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ABSTRACT:
Aflatoxin M1 (AFM1) is a secondary metabolite in the breast milk of lactating mothers who consume foodstuffs infected by the fungi *Aspergillus flavus* and *Aspergillus Parasiticus*. The concentration of AFM1 in breast milk of lactating mothers is of major public health concern, because it can negatively affect the health of their babies. The major objective of this study was to assess the AFM1 concentration in the breast milk of lactating mothers in Papua New Guinea (PNG). This was a prospective cross-sectional study carried out between 2011 and 2015 in three of the four Regions in PNG: the National Capital District (NCD) in the Southern Region; Eastern Highlands (EHP) and Western Highlands (WHP) provinces in the Highlands Region; and East New Britain (ENB) and Manus provinces in the Islands Region. The Susu-Mama, Well-Baby and Paediatric clinics in the General Hospitals in each of the selected provinces in the three regions were the primary sites for this study. A solid phase competitive Enzyme-Linked Immunosorbent Assay (ELISA 96 Microwell plates) was used for the quantification of AFM1 in breast milk from consented lactating mothers. A total of 874 lactating mothers and their babies participated in this study. The mean age of the mothers was 28.0 ±5.5 years. The age range of all the babies was 2 to 6 weeks. 76.1% (665/874) of all breast milk samples analyzed had detectable levels of AFM1. The concentration of AFM1 was above 10.00ppt in 89 (10.2%) of the 874 breast milk samples (which, according to the Australia / New Zealand / Austria safe cut-off limits for AFM1, makes them unsafe for consumption by the babies). The mean AFM1 concentration in the breast milk samples from lactating mothers in EHP (7.99ppt) was higher than that in the samples from the other 4 provinces in the present study. AFM1 concentration was above 10.00ppt in 14 (4.6%) of the 300 breast milk samples from NCD, in 62 (31.0%) of the 200 samples from EHP, in 10 (4.5%) of the 220 samples from ENB and in 3 (3.0%) of the 100 samples from WHP. In order to reduce the AFM1 concentrations in breast milk of lactating mothers, basic nutrition education, aggressive advocacy, social mobilization, awareness campaigns, including communication with all relevant target groups and the relevant policy makers are urgently required.

Keywords: Breast milk, Aflatoxin, Aflatoxin M1, Lactating mothers, Papua New Guinea

INTRODUCTION:

Mycotoxins are toxic metabolites produced by fungi. One of their major functions is protection of the environment of the fungi [1]. Aflatoxin (AF) is one of the most widely occurring mycotoxins produced by the fungi, *Aspergillus* species that grow in a variety of food crops in temperate climates during storage. AF is a secondary metabolite of the frequently occurring *A. flavus*, *A. parasiticus* and *A. Nominis* [1 – 3]. Other AF-producing Aspergillus species that are encountered less frequently are *Aspergillus bombycis*, *Aspergillus ochraceoroseus* and *Aspergillu spseudotamari* [4 – 6]. There are over 30 Aflatoxins already identified [7, 8]. The specific Aflatoxins that are of public health concern and are, therefore, monitored in the food chain, are Aflatoxin B1 (AFB1), B2 (AFB2), G1 (AFG1), G2 (AFG2) and M1 (AFM1) [7, 8]. Aflatoxin B1 (AFB1) is the most toxic, compared to all the others [3, 4]. AFB1 is metabolized to Aflatoxin M1 (AFM 1) in the liver, and is then excreted in the breast milk of any lactating mammals (including humans) that consumed foods contaminated by AFB1 [5, 8, 9]. Thus, AFM1 is a biomarker of recent dietary exposure of lactating mothers to AFB1 contaminated foodstuffs. AFB1 and AFM1 are classified as class 1 human carcinogens by the International Agency for Research on Cancer (IARC) [3, 10, 11]. They are hepatotoxic, carcinogenic, mutagenic, teratogenic and immunosuppressive. AFM1 causes birth defects, malnutrition and growth retardation in neonates [3, 5, 7, 10, 11].

