Factors contributing to urinary aflatoxin M_1 occurrence among residents in Hulu Langat district, Malaysia

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ABSTRACT

Introduction: Chronic exposure to aflatoxin can lead to complications such as liver failure and cancer. There are many factors that affect aflatoxin occurrence. This study aimed to assess the association between sociodemographic factors and the knowledge, attitude and practice towards aflatoxin with urinary aflatoxin M_1 occurrence among residents in Hulu Langat district, Malaysia. **Methods:** This was a cross-sectional study conducted among healthy Malaysian adults aged 18 to 60 years residing in Hulu Langat district, Malaysia. Socio-demographic background and the knowledge, attitude and practice of respondents towards aflatoxin were assessed through questionnaires. Non-fasting urine sample (15 ml) was collected in the morning and urinary aflatoxin M_1 level was quantified. **Results:** Of the 444 healthy Malaysian adults, 199 urine samples were detected with aflatoxin M_1. From 37 positive samples with aflatoxin M_1 level above detection limit (0.64 ng/ml), mean value was 1.23±0.91 ng/ml (range = 0.65-5.34 ng/ml). Urinary aflatoxin M_1 occurrence was significantly different across ethnicity, age group, monthly household income, attitude and practice towards aflatoxin. Binomial logistic regression confirmed ethnicity and monthly household income as factors contributing to urinary aflatoxin M_1 occurrence. Chinese were 3.20 times more likely to have aflatoxin exposure than non-Chinese. Detected urinary aflatoxin M_1 was more common among household with a monthly income above RM1,500. **Conclusion:** The results provided an insight to explain the variation in aflatoxin occurrence among the population.

Keywords: aflatoxin, attitude, ethnicity, household income, practice

INTRODUCTION

Aflatoxins are highly toxic secondary metabolites produced by the *Aspergillus* species fungi (Reddy, Farhana & Salleh, 2011). They are mainly present in foods, including grains, nuts and legumes,

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eggs, and dairy products, which are not properly stored. This toxic compound produced by fungi and moulds are chemically stable and unable to be destroyed through food processing (Bezerra da Rocha *et al.*, 2014). Chronic exposure to aflatoxin can lead to aflatoxicosis and cause serious health threats, such as hepatic cirrhosis and carcinoma, as reported in Asia and Africa (Bosetti, Turati & La Vecchia, 2014).

Storage, environmental and ecological conditions are the main contributors to the growth and development of fungus in foods and feeds (Hussein & Brasel, 2001). Warm and humid climates prevailing in the tropical and sub-tropical developing countries have been shown to favour the production of aflatoxin by fungus (Leong *et al.*, 2011). As one of the countries that experiences tropical climate (Abdullah, Nawawi & Othman, 1998), Malaysia has a higher risk of experiencing aflatoxicosis if storage and production process of foods and feeds are poorly managed.

The outbreak of aflatoxicosis in Malaysia was first recorded in 1960, whereby over 50 pigs from two farms in Malacca died upon consuming aflatoxin-contaminated feeds (Lim & Yeap, 1966). In October 1988, 13 children of Chinese ethnicity aged 2.5-11 years died of acute hepatic encephalopathy in Perak, Malaysia. The deaths of these children were confirmed due to the intake of a type of Chinese noodle, *Loh See Fun*, which had been contaminated by aflatoxin (Lye *et al.*, 1995). Based on existing data, dietary aflatoxin exposure may contribute up to 17.3% towards liver cancer occurrence in Malaysia (Chin, Abdullah & Sugita-Konishi, 2012). Although the figure may not seem big, its potential adverse effects cannot be ignored.

Aflatoxin M_1 (AFM$_1$) is a sensitive biomarker of human exposure to aflatoxins since it can be detected in urine sample (Jager *et al.*, 2016). With a biological half-life of about one day, the urinary excretion of AFM$_1$ reflects the actual dietary aflatoxin exposure for the past 1-2 days (Cheng *et al.*, 1997; Kensler *et al.*, 2010). It has been widely used to monitor aflatoxin exposure among various populations including children, lactating women, and the general population (Ali *et al.*, 2017; Chen *et al.*, 2018; Gebreegziabher, Tsegaye & Stoecker, 2016). In the liver, AFM$_1$ is metabolised from aflatoxin B$_1$ (Marchese *et al.*, 2018), which is the most toxic aflatoxin and of greatest significance (Pearce *et al.*, 2015). However, the potency of AFM$_1$ is only approximately one order of magnitude lower than that of AFB$_1$ (Prandini *et al.*, 2009).

