the aflatoxins.

Aim: to determine the level of exposure of aflatoxin from mother to child and its mode of transfer.

Patients and Method: 570 pregnant women in the labour ward of The Federal Medical Centre Yola were investigated for their aflatoxin content by using the velasco fluorotoxin meter which comprised of 89 samples of amniotic fluid, 213 of serum from maternal blood and 211 serums from neonatal cord blood; 57 of those were controls.

Results: The Aflatoxin values of G1, G2, and M2 above 20 ppb were obtained in 66 samples of the amniotic fluid (74.1%); 133 from venous maternal blood (62.4%) and 142 from neonatal cord blood (67.2%) blood.

Conclusion: This results is suggestive that aflatoxin present in maternal blood crosses the transplacental barrier and accumulates in the foetus which further explains the high concentration of aflatoxin in the amniotic fluid and the in utero exposure to these toxins.

Keyword: Aflatoxin; Transplacental; Velasco fluorotoxin; Foetus

Introduction

Aspergillus is a fungus that essentially belongs to grains storage flora. It grows optimally at 25°C with a minimum necessary water activity of 0.75. It starts to produce secondary metabolites at 10-12°C, but the most toxic ones are produced at 25°C with a water activity of 0.95 [1,2]. Those toxic secondary metabolites named aflatoxins (AF) is a group of mycotoxins produced by a large number of Aspergillus species, basically by three phylogenetically distinct sections. The main producers are A. flavus, and A. parasiticus, but it has been demonstrated that A. nomius, A. pseudotamarii, A. parvisclerotigenus, and A. bombycis of section Flavi, A. ochraceoroseus and A. rambellii from section Ochraceorosei and Emericella astellata and E. venezuelensis from Nidulatans section also generate aflatoxins [3-5]. All of them contaminate a large fraction of the world’s food, including maize, rice, sorghum, barley, rye, wheat, peanut, groundnut, soya, cottonseed, and other derivative products made from these primary feedstuffs in low-income countries [4,6,7]. Although aflatoxins have been a problem throughout history, until 1960 they have been recognized as significant contaminants within agriculture, because in this year they were initially isolated and identified as the causative toxins in “Turkey-X-disease” after 100,000 turkeys died in England from an acute necrosis of the liver and hyperplasia of the bile duct after consuming groundnuts infected with Aspergillus flavus [8-10].

Mycotoxins have been the subject of intense research efforts [10,11]. All fungi, including Aspergillu produce a large number of metabolites which are divided arbitrarily into primary and secondary metabolites [8]. Primary metabolites are those which are concerned with the fundamental life processes of the organism while secondary metabolites cover the remaining compounds for which there is no such function. Many of the secondary metabolites are of complex chemical composition and are extremely toxic to other forms of life. E.g. the antibiotics and aflatoxins which is the subject of this work. Of the known mycotoxins, the most important in relation to direct hazard to human health are the aflatoxins [2,12].

Materials and Method

Sample Collection

Serum

Whole blood samples of 3-5 ml were collected from pregnant women in the labour ward either before or after delivery. The venous maternal blood was taken using disposable syringes, and collected in universal bottles for centrifugation. Cord blood sample of 10 ml was collected into universal blood containers directly from the umbilical cord immediately after delivery. Serum was collected using a drawn-out pipette after centrifugation with a bench centrifuge at 5000 rpm for 5 mins. The sera were frozen and analyzed later.
Amniotic fluid

Amniotic fluid was collected in a sterile bed pan by either artificial or natural rupture of the membrane immediately before birth. If membrane ruptures before patient reaches labour ward, residual fluid is collected after birth. If badly stained with meconium it is discarded. Hence amniotic fluid was not got from all the mothers, especially in cases of old still birth. Amniotic fluid in sterile bed pan was then poured into McCartney bottles and kept frozen till analysis.

Reagents

Aflatoxin G1, G2 and M2 mixed standards (RIVM Bithoven, Netherlands). Aflatoxin G1, G2, and M2 (makor chemicals. Jerusalem. Isolates). Aflatoxin G1, G2, and M2 were used for the detection of aflatoxin in serum and amniotic fluid. Trichloroacetic acid (BDH), Alumina-Woelm Pharma (GMBH Co. Eschevege), Florisil-BDH (about 60-100 U.S. mesh), Sea sand-Fisher Scientific Co. N.J. U.S.A.) Silicar Gel-Carlo Erba milano (Omm 0.05-s-20), Methanol-M and M Ltd. England.