Some common foodstuffs contaminated with AFB1 are peanuts, maize, cottonseeds, grains, rice, cassava, cereals, sago, coconuts, ginger, dried fruits, wheat and chillies [10 – 13]. These are foodstuffs frequently consumed by humans. Some of these foodstuffs are the staple foods in most countries worldwide. AFB1 contamination of food crops may occur before harvest and during storage [13, 14]. Postharvest Aflatoxin contamination may occur when the crops are not properly treated during the drying and storage processes. Some of the important factors that should be considered during storage include the moisture content of the crops and the relative humidity of the surroundings [13 – 17]. The favorable conditions for growth of the Aspergillus fungi are storage temperatures between 27°C and 35°C and moisture content exceeding 7.0% (10.0% with ventilation) [13 – 17]. Timely harvesting of crops, rapid and adequate drying prior to storage are important in preventing fungal growth and, consequently, Aflatoxin contamination [14 -16]. AFB1 contamination is particularly severe in resource limited countries in Asia, Africa and the South Pacific, where

there is little consumer awareness of food safety issues related to Aflatoxins [5, 13].

The levels of Aflatoxins considered safe for human consumption, vary from country to country. In most developed countries, the generally acceptable levels range from 0 to 30.0ppb [17 – 19]. The Codex Alimentarius Commission (CAC) has established a limit of 15.0ppb for total Aflatoxins in all foods worldwide [17 - 19]. However, some countries and organizations have set their own cut-off limits. These levels are to be monitored by consumer protection agencies that regulate the importation of foodstuffs [17 – 19]. The upper limit of AFM1 in breast milk is set at less than 0.05ppb (50.0ppt) by the CAC and the European Community [17 – 19].

In some resource limited countries like Papua New Guinea (PNG), although the legislation is in place to regulate the AFB1 levels in foodstuffs, the laws are not strictly implemented. According to reports from the CAC, most people living in resource limited countries are typically exposed to Aflatoxins in their diets, largely because their locally produced food crops are not effectively monitored to ensure proper implementation of guidelines for harvesting, drying and storage [18, 19].

In 1972, of the 20 food samples collected from Kundiawa, Kaiapit, Koki, Lae, East New Britain (ENB) and Markham Valley in PNG, 16 (80.0%) were contaminated with AFB1 [20]. In surveys, conducted in 1996 and 2002 by the National Agricultural Research Institute (NARI), 34.4% of the foodstuffs collected from markets in Western Highlands, Sepik, Morobe, East New Britain (ENB) Provinces and the National Capital Districts (NCD) were contaminated with AFB1 in levels between 5.0 to 20.0 ppb [21]. In a recent study, AFB1 contamination of foodstuffs was reported in some major cities in PNG [22].

Despite the cumulative evidence of the prevalence of AFB1 contaminated foodstuffs in the various provinces, there is no published data on the AFM1 levels in the breast milk of lactating mothers in PNG.

Breastfeeding is a common practice in most of the provinces in PNG; thus, there is a high risk of exposure of neonates to AFM1 in the breast milk of lactating mothers, if they have consumed AFB1 contaminated foodstuffs. However, breastfeeding of the neonate for the first six months of life, with continued breast feeding for up to two years of age, is very important and must be encouraged. Breast feeding promotes mother-child relationship and guarantees the effective growth and development of the infant by providing adequate nutrients and the required antibodies for control of infections [23]. There is, therefore, the need to regularly assess the AFM1 concentration in the breast milk of lactating mothers, in order to obtain data that can be

used for formulation of policies and programs for the systematic monitoring of Aflatoxin levels in foodstuffs in PNG.

The major objective of this study was to assess the AFM1 concentrations in breast milk of lactating mothers in PNG, using breast milk of lactating mothers resident in three of the four regions in PNG.

METHODOLOGY:

Study sites:

This was a prospective study, carried out between 2011 and 2015 in three of the four Regions in PNG: the National Capital District in the Southern Region; Eastern Highlands and Western Highlands provinces in the Highlands Region; and East New Britain and Manus provinces in the Islands Region. The Susu-Mama, Well-Baby and Paediatric clinics in the General Hospitals in each of the selected provinces in the regions were the primary sites for this study.

