Is Aflatoxin a Threat to Human-Health in Ethiopia? A Systematic Review

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Abstract

Background: *Aspergilli* are one of the major causes of degradation of agricultural products, as they can contaminate foods and feeds at different stages including pre- and post-harvest, processing, and handling. Besides, mainly *A. niger, A. flavus, and A. fumigatus* species are also causes of animal and human diseases, like mycotoxicosis, noninvasive, and invasive infections in immune-compromised patients, and hypersensitive reactions (e.g., asthma, allergic alveolitis) due to exposure to fungal fragments. Aflatoxin is one of the causes of the deadly hepatocellular carcinoma.

Methods: Electronic search in Pub-Med /MEDLINE and Google was conducted to find published literature with the English language. Reference lists of relevant articles were searched manually. We used search terms like "aflatoxin AND Ethiopia", "mycotoxin AND Ethiopia", and "aflatoxin AND Hepatocellular carcinoma AND Ethiopia". Titles and abstracts were initially screened for eligibility. The full texts of articles judged to be eligible were reviewed if they meet the inclusion criteria. Data were extracted on important variables like the sample size, region of the study, the food commodities studied, level of aflatoxin detected, aflatoxin detection rate, and more other variables. Microsoft Excel was used for data extraction.

Results: The electronic searches identified 52 articles of which 19 were found eligible. Most parts of the country were covered by the articles. The food commodities that were assessed by the studies include cereals like maize, sorghum, teff, wheat, barley, peas, beans, and groundnut. Milk was also assessed for aflatoxin. Similarly, beer samples were assessed for aflatoxin content in one article. Most of the studies reported aflatoxin contamination in the food items studied. Parts of the food items contained aflatoxin beyond the limit put by the different regulatory bodies like the East African standard, European commission, and food and drug administration (FDA). We were not able to retrieve articles that assessed hepatocellular carcinoma and its association with aflatoxin in Ethiopia.

Conclusion and recommendation: Aflatoxin contamination of food commodities intended for human consumption is a vast problem. Though the association of aflatoxin contamination in food commodities and hepatocellular carcinoma was not assessed in Ethiopia, it was confirmed elsewhere to be associated with hepatocellular carcinoma. Hence, the regulatory bodies in Ethiopia and policymakers shall work on creating awareness, putting ways of limiting and avoiding aflatoxins in food commodities intended for human consumption.

Keywords: Aflatoxin; Hepatocellular carcinoma; Ethiopia

Abbreviations:

AFB: Aflatoxin B; AFM: Aflatoxin M; *A. aculeatus: Aspergillus aculeatus; A. carbonarius: Aspergillus carbonarius; A. flavus: Aspergillus flavus; A. niger: Aspergillus niger; A. oryzae: Aspergillus oryzae; A. parasiticus: Aspergillus parasiticus; A. sojae: Aspergillus sojae; A. terreus: Aspergillus terreus;* EAS: East African Standard; FDA: Food and Drug Administration; HCC: Hepato-Cellular Carcinoma; kg: kilogram; L: liter; ppb: parts per billion; µg: microgram

Introduction

The genus *Aspergillus* is among the most abundant and widely distributed organism on earth, and at the moment comprises 339 known species. Although they are not considered to be a major cause of plant diseases, *Aspergillus* species are responsible for several disorders in various plants and plant products, especially as opportunistic storage molds. The notable consequence of their presence is contamination of foods and feeds by mycotoxins, among which the most important are aflatoxins, ochratoxin A, and to a less extent, fumonisins. Aflatoxins B1, B2, G1, and G2 are the most toxic and carcinogenic mycotoxins, due to their extreme hepatocarcinogenicity [1].

Aspergillus species are widespread geographically and can be either beneficial or harmful microorganisms; however, they have mainly a saprophytic lifestyle and predominantly grow on plant decaying materials. Many *Aspergilli*-related patents have been issued for medical compounds, such as lovastatin, produced by *A. terreus*. Several antibiotics, antitumoral, and antifungal agents have been derived from *Aspergillus* metabolites. Strains of *Aspergillus* are diffusely used in industrial products like soy sauce, miso, sake *(A.oryzae and A. sojae)*, several organic acids and enzymes *(A. niger, A. aculeatus, and A. carbonarius)*. Two of the most important industrial products produced by Aspergilli are amylase and citric acids [2,3].