Several factors have been shown to affect aflatoxin level in humans. For example, a field study conducted among rural Ghanaians reported significant associations between education level, ethic group, and number of households with AFB$_1$-albumin level (Jolly *et al.*, 2006). In Malaysia, Leong *et al.* (2012) found that Chinese and older people were more likely to be exposed to aflatoxin. The current study previously demonstrated that the consumption of eggs and dairy products were positively associated with urinary AFM$_1$ (Siti Husna, Rosita & Mohd Redzwan, 2018). However, the relationships between factors such as knowledge, attitude, and practice (KAP) with aflatoxin that may lead to the increase in urinary AFM$_1$ occurrence have not been thoroughly examined. In this paper, we explored the associations between sociodemographic factors and KAP towards aflatoxin with urinary AFM$_1$ occurrence among residents in Hulu Langat district, Malaysia.

MATERIALS AND METHODS

Study design and population
This was a cross-sectional study conducted among adults aged 18 to 60

years residing in six subdistricts of Hulu Langat, Malaysia, including Beranang, Cheras, Hulu Langat, Hulu Semenyih, Semenyih, and Kajang. Ethical approval was granted by the Ethics Committee for Research Involving Human Subjects, Universiti Putra Malaysia (JKEUPM) (Project number: FPSK (EXP16) P047). Written informed consent was obtained from respondents prior to the study. Respondents were required to complete a set of questionnaires. A non-fasting urine sample was collected in the morning and stored at -80°C until further analysis.

Respondents were recruited using multistage sampling design. In the first stage, all subdistricts within Hulu Langat were divided into three categories based on their development status (countryside, developing, and developed areas). In the second stage, systematic random sampling was applied to select 2-8 residential areas from each category. Lastly, residents of the selected residential areas were randomly approached and screened to recruit for eligible respondents in the study.

There were 455 out of 468 respondents who met the following inclusion criteria: (1) in good health; (2) not taking any medications or supplements; (3) not smoking; (4) not following a restricted diet; and (5) not pregnant and not in postpartum period. Eleven respondents were further excluded from the study due to missing questionnaires (*n*=5), out of age range (*n*=2), and unreturned urine containers (*n*=4). Thus, the response rate of this study was 94.9%.

Socio-demographic factors

Socio-demographic factors including gender, age, education level, marital status, monthly personal income, and monthly household income were assessed via a self-administered close-ended questionnaire.

KAP towards aflatoxin

The KAP towards aflatoxin and its contamination in foods and feeds were assessed using a questionnaire adapted from a Ghanaian study (Jolly *et al.*, 2006). Prior to data collection, pre-testing was carried out on 31 respondents from Serdang, Selangor who met the inclusion and exclusion criteria. Based on pre-testing results, all the questions from Jolly *et al.* (2006) were retained.

The knowledge domain consisted of eight multiple choice items. Each item provided 'correct', 'wrong', and 'do not know' options. Each 'correct' answer was scored one, whereas 'wrong' and 'do not know' scored zero. The total score of knowledge ranged from 0 to 8. The cut-off value was determined based on the calculated median value to classify knowledge level into low (≤1.00) and high (>1.00).

There were four items in the attitude domain, defined as respondent's awareness and belief towards aflatoxin and its contamination in foods and feeds. The degree of agreement was indicated based on a four Likert scale (1=strongly disagree, 2=disagree, 3=agree, 4=strongly agree), with a total score ranging from 4 to 16. The median value calculated statistically was used as a cut-off point to differentiate negative (≤11.00) and positive (>11.00) attitudes.