Preparation of Microcolumn for velasco fluorotoxin meter

One end of the column was plugged not too tightly with glass wool 2-3 mm in depth. At the top of the column was placed a small funnel fitted with plastic tip. 5-7 mm in depth of sand was added using the scoop. A layer of florisil was then added, of depth not more than 5-7 mm. Another layer of sand 5-7 mm was again added. Next was added, the silica gel about 15 mm in depth. Finally a layer of neutral alumina was added about 15 mm in depth.

Development of Aflatoxin on Microcolumn

The prepared microcolumn was wetted with chloroform. This was done by lowering the bottom of the column into a vial containing chloroform, which was allowed to seep up through the various layers. By using a syringe, 1 ml of the sample solutions previously prepared or standard aflatoxin solutions-50 ng/ml from the vial (or bijou bottles) was transferred into the prewetted columns and allowed to drain. The drain time for 1ml of solution in the columns was about 5 mins. Pressure or vacuum was applied to reduce the drain time. A simple and effective vacuum arrangement was to place the column in the test tube, with the side arm, and to attach the small rubber bulb on the side arm as a source of vacuum, by varying the amount of pressure exerted on the bulb before attaching to the side arm the flow rate was varied. This vacuum technique became easier to control after a few runs. It was necessary to avoid very fast flow rates (i.e. 1 ml drained in less than 2 mins, with attendant loss of aflatoxin). After the solution had drained, 1 ml of chloroform-methanol (96+4) was added to the column and allowed to drain. While the column was still wet it was placed in the velascb fluorotoxin meter (VFM) instrument and the reading taken.

Calibration of Velasco Fluorotoxin Meter

The velasco fluorotoxinmeter (VFM) was calibrated using the standard microcolumns developed and a blank column. The operational procedure for the calibration was as follows:-

The VFM was plugged into an alectic source and the POWER switched moved to the ON position. The LAMPSTART push-bottom was depressed and held in this position for approximately 20 seconds then released. The lamp operation was verified by ensuring that the bluish-white glow from the hole on the rear access cover of the VFM was on. When the lamb did not start, the starting operation was repeated.

One hour was allowed for the warm-up and stabilization of the instrument. Then the blank micro column was inserted into the sample holder and the micro column cap placed over the exposed portion of the micro column. The VFM was adjusted to read zero using the ZERO control knob, and the blank micro column was removed.

A wetted standard aflatoxin microcolumn (50 ng per ml) was inserted into the sample holder and the micro column cap placed in position. The Calibration control knob was used to adjust the VFM to read 20 ppb. By alternatively using the blank and standard micro columns repeatedly (about twice each) the zero and 20 ppb readings were obtained. The 20 ppb setting was the average of two standard micro column readings. The aflatoxin standards used were G1, G2 and M2.

Estimation of Aflatoxin from samples–VFM Analysis

The estimation of aflatoxin in the serum, and amniotic fluid required that the frozen previously extracted sample solutions be defrosted.

Then, using a syringe, 1 ml of sample solution was transferred into a pre-wetted column and allowed to drain. After the sample had drained 1ml of chloroform-methanol (96:4) was applied and allowed to drain. While the column was still wet, it was placed in the VFM instrument and the reading taken. The column was turned around 180°C and the second reading was taken. The final aflatoxin reading was the average of the two.

Aflatoxin was detected by trapping it on a special layer of florisil in the microcolumn. Calculation of free aflatoxin, incorporating the dilution factor were made and expressed as ng per ml.
Discussion

A consideration of the difference in the percentage of positive detection of aflatoxin between the controls (59.6%) and other serum samples and amniotic fluid (74.1%, 62.4% and 67.2 %.) Which further implies that the controls either did not ingest high levels of Aflatoxin or that it had been eliminated from these sera through the activity of their microsomal mixed function oxidase system (MFO), which has the capacity to metabolize aflatoxin [2,8,12]. This fact therefore could account for the reduction of aflatoxin to the low levels observed in the controls. The metabolic implication is that the presence of this high percentage of aflatoxin above 20 ppb as shown in the results (appendix) of the sera and amniotic fluid, if not eliminated from the liver, might lead to accumulation and consequently toxicity, if such levels exceed the metabolic capacity of the liver.

A comparison of the percentage positive aflatoxin detection levels in Zimbabwe (31.42%); Sudan (23.2%) and Nigeria (83.64%) suggest a higher level in Nigeria than in the other countries. One of the factors contributing to this difference could be based on agriculture-farming system staple foods, storage and processing of foods. Climatic differences could also have influence on these differences.

The biochemical effects of aflatoxin have been attributed to the interaction of the compounds with DNA, RNA and proteins, also to the inhibition of protein and nucleic acid synthesis [13,14].

