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Aflatoxin Contamination of Foods in Mozambique: Occurrence, Public Health Implications and Challenges

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ABSTRACT

Aflatoxins have gained increased recognition worldwide as several researches reveal the negative impacts on health, food security and trade. Major staple foods in Mozambique are prone to aflatoxin contamination, posing health risks to consumers, including the development of liver cancer and the progression of some infectious diseases. Aflatoxin contamination is mainly reported in peanuts, maize and their products. Nevertheless, some studies had reported the presence of aflatoxins and its metabolites in some foodstuffs of animal origin. Surprisingly, some of the contaminated foods had levels greater than the Codex permissible limits adopted by the Mozambican Government Authorities. Lack of awareness of occurrence and risks of mycotoxins, legislation enforcement, poor agricultural practices and undiversified diets predispose populations to dietary aflatoxin exposure. Regular surveys on aflatoxin contamination of food and exposure assessment through the measurement of aflatoxin biomarkers in human biological samples are not yet being performed. Regardless of these findings, the more important task is to monitor and control humans from being exposed to aflatoxins. Dietary assessment, clinical measurements and the enforcement of law should be immediately implemented as preventive strategies. With the current research on aflatoxin in Mozambique, both national and global networking for research collaboration is needed to expand the knowledge and disseminate the information to the global scientific community.

Introduction

Aflatoxins are secondary fungal metabolites known to cause serious health effects to both humans and animals. They are produced primarily by *Aspergillus flavus*, *A. parasiticus* and *A. nomius*, especially under hydrous stress[1,2]. Hot temperatures (36 to 38ºC) and high humidity (above 85%) are among the favorable environmental conditions which promote fungal and aflatoxin production[1-3].

Aflatoxins are ubiquitously found in foodstuffs, affecting a large portion of world food crops, particularly maize, groundnut, sorghum and their derivative products[2-5], and potentially exposing up to 5 billion people in the developing countries[2,6]. In most areas of these countries, especially in Africa, predisposing conditions that contribute to proliferation of fungi and increase the risk of aflatoxin production such as poor harvesting practices, and improper practices during transportation, storage and marketing are commun[2-5].

Eighteen different aflatoxin species have been identified. Nevertheless, aflatoxin B1 (AFB1), B2, G1, G2 and aflatoxin M1

(AFM1) are the best-known and of the greatest concern [1,7]. AFB1 is usually the most common, the most toxic and the most carcinogenic[1,7]. AFM1 is normally found in milk and urine, as the principal hydroxylated AFB1 metabolite produced by humans and other animal consuming a diet contaminated with AFB1[1,7]. All aflatoxins (AFB1, AFB2, AFG1, AFG2, AFM1) have been classified as carcinogenic to humans (Group 1) by the International Agency for Research on Cancer (IARC)[2,7,8].

Aflatoxins generate the greatest losses and the highest management costs due to their extremely high toxicity on a unit basis[1-3,9,10] and their long history of stringent regulations[11,12]. Global prevalence data suggests that aflatoxin, particularly AFB1, is 16 to 32 times more common in developing countries than in developed countries[8]. Human exposure to aflatoxins usually results from ingestion of contaminated foods, with indirect exposure through consumption of foods from animals previously exposed to aflatoxins in feeds much less[1,2,5,6,9].

Aflatoxins are fluorescent under ultraviolet (UV) light[7,13-15]. Initial attempts to detect aflatoxin were based on this criteria[13], where resulted the "B" and "G" classes, i.e., blue and green, respectively[7,13-15]. Subsequently, the purification of aflatoxin to the associated metabolites with and the same chemical proprieties became an important approach in scientific research on aflatoxins[7,13,14].

At present, the development of analytical methods to detect and quantify aflatoxins in foodstuffs[7,13,14], assessment of health risks from aflatoxin contamination of human food sources[8,9,12], and reduction of exposure to aflatoxin through various preventive strategies[2,3,7,16-18] are the major research areas. In parallel with improved technology, metabolomics studies, including the structural characterization and synthesis of the major aflatoxins also have expanded, and led to a better understanding of their toxicology and metabolism[13,20]. For example, the study of aflatoxin-related disease in human populations is now possible through the development and use of aflatoxin biomarkers[19-27]. The isolation of aflatoxin biomarkers, such as serum Aflatoxin-albumin, AFB1-DNA adduct, AFB1-lysine adduct and other AFB1 metabolites, in urine, breast milk and feces has enabled progress beyond the determination of toxin levels in food and feeds, and provides stronger evidence on the extent and severity of aflatoxin exposure within human population[15,28,29].