Study Design and Sampling:

This was a prospective hospital out-patient based cross-sectional study, because of the difficulty obtaining ethical clearance and permission to collect biological samples from healthy individuals in PNG for research. All lactating mothers that attended the Susu-Mama clinics for guidance on breastfeeding of their babies, and those that attended the Children's Outpatient clinics including the Well-baby clinics for routine check-up and vaccination during the study period were eligible for enrolment. Simple random sampling, using a table of random numbers, was used to select the lactating mothers that participated in this study.

Exclusion criteria:

Lactating mothers with malaria, high fever, any other significant illness and those with infants admitted in the paediatric wards were excluded from the study.

Collection of breast milk samples and questionnaires:

Each of the selected lactating mothers was briefed on the purpose of the study, before asking her or the accompanying relative to read and to sign an informed consent form. The mothers were at different stages of lactation. The consented lactating mother was then requested to donate about 5.0ml of breast milk during regular feeding of her baby; by hand expression the milk was put directly into a labeled sterile container. The breast milk samples were kept inside a cool-box at 4 to 8ºC, before they were moved to the laboratory in the Hospital and kept frozen in a freezer at –15ºC. All the breast milk samples from the provincial hospitals were kept frozen and transported by air to the Micronutrient Research Laboratory (MRL), in the Division of Basic Medical Sciences (BMS) in School of

Medicine and Health Sciences (SMHS) University of Papua New Guinea (UPNG). The samples were kept frozen at – 70ºC until required for analysis.

A self-designed pretested questionnaire was used to collect specific information about the lactating mothers and their babies. The information collected included, residential location, age, time of last meal eaten, type of meal eaten, type of nuts consumed regularly and knowledge of Aflatoxin. For the babies the gender, date of birth, birth weight and length were obtained mainly from the baby book.

Sample preparation and analysis:

Each of the breast milk was gradually thawed from – 70ºC to – 20ºC and then to 4ºC. They were centrifuging at 2000g for 10 minutes in a refrigerated centrifuge to induce separation of the upper fatty layer in each of the samples. The fatty layer was later removed by aspiration with sterile pasture pipette and the lower plasma used for the assay of AFM1 [24].

A commercial Enzyme-Linked Immunosorbent Assay (ELISA) from HELICA Bio-systems Inc was used for the quantification of AFM1 in the breast milk. The HELICA Aflatoxin M1 Assay is a solid phase competitive enzyme immunoassay (ELIZA 96 Microwell plates). An antibody with high affinity for Aflatoxin M1 is coated onto polystyrene microwells. When standards or samples containing AFM1 are added to the appropriate microwells, the AFM1, which is the antigen, binds to the coated antibody [24].

The complete analytical procedure, using the breast milk samples, commercial standards and quality control samples, were carried out as indicated in the instructional protocol of the manufacturer [24]. All tests were done in duplicate; a Microplate washer and multi-channel semi-automated pipettes were used as appropriate. After the addition of the stop-solution, a Microplate reader (RT-2000C fully integrated with a read-out panel) with an absorbance filter set at 450 nm and a differential filter set at 630 nm was used to measure the optical density of the microwells. The limit of detection was 2.00ppt, mean recovery was 95.5 ±2.5% and Coefficient of Variation (CV) was 3.0%. All reagents used were of analytical grade.

Data analysis and interpretation:

The statistical package for social sciences (SPSS) version 20 for Windows and Excel MS data pack software were used for statistical analysis of the data. The Kolmogorov-Smirnov test was used to assess distribution of the data; Mann-Whitney U test, Wilcoxon rank sum tests and Chi-square test (Fisher's exact test), were used as appropriate.

Currently in PNG there are no recommended cut-off limits to indicate the safe concentration of AFM1 in breast milk. In Australia, New Zealand and Austria the recommended safe

cut-off limit for AFM1 in breast milk is AFM1 < 10.00 pg/ml (10.00ppt or 10.00ng/L). The Codex Alimentarius Commission (CAC), European Union (EU) and the United States of America (USA) recommended the safe cut-off limits are AFM1 <25.00ppt for breast milk and AFM1 < 50.00ppt for AFM1 in milk powder and other milk products [3, 4, 13, 19, 25]. In the present study both recommended cut-off limits were used for interpretation of the results.