The impairment of nucleic acid synthesis should alter protein synthesis for example, the incorporation of 14 C-leucine into rat liver cytoplasm and an apparent loss of chromatin from the nucleus [7,17].

The findings in this investigation provide suggestive evidence that these pregnant women were exposed to aflatoxins in their diets during gestation period and that these substances can be detected in the serum of both maternal blood and neonatal cord blood and amniotic fluid, in a higher population of these women.

Circumstantial evidence of the role of aflatoxins in human disease includes epidemiological correlation between aflatoxins in food and hepatoma in Africa and Thailand, [18] severe liver disease in India after ingestion of aflatoxin-contaminated foods [12,19] and epidemiological and clinical correlations between eye's syndrome in children and dietary exposure to aflatoxin in Thailand [20,21].

These reports have confirmed vertebran observations of the vulnerability of the liver to injury by aflatoxins. Apart from the evidence concerning hepatoma, clinical evidence of aflatoxin poisoning in man is based mainly on acute pathology precipitated by short exposure to aflatoxins [5,22,23]. The two findings which call for some explanation are the growth retardation and the enhancement of the maternal liver injury both resulting from aflatoxin administration in late pregnancy. In their experiment they also reported that reduction of maternal food intake is not an adequate explanation for the foetal stunting as complete absence of dietary protein in the last 6 days of pregnancy and causes only moderate foetal growth and retardation in the rat [24,25].

Other possibilities considered included direct action of aflatoxin on the placenta or foetus or impairment of foetal nutrition secondary to the effect of the toxin on the mother. There was no evidence of placental damage in any of the pregnancy and in particular, they did not find the hemorrhages at the utero-placental junction reported by other investigators in this field.

Foetal tissues are most susceptible to the action of toxicagents or environmental alterations during the period of organogenesis (days 6-12 in the rat). Substances causing foetal growth retardation in late pregnancy would be expected to have a more severe effect in early pregnancy with resultant foetal mitotic drugs (Murphy, 1960), x-irradiation [6,8,13] and uterine ischaemia [9,16,26]. Butler's and Wigglesworth's experiments do not suggest a direct action of aflatoxin on the foetus as administration in early pregnancy failed to cause either foetal death or malformation.

Several findings support the hypothesis: that the foetal growth retardation in their experiments was secondary to the effect of aflatoxin on the mother, only when the rat was conditioned during the time of most rapid foetal weight gain (days 17-21) was foetal growth retarded. Doses of aflatoxin which failed to produce recognisable lesions in the maternal liver also failed to cause foetal stunting; where liver lesions were produced the severity of foetal stunting appeared proportional to the extent of the maternal liver injury. The single rat dosed in early pregnancy which had stunted foetuses showed evidence of persisting liver damage with widespread severe fatty infiltration [4,12,19].

There is no evidence as to the mechanism by which maternal liver injury might impair foetal growth. It is, however, known that the foetus obtains its protein requirements in the form of amino-acids and it would seem reasonable to speculate that damage to the protein synthetic mechanisms of the maternal liver might well alter the amino acid composition of the maternal plasma to the disadvantage of the foetus [19,20]. Experiments using carbon tetrachloride as a hepatotoxic agent have not caused foetal growth retardation (Wigglesworth-unpublished observation) but recovery from carbon tetrachloride liver necrosis is very much more rapid than that following aflatoxin administration.

Conclusion

The phenomenon where by aflatoxin possesses carcinogenic and toxicogenic and biochemical activities affecting the genetic apparatus in one way or the other may be obscure, but nevertheless, the fact that aflatoxins are naturally occurring environmental pollutants found intimately associated with human food and animal feed should be of great concern due to the hazards they pose to human and animal health.

The effects of these factors on the human host are generally mutually reinforcing. Most of the adverse ecologic factors which are probably of significant importance in the aetiology and pathogenesis of certain disease, flourish best in the permissive situation of extensive malnutrition.
The possibility of removing the potential carcinogen from the environment of a selected population is certainly difficult, but the progressive urbanization which is occurring in developing countries, may afford an opportunity for determining the effect of urbanization on aflatoxicosis, changes in food habits and sources of dietary staples.

In Nigeria as it is obtainable in other developing countries where fungal infestation and aflatoxin contamination of foods is very common, it is essential that the potential hazards of the problem can be recognized. The food industry and research agencies should make every effort to maintain and improve on the efficiency of the agricultural practices, adopt effective sun or mechanical drying and sound storage of foods. These would appear to offer the most effective method in the prevention of aflatoxin contamination.

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