Unfortunately, *Aspergilli* are one of the major causes of degradation of agricultural products, as they can contaminate foods and feeds at different stages including pre- and post-harvest processing, and handling. Besides, mainly *A. niger, A. flavus, and A*. fumigatus species are also causes of animal and human diseases, like mycotoxicosis, noninvasive, and invasive infections in immune-compromised patients, and hypersensitive reactions (e.g., asthma, allergic alveolitis) due to exposure to fungal fragments. *Aspergillus* species are opportunistic pathogens without host specialization and frequently isolated as food contaminants. Only a limited number of *Aspergillus* species can invade living plant tissues, while most of the species are storage mold on plant products [4,5].

Agricultural products can be contaminated by *Aspergillus* species, with changes of sensorial, nutritional, and qualitative nature like pigmentation, discoloration, rotting, and development of off-odors and off-flavors. The most notable consequence of their presence is mycotoxin contamination of foods and feeds [1].

Aflatoxins are decaketide-derived secondary metabolites produced by a complex biosynthetic pathway which could lead to four different metabolites: aflatoxin B1, B2, G1, and G2 (AFs). Aflatoxins, mainly AFB1, are the most toxic and carcinogenic naturally occurring mycotoxins. Aflatoxin B1 exhibits hepatocarcinogenic and hepatotoxic properties and epidemiological data implicate AFB1 as a component of liver cancer in humans in certain parts of the world [1]. Prospective studies have shown a strong association between biological markers of AFB1 exposure in serum or urine and the risk of subsequent hepatocellular carcinoma (HCC) [6].

Exposure to Aflatoxin is a major risk factor for the development of hepatocellular carcinoma (HCC), which is one of the most common malignancies worldwide [7,8]. Aflatoxins are secondary metabolites of *Aspergillus flavus* (AF) and *Aspergillus parasiticus* (AP), fungus that develops under characteristic environmental conditions that are related to high moisture and temperature, and is associated to the storage of maize, groundnuts, wheat, soy and rice [9]. The Food and Drug Administration (FDA) has regulated aflatoxin levels for human consumption establishing a level of 20 µg/Kg total aflatoxin (B1, B2, G1, and G2) [9,10].

Aflatoxin and human health

Prospective studies have shown a strong association between biological markers of AFB1 exposure in serum or urine and risk of subsequent HCC. The interaction between AFB1 exposure and HBV infection in HCC risk was replicated in different cohorts [6]. Aflatoxins commonly contaminate foods such as peanuts, grain, legumes, and corn. They are carcinogenic in experimental animal models and aflatoxin B1 (AFB1) is the most potent hepatocarcinogen [11]. Worldwide AFB1 exposure correlates with a specific mutation at codon 249 in the p53 tumor suppressor gene in liver tumors and suggested interaction of aflatoxins with hepatitis B virus infection in the development of hepatocellular carcinoma [12-14].

A study in the early 1970s measured aflatoxin B1 levels in major diet components from different parts of Uganda over one year and found that the frequency of aflatoxin B1 contamination was particularly high in provinces with high HCC incidence [15]. Similar associations were reported in Kenya [16], Swaziland [17], and Thailand [18].

The maximum limit set by the East African Community (EAC) for cereal flours intended for human consumption is 10 µg/kg for total aflatoxin content; and 5 µg/kg for aflatoxin B1 content [19].

Data was analyzed on SPSS-16. Descriptive statistics were applied on the available data. Mean ± SD was presented for age, height, right renal length (RR L), left renal length (LR L), right parenchymal thickness and left parenchymal thickness. Frequencies and percentages were computed for gender and age groups.

