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Aflatoxigenic Feeding and its Possible Implications After Pregnancy

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ABSTRACT

Aflatoxins are secondary metabolites produced by the fungi *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius.* It is known that they generate mycotoxins capable of causing carcinogenic, mutagenic or teratogenic damage which have been detected in corn, peanuts and grains in general. The molecular mechanism by which aflatoxins are produced comes from the teratogenic effect and inhibition of cells division at the level of metaphase. Evaluate the association between the fetus' exposure to aflatoxigenic food and risk of death or low weight at birth. A case –control study was conducted among 513 women in a postpartum situation. They were interviewed retrospectively about their diet during the first two months of pregnancy. Daily intake denotes meaningful differences between the case and control groups over junk foods and corn tortilla respectively with $p < 0.05$, and between the quality of the corn tortillas and peanuts, which apparently cause synergism to the variable: smoking during pregnancy. Poor quality tortilla consumption and junk food are apparently involved in the mortality of the fetus, it is considered as one of the possible causes of this event. Risk exists in both cases, high-risk foods are corn tortillas with filaments between them (when separating them) and the unpleasant taste of peanuts.

Key words: Aflatoxigenic food, High-risk, Low birth weight, Mortality of the product, Postpartum.

INTRODUCTION

In 1960, the death of thousands of poults was registered in England; such event happened a week after the first symptoms appeared. Necropsies revealed hemorrhages and necrotic areas in the poult's liver; later the correlation between the death of the turkeys and their consumption of peanut from Brazil was established. It is worth saying that such peanuts were invaded by *Aspergillus flavus*[1]. Aflatoxins are secondary metabolites produced by the fungi *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius*[2-4]*.* It is known that they generate mycotoxins capable of causing carcinogenic, mutagenic or teratogenic damage[3-5]. Those have been detected in corn[3], peanut products and grains in general[2].Aflatoxins; B1 (AFB1), B2 (AFB2), M (AFM), G1 (AFG1) and G2 (AFG2). The most important is the AFB1 regarding its toxigenic degree[3-5]. It was possible to detect the AFM as one of the secondary metabolites of AFB1 in blood and urine[5] and in cord blood[3-5]. It is certain that these aflatoxins have causal association with liver cancer[6].

The molecular mechanism by which aflatoxinsare producedderivatesfrom the teratogenic effectand inhibition of cell division at the level of metaphase[7]. It is known that the genotoxic effect depends on the metabolic activation of the aflatoxin B1 to cause translocation of guanine thymine. Epidemiological studies show that these cause translocation and mutation at codon 249 of the p53 tumor suppressor gene, region 22 to 25 of DNA. Thus, aflatoxigenic food intake in humans has effects (8-11). In other words, exposure to aflatoxins on one's diet is critical. Apparently,this topic has not been systematically studied in humans[8,9]. Moreover, the effects of this type of diet have not been studied as well as the effects to the fetus regarding risk to mortality, low weight at birth and teratogenic damage.

There is evidence of the effect of aflatoxins in animals, both mutagenic and carcinogenic[12]. We know for example that in acute outbreaks these can cause embryonic death, toxicity for the fetus, decrease eggs production and the sizes of these. In 1999, the FAO and WHO noted that food for human consumption must remain free of mycotoxins du to the fact that these can decrease productivity of ruminants and occasionally causedeath[13].

Several experiments have been conducted in order to diagnose the effects of aflatoxins to animals. For example, in rabbits it was found that after being administered with 100 micrograms of aflatoxins per every kg of pregnancy weight, 47% showed to have a high mortality rate. Some of the rabbits who remained alive experienced liver damage. However, those who were administered 50 micrograms per kg during 10 days had a birth weight rate of zero[14].Testicular steroid genesis has also been observed in rats[15].It has been observed that aflatoxins can also drastically affect trout[16,18] as well as humans; in 1974 there was a food poisoning outbreak where 397 cases were recorded and 106 died[11].

Risk factors for hepatocellular carcinoma[4,11,18]. Were also detected as well as aflatoxigenic milk[6, 16, 19], affecting umbilical cords[3, 17], which are the result of ingesting aflatoxigenic food during pregnancy obtaining low weight at birth as an adverse effect[19,20].Corn and other foods[21], such as fruit juice[22] represent a substrate for the growth of Aspergillus. Corn is one of the most common foods of the Mexican population; it represents one of the fundamental means for the development of aflatoxins, including cereals[2,3,23,24].