In Mozambique, the information on aflatoxins, particularly related to exposure are clustered in sporadic and few scientific reports, with a very low awareness outside academic sector[30]. Most of these reports were published over three decades ago, covering a few areas in the south of Mozambique. Furthermore, these reports might be outdated at some extent due to several socio-cultural, demographic, economic and politic changes that Mozambique has undergone. Recently, some updates reflecting new challenges in health sciences and agriculture have been reported. Therefore, the use of biomarkers measurement approach could be an asset to provide essential data on aflatoxin human exposure and health risk assessment in Mozambique.

This review presents an up-to-date documentation of the aflatoxin contamination of food commodities and discusses the current research, highlighting the mycotoxin menace in Mozambique with respect to health impacts, exposure and risk, and challenges on the way forward.

Occurrence of aflatoxins in food commodities and human exposure

Aflatoxin contamination of foodstuffs has been well documented in most of African countries. Groundnuts, maize and their products are among the commodities most susceptible to aflatoxin contamination, and are the major sources of human exposure to aflatoxins[2-6].

Although there are few scientific reports in Mozambique, aflatoxin contamination has been reported in a range of foods, including maize, groundnuts, sorghum, cassava, beans, rice, prepared foods, feed, poultry giblets and other products, showing it's the ubiquitous occurrence patterns in food commodities. Table 1 documents the reported occurence of aflatoxin in food commodities in Mozambique. Grains, including maize and groundnuts have been the most studied and considered the most concerning food commodities in regard to incidence and toxicity. These food commodities are used in many Mozambican diet either as a main ingredient or a base material[30-36], and also are key staples for feed and industrial use[36,39,40]. In fact, these crops are among the most widely planted commodities and are almost universally available in retail shops as they usually are inexpensive[31-37].

Most of the studies carried out do not distinguish the aflatoxins or are focused on AFB1. Few such as the studies by van Rensburg et al. [32] and Warth et al.[36], Mondlane et al.[39] also detected AFB2, AFG1 and AFG2. For instance, not all registered the sample size or the frequency of aflatoxin contamination. Yet, the results allow some comparisons. In general, the frequency of aflatoxin contamination ranged from zero (0%) up to 95%, with detectable levels reaching up to 2740 μg/kg, exceeding the most aflatoxins legal limits, including the limit recommended by Codex Alimentarius (10 μg/kg) and adopted in Mozambique[30,41,42]. For example, a survey during 1968-1974 by van Rensburg et al.[31] on aflatoxin contamination in dray stored raw cereals identified groundnuts as the main source of aflatoxins with an average concentration of 1036 μg/kg, whereas maize was much less contaminated with an average concentration of 2.4 μg/kg. As complementary part of the early mentioned

Table 1. Occurrence of aflatoxins in some Mozambican commodities and foodstuffs

Foodstuff	Type of aflatoxin	Sample size (*n*)	Positive samples *n (%)*	Aflatoxin level* *(µg/kg)*	Reference
Groundnuts	Total	-	153 (-)	1036	van Rensburg et al.[31]
	Total	-	-	2740	van Wyk et al.[33]
	B1	23	3 (14)	3.4–123	Warth et al.[36]
	B2		1 (5)	19.5	
	G1		1 (5)	30.3	
	G2		-	-	
	Total	-	-	5,7	Augusto et al.[37]
	Total	-	-	>20	Zuza et al.[38]
	B1	57	57 (100)	<LOD–73	Hlashwayo[42]
Maize	Total	-	168 (-)	2.4	van Rensburg et al.[30]
	B1	13	6 (46)	16.3–360	Warth et al.[36]
	B2		4 (31)	6.9–31	
	G1		6 (46)	19.7–256	
	G2		4 (31)	9.6–40	
	Total	-	-	690	Augusto et al.[37]
Beans	Total	-	65 (-)	13	van Rensburg et al.[31]
Cassava	Total	-	89 (-)	0.1	
Rice	Total	-	34 (-)	4	
Prepared food	B1	2183	174 (8)	132	van Rensburg et al.[32]
	B2		61 (2.8)		
	G1		17 (0.8)		
	G2		15 (0.7)		
Feed	B1	19	6/10 (60)	24.0–300	Warth et al.[36]
	B2		5/10 (50)	21.7–30	
	G1		5/10 (50)	24.4–240	
	G2		5/10 (50)	8.7–48	
Poultry feed	B1	-	- (65.5)	31.1	Mondlane et al.[39]
Chicken liver	B1	100	39 (39)	0.57–3.8	Sineque et al.[41]
Chicken gizzard	B1	80	11 (13.8)	0.68–2.1	
Others	B1	7	3 (43)	3.8–430	Warth et al.[36]
	B2		1 (14)	51.3	
	G1		1 (14)	382	
	G2		1 (14)	48.6	

