The contamination rate of Aflatoxin M1 in milk samples of Iran: A systematic review and meta-analysis protocol

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Background

Aflatoxin M1 (AFM1) as a 4-hydroxylated metabolite of carcinogenic mycotoxin Aflatoxin B1 (AFB1) is considered as an acute toxic compound found in milk and its derived products (1). Approximately, 0.3-6.2 % of ingested AFB1 (received by contaminated feeding) has been claimed to be transformed to AFM1 through cytochrome P450 associated enzymes and consequently released into the milk (2). It is classified as group 1, carcinogenic to human. Mycotoxins intake is found to be severely lead to adverse health effects including reproductive tract child growth impairment and hepatitis B virus infection (3). Despite the significantly lower carcinogenic characteristics of AFM1 compared to AFB1, its genotoxic, carcinogenic and cytotoxic effects have been well documented in different studies and consequently aflatoxicosis is considered as a food-borne disease.

Regarding the critical public health issue induced by AFM1 and as milk is essential in human food chain, its maximum limit has been set by regulatory agencies considering the economic status and development of the countries (4). The maximum permissible limit for AFM1 is 50 ng/kg to 500 ng/kg (5). In Iran, it has been regulated as 100 ng/kg (6).

To the best of our knowledge, there is several similar systematic review and meta-analysis about the contamination of AFM1 in milk, globally and specifically in Iran (4, 5, 7-10). However, we will try to examine the mean and prevalence of AFM1 in different type of milk (cow, sheep, goat, raw, processed) in different region of Iran with long time interval (1974-2021). Moreover, more general and specific databases and grey literature with excellent eligibility criteria will be applied to have a more comprehensive review leading to an interesting finding by techniques such as risk of bias assessment (for assessing quality of included studies), publication bias assessment, finding the source of heterogeneity by sub-group analysis in addition to meta-analysis process.

Objectives

Primary objective: Estimation of Aflatoxin M1's contamination level in milk samples of Iran.

Secondary objectives:

- Calculation of the mean and prevalence of Aflatoxin M1 by milk types (cow, goat, sheep, etc.)
- Calculation of the mean and prevalence of Aflatoxin M1 by milk types (raw, pasteurized, UHT)
- Calculation of the mean and prevalence of Aflatoxin M1 by Province
- Calculation of the mean and prevalence of Aflatoxin M1 by determination technique (HPLC, ELIZA, etc.)
- Heterogeneity assessment and finding its potential reasons
- Calculation of trends of Aflatoxin M1 in milk samples of Iran
- Risk assessment of Aflatoxin M1 exposure through milk consumption among Iranian population
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Methods

Inclusion and exclusion criteria (eligibility criteria):

Possible and suitable eligibility criteria regarding the objectives of the study will be considered in the screening and selection phases.

This review will consider studies, conducted in Iran, that include presence and contamination of aflatoxin M1 in all kinds of animal milk (raw or heat processed with all heat-treatment methods).

It explicitly excludes any data and study that reports on human milk and other dairy products. If a

study reported all dairy products and includes clearly reported data on milk as a subgroup, it will be included. Studies investigated aflatoxin M1 in milk of other countries (except Iran) will be excluded. This review will include only studies conducted in the occurrence of aflatoxin M1 in milk samples and other studies conducted on samples covering other aflatoxins (like B1, B2,...) or other mycotoxins will be excluded. Any cross-sectional studies (descriptive or descriptive-analytical) reporting prevalence of aflatoxin M1 in milk samples will be included. Studies have to specifically provide mean and range of contamination in their results, or the prevalence has to be calculated using the reported data. Clear presence of Aflatoxin M1 detection technique in milk is other inclusion criteria. Method validation studies, books and review articles as well as genetic, ecological, and animal studies will be excluded. Furthermore, inaccessibility of the full text of the research studies is another exclusion criteria. There is no limitation for sampling method or sample size of researches in this review.

It is worthy to note that the steps of selection, data extraction, and risk of bias assessment will be performed by two authors independently and disagreements are supposed to be resolved by consensus.

Search strategy (search method and sources)

Search terms/keywords

To achieve the most inclusive search, the search strategy will be based on two components (outcome, participant) and include both official and unofficial databases (Gray literature). To find Keywords for the search two components (aflatoxin M1 and milk), thesaurus systems, like MeSH, the free text method (like question from experts) will be used. Further keywords obtained from related articles and abstracts identified through a scoping search in published, peer-reviewed literature (the first step of three phased method). We will also scope for published systematic

reviews in the field of aflatoxin M1 occurrence in milk products to identify more keywords for use in the search strategy. A pilot search strategy will be developed based on Scopus and PubMed tested to find if it identifies relevant studies in the field and will be finalized. This search syntax will then be translated for use with different databases and the final search strategy used in every database will be reported. If additional resources or databases are identified from included studies and their reference list, they will be searched as well using appropriate search strategy. The search strategy used SCOPUS is shown in the following:

(ALL (milk) OR ALL (dairy) OR ALL ("dairy product") OR ALL ("milk product") OR ALL (dairying) OR ALL ("dairy cattle") OR ALL ("Dairy farm")) AND (ALL ("aflatoxin M1") OR ALL (aflatoxin) OR ALL ("Aflatoxin-M1") OR ALL (afm1)) AND (TITLE-ABS (Iran) OR AFFIL (Iran) OR PUBLISHER (Iran)) AND ((PUBYEAR < 2022 AND PUBYEAR > 1974) AND NOT PUBDATETXT (December 2021))

Sources of primary research

To achieve the study objectives, searches will be carried out in the following electronic databases: General databases: Scopus, Google Scholar (search engine), PubMed, Web of Science (WOS), and ProQuest; specific databases: Agricola, and Agris without language restriction for studies on the prevalence of Aflatoxin M1 in milk marketed in Iran. Furthermore, Iranian academic databases like SID and Magiran will be explored (in Persian language). Relevant gray literature including thesis (dissertations), technical reports and conference papers will be included by searching in the electronic databases of ProQuest, Scopus, Web of Science, Google Scholar, as well as Irandoc, civilica.com and research.ir (Iranian databases). We will also check the reference list of included studies to check no missing document. Furthermore, the reference list of similar previous

systematic reviews will be investigated to find probable missed article. Other sources like organizational websites and contact to relevant expert will may be performed in this review.

Meta-analysis

Important data (author name, year of publication, detection techniques, type of milk, and type of livestock, sample size, mean of AFM1 in milk, city, etc.) will extract from included studies and collect in Excel. Meta-analysis will evaluate considering parameters like effect size (key measure), heterogeneity, publication bias, and risk of bias assessment using STATA.

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