A mechanism to decrease the concentration of aflatoxins is provided by the lime, which during nixtamalization changes the pH[25,26]. Aflatoxins have a half-life in the human body among 2 to 3 months. The effects ofaflatoxins in humans have not been studied yet in a systematic way[15,16,27]. Consumption of these toxins has been associated to some of the chronic malnutrition effects in children undergoing growth; among which we find liver and kidney damage, carcinogenesis and immunosuppression[28-33],wasting and stunting have also beenimplicated in the pathogenic process of aflatoxins and[32, 34].

Aflatoxins have been detected in body fluids including urine and have been associated with malnutrition[35-36]. In the study entitled "Aflatoxins in body fluids and food of Nigerian Children with protein-energy malnutrition"[37] Several types of aflatoxins were detected in urine samples from patients and controls in varying concentrations, which later were analyzed and showed to have high levels of those (90.9%), followed by kwashiorkor (84.6%), marasmus (81.8%) and marasmus kwashiorkor (60%), in descending order. However, the observed differences in detection rates in the urine were not significant ($p > 0.05$) (37).The association between aflatoxigenic food and their impact on the fetus has not apparently been studied, which highlights the importance and meaningfulness of studying the effects of those substances on pregnant women during the first quarter of pregnancy and compare rates to fetal mortality, low birth weight and even in children who are born with apparent congenital malformations. This research was conducted in order to answer the following question. Is there an association between intake of aflatoxigenic food during the first two months of pregnancy specifically in reference to death of the fetus and low weight at birth?

Hypothesis

HI there is an association between aflatoxigenic food intake during the first two months of pregnancy, death of the product and low weight at birth.

H0 There is no association between aflatoxigenic food intake during the first two months of pregnancy, death of the product and low weight at birth.

MATERIAL AND METHODS

Study Design

A case-controlled study was conducted by analyzing the postpartum situation and conditions of women above 30 years old and who gave birth at the Gynecology and Obstetrics Civil Hospital of Guadalajara"Dr. Juan I. Menchaca", Jalisco, Mèxico where interviews were conducted.

Sample size

171 cases of product mortality were studied as well as 171 cases of low birth weight and 171 controls for the first two events. The calculation of the sample numbers was performed in 2.0 for a win episcope overcome OR2.0

Sampling

No probabilistic, it was carried out by including consecutive cases of postpartum women who were classified for having had products with low weight at birth or congenital apparent malformations. Women who consumed or not aflatoxigenic food during the first two months of pregnancy as well as postpartum women who did not have events such as death of the product, low weight at birth or apparent congenital malformations.

Validation of the measuring instrument

Application of the structured interview is based on several aspects; First of all, in regards to food history, the questions collected information about the cultural impact of nutrition, collecting data through a 24-hour recall a food frequency questionnaire and information listed elsewhere, appetite and perception of tastes and aromas, quality of foods, possible changes in the last 12 months. (Dietary Anamnesis)[30]. The dietary history should provide data with which to obtain accurate nutrient intake by the patient for an appraisal. This provides information on the long-term diet , does not require a great effort from the participants, provides information on the relative consumption of groups of individuals, and their distribution in consumption; individuals can be classified according to relative consumption levels; it is useful for retrospective investigations relatedto dietary intake and the incidence of the disease. With respect to food's intake frequency honest answers are more likely than a 24-hour recall, but these are as good as what the patient's memory is. This method is important to estimate association between chronic diseases and diet, and it is important in case-control studies[39].

The application of the interview was conducted in the presence of a second observer, the end of the interview voluntarily requested data such as: name, address and telephone number to perform validation by matching two measurements at the same address or subject via telephone was given as appropriate. To this end Test -Retest and Kappa coefficient, threw themselves employment 0.98 and 0.93 respectively.