*The single values represents the average of contamination levels reported in the study – the range is not specified; *(-)* = data not specified/ presented; LOD = Limit of detection.

survey, van Rensburg et al.[32] reported that aflatoxins were detected in 8% of all prepared food samples, with a mean value of 38 µg/kg for positive samples (maximum of 1317 µg/kg). In this case, aflatoxins B1 (89%) and B2 (6%), were the most predominant whereas aflatoxins G1 and G2 were much less frequently detected[32]. In recent studies by Warth et al.[36], Augusto et al.[37], Zuza et al.[38], and Hlashwayo[42], high frequency (up to 50%) of aflatoxin contamination and contamination levels exceeding the codex limits has also reported. Additionally, rejections and alerts of due to high aflatoxin contamination levels in groundnuts and their products has been notified by the European market[35,36]. For example, in 2007, aflatoxins contamination (AFB1 = 4.8 µg/kg and total aflatoxin = 7.5 µg/kg) of groundnut kernels from Mozambique were notified by the United Kingdom and Netherlands via the Rapid Alert System for Food and Feed (RASFF)[35].

These findings do not represent the whole scenario of aflatoxin contamination in Mozambique. However, such information shows the pervasiveness of human exposure to this food contaminant. Based on data from 1993 that was gathered by estimates of typical maize and groundnuts, including derivatives products consumption, contamination levels and body weight, the estimated daily exposure to aflatoxins in Mozambique is between 20-180 ng/kg bw perday[2,43,44], which is much higher than those in Western Europe and North America (0–1ng/kg bw per day) and exceed the provisional maximum tolerable daily intake (PMTDI) for aflatoxins set by the Joint FAO/WHO Expert Committee on Food Additives (JECFA)[2,29,45].

In fact, in Southern Africa countries, an ample, mounting evidence showing that the inhabitants, particularly from rural subsistence farming communities are at a high risk

of exposure and negative health impacts of mycotoxins has been documented[2,45]. These high levels of mycotoxin exposure in this region have been directly related to a lack of dietary diversity[2,3,45]. In addition, due to stringent mycotoxin standards imposed in developed countries its common in many Africans countries, including Mozambique, that rejected food usually used either as animal feed or in some circumstances for the production of local products[47] as well as the preparation of traditional crop-based beer[2]. In these countries, there is less emphasis on legislating maximum levels and even when such legislation exists, the capacity to enforce it is frequently lacking[2,11,12]. The least contaminated foods are destined for export, whereas highly contaminated products are retained for local consumption, exacerbating the high exposure levels of local populations[2,45-48].

Thus, it have been reported that access to a greater variety of foods and replacement of those at high risk of contamination will lower the risk of exposure by lessening the intake of these commonly contaminated foods[2,3,45]. According to Chen et al.[49], increased dietary diversity is one intervention for which the strongest evidence of improvement of health exists, but which is also the most difficult to achieve. Challenges to implementing dietary diversity in Mozambique may include environmental factors, food insecurity, cultural traditions and economic constraints[2,3].

Although the contamination of aflatoxins in groundnut and maize have been detected in high levels, in Mozambique research's focusing its derivative products, particularly destined for livestock animals are still lacking. Aflatoxins from animal products, including eggs, milk and meat of livestock consuming aflatoxin, especially aflatoxin B1 contaminated feed is a further source of human exposure that is often neglected or under-represented[2,30,40,41,45]. Studies by Warth et al.[36] and Mondlane et al.[39] in feed, and Sineque et al.[41] in poultry giblets reported aflatoxin contamination in these products, also exceeding the aflatoxin legal limits. Data on aflatoxin contamination and exposure from either livestock milk and milk products, and human breast milk does not exist. However, the aflatoxin carryover to human breast milk in Africans countries has been estimated at 0.1–0.4%[2].