Ethical clearance:

Ethical clearance and approval for this study was obtained from the Ethics and Research Grant Committee in the SMHS UPNG, and the Medical Research Advisory Committee (MRAC), National Department of Health (NDOH) PNG. Permission was obtained from the Chief Executive Officer and Director of Medical Services of PMGH and the appropriate authorities in the various Provincial General hospitals. The significance of the study was explained to each of the selected lactating mothers. Oral and signed informed consents were obtained from each lactating mother before receiving the breast milk sample.

RESULTS:

A total of 1000 lactating mothers were recruited for this study. Consent was obtained from 900 lactating mothers, which gave a non-consent rate of 10.0%. Of the 900 breast milk collected, 26 were discarded because of spillage (10 samples), non-availability of the questionnaires (6 samples) and very small amount of breast milk collected (10 samples). Six of the 26 samples discarded were from Manus province and 20 from the NCD.

The data obtained for the 874 lactating mothers is presented as the PNG data. The mean age of all the 874 lactating mothers was 26.0 ± 5.5 years (mean ± standard deviation), the age range was 15.0 to 40.0 years (Table 1). The distribution of all the mothers according to their age groups is presented in Table 2. A total of 304 (34.0%) lactating mothers were in the 20 to 24 years age group, followed by 264 (30.2%) in the 25 – 29 years age group.

All the 874 babies were full term; their age range was 2 to 6 weeks and they were still breast feeding at the time of the visit to collect the breast milk. The mean birth weight of the babies was 3.1 ± 0.62 kg, the range was 1.3 to 4.7 kg and the median was 3.10 kg. The birth weights of 25 (2.9%) babies were below 2.0kg, characterized as Very low birth weight (VLBW); the birth weights of 53 (6.1%) were between 2.0 to 2.49kg, characterized as Low birth weight (LBW) and the birth weights of 796 (91.1%) babies were above 2.5kg, characterized as normal birth weight. The mean birth length of all the babies was 48.0 ± 4.3 cm and the range was 35.0 – 63.0 cm.

Bivariate correlation analysis was used to test the relationship between the birth weights and

birth lengths of all the babies. The Spearman's rho correlation coefficient indicated strong direct correlation between the birth weights and birth lengths of all the babies (rho = 0.378; p = 0.01, 2-tailed). This implies that the taller babies were heavier than the shorter babies.

Of the 874 breast milk analyzed, AFM1 was detected in 665 (76.1%) of them. The Shapiro-Wilk test (p = 0.0001; df = 665) indicated that the AFM1 (ppt) concentration was not normally distributed in the breast milk from the 665 lactating mothers. The box-plot (Fig. 1) of the AFM1 concentrations also indicates that the values were not normally distributed.

The summary statistics of the AFM1 concentrations in all the breast milk samples from all the mothers are presented in Table 3. The median AFM1 concentration was 4.04 ppt and the Interquartile Range (IQR) was 2.05 to 6.62 ppt.

Table 1: Some characteristics of the lactating mothers that participated in the study

	PNG	**NCD**	**EHP**	**ENB**	**Manus**	**WHP**
N	874	300	200	220	54	100
Mean age (yrs)	26.0	25.6	24.6	27.1	26.9	25.7
Standard Dev (SD)	5.5	5.6	4.9	5.7	6.4	5.1
Median age (yrs)	25.4	25.0	24.0	26.0	27.0	25.0
Age range (yrs)	15.0 – 40.0	15.0 – 40.0	15.0 – 40.0	17.0 – 40.0	18.0 – 40.0	17.0 – 40.0
AFM 1 detected in breast milk	665 (76.1%)	174 (58.0%)	155 (77.5%)	220 (100.0%)	52 (96.3%)	64 (64.0%)

Table 2**:** Distribution of all the lactating mothers according to age groups

Age groups (years)	**PNG 874 (%)**	**NCD 300 (%)**	**EHP 200 (%)**	**ENB 220 (%)**	**Manus 54 (%)**	**WHP 100 (%)**
15 – 19	90 (10.3)	38 (12.7)	27 (13.5)	10 (4.5)	6 (11.1)	9 (9.0)
20 – 24	304 (34.8)	109 (36.3)	77 (38.5)	69 (31.4)	15 (27.8)	34 (34.0)
25 – 29	264 (30.2)	83 (27.7)	54 (27.0)	73 (33.2)	17 (31.5)	37 (37.0)
30 – 34	141 (16.1)	43 (14.3)	33 (16.5)	43 (19.5)	10 (18.5)	12 (12.0)
35 – 40	75 (8.6)	27 (9.0)	9 (4.5)	25 (11.4)	6 (11.1)	8 (8.0)

