Aflatoxin M1 Exposure and Health Risk Assessment in Children and Adults due to Pasteurized Milk Consumption in Addis Ababa, Ethiopia

Hiwot Tadesse¹,*, Aynadis Tamene¹, and Gulelat Dessie²

ABSTRACT

Milk and dairy products stand as essential sources of nutrition across all age groups. However, the presence of Aflatoxin M1, particularly common and risky for young children, necessitates careful assessment of milk. This study, conducted in Addis Ababa, Ethiopia, aimed to evaluate the prevalence of Aflatoxin M1 using High-Performance Liquid Chromatography in pasteurized milk and assess associated health risks using deterministic techniques. The prevalence of Aflatoxin M1 in pasteurized milk was 56.6%, with Aflatoxin M1 levels ranging from 0 to 6.27 μg/L and a mean value of 1.237 μg/L. The risk assessment revealed substantial Aflatoxin M1 exposure risk from pasteurized milk consumption, as reflected by margin of exposure values <10,000, specifically 25.9, 109.6, and 380 for children aged 2–5, 6–9, and adults, respectively. The hazard index values for pasteurized milk consumers were 110, 26, and 7.5 for children aged 2–5, 6–9, and adults, respectively. Furthermore, cancer risk calculations yielded values of 0.18, 0.04, and 0.01 per 100,000 population for children aged 2–5, 6–9, and adults, respectively, with an average potency of 0.007978 cases per 100,000/yr ng Aflatoxin M1/kg bw/day. Notably, children aged 2–5 and 6–7 face elevated risks, whereas adults face relatively lower risks.

Keywords: Aflatoxin M1, Exposure assessment, Pasteurized milk, Risk assessment.

1. INTRODUCTION

Milk, a nutrient-rich food, plays a pivotal role in promoting human health and facilitating bodily growth and development. It boasts an array of essential macronutrients and micronutrients crucial for sustaining growth, providing energy, supporting reproductive functions, facilitating maintenance and repair processes, curbing appetite, and upholding overall well-being [1]. However, despite the numerous health benefits attributed to milk and dairy products, they often fall prey to contamination by microbes and toxins, among which aflatoxins take the spotlight [2]. Aflatoxins, a group of hazardous and carcinogenic metabolites, are produced by specific species of Aspergillus fungi thriving on various plants and plant-based products [3]. These naturally occurring mycotoxins are potent, bearing both genotoxic, chronic, and acute liver toxic properties, particularly when coupled with hepatitis [3]. Aflatoxin B1 (AFB1), a subtype of aflatoxin, stands as the most prevalent and potent carcinogenic toxin [4]. Epidemiological studies have hinted at a possible link between AFB1 exposure and human liver cancer, as well as acute hepatitis [5]. The International Agency for Research on Cancer has categorically classified this toxin as a Group 1 carcinogen, signifying its unequivocal carcinogenicity to humans [6], [7].

Highlight a section that you want to designate with a certain style, then select the appropriate name on the style.
stands as the most significant toxin found in milk and dairy products [11]. It originates in the livers of lactating animals and is subsequently discharged into raw milk by dairy cows that have ingested a feed containing AFB1. Importantly, AFM1 exhibits stability even at high temperatures (≥250 °C), rendering it resistant to removal through heating processes. Consequently, this toxin can persist in milk and its derived products, including pasteurized milk [12].

Children heightened metabolic rates, smaller body weights, underdeveloped metabolic pathways, and incomplete tissue and organ maturation render them more susceptible than adults to acute hepatotoxicity induced by aflatoxins and other toxic substances [9]. This vulnerability is particularly pronounced in developing nations. Among the human population, children—especially infants rely on milk for proper growth and development, often consuming it in substantial quantities are more vulnerable [13]. Additionally, their exposure to aflatoxins is exacerbated by the consumption of complementary foods crafted from cereals and legumes [13], [14], which are susceptible to contamination by aflatoxin-producing fungi. These complementary foods may be either mixed with or consumed alongside milk [1]. Furthermore, because children are expected to live longer than adults, they face an elevated risk of developing chronic ailments [8], [15], [16]. Moreover, their comparatively weaker detoxification capacity, swifter metabolic rates, and lower intake-to-body weight ratios heighten their susceptibility to the adverse effects of AFM1 [17].