Health impacts, risks and exposure assessment of dietary aflatoxins

The presence of aflatoxins in the food chain is a serious matter but not knowing its impact to the health is a big problem and should be a public concern, because even aflatoxin exposure at low levels can result in measurable human health impacts[6-9,15].

Aflatoxin contamination of predominantly consumed food commodities can exert serious health problems in consumer populations, directly, and also contribute to the increased incidence and severity of many infectious and non-infectious diseases[6-9,15]. Aflatoxins are known as carcinogenic, mutagenic, teratogenic, estrogenic, neurotoxic, hepatotoxic, nephrotoxic and cytotoxic agents, and may induce immunosuppression in humans[1,6-9,19-23]. They can bind to DNA and thereby promote cancer[1,6,7,15,19-23].

In Mozambique the chronic health risks of aflatoxin are prevalent because aflatoxin occurs more frequently under tropical conditions and staple diets in many areas of the country are often constituted by aflatoxin susceptible crops[45]. Table 2 presents some aflatoxin-related public health problems in Mozambique and other African countries.

Aflatoxins, especially aflatoxin B1 has been extensively linked as major risk factor to human primary liver cancer in Mozambique[31,32,43,44] and elsewhere in Africa[27,31,32,50] and Asia[21,41], in which it acts synergistically with hepatitis B virus (HBV)[6-8,19-23]. In Africa, acute exposure to high doses of aflatoxins have caused deaths from aflatoxicosis[6-8,24].

Other evidence in Africa suggests that there may be an interaction between chronic aflatoxin exposure and malnutrition[6,9,15,17,27,51,52], immunosuppression[6,9,15,53], anemia[6,25,54], impaired growth[6,9,15,17,26,55], infertility[6,9,15,56] and diseases such as malaria, HIV/AIDS[6,9] or certain respiratory diseases[2,8,45]. In young children the risk of growth delay increases after exposure to aflatoxins[15,26]. Aflatoxins interferes with micronutrient metabolism and may contribute to growth stunting during early childhood and together with other mycotoxins, are commonly suspected to play a role in the pathogenesis of kwashiorkor, a frequent condition in African children[2,6,15,26]. A review study by Katerere et al.[51] to assess the link between chronic aflatoxicosis and infant malnutrition in Southern Africa concluded that there is mounting evidence implicating aflatoxin contamination as an important factor in infant under-nutrition, increased morbidity and mortality due to negative impact on immune function and micronutrient absorption.

Table 2. Aflatoxin-related public health problems in Mozambique and other African countries

Health problem	Country	Reference
Primary hepatocellular carcinoma	Mozambique	Rensburg et al.[31,32]
	Egypt	Tumer et al.[50]
	Cameroon	Tchana et al.[27]
Aflatoxicosis	Kenya	Azziz-Baumgartner et al.[24]
Growth faltering	Gambia	Tumer et al.[26] Gong et al.[55]
Malnutrition	Nigeria	Onyemelukwe et al.[52]
Immunodeficiency	Ghana	Jiang et al.[53]
Anemia	Ghana	Shuaib et al.[54] Smith et al.[25]
Infertility	Nigeria	Uriah et al.[56]

In Mozambique, the true level of exposure and impact of aflatoxin intake are not clear. The recent information covers very few foods, lacks information on the frequency and quantity consumed and the health effects in the population. These limited data on mycotoxin exposure in general and risk assessment exists, primarily due to a lack of country specific data on food consumption patterns, limited human resource capacity and technical expertise to effectively monitor and evaluate mycotoxin levels[2,15,30,45]. In general, data on the likely human exposure to mycotoxins is still challenging to collect due to variation in food contamination levels and intake amounts in subsistence farming situations, as well as the differences and variations in toxicokinetics and toxicodynamics of individuals in these rural communities, which may simultaneously be suffering poor overall nutrition[1,2,6,15,45].