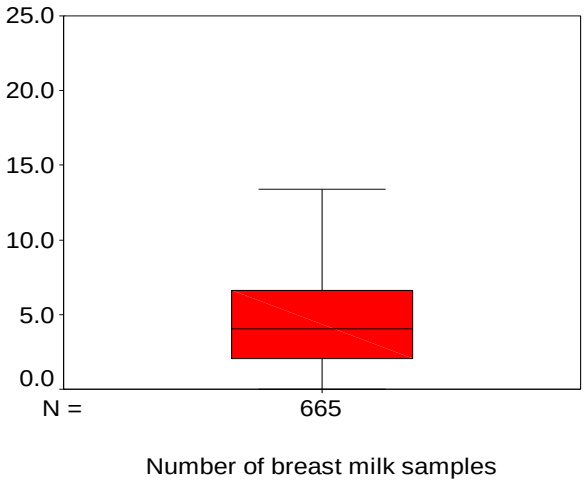

Fig. 1: Box-plot of AFM1 concentrations (ppt) in breast milk of lactating mothers

Table 3: Summary statistics of AFM 1 concentrations (ppt) in the breast milk of lactating mothers

Parameters	PNG	NCD	EHP	ENB	Manus	WHP
N	665	174	155	220	52	64
Median (ppt)	4.04	2.47	7.99	4.20	4.60	2.45
Interquartile Range (IQR) (ppt)	2.05 – 6.62	0.84 – 5.79	2.98 – 13.46	2.79 – 5.74	3.09 – 5.75	1.07 – 3.59
Mean (ppt)	6.51	5.65	9.09	6.62	4.28	3.88
Range (ppt)	0.01 – 93.04	0.01 – 90.71	0.01 – 43.38	0.29 – 93.00	0.9 – 7.69	0.02 – 41.02

Conversions: ppt = ng/kg = pg/g = ng/L = pg/ml

Table 4: Distribution (%) of AFM1 in breast milk of lactating mothers according to the different AFM1 safe cut-off limits

AFM1 cut-off limits	PNG 874 (%)	NCD 300 (%)	EHP 200 (%)	ENB 220 (%)	Manus 54 (%)	WHP 100 (%)
AFM1 = 0.0ppt	209 (23.9)	126 (42.0)	45 (22.5)	0	2 (3.7)	36 (36.0)
AFM 1 > 10.0ppt	89 (10.2)	14 (4.6)	62 (31.0)	10 (4.5)	0	3 (3.0)
AFM1 >25.0ppt	25 (2.9)	10 (3.3)	7 (3.5)	6 (2.7)	0	0

NB: Figures and % are cumulative; thus % do not add up to 100

The 874 breast milk samples were separated according to the recommended AFM1 safe cut-off limits; the distribution obtained is presented in Table 4. AFM1 concentrations were above 10.00ppt in 89 (10.2%) of the 874 breast milk. Thus, according to the Australia / New Zealand / Austria safe cut-off limit, these 89 breast milk were contaminated with AFM1 at the concentration that makes them unsafe for consumption by the babies at the time of collection of the breast milk. Using the recommend cut-off limit proposed by the Codex Alimentarius Commission (CAC), European Union (EU) and the United States of America (USA), the AFM1 concentrations in 25 (2.9%) breast milk were about 25.00ppt. The breast milk samples from these mothers were unsafe for the babies to consume at the time of collection of the breast milk.

Bivariate correlation analysis indicated a very weak non-statistically significant relationship (Spearman's rho = 0.064, p = 0.345, 2-tailed) between the AFM1 concentrations in the breast milk of all the mothers and the birth weights of their babies.

The Spearman's rho coefficient of correlation (rho = -0.102, p = 0.131, 2-tailed) also indicated weak inverse non-statistically significant relationship between the AFM1 concentrations in breast milk and the birth lengths of the babies.