A total of five items was listed in the practice domain focusing on the practices in gaining information related to aflatoxin and controlling aflatoxin contamination in food products. Respondents were asked to describe the frequency of experiencing the listed situations based on five categories: 1=never, 2=seldom, 3=sometimes, 4=frequently, 5=always. Only one question *'I avoid buying expensive food products to save money'* had reverse scoring as it was a negative statement. The total scores ranged from

5 to 25 and it was classified into poor (≤13.00) and good (>13.00) practices.

Urinary AFM$_1$ analysis

The analysis of urinary AFM$_1$ has been described previously (Siti Husna *et al.*, 2018). Briefly, morning urine samples (15 ml) were collected and kept frozen at -80°C until further analysis. Prior to the analysis, urine samples were centrifuged at 3000×*g* for five minutes and supernatants were collected. The AFM$_1$ level was quantified using Helica Aflatoxin M$_1$ ELISA kit (Helica Biosystem, Inc., Santa Ana, CA). The absorbance was measured at 450 nm using a microplate reader (SIRIO S Microplate Reader, RADIM, Italy). The limit of detection was 0.064 ng/ml and the recovery range was 73.0% to 88.0%. The concentration of urinary AFM$_1$ was corrected for variations in urine dilution among individual samples and expressed in ng/ml.

Statistical analysis

Data were analysed using IBM SPSS Statistics version 22. Non-parametric tests were used since data were not normally distributed. Chi-square test for association was carried out to determine the relationships between independent variables and urinary AFM$_1$ occurrence; whereas binomial logistic regression was run to identify the possible predictors that were found to be significant in bivariate analysis. Statistical significance was set at *p*<0.05.

RESULTS

Urinary AFM$_1$ level

Table 1 describes the urinary AFM$_1$ level of respondents. Of the 444 urine samples, 44.8% (*n*=199) had detectable urinary AFM$_1$ biomarker, which was an AFM$_1$ concentration of >0. From the positive samples, only 37 samples (18.6%) contained a urinary AFM$_1$ level above the detection limit of 0.64 ng/ml. Data from these 37 samples were further analysed. Urinary AFM$_1$ level of respondents ranged from 0.65 to 5.34 ng/ml, with a mean of 1.23±0.91 ng/ml and median of 0.89 ng/ml.

Table 1. Descriptive statistics of urinary AFM$_1$ level among respondents (*n*=37)

	Urinary AFM$_1$ level (ng/ml)
Mean±standard deviation	1.23±0.91
Median	0.89
Range	0.65 – 5.34
Percentile	
25th	0.76
50th	0.89
75th	1.20
95th	3.19

Associations between sociodemographic factors and urinary AFM$_1$ occurrence

When sociodemographic factors were examined based on the presence or absence of urinary AFM$_1$ biomarker, the biomarker was significantly different across ethnicity, age, and monthly household income (Table 2). However, no significant difference was found between urinary AFM$_1$ level with gender, marital status, education level, and monthly personal income. Respondents of Chinese ethnicity had a higher urinary AFM$_1$ occurrence compared to non-Chinese. More respondents aged 18 to 24 years had detectable urinary AFM$_1$ level, but the effect size of the association was small with a *Phi* value of 0.09. Those who had a household income of >RM1,500 monthly were more likely to have detectable urinary AFM$_1$ biomarker as compared to those with ≤RM1,500 in monthly household income.

Table 2. Associations between sociodemographic factors and urinary AFM_1 occurrence among respondents (*n*=444)

Sociodemographic factors	AFM_1, n (%)		χ^2	Phi	p-value
	Positive	Negative			
Gender			0.06	0.01	0.81
Male	90 (45.5)	108 (54.5)			
Female	109 (44.3)	137 (55.7)			
Marital status			1.69	0.06	0.19
Single/ divorce/ others	153 (46.6)	175 (53.4)			
Married	46 (39.7)	70 (60.3)			
Ethnicity			56.77	0.36	0.01**
Chinese	134 (63.5)	77 (36.5)			
Non-Chinese	65 (27.9)	168 (72.1)			
Age group†			3.92	0.09	0.05*
18 - 24 years old	113 (49.3)	116 (50.7)			
25 - 60 years old	86 (40.0)	129 (60.0)			
Education level			0.25	-0.02	0.62
None/ primary	51 (42.9)	68 (57.1)			
Secondary/ tertiary	148 (45.5)	177 (54.5)			
Monthly personal income‡			0.10	0.02	0.75
≤ RM1,500	136 (45.3)	164 (54.7)			
> RM1,500	63 (43.8)	81 (56.3)			
Monthly household income‡			32.70	-0.27	0.01**
≤ RM1,500	31 (23.8)	99 (76.2)			
> RM1,500	168 (53.5)	146 (46.5)			