While the potential for AFM1 contamination in milk and dairy products is a significant safety concern, there remains a notable dearth of information regarding the prevalence of AFM1, specifically in pasteurized milk. Prior risk assessment studies related to milk consumption, including the one published [18], have indeed been conducted; however, these studies did not account for children as a distinct population group, nor did they focus on pasteurized milk as a specific food product. Addressing this critical gap in knowledge, our study set out to assess exposure and prevalence of AFM1, each of the 66 samples underwent individual analysis. As the project delved into risk characterization, the mean values derived from all the samples were considered.

2.3. Determination of AFM1 Occurrences in Pasteurized Milk

For the quantification analysis of AFM1, the Agilent High-Performance Liquid Chromatography system (HPLC 1260 Infinity series) was utilized, which featured a quaternary pump and fluorescence detection. The analysis was conducted in accordance with the method specified in EN ISO 14501:2007. Data acquisition and quantification were carried out using Chem Station (Open Lab edition). The Agilent HPLC, equipped with a fluorescence detector, was configured with an excitation wavelength of 360 nm and an emission wavelength of 440 nm. The HPLC column employed was TC-C18 (2), measuring 4.6 mm × 250 mm, with a pore size of 170, particle size of 5.0 μm, inner diameter of 4.6 mm, and a carbon load of 12%. The column compartment was maintained at a temperature of 35 °C. For the mobile phase, a mixture of water and acetonitrile was used at a ratio of 25:75 (v/v), respectively, employing an isocratic delivery mode with a flow rate of 0.8 mL/min and an injection volume of 10 μL [22].

2.2. Sample Size and Sampling

A thorough risk assessment was conducted for AFM1 by analyzing its concentration in pasteurized milk. To gather samples, a cross-sectional study design was employed that encompassed all the sub-cities of Addis Ababa. In total, 60 pasteurized milk samples were collected, with representative samples obtained from each sub-city. The sampling approach involved simple random selection. Each sample was diligently collected in its original sachet packaging, ensuring proper preservation using cold storage. Subsequently, these samples were transported to the food laboratory of the Ethiopian Food and Drug Authority for analysis. In a quest to determine the occurrence and prevalence of AFM1, each of the 66 samples underwent individual analysis. As the project delved into risk characterization, the mean values derived from all the samples were considered.

2.1. Study Area

The study was carried out in Addis Ababa, the capital city of the Federal Democratic Republic of Ethiopia, where pasteurized milk is a staple in most households, ranking closely behind cottage butter [19]. Addis Ababa is positioned between latitudes 8° 55’ and 9° 3’ North and longitudes 38° 43’ and 38° 50’ East, covering an expanse of 51,000 hectares in the central highlands. It graces an average altitude ranging from 2000 to 2560 meters above sea level and is divided into 11 sub-cities [20]. Presently, Ethiopia is home to 32 milk processing industries scattered across various regions. The urban populace constitutes a significant market for dairy products, with Addis Ababa and its neighboring districts playing a pivotal role. The primary dairy processing plant offerings encompass pasteurized milk, yogurt, and an array of cheese varieties, including Provolone, Mozzarella, Gouda, and Feta. Among the diverse dairy products produced throughout the country, pasteurized milk accounts for approximately 83.4% of the total milk production, while yogurt makes up about 12.69% [21].

2. MATERIALS AND METHODS

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2.3.1. Preparation of Standard Solutions

A mother stock solution was prepared with a concentration of 0.1 μg/mL using a standard solution of AFM1 (with a concentration of 0.993 μg/mL in acetonitrile). This mother stock solution was stored in a freezer for preservation. To create a series of working solutions for calibration, a working stock solution of 0.01 μg/mL was taken and diluted step by step using a combined solution (acetonitrile/water, 75/25, v/v). These working solutions were then stored in vials at temperatures below 4 °C. The
calibration curve was constructed using calibration solutions with concentrations of 0.02, 0.04, 0.06, 0.08, and 0.10 μg/kg. In instances where samples had AFM1 concentrations exceeding the calibration range, we applied dilution techniques, factoring in dilution factors as stipulated by EN ISO 14501:2007 [22].