When asked about their knowledge of AFM1, 78.4% (685) of the 874 lactating mothers do not have any knowledge about AFM1. However, most (85%) of the mothers were positive about eating peanuts with mould after wiping them. Some of the mothers (78.0%) were not aware of eating any other foodstuffs with mould, because they do not think mould can appear in any other foodstuffs apart from peanuts.

For more detailed analysis of the data, the 874 lactating mothers were separated according to their locations; the National Capital District (NCD) representing the Southern region; Eastern Highlands province (EHP) and Western-Highlands province (WHP) representing the Highlands region; East New Britain province (ENB) and Manus province representing the Islands region. Of the 874 mothers, 300 (34.3%) were from NCD, 200 (22.9%) from EHP, 100 (11.4%) from WHP, 220 (25.2%) from ENB and 54 (6.2%) from Manus province.

The descriptive statistics of the age of the mothers in the NCD and the four provinces are presented in Table 1. There were no statistically significant differences in the mean ages of the mothers. Table 2 shows the distribution of the lactating mothers into age groups. The highest number was in the 20 – 29 years age group, in NCD 64.0% (192/300), in

EHP 65.5% (131/200), in ENB 64.5% (142/220), in Manus 59.3% (32/54) and in WHP 71.0% (71/100).

The mean birth weight for babies in NCD was 3.0 ± 0.5kg and the range was 1.5 – 4.3kg; for EHP the mean birth weight was 3.4± 0.5kg and the range was 1.9 – 4.4kg; for ENB it was 3.2 ± 0.5kg and the range was 1.3 – 4.2kg and for WHP it was 3.5 ± 0.6kg and the range was 1.5 – 4.7kg.

The proportion (3.0%, 6.0% and 91.0% respectively) of birth weights classified into VLBW, LBW and Normal birth weight, were almost similar among the babies in the NCD and in each of the four provinces.

AFM1 was detected in 174 (58.0%) of the 300 breast milk samples from NCD, in 155 (77.5%) of the 200 samples from EHP, in 220 (100.0%) of the 220 samples from ENB, in 52 (96.3%) of the 54 samples from Manus province and in 64 (64.0%) of the 100 samples from WHP.

The distributions of the AFM1 (ppt) concentrations in the breast milk samples from NCD and the four provinces presented in the box-plots in Fig 2 show that the concentrations were not normally distributed. The Kolmogorov-Smirnov tests for normality of distribution also showed that the AFM1 concentrations were not normally distributed (p = 0.001). Thus, non-parametric statistics were used for analyses of the data. Table 3 shows the summary statistics of the AFM1 concentrations in the breast milk of lactating mothers from NCD and the four provinces.

The Mann-Whitney U and Wilcoxon W tests indicated that the AFM1 concentrations in breast milk from NCD was significantly lower (p=0.001, 2-tailed) than the AFM1 concentrations in breast milk from EHP, ENB and Manus provinces. There was no statistically significant differences in the AFM1 concentrations in the breast milk from Manus and ENB (p = 0.828, 2-tailed).

Table 4 shows the distribution of the breast milk from lactating mothers in NCD and the four provinces according to the recommended AFM1 safe cut-off limits.

Concentration of AFM1 was greater than 10.00ppt in 14 (4.6%) of the 300 breast milk samples from NCD, in 62 (31.0%) of the 200 samples from EHP, in 10 (4.5%) of the 220 samples from ENB and in 3 (3.0%) of the 100 samples from WHP. This, according to the Australia / New Zealand / Austria safe cut-off limit, indicates AFM1 contamination of the breast milk samples, making then unsuitable for consumption by the babies at the time of collect of the breast milk.

The number (%) of breast milk from mothers in NCD, EHP and ENB with AFM1 concentrations above 25.00ppt is also shown in Table 4.