Rationale of the systematic review

The rationale of this systematic review is to evaluate the magnitude of aflatoxin food contamination and the potential consequent influences of aflatoxin on human health in Ethiopia. Aflatoxin related hepatocellular carcinoma is preventable cancer thus identification of its commodities is important in minimizing the incidence of aflatoxin induced hepatocellular carcinoma. This may be achieved by establishing important interventions and adopting dietary changes. Aflatoxin is a potent hepatocarcinogen. Aflatoxin is reported to be strongly associated with hepatocellular carcinoma. This systematic review will help policymakers and regulatory bodies to take action and formulate regulations that will help control the problem.

Methods

This study was conducted according to the guideline of the PRISMA group (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) [20]. The PRISMA checklist was used to ensure the inclusion of relevant pieces of information. We aimed to describe the magnitude of aflatoxin contamination of human food in Ethiopia. The other objectives of this review were assessing knowledge and awareness of the different stakeholders regarding aflatoxin. Similarly, we wanted to assess the association of aflatoxin contamination of food commodities and hepatocellular carcinoma in Ethiopia.

Search strategy

Electronic search in Pub-Med/MEDLINE and Google was conducted to find published literature with the English language. Reference lists of relevant articles were searched manually. We used search terms like “aflatoxin AND Ethiopia”, “mycotoxin AND Ethiopia”, and “aflatoxin AND Hepatocellular carcinoma AND Ethiopia”. The last search was conducted on September 4, 2019.

Study selection

We selected articles that have addressed aflatoxin in Ethiopia. There was no restriction on the year of publication. We did not put any limitations on the study design and sample size as well. To minimize selection bias, all possible relevant articles were evaluated critically and those that meet the inclusion criteria were selected. Abstracts were examined with full articles obtained if the study looked relevant.

However, articles written only in the English language were selected. Similarly, all the articles retrieved were only cross-sectional studies.

Inclusion and exclusion criteria

Articles that assessed aflatoxin contamination of food commodities and items intended for human consumption or those articles that assessed knowledge, awareness, and attitude of the various stakeholders regarding aflatoxin contamination in Ethiopia are included in this systematic review.

We excluded review articles, articles that assessed only the performance of laboratory methods for aflatoxin detection, articles done outside Ethiopia, articles that assessed fungal contamination without aflatoxin analysis, and articles that assessed mycotoxins other than aflatoxin. The details of the inclusion and exclusion criteria are given in supplementary file 1.

Data extraction

Full articles were examined for quality, and data were extracted by the authors using forms that were built for this purpose listing the relevant variables. The first author, publication year of the study, study area, study design, the food commodity assessed, the aflatoxin type, the aflatoxin content in micrograms (µg) per kilogram (Kg) or in parts per billion (ppb) or micrograms per liter (l) were extracted. The data were extracted by AO and BA.

Results

Literature search and article characteristics

A total of 52 studies were retrieved from Pub-Med/MEDLINE databases, Google as well as through manual search of reference lists. Thirty-six were excluded; 16 were duplicates, 1 was only about laboratory technique, others were about other forms of mycotoxins than aflatoxins. The details of inclusion and exclusion are shown in the flow chart below (Figure 1).

We included nineteen articles that addressed aflatoxin in this systematic review [21-39]. Four of the articles have addressed knowledge of different stakeholders about aflatoxin [25,27,30,35].

Food items assessed for aflatoxins

Various commodities of food items intended for human consumption were assessed for the presence of aflatoxins in the articles included in this systematic review. The commodities of food items included sorghum [21-23,28,31], groundnut (peanut) [28,38-40], maize [28,32,36,37], wheat [21,28], barley [21,28], millet [28], teff [28], broad beans [28], dry peas [28], pepper [28], and coffee [26]. Milk was also assessed for contamination by aflatoxin M in one study [24]. However, two of the articles included in this systematic review did not assess food items, one of the articles assessed urinary aflatoxin [33] while the other assessed knowledge and awareness of the various stakeholders about aflatoxin contamination of food items [35].

Areas of the country where the articles were conducted

Most regions of the country were covered by the studies included in this systematic review. These includes the Amhara national regional state [21,23,25,33,34], the Oromia national regional state [21,25,26,31,32,34], Harari regional state [22,38], Dire Dawa city administration [32,38], Southern nations nationalities and peoples regional state (SNNPR) [25,28,34,36,37], Tigray regional state [25,33,34], Somali regional state [38], and Addis Ababa city administration [30]. We were not able to retrieve articles done in other regions of the country like Afar, Benishangul-Gumuz, and Gambella.