Inclusion criteria of postpartum woman of the New Civil Hospital of Guadalajara

Inclusioncriteria.	Not inclusion criteria	Exclusion criteria
Postpartum woman in Gynecology And Obstetrics of the New Civil Hospital of Guadalajara, 30 years of Age or younger.	Postpartum woman whose state of Health is not possible for participating in the study. Maximum 30 years old.	History of preeclampsia, eclampsia (toxemia), comprehensive interview

Criteria for diagnosing exposure to dietary aflatoxigenic dependents

The measuring instrument detects the food frequency, determines the quality of aflatoxigenic foods and evaluates the possibility of bias information from the interviewee's memory. To set the exposure

criteria it is essential to consider the second aspect, which refers to the same quality of food that the mother used in order to estimate the risk such food represents. Since the majority could recall their food intake habits, bias information was minimal; 2 cases were detected for having had difficulties to remember their eating habits, while 513 could perfectly remember their first two months of pregnancy diet.

RESULTS

Regarding the food group there is a statistically significant difference between the type and frequency of foods that the mother ingested during the first two months of pregnancy in cases of death of the product in terms of consumption of oats, corn tortillas, beans and junk foods. In cases of

Table 1: Food frequency of cases and controls

N	Food Ji² (*p < 0.05)	Controls	%	Cases Mortalities	%	Cases of low weight at birth	%
1	Rice	161	94.2	156	91.2	157	91.8
2	Oat	95	55.6	72*	42.1	98	57.3
3	Corn gruel	84	49.1	54*	31.6	65*	40.4
4	Corn tortilla	158	92.4	168*	98.2	169*	98.8
5	Flour tortilla	89	52.0	93	54.4	84	49.1
6	Tejuino.	55	32.2	52	30.4	63	36.8
7	Tamales.	98	57.3	94	55.0	103	60.2
8	Cofundas.	16	9.4	13	7.6	14	8.2
9	Bread	156	91.2	157	91.8	163	95.3
10	Dough	161	94.2	160	93.6	162	94.7
11	Peanuts.	69	40.4	74	43.3	83*	48.5
12	Nuts.	51	29.8	60	35.1	56	32.7
13	Almonds	47	27.5	45	26.3	39	22.8
14	Pistachios.	51	29.8	52	30.4	71*	41.5
15	Vegetables.	157	91.8	160	93.6	165	96.5
16	Fruit Juice	166	97.5	167	97.7	168	98.2
17	Red meat	151	88.3	150	87.7	143	83.6
18	Fish	101	59.1	106	62.0	102	59.6
19	Cream.	139	81.3	140	81.9	133	77.8
20	Milk.	162	94.7	160	93.6	164	95.9
21	Cheese.	150	87.7	158	92.4	148	86.5
22	Yogurt.	147	86.0	137	80.1	135	78.9
23	Chicken	156	91.2	157	91.8	150	87.7
24	Sweets	85	49.7	92	53.8	83	48.5
25	Chocolates.	78	45.6	92	53.8	76	44.4
26	Mazapan.	54	31.6	52	30.4	66	38.6
27	Cookies.	106	62.0	115	67.3	119	69.6
28	Scrap.	89	52.0	111*	64.9	115*	67.3
29	Beans.	168	98.2	157*	91.8	165	96.5
30	Raw milk fight.	9	5.3	14	8.2	11	6.4
31	Boiled milk brawl	36	21.1	33	19.3	39	22.8

(*p < 0.05)

Direct Source: Structured Interview applied to women in childbirth situation post, Civil Hospital of Guadalajara Dr. Juan I. Menchaca

low weight at birth there is a significant difference in terms of intake of corn gruel, corn tortilla, peanuts, pistachios and junk foods (Table 1).

By forming groups organizing food in the group to which they are cereals, oilseeds, meat, milk and fruits, for both the controls and pair cases, the following was found: there is a significant difference between controls and cases in reference to product mortality compared to cereals, particularly oats. In the case of controls and cases of low weight at birth a significant difference was detected as well as with oatmeal and pistachios (Table2).

On the consumption of cereals and oilseeds,it can be seen that both the controls and the cases consumed virtually the same types of food, the minimum important differences represent the type of the food eaten.