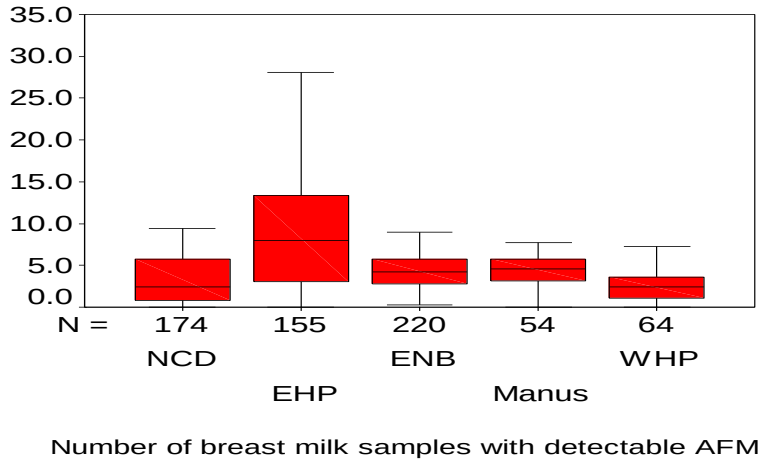

Fig. 2: Box-plots of AFM1 concentrations (ppt) in breast milk of lactating mothers in NCD and the four provinces that participated in this study

Table 5: Aflatoxin M1 levels (ppt) in breast milk of lactating women in different countries

Countries	No of samples	Percent (n) of contaminated breast milk	Mean AFM1 (ppt)	Range AFM1 (ppt)	Median AFM1 (ppt)	Interquartile Range (IQR) (ppt)	Ref
Iran	132	6.0% (8)	9.45	7.10 – 10.80	9.95	--	[26]
Iran	160	98.1% (157)		0.30 – 26.70	--	--	[28]
Brazil	94	5.3% (5)	18.00	13.0 – 25.00	--	--	[29]
Egypt	388	35.6% (138)	--	--	13.50	10.27 – 21.43	[30]
Colombia	50	90.0% (45)	5.20	0.9 – 18.50	--	--	[31]
Iran	87	27.6% (24)	0.56	0.13 – 4.91	--	--	[32]
Isfahan	80	1.3% (1)	6.80	--	--	--	[33]
Turkey	74	89.2% (66)	19.0	9.60 – 80.00	--	--	[34]

DISCUSSION:

The data obtained in the present study indicated that 76.1% (665/874) of the breast milk samples collected from lactating mothers in three of the four regions in PNG have detectable levels of AFM1. This strongly suggests the exposure of some babies to AFM1 within the first few weeks of life. The major source of the AFM1, which is the biomarker of AFB1, was the consumption of Aflatoxin contaminated foodstuffs by the lactating mothers. Since exclusive breast feeding for the first six months of life, and continued thereafter for up to two years, is one

of the major requirements that must always be advocated and implemented, the need to reduce the availability of Aflatoxin contaminated foodstuffs in the markets should be among the top priorities of program planners in the NDOH in PNG.

The high prevalence (76.1%) of AFM1 contaminated breast milk from lactating mothers in PNG in the present study was lower than the 98.1%, 90.0% and 89.2% contaminations reported for breast milk from lactating mothers in Iran [28], Colombia [31] and Turkey [34].

The median (4.04ppt) and IQR (2.05 – 6.62ppt) concentrations in the breast milk of all the lactating mothers in our present study were significantly lower than the corresponding 13.50ppt and 10.27 – 21.43ppt respectively in breast milk from lactating mothers in Egypt [30]. Table 5 shows the AFM1 concentrations reported for breast milk in lactating mothers from various countries.

The relatively high prevalence of AFM1 contamination of breast milk from lactating mothers in NCD (58.0%) was lower than the 77.5%, 100.0%, 96.3% and 64.0% obtained for EHP, ENB, Manus and WHP in the present study, and the 98.1%, 90.0% and 89.2% obtained in Iran [28], Colombia [31] and Turkey [34], respectively. The mean AFM1 concentration in the breast milk from lactating mothers in EHP (7.99ppt) was higher than the mean AFM1 in breast milk from lactating mothers in the other 4 provinces in the present study, and also the values reported for Colombia (5.20ppt), Iran (0.56ppt) and Isfahan (6.80ppt) [31, 32, 33]. The mean AFM1 for EHP (7.99ppt) is, however, lower than the cut-off limit of 10.00 ppm, the mean AFM1 concentrations reported for breast milk from lactating mothers in Iran (9.45ppt), Brazil (18.00ppt) and Turkey (19.00ppt) [26, 29, 34]. The 31.0% (62/200) breast milk from lactating mothers in EHP with AFM1 concentration above the 10.00ppt cut-off limits should be of great concern to the authorities in the EHP and NDOH in PNG, because it indicates availability of foodstuffs with high levels of AFB1 contamination in the markets in EHP.