Characteristics of food commodities studied (Moisture, storage)

The moisture of the groundnut seeds ranged from 3.4% to 10.2% [38]. Similarly, the moisture content of sorghum and other cereals was in the range of 8.8%-11.86% in the four larger regions of the country [23,34]. The moisture content of the various cereals and food commodities (barley, wheat, teff, sorghum, pepper, millet, maize, broad beans, dry peas, and peanut was 12.8%-15% in the study

conducted in parts of the Oromia, southern nations, nationalities and peoples, and Harari regions [28,37]. The moisture content of the majority of the food commodities was in the recommended level 10%-13% [40,41].

With regards to storage, the cereals and the different cash crops assessed by the studies included in this review were stored in various storages. The commonly used stores were above ground pits ("Gotera"), underground pits ("Gota") [21-23,28,36,37]. "Gota" is a cylindrical chamber made of mud mixed with straw, whereas "gotera" is 'basketwork' with thatched conical roofing [21]. Groundnuts were stored in polyethylene bags or sacks, 58% of farmers used old bags and sacks for storing the groundnut seeds [38]. The coffee grains were stored in various storages like standard sacks, fertilizer bags and poly-bags [26]. The storage duration ranged from 03 to 10 months of post-harvesting [32,37]. The utilization of these stores was not optimal. However, adequate storage conditions with optimal moisture, temperature, and aeration are vital for the prevention of aflatoxin contamination [42].

Knowledge, awareness and attitude of stakeholders about aflatoxin

In a research conducted with the objective of determining the stakeholders' perceptions about groundnut qualities with respect to aflatoxin contamination and pre- and post-harvest practices affecting the development of aflatoxigenic fungi and aflatoxin contamination among 165 respondents, 98.7% farmers and 93.3% traders disagreed on the indices of groundnut quality assumed of promoting aflatoxin contamination. Similarly, 70% of the consumers were unaware of aflatoxin contamination and its consequence [27]. About 78% of interviewed farmers did not properly dry their harvested groundnut and lacked awareness about increasing fungal invasion in relation to improper drying and storage methods [38].

Aflatoxin content of the food commodities

Sorghum: Six to 96.7% of the sorghum samples assessed for aflatoxin were contaminated with variable amounts of aflatoxin [21-23,28,39]. The total aflatoxin B1, B2, G1, and G2 in sorghum grains ranged from 11.4 to 344.3 µg/kg, 3.9 to 153.7 µg/kg, 1.2 to 91.8 µg/kg, 9.9 to 139.6 µg/kg, and 3.2 to 52.02 µg/kg, respectively [22,23]. Sorghum stored for longer duration had higher aflatoxin content than freshly collected and that stored for a shorter duration [22,23].

Maize: Eight to 100% of the maize samples assessed for aflatoxin were contaminated with variable amounts of aflatoxin. The aflatoxin content in the maize samples ranged from 5-513 µg/kg

[28,32,36,37]. This review revealed Maize was found to be one of the highly aflatoxin-contaminated food commodities. Similarly, maize was one of the two (along with groundnut) highly aflatoxin contaminated food commodities in the world [43].

Groundnut: A total of 22.5 to 77.5% of groundnut samples were contaminated with the hepatocarcinogen aflatoxin [28,38,39]. Similarly, 50% groundnut cakes locally known as "Halawa" samples were contaminated with aflatoxin [38]. The aflatoxin content in the groundnut samples was in the range of 15 and 11,900 µg/kg [28,38,39]. The aflatoxin content in the groundnut cakes reached to 158.1 µg/kg [38]. Among the various cereals and cash crops that were assessed for aflatoxin contamination, groundnut and sorghum had higher aflatoxin content [28]. Compared to other food commodities groundnut was found to be the most aflatoxin-contaminated. This is consistent with one study that reported groundnut and maize to be contaminated highly with aflatoxin in the world [43].