Statistical association

Concerning the frequency of the intake of quality food that the mother ingested during the first two months of pregnancy in cases of death of the product, there is a significant difference in the consumption of dried fruit and peanut caramels. In cases of low weight at birth, a significant difference was also detected in terms of eating tortilla of poor quality, which means that were wet and sticky, that by separating them whitish filaments could be observed and the taste was not pleasant, as well as; some peanuts tasted bad, the unpleasant taste of marzipan and flavor of dried fruits (Table 3).

It is important to note that the frequency of exposure to aflatoxigenic food both in controls and in cases of death of the product and low weight at birth, there is a significant difference in the intake of corn tortilla and peanuts (Table 4).

Concerning the concentrated exposure to frequencies regarding mortalities of the product, it was found that 70 cases were exposed and 101 other cases were not exposed. In 39 other controls were exposed and 132 were not exposed, which allowed detecting an OR Odds ratio = 2.35 with confidence

Table 2: Intake of grains and oilseeds in controls and cases of mortality of the product and low weight at birth

N	**Cereals**	**Controls**	**Cases Mortalities**	**Cases of low weight at birth**
1	*Rice*	161	156	157
2	*Oat*	95	72*	98
3	*Corn gruel*	84	54*	69
4	*Corn tortilla*	158		168* 169*
5	*Flour tortilla*	89	93	84
6	*Tejuino.*	55	92	63
7	*Tamales.*	98	94	103
8	*Corundas.*	16	13	14
9	*Bread*	156	157	163
10	*Dough.*	161	160	162
11	*Cookies.*	106	115	119
	Oiseeds	Controls	Cases Mortalities	Cases of low birth weight.
1	*Peanuts*	69	74	83
2	*Nuts*	51	60	56
3	*Almonds.*	47	45	34
4	*Pistachios.*	51	52	71*
5	*Mazapan.*	54	52	66

($^{*}p < 0.05$)

Direct Source: Structured Interview applied to women in postpartum situation, Civil Hospital of Guadalajara Dr. Juan I. Menchaca

Table 3: Frequency of food quality control and food product mortalities and low weight at birth

Ji²	Food(*p < 0.05)	Controls	%	Mortalities	%	Cases of low weight at birth	%
1	Consumed corn tortilla.	169	98.2	167	97.7	168	98.2
2	Clammy.	18	10.5	27	15.8	36*	21.1
3	Orange to Green tones.	5	2.9	9	5.3	4	2.3
4	Filaments were observed.	24	14.0	36	21.1	47*	27.5
5	Unpleasant odor or taste.	15	8.8	19	11.1	34*	19.2
6	Consumed wheat tortillas.	83	48.5	91	53.2	80	46.8
7	Green-blue tones	7	4.1	3	1.8	7	4.1
8	Ate peanuts	70	40.8	68	39.8	76	44.4
9	Some tasted bad. unpleasant	20	11.7	31	18.1	38*	22.2
10	Ate nuts.	45	26.3	60	35.1	51	29.8
11	Dull and parchedappearance.	11	6.4	17	9.9	7	4.1
12	Some were sorry.	13	7.6	20	11.7	15	8.8
13	Ate pistachios.	50	25.2	49	28.7	61	35.7
14	sometastedbadly, disgusting.	10	5.8	12	7.0	14	8.2
15	Ate mazapán.	46	29.9	51	29.8	58	33.9
16	The flavor was nice.	1	26.3	2	28.7	15*	25.9
17	Ate driedfruit.	33	19.3	20*	11.7	20*	11.7
18	The appearance was nice.	2	18.3	2	10.5	3	9.9
19	The flavor of dried fruit was nice.	0	19.3	0	11.7	3	9.9
20	Ate peanut caramel.	7	4.5	20*	11.7	13	7.6
21	I drink raw milk.	32	18.7	38	22.2	36	21.1

Direct Source: Structured Interview applied to women in postpartum situation, Civil Hospital of Guadalajara Dr. Juan I. Menchaca

Table 4: Aflatoxigenic Frequency of exposure to food, controls and cases product with mortality and low weight at birth