*p<0.05; **p<0.01 from Chi-square test for association
†Classification of age based on median=24 years old
‡Classification of income based on Malaysian Economic Planning Unit (Malaysian Economic Planning Unit, 2012)

Associations between KAP towards aflatoxin with urinary AFM₁ occurrence

Significant differences were demonstrated between attitude and practice, instead of knowledge, towards aflatoxin with urinary AFM₁ occurrence as presented in Table 3. Regardless of education level, more than half of the respondents (52.0%) did not have knowledge about aflatoxins and were not aware of aflatoxin contamination in products. Similarly, less than half of the respondents had positive attitude (51.5%) and good practice (46.4%) in controlling aflatoxin contamination in their foods. Interestingly, the likelihood of having detected urinary AFM₁ was higher among those with positive attitude and good practice as compared with those with negative attitude and poor practice.

Binomial logistic regression model

A binomial logistic regression was performed to ascertain the effect of independent factors on the likelihood of having detectable urinary AFM₁ among respondents (Table 4). Based on the results, ethnicity and monthly household income were the independent variables contributing to aflatoxin occurrence in

Table 3. Associations between knowledge, attitude, and practice (KAP) towards aflatoxin and the occurrence of urinary AFM_1 biomarker among respondents (n=444)

Variables	AFM_1, n (%)		χ^2	Phi	p-value
	Positive	Negative			
Knowledge			3.32	-0.09	0.07
Low	94 (40.7)	137 (59.3)			
High	105 (49.3)	108 (50.7)			
Attitude			8.61	-0.14	<0.01**
Negative	81 (37.7)	134 (62.3)			
Positive	118 (51.5)	111 (48.5)			
Practice			4.99	0.05	0.03*
Poor	95 (39.9)	143 (60.1)			
Good	104 (50.5)	102 (49.5)			

*p<0.05; **p<0.01 from Chi-square test for association

urine. Indeed, ethnicity had the highest Wald's value, indicating it as the main predictor of aflatoxin occurrence among the respondents. Those of Chinese ethnicity were 3.20 times more likely to exhibit aflatoxin occurrence in urine as compared with non-Chinese. Higher monthly household income was also associated with an increased likelihood of exhibiting aflatoxin occurrence.

DISCUSSION

Malaysia is a multiracial and multi-religious Southeast Asian country with different cultures among the ethnic groups. Therefore, populations living in the country may have different food preferences and lifestyles, leading to different aflatoxin exposure. This study found that people of Chinese ethnicity were more likely to be exposed to aflatoxin as compared to non-Chinese, including Malay, Indian, and other smaller ethnic groups. This finding is consistent with a previous local study conducted by Leong *et al.* (2012), which showed a 3.05-fold higher risk of aflatoxin exposure among the Chinese as compared with other ethnicities. The observation is plausible as Chinese tend to have higher intakes of dairy products, nuts, and cereal-based foods, and these food commodities are at high risk of being contaminated with aflatoxin (Nurul Fadhilah, Teo & Foo, 2016). In fact, Leong *et al.* (2012) claimed that aflatoxin metabolism may be influenced by genetic characteristics that vary across ethnic groups. Future study should explore this hypothesis.