2.3.2. Preparation of Samples
Following a gentle warming at approximately 37 °C in a water bath, the samples underwent centrifugation at 2000 g to facilitate the separation of the fat layers, after which they were filtered. A precisely measured test portion of 50 mL was then transferred into a syringe barrel connected to an AFM1 immunoaffinity column. The sample was then passed through the column at a controlled, slow flow rate of 1–2 mL/min. Subsequently, the columns were washed with 20 mL of deionized water, and air was passed through the columns to ensure thorough drying. To elute the AFM1, 4 mL of pure acetonitrile was introduced and allowed to interact with the column for a minimum of 60 s. The resulting eluate was then evaporated to dryness using a gentle stream of nitrogen. The residue obtained was dissolved in 500 μl of the mobile phase and subjected to filtration using a membrane filter before being injected into the HPLC system for quantification, following the protocol outlined in EN ISO 14501:2007 [22].

2.3.3. Instrumentation
An HPLC system featuring a quaternary pump and fluorescence detection for the quantification analysis of AFM1, following the established protocol outlined in EN ISO 14501:2007 was utilized. Data acquisition and quantification were seamlessly managed through ChemStation (Open Lab edition). The Agilent HPLC system, equipped with a fluorescence detector, was finely tuned for optimal performance. It was configured with specific parameters, including an excitation wavelength set at 360 nm and an emission wavelength at 440 nm. The column compartment maintained a constant temperature of 35 °C. For chromatographic separation, we employed an HPLC Column with the specifications TC-C18 (2), featuring a pore size of 170, particle size of 5.0 μm, inner diameter of 4.6 mm, and a length of 250 mm, with a carbon load of 12%. In the mobile phase, water and acetonitrile at a consistent ratio of 25:75 (v/v) were blended. The analytical approach adopted an isocratic delivery mode, with a steady flow rate of 0.8 mL/min, and sample injection was standardized at 10 μL. This accurately configured instrumentation ensured the precision and accuracy required for AFM1 quantification [1].

2.3.4. Validation
The validation process for the HPLC-FLD method adhered to the stringent criteria outlined in European Commission Decision 657/2002/EC, specifically tailored for confirmatory analytical methods. Several critical parameters underwent thorough evaluation, including linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and selectivity. To assess linearity, we constructed solvent-matched calibration curves with five data points, and each calibration was accurately performed in triplicate using AFM1 standard solutions spanning concentrations from 0.05 to 0.8 mg/L. These calibration curves were generated by plotting peak areas against the corresponding AFM1 concentrations. The robustness of linearity was judiciously examined through linear regression analysis, expressed as the coefficient of determination ($r^2$). The precision evaluation was conducted by gauging the percent of Relative Standard Deviation (RSD) derived from three identical extractions of milk samples spiked with AFM1, both at consistent spiking levels and across three distinct levels of spiking. This comprehensive precision assessment ensured the reliability and repeatability of our method.

Furthermore, a method selectivity assessment was employed to scrutinize potential interference from endogeneous substances around the retention time of the target analyte. This was achieved by scrutinizing AFM1 in known negative milk matrices and reagent blanks, fortifying the confidence in the method’s specificity and ability to discern the target analyte from background noise and contaminants [1].

2.4. Pasteurized Milk Consumption Survey and Ethiopian Food Based Dietary Guideline (EFBDG)
For the risk assessment purpose, consumption of pasteurized milk by the target population was assessed using their dietary habits. To assess the dietary habits within our target population, a comprehensive survey by distributing standardized questionnaires to 160 participants was conducted. Among them, 80 were children aged 2 to 9 years, while the remaining 80 were adults between the ages of 18 and 65. The survey of children was done through their mothers or caregivers. Importantly, we collected data on an individual basis, ensuring that household dynamics did not influence the results.

Each participant was kindly requested to provide a 24-hour dietary recall, with a specific focus on their consumption of pasteurized milk and dairy-based products. The primary objective was to quantify the extent of pasteurized milk intake within these specified age groups. It is noteworthy that this survey was carefully conducted across all eleven sub-cities, ensuring a balanced representation of both genders.