RiskfoodJi² .(*p < 0.05)	Frequency control	%	Mortality rate of the product	%	Frequency in low weight at birth	%
tortilla	15	38.4	27*	38.5	25	36.2
peanut	10	25.6	20*	28.5	16	23.1
Pistache	2	5.1	4	5.7	1	1.4
walnut	3	7.7	9	12.8	3	4.3
Tortilla-peanut.	2	5.1	3	4.2	6	8.9
Tortilla-nut	1	2.6	0	0	4	5.7
Tortilla-pistachio.	0	0	0	0	1	1.4
Tortilla marzapan.	0	0	0	0	3	4.3
Peanut-nut	3	7.7	3	4.2	4	5.7
Peanut-pistachio	1	2.6	2	2.8	0	0
Three or more	2	5.1	2	2.8	6	8.9
Total exposed	39	100	70	100	69	100

Direct Source: Structured Interview applied to women in childbirth situation post, Civil Hospital of Guadalajara Dr. Juan I. Menchaca.

intervals (1.43 <OR < 3.86) and confidence limits for the OR of 95 %, with a value of p < 0.05 by Chi 2. (Table 5).

The results obtained in regards to low weight at birth, it was detected that 69 cases were exposed to poor quality food referrals, 102 were not exposed, 39 controls themselves were exposed and 132 were unexposed. This also allowed us to determine that there is an association with an OR Odds ratio = 2.29 (1.39 <OR < 3.77) with confidence limits of 95 % for OR and a value of p < 0.05 by Chi 2 which, in the same way influenced mortality of the product. (Table 5).

This situation can also be detected in everyday foods such as tortillas, peanuts, pistachios and walnuts OR for suggesting significant association with mortality events of the product and low weight at birth, even if they pooled cases where exposure plus Tortilla included other foods, and even as total number of exposed detect the OR is greater than 2, with a value of 2.35 in the case of death of the product and 2.29 respect to low weight at birth.

Table 5: Association between food intake and aflatoxigenic mortality of the product, low weight at birth

Variable Aflatoxigenic food		Cases		Controls.	OR Crude	IC 95%.
MortalitiesProduct *	F	%	F	%	OR crude	IC 95%
Ji ^{2} 12.94						
0.0003215 (*p < 0.05)						
Exposed	70	40.9	39	22.8	2.35	(1.43 <OR< 3.86)
Notexposed	101	59.0	132	77.1		
Cases of low weight at birth.*Ji ^{2} 12.18						
0.0004832 (*p < 0.05)						
Exposed	69	40.3	39	22.8	2.29	(1.39 <OR< 3.77)
Notexposed	102	59.6	132	77.1		

Direct Source: Structured Interview applied to women in postpartum situation, Civil Hospital of Guadalajara Dr. Juan I. Menchaca.

Table 6: Binary logistic regression for case-control mortality between the product and the variables that may influence the effect organized by category

S. No	Variable	OR thight	IC 95%	Value of p
1	Age-smoke during pregnancy	1.0	.952-1.050	.987
2	Age-consumption of junk foods.	1.0	.956-1.056	.849
3	Scrap-smoke during pregnancy	1.612	1.037-2.504	.034*
4	Corn-Tortilla smoked during pregnancy	5.599	1.404-22.324	.015*
	Including variables in the model: Corn tortilla, junk foods and smoked during pregnancy			
1	Corn Tortilla	5.27	1.336-20-827	.018*
2	junk food	1.57	1.006-2-450	.047*
3	Smoke during pregnancy	17.0	2.038-142.02	.009*

Direct Source: Structured Interview applied to women in postpartum situation, Civil Hospital of Guadalajara Dr. Juan I. Menchaca

These results suggest that considering the quality of food consumed there is an association between aflatoxigenic food intakes during the first two months of pregnancy, product with mortality probabilities and with low weight at birth. It is important to note that in terms of food intake, the interviews mentioned to have eaten non varied foods, on the opposite they mentione they would always eat the same types of food. The differences that exist regarding the quality of the diet made the difference between the studied events compared to controls.

In the binary logistic regression analysis, focusing on the quality of each aflatoxigenic food it was detected that in reference to mortality of the product, there is no significant relation to age, with a crude OR .994 , 95 for IC % of 947-1043 p of .797, in addition to not influence the results for the event. For foods that have significance if this event include; junk food consumption, with crude OR 1.704, 95% CI for 1104-2632 and p .016. As for the set itself OR smoked during pregnancy was OR 1.612 , 95% CI for 1037-2504 , and a p of .034 and corn tortilla from the output of crude OR 4.607 , IC for 95 % of 1.289-16.47 and p-value of .019 , which regard themselves smoked during pregnancy yields an adjusted OR 5.59 , 95% CI for 1.404-22.32 and p .015 . (Table 6).