Table 4. Binomial logistic regression predicting independent factors with the occurrence of urinary AFM_1 biomarker among respondents (n=444)

Variables	Exp B (95% CI)	Wald (df)	p-value
Sociodemographic factors			
Ethnicity	3.20 (2.04-5.03)	25.57 (1)	0.01**
Monthly household income	0.32 (0.19-0.57)	15.39 (1)	0.01**
Knowledge	0.86 (0.54-1.37)	0.41 (1)	0.52
Attitude	0.68 (0.42-1.10)	2.42 (1)	0.12
Practice	0.73 (0.45-1.19)	1.59 (1)	0.21

*p<0.05; **p<0.01

It is of interest to note that people with a monthly household income of RM1,500 or less were more protected from aflatoxin exposure than those with >RM1,500. According to the Economic Planning Unit (2013), the average monthly income of the urban households was almost twice higher than that of rural families. Majority of the study areas covered in this study were urban areas. The lifestyle of the urban population is usually more hectic than in rural areas. As such, urban dwellers tend to practise eating-out (Noraziah & Mohd Azlan, 2017), which makes the exposure of aflatoxin harder to be controlled since foods are managed and prepared by external food handlers. Therefore, it is reasonable to postulate environmental and lifestyle factors as possible inter-related factors to aflatoxin occurrence.

The evidence of aflatoxin occurrence trending across different age groups remains controversial. The current results reported to have younger people aged 18 to 24 years experiencing aflatoxin occurrence more than older people aged 25 to 60 years. This is in line with findings from Mohd Redzwan, Rosita and Mohd Sokhini Abdul (2012), whereby respondents aged 35 years and below had slightly higher AFM_1 level than those aged 36 years and above. However, Leong *et al.* (2012) indicated a reverse trend as higher risk of aflatoxin exposure was observed among older age group of 31 to 50 years as compared with younger age group of 18 to 30 years. On the other hand, earlier research done by Sun *et al.* (1999) showed high aflatoxin occurrence among males aged 30 to 45 years. Aflatoxin metabolism is correlated with liver and kidney functions (Mohd Redzwan *et al.*, 2014). A deterioration in liver and kidney function due to ageing may affect the production of aflatoxin metabolites in human. It would be interesting to explore further on this observation.

There is no specific antidote discovered yet to overcome aflatoxicosis, thus it is important to create awareness about aflatoxin among the people in order to control its exposure to our body. However, the awareness of people is still below satisfactory level as the outbreak of aflatoxicosis is still reported around the world (Kamala *et al.*, 2018). Indeed, the present study demonstrated that majority of the respondents did not know about aflatoxin and had negative attitude and poor practice in controlling aflatoxin contamination of foods. To the best of our knowledge, the association between KAP towards aflatoxin and urinary AFM_1 occurrence has not been reported in the literature. Interestingly, the current results found that those with positive attitude and good practice towards aflatoxin were more likely to have detected urinary AFM_1 as compared to those with negative attitude and poor practice. However, it should be noted that the attitude- and practice-related questions were focused on preventing the procurement of aflatoxin-contaminated foods rather than food storage and handling at home. There is a likelihood that respondents had negative attitude and poor practice in food storage and handling at home that exposed them to mould and aflatoxin, leading to the presence of AFM_1 biomarker in their urine samples.

There are several limitations to this study. First, the measurement of AFM_1 biomarker in the urine only reflected aflatoxin exposure for the past 1-2 days (Kensler *et al.*, 2010). The biomarker level may vary from day to day depending on the dietary intake of individuals, thus it is not suitable for assessing long term exposure. Therefore, it is advisable to assess other aflatoxin biomarkers, such as serum aflatoxin B_1-lysine adduct, that

represents long term exposure (Kensler *et al.*, 2010). Besides, this study only covered the Hulu Langat district, which is only one part of the Selangor state in Malaysia. Therefore, the results cannot be generalised to the whole population.

CONCLUSION

Ethnicity and monthly household income were the major contributing factors to aflatoxin exposure in the present study. People of Chinese ethnicity were 3.20 times more likely to have aflatoxin exposure as compared to non-Chinese. An increase in household income was associated with the likelihood of exhibiting AFM_1 occurrence in urine. The findings are believed to be useful in explaining the variations in aflatoxin exposure among the population of this country.

Authors' contributions

SHS, conducted the study, data analysis and interpretation; CWL, reviewed the study and prepared the draft of the manuscript; RJ, principal investigator, conceptualised the study and reviewed the manuscript; MRS, co-supervised the study.

Conflict of interest

The authors declare that there is no conflict of interest.

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