In the analysis, we also considered the EFBDG for the year 2022. These guidelines provided Recommended Daily Intake (RDI) values for pasteurized milk consumption, which were determined to be 0.25 kg/day for children aged 2–5 years, 0.2 kg/day for children aged 6–9 years, and 0.2 kg/day for adults aged 18 and above [23]. By aligning our findings with these dietary guidelines, we gained valuable insights into adherence to recommended milk consumption patterns.

2.5. Exposure Assessment Due to Consumption of Pasteurized Milk
Exposure assessment was conducted by employing deterministic methods (or single data points) [24]. The aim was to determine the chronic exposure to AFM1 in two distinct population groups: children and adults. To highlight the differences in exposure levels, the exposure to AFM1 in pasteurized milk for both groups of consumers...
was computed. The daily intake of AFM1 (expressed as ng/kg bw/day) was computed by taking into account the average concentration of AFM1 in pasteurized milk, the daily consumption rate of pasteurized milk, and the body weight of the individuals within the target populations [18], [25]:

$$EDI = \frac{Ac \times DAC}{bw}$$

Equation (1) represents the calculation of the Estimated Daily Intake (EDI), which was determined using the following variables: Ac (the average concentration of AFM1 in μg/L), DAC (the daily average consumption of pasteurized milk in kg/day), and bw (body weight) for both children and adult populations.

In this calculation, various concentration levels of AFM1 in pasteurized milk were considered, including the mean, upper bound, and lower bound concentrations. The daily average consumption of pasteurized milk is derived from a calculated value, while the body weights are determined using the WHO Weight for Age Standard. For toddlers, the weight is 9.6 kg, for older children, it is 23.5 kg, and we took the average value of these two figures, which amounts to 16.6 kg [1], [26], [27]. For adults, the average body weight used is 65.5 kg [18].

2.6. Risk Characterization

A thorough health risk assessment for AFM1 was conducted associated with the consumption of pasteurized milk. This analysis encompassed multiple approaches, including the Margin of Exposure, assessment of its potential impact on liver cancer, and the Hazard Index methodology.

2.6.1. Margin of Exposure (MOE) Characterization for AFM1

The MOE is a measure employed to assess the potential health risks associated with AFM1 exposure. It is determined by dividing a reference value of 570 ng/kg bw/day (established through a 2-year study in male Fischer rats, specifically regarding AFM1’s potency in inducing hepatocellular carcinoma or HCC) by the EDI [18]. A MOE value equal to or greater than 10,000 signifies minimal concern from a public health standpoint.

In MOE calculations, the use of the Benchmark Dose Level with a 10% confidence limit (BMDL10) is recommended. BMDL10 represents the lowest dose at which there is 95% certainty that it will not result in more than a 10% incidence of cancer [1], [18]. This benchmark dose is the dosage level at which a detectable but limited response is observed [1], [18].

$$MOE = \frac{BMDL}{EDI}$$

In the context of risk assessment, the European Food Safety Authority [28] has identified liver carcinogenicity, specifically the development of HCCs in male rats, as the most critical consequence. Therefore, the BMDL10, which is the lower confidence limit for a benchmark response of 10% (BMDL10) related to the occurrence of HCCs, has been considered. Since a specific BMDL10 value for AFM1 is not available, the BMDL10 for HCCs induced by the ingestion of AFB1 (0.4 μg/kg or 400 ng/kg body weight per day) [1] has been utilized in this study. This approach involves the application of a potency factor for defining MOE.

2.6.2. Estimated Liver Cancer Risk Due to Consumption of Pasteurized Milk

The Joint FAO/WHO Expert Committee on Food Additives conducted a comprehensive assessment of cancer risk associated with exposure to 1 ng of AFB1 per kilogram of body weight per day in a group comprising 100,000 individuals. The findings revealed that the upper limits of risk were 0.049 additional cancer cases per 105 people in populations with HBsAg-negative status and 0.562 additional cancer cases per 105 in populations with HBsAg-positive status [29].

In carcinogenicity experiments, it was observed that AFM1 exhibited approximately one-tenth of the carcinogenic potency of AFB1, even in sensitive species such as Fischer rats and Rainbow trout. Consequently, the predicted carcinogenic potency of AFM1 was estimated to be 0.0049 additional cancer cases per 105 individuals in HBsAg-negative populations and 0.0562 additional cancer cases per 105 individuals in HBsAg-positive populations.