The variables that refer to whether they used insecticide and live near an industry are not significant and do not cause effects on the variables of interest. As to whether smoked during pregnancy an interesting effect, with crude OR 15.155, 95% CI for 1970-116563 and detected p .009, significance and apparently has the effect of synergism is observed risk by smoking during pregnancy when the model with significant variables was implemented, as they are eating junk foods and corn tortilla, with an OR of 17.0 , 95% CI for 2.038-142.02 and p .009 (Table 6). From the results obtained it accepts and rejects HI, H0.

DISCUSSION

The results obtained in this study show areas of opportunity in the field of public health, not only for research but to take actions to improve the quality of life and impact on life itself by simply recommending eating tortilla that is daily made, avoiding cereals and oilseeds that have an unpleasant smell and taste as well as analyze their characteristics.

By eating tortillas that are daily prepared aflatoxigenic exposure can be reduced in relation to concentrations of aflatoxins. Some investigations were performed in the laboratory where the inactivation of these is evaluated and found to be inactivated in up to 96 % aflatoxin B1, and 70 % aflatoxicol (26,30),this indicates that tortillas are not totally free of Aspergillusspp so there is the possibility that the type of risk is revealed, in regard to bioaccumulation of aflatoxins and even if tortillas are consumed after several days of being made, this obviously gives exposure to a higher concentration and thus their effects will be of greater magnitude, that if one takes into account that facilities, transport of inputs (mass) - distribution and sale of tortillas is not normally carried out under adequate hygienic sanitary conditions, also considering that sometimes such product is also dealt with products derived from peanuts in various presentations, but do not have the endorsement of health department and quality control without the potential for adverse human health damage increases.

It is relevant for our study to know that there have been studies aimed at assessing human aflatoxigenic effects, as shown for developing countries[33], which statethat : Aflatoxins are well recognized to cause liver cancer, besides to present significant additional toxicity. In a cross sectional study in Benin and Togo[29, 31, 32], it is stated that aflatoxins can affect since fetal life and early childhood, with the first use of solids, the effect of aflatoxigenic foods consumption depends on the mother'sdiet during pregnancy. The study reveals the association between aflatoxigenic exposure and low weight at birth as an indicator of chronic or acute malnutrition, by being exposed to those substances in the uterus continuing to be exposed throughout his life, besides the association found between poor nutritional status and aflatoxins - serum albumin[28, 33, 35-37].

This is one of the studies that is consistent with the results obtained in the present investigation, especially regarding low weight at birth, but also emphasizes almost more importantly solid foods and the concentrations of aflatoxins in those as well as in breast milk, situation which can increase the risk of suffering the effects of aflatoxigenic food intake for the product.

CONCLUSIONS

1. Both cases and controls consumed the same type of food, but the difference was in the quality of some foods such as tortillas and peanuts, which determined the same difference from the events and outcomes studied.
2. The tortilla consumed was described to have poor quality, with whitish filaments or yarns when separating them, and the unpleasant taste of peanuts represents risk for low weight at birth if ingested during the first two months of pregnancy; synergism is even possible for the mothers who smoke.
3. Poor quality tortilla consumption and junk food are apparently involved in the mortality effect of the product, as one of the possible causes of this event.
4. The foods that mostly impact the outcomes of pregnancy in terms of aflatoxigenic effects are corn tortillas, peanuts, pistachios and junk foods.

Suggestions and recommendations.

In order to avoid risks associated with aflatoxigenic intake in one's diet, particularly regarding dangerous food groups during pregnancy, it is recommended to consume tortillas that are daily made, cereals and oilseeds with a nice appearance and avoid those that show to have anunpleasant smell and taste. It is necessary for health authorities to perform epidemiological surveillance under these matters. It is highly suggested to have monitoring systemregarding the transportation of tortilla dough, such as the facilities for stocking it, transportation of this product to distributors, because this is normally not carried out under suitable, hygienicand sanitary conditions.

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