Exposure assessment of aflatoxin for both humans and animals has been based on data from the analysis of aflatoxins in food and feed samples collected from farms, markets, mills and stores[13,15,28]. These data were obtained by measuring aflatoxin levels in food samples and extrapolating the results to estimate average intake at the population level[15,28].

Measuring aflatoxins in food and feeds has provided initial estimates of aflatoxin exposure[13], but this approach is not reliable for determining an individual exposure for human and livestock animals, especially in developing countries[15,19,28]. The limitation of such data is that aflatoxin exposures vary with diet and with the level of contamination in the particular foodstuff consumed which also can vary widely[15,17-19]. The amount of aflatoxins present in raw foods may not be the same as that in food that's ingested[15]. In most cases, grains are sorted to some extent to remove kernels that are considered unfit to eat[2-5,16-18]. Thus, the most reliable measure of exposure may be through analysis of samples of prepared meals[15].

The expansion of metabolomics and the availability of multiple aflatoxin biomarkers for aflatoxin enables molecular epidemiology and direct measures of aflatoxin exposure of individuals in human populations[15,28,29]. Aflatoxin biomarkers such as AFM1 and AFB1-N7-guanine in urine and AFB1-albumin adducts in serum are all well documented for measures of aflatoxin exposure, especially in African countries and China[6,15-29,51-55]., where aflatoxin contamination is ubiquitous. These biomarkers are very useful for epidemiologists and public health workers and are being used to assess the extent and severity of aflatoxin exposure in the population. They also can be used to rapidly screen samples for acute exposures[15]. More importantly, they can assess chronic exposure which is not possible with other markers, e.g., aflatoxin-N7-guanine adduct in urine[6,15,19,28,29].

In Mozambique, exposure assessment by measuring aflatoxin biomarkers in human biological samples is in its early days compared to other African countries. The existing data is based on the food intake assessment approach[32,45]. Surveys of aflatoxin exposure with biomarkers in Africa have found that these biomarkers are present in the general populations (Table 3). Most of the these surveys has focused on children exposure with 85-100% of children have either detectable levels of serum aflatoxin-albumin (AF-alb) or urinary aflatoxins resulting presumably from high exposure levels of AFB1 in food and AFM1 in human breast milk. Aflatoxin exposure begins from utero and continues through breastfeeding in the post-natal period. Aflatoxin was found in umbilical cord blood samples in Ghana, Kenya, Nigeria and Sudan. The mothers of these infants had aflatoxins in their blood at the time of delivery[15]. Therefore, considering these evidences, the scenario of aflatoxin exposure in infants can be exacerbated with the co-occurrence of other mycotoxins[2,6,9,15].

Outstanding scientific gaps and challenges

The problem of aflatoxins is most acute in developing countries, including Mozambique, which lack resources and analytical capacity for analyses. Research on mycotoxins does not appear on top of the agenda in these countries as they prioritizes research on more pressing human health issues such as HIV/AIDS, malaria and infant mortality[2,6,9,30,45].

Although Mozambique has joined Codex Alimentarius and adopted their guides, country specific data on occurrence and exposure to aflatoxins, general awareness, measures to limit contamination in the field and in storage, and the negative health effects of aflatoxin consumption is still very limited. As a result of these limitations, few data are reported from Mozambique, and usually based on only a limited number of samples of uncertain quality especially in terms of robust sampling design[30,45]. These data are from few, mostly old studies and are based on estimates of food consumption. Thus, there is a widening gap between the quality and quantity of data generated by the laboratories in developed countries compared and those in developing countries.

It is a very important task to monitor and control aflatoxin contamination in the human food resources as it involves many aspects. The enforcement of legislations, general awareness on the occurrence and toxic effects of mycotoxins, surveillance and introduction appropriate control measures are critical initial steps towards food safety, economic sustainability and public health promotion. To date, there have been limited research and efforts to compare methods from different laboratories. Variation on the quantification of aflatoxins is influenced by the quantification technique, sample size, replicate

Table 3. Human exposure to aflatoxins in Africa – some results from biomarker studies