The correlation between the concentration of AFM1 in breast milk and amount of peanut and other foodstuffs consumed by the lactating mothers was not assessed because of difficulties in recording the quantity of the different foods eaten per day. In addition, a 24-hour dietary recall is needed to obtain such data. However, based on the information from other researchers, it is logical to assume that the AFM1 concentrations in the breast milk of the lactating mothers were due to consumption of AFB1 contaminated foods several hours before the breast milk samples were collected [35, 36]. Studies in African, Asian and other regions have demonstrated correlation between the quantities of AFB1 contaminated

foods eaten and AFM1 concentrations in breast milk of lactating mothers [35, 36].

In the present study, the data obtained from the questionnaires indicated popular consumption of foodstuffs such as peanuts, tubers, root crops, legumes and cereals. Peanut is among the five major cash crops cultivated by small and medium scale farmers in PNG. Peanut is a major component of the diet consumed in both rural and urban households in most of the provinces. The practice of washing Aflatoxin infected peanuts before eating them is of major concern. It indicates the urgent need for intensive nutrition education, food safety information and awareness campaigns to advocate for proper implementation of recommended guidelines to reduce the infestation of peanuts by fungi.

In an effort to improve the export quality of peanuts grown in PNG, the National Agricultural Research Institute (NARI) in PNG in collaboration with stakeholders implemented the "Aflatoxin Contamination and Public Awareness Program on Better Handling Practices" in 2003. This major project was funded by PNG Agricultural Innovations Grant Facility (AIGF) between 2003 and 2005 [37]. The project involved bimonthly collection of peanut samples from registered small and medium scale farmers for analysis of AFB1, production of advocacy materials and newsletters in the local PNG language [38, 39] that were distributed locally to the farmers. The long term impact of this project has not been fully assessed because the project was not accompanied by effective monitoring [37]. There is an urgent need to develop similar programs that can be monitored with the use of mobile phones and other recently developed methods for monitoring such programs among farmers in the rural areas.

In the present study, the age range of the babies was 2 to 6 weeks and they were all breast feeding. Breast milk is the ideal quality food for all babies in this age group, because it provides all the required macro and micronutrients in adequate amounts with high bioavailability. In addition, it provides immunological protection against infections and promotes healthy growth and development. Prolonged exposure to AFM1 may cause stunting and underweight and negatively impact the immune status causing the infant to become prone to infectious diseases [23, 29, 40]. Thus, the need to ensure safe and effective breast feeding of the babies cannot be overemphasized. As already stated, good health, optimal growth and development of a child can be achieved with exclusive breastfeeding for the first six months of life and, continued thereafter for up to two years of age [23, 40]

Thus, to effectively reduce the AFM 1 concentrations in the breast milk of lactating mothers, social mobilization, intensive nutrition education and awareness campaigns, including

communication with all relevant target groups and agencies, like Susu-mama, and the relevant policy makers, are urgently required.

CONCLUSIONS:

The results obtained in the present study indicated that 76.1% (665/874) of the breast milk samples, collected from lactating mothers in three of the four regions in PNG, have detectable levels of AFM1. The concentration of AFM1 was above 10.00ppt in 89 (10.2%) of the 874 samples, which, according to the Australia / New Zealand / Austria safe cut-off limit, make them unsafe for consumption by the babies at the time of collection of the breast milk. The mean AFM1 concentration in the breast milk from lactating mothers in EHP (7.99ppt) was higher than the mean AFM1 in breast milk from lactating mothers in the other 4 provinces.

AFM1 concentration was above 10.00ppt in 14 (4.6%) of the 300 samples from lactating mothers in NCD, 62 (31.0%) of the 200 samples collected in EHP, 10 (4.5%) of the 220 samples from ENB and in 3 (3.0%) of the 100 samples in WHP. In order to reduce the AFM1 concentrations in breast milk of lactating mothers, basic nutrition education, aggressive advocacy, social mobilization, awareness campaigns, including communication with all relevant target groups and the relevant policy makers are urgently required.

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