Other cereals: Four to 30% of wheat, barley, teff, and millet samples were found contaminated with aflatoxin [21,28]. Aflatoxin content up to 283 µg/kg was detected [28,29,34].

Milk and Beer: One hundred ten milk samples were assessed for aflatoxin M contamination in the greater milk shed in Addis Ababa city, where all the milk samples were found to be contaminated with aflatoxin M (AFM) in the range of 0.028-4.98 µg/L. Overall, only nine (8.2%) out of a total of 110 milk samples contained less than or equal to 0.05 µg/L of AFM1 [24]. AFM limit of 0.05 µg/L is put by the European Commission [44]. Similarly, from the twelve alcoholic domestic beers that

were assessed for aflatoxin content in Addis Ababa city administration, eleven brands were positive with a range of total aflatoxin between 1.23 and 12.47 μg/L [30].

Comparisons of the aflatoxin content of the food commodities with standards

Almost all the studied food commodities and items assessed for aflatoxin content with variable proportions had crossed the limit put by the different standards and regulatory bodies such as East African standards, the European Union and the FDA [19,44,45]. The aflatoxin limit put by the east African standard is 10 μg/kg total aflatoxin content and 5 μg/kg for aflatoxin B1 content [19].

Implications

The results of this systematic review have several implications in clinical practice and in policy issues. There is a high burden of aflatoxin contamination of staple food commodities and cash crops. This systematic review had revealed that the commonly used food commodities for human consumption like sorghum, maize, and Groundnut were highly contaminated with aflatoxin in Ethiopia. Similarly, the knowledge, awareness, and perception of the different stakeholders regarding aflatoxin contamination and its untoward effect on health were found to be poor in Ethiopia that will eventually result in increased aflatoxin contamination of food commodities and consumption of the contaminated food commodities. Hence, awareness creation among the different stakeholders like farmers, traders, and consumers about aflatoxin contamination and its health impacts is essential. Similarly, it will be better to have periodic measures on ensuring that aflatoxin-contaminated food commodities are prohibited from distribution and use.

Strength and limitations

This study has the following strengths. This is a systematic review that has addressed aflatoxin contamination of food commodities intended for human consumption in Ethiopia. It has analyzed articles from most parts of the country where eligible studies have been retrieved from. It has analyzed various food commodities including milk and beer as well.

Nevertheless, this study has several limitations. The synthesis we performed was only qualitative; we have not performed quantitative analysis. The studies used for analysis were heterogeneous as different techniques were used for extracting the aflatoxin. There was no article retrieved from three regions of the country which could raise a representativeness concern. The other major limitation of this study is the lack of data on the association of aflatoxin exposure and hepatocellular carcinoma in Ethiopia.

Conclusion

Aflatoxin contamination of food commodities intended for human consumption in Ethiopia is a huge problem. Similarly, the knowledge, awareness, and perception of the different stakeholders about aflatoxin contamination of food commodities are poor in Ethiopia. It is essential to make concerted campaigns to create and raise perception among farmers, traders, and consumers about pre- and post-harvest practices affecting food commodities quality and aflatoxin contamination. Policymakers and regulatory bodies shall better work in ensuring low or no aflatoxin contamination in food commodities intended for human consumption.

Data Availability

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Nevertheless, most of the important data and the included articles are given in the supplementary materials.

Conflicts of Interest

The authors declare that there are no conflicts of interest.

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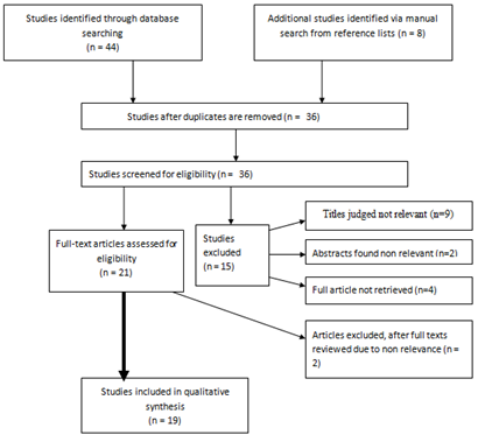

Figure 1: Flow chart of the studies included.