Country	Subject	Samples		Type of biomarker (rangue level)	Reference
		Total (n)	Positive (%)		
Benin	Childrens (16–37 months of age)	200	99	AF-alb adduct (mean 86.8 pg/mg)	Gong et al.[55]
Egypt	Lactating mothers	10	20	Breast milk AFM1 (mean 2.75 µg/l)	Alla et al.[57]
	Lactating mothers	443	56	Breast milk AFM1 (6–500 pg/ml)	Polychronaki et al.[58]
	Childrens (1–2.5 years of age)	50	38	Urinary AFM1 (2.5–2.8 pg/ml)	Polychronaki et al.[59]
	Lactating mothers	125	>50	Breast milk AFM1 (mean 9.8 ng/l)	El-Tras et al.[60]
	Pregnant women	98	35	AF-alb adduct (mean 4.9 pg/mg)	Piekkola et al.[61]
			48	Urinary AFM1 (mean 19.7 pg/mg)	
Guinea	Childrens (2–4 years of age)	50	86	Urinary AFM1 (10–27 pg/ml)	Polychronaki et al.[59]
Kenya	Women	884	100	AF-alb adduct (mean 7.47 pg/mg)	Leroy et al.[62]
Cameroon	Adults (83% HIV-positive)	175	83	Urinary AFM1 (*detected – level "ns"*)	Abia et al.[63]
	Kwashiorkor childrens	31	35.5	Urinary AFM1 (0.11–2.8 µg/l)	Tchana et al.[27]
	Marasmic childrens	11	45.5	Urinary AFM1 (0.11–0.86 µg/l)	
	Lactating mothers	62	4.8	Breast milk AFM1 (0.005–0.65 µg/l)	
Ghana	Adults	140	-	AF-alb adduct (0.12–3 pmol/mg)	Jolly et al.[64]
		91	-	Urinary AFM1 (*nd*–11,500 pg/mg)	
	Childrens	28	100	Urinary AFM1 (25–8,400 pg/mg)	Kumi et al.[65]
	Pregnant women*	246*	99.4**	AF-alb adduct (mean 1.2 and 1.9 pmol/mg)***	Natamba et al.[66]
Nigeria	Lactating mothers	50	82	Breast milk AFM1 (3–35 ng/l)	Adejumo et al.[67]
	Children, adolescents, adults	120	50.8	Urinary AFM1 *detected*	Ezekiel et al.[68]
Gambia	Childrens (3–9 years of age)	444	100	AF-alb adduct (2–459 pg/mg)	Turner et al.[69,70]
	Maternal blood at pregnancy	119	100	AF-alb adduct (4.8–260 pg/mg)	Turner et al.[26]
	Cord blood	99	48.5	AF-alb adduct (5–90 pg/mg)	
	Childrens	118	11	AF-alb adduct (5–30 pg/mg)	
Uganda (1999–2003)	All ages and both sex	374	92.5	AF-alb adduct (0.4–120 pg/mg)	Kang et al.[71]
Uganda (1989–2010)	All ages and both sex	713	90	AF-alb adduct (0.4–170 pg/mg)	
Sudan	Lactating mothers	94	54.2	Breast milk AFM1 (nd–3 µg/kg)	Elzupir et al.[72]
Tanzania	Lactating mothers	143	100	0.1–0.55 ng/ml	Magoha et al.[73]
	Children (6–14 months of age)	166	67	AF-alb (mean 4.7 pg/mg)	Shirima et al.[74]

(-) = data not specified/presented; *nd* = not detected; *HIV- uninfected and infected women during pregnancy and early lactation; **For all patients – in HIV-infected aflatoxins were detected in 100% of samples, with levels increased as pregnancy progressed; ***For HIV- uninfected and infected women, respectively.

number and laboratory where analyses are conducted[14]. Mozambique needs to acquire sampling and analytical tools that can be used to:

- Rapidly and inexpensively screen at the field or laboratory level, across broad level of contamination. Such screening would support a rapid alert system that informs responses and appropriate actions for food safety.
- Make risk assessments based on biomarkers. Such assessments provide sustainable information on the extent of human exposure to aflatoxins and a reliable toll for appropriate interventions strategies.

Furthermore, future research should be focused on the generation of data dealing with epidemiological, exposure and toxicity effects. On the global scale, a networking with other nations is needed and essential to improve research and evidence base on aflatoxin contamination and exposure, including expanding the knowledge and disseminating the information to the global scientific community.

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Conflict of interest

All authors contributed to writing the manuscript. There are no conflicts of interest.

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