To account for variations in hepatitis B virus (HBV) prevalence, this investigation considered an overall pooled prevalence of HBV-positive populations at 6%. This percentage difference was calculated as 94% for HBsAg-negative populations (100%–6%). The prevalence data were gathered through a systematic review and meta-analysis of studies published over a nine-year period from 2010 to 2019 [30].

The calculation of average cancer potency is defined by (3), where it is determined as follows:

$$Average\ cancer\ potency\ =\ (0.0562\times\ HBsAg\ positive\ population)\ +\ (0.0049\times\ HBsAg\ negative\ populations)$$

$$/\text{Prevalence\ in\ Ethiopia}$$

Substituting the values:

$$Average\ cancer\ potency\ =\ (0.0562\times\ 0.06)\ +\ (0.0049\times\ 0.94)$$

This results in an average cancer potency of 0.007978 cases per 100,000 individuals per year per nanogram of aflatoxin per kilogram of body weight per day (ng aflatoxin/kg bw/day).

Consequently, to assess the population cancer risk of AFM1-induced HCC, it involves multiplying the estimated mean AFM1 exposure by the probability of average cancer potency, as described in (4):

$$Cancer\ risk\ =\ (EDI)\times\ Average\ cancer\ potency$$

This formula helps determine the cancer risk in terms of cases per 100,000 individuals per year due to AFM1 exposure.
2.6.3. Hazard Index

To further assess public health concerns related to AFM1 from the consumption of pasteurized milk, a Hazard Index (HI) was employed. The Tolerable Daily Intake (TDI) for AFM1, set at 0.2 ng/kg bw/day based on the recommendations of [31], was derived by dividing the Threshold Dose per body weight (TD50) by 5000. Subsequently, the HI was calculated by dividing the EDI by the TDI value for AFM1 (0.2 ng/kg bw/day).

In the context of HI calculations, if the HI for AFM1 remains below 1, it is generally considered safe for consumers. However, if the HI surpasses 1, it raises concerns about potential risks of liver cancer [19], [32], [33]. The formula for HI is expressed as follows:

\[ HI = \frac{EDI}{Tolerable \ Daily \ Intake \ for \ AFM1} \] (5)

Equation (5) allows for an evaluation of the potential health risks associated with AFM1 intake through pasteurized milk, with an HI value exceeding 1 indicating a potential risk of liver cancer for consumers.

2.7. Statistical Analysis

AFM1 levels in the pasteurized milk samples were assessed in triplicate, and the findings were presented as the mean values alongside their corresponding standard deviations and standard errors. Exposure and risk assessment data were reported in terms of both mean and median values. Statistical analysis was conducted using Microsoft Windows Excel (version 16), Minitab software (version 14), and Tibico Statistica software (version 13.5).

To determine the health risks associated with AFM1 exposure, deterministic health risk assessment models were applied to calculate values for the mean and median of EDI, MOE, HI, average cancer potency, and cancer risk. Various formulas were employed in these calculations to comprehensively assess the potential health impacts.

3. Results

3.1. Occurrence and Prevalence of AFM1 in Pasteurized Milk

The average AFM1 concentration was 1.237 μg/L, with a standard deviation of 1.317 μg/L and a standard error of 0.184 μg/L. In a 95% confidence interval, the upper bound (UB) and lower bound (LB) of AFM1 concentrations were determined to be 0.867 and 1.608 μg/L, respectively (Table I). It is worth noting that AFM1 was detected in 56.6% of the pasteurized milk samples analyzed.

3.2. The Pasteurized Milk Consumption

Pasteurized milk consumption in Addis Ababa was reported to be 0.19, 0.1, 0.082 lit/day for children 2–5 years, 6–9 years, and adults (≥18 years), respectively (Table II).

3.3. Exposure Assessment

3.3.1. Estimated Daily Intakes

According to Ethiopian food-based dietary guidelines, the EDI values were determined as 25.2, 10.5, and 3.8 for children aged 2 to 5, 6 to 9, and adults over 18 years of age, respectively. These values were established when individuals adhered to the recommended daily milk consumption.

The survey results, which encompassed various age groups, revealed different EDI figures. Specifically, for children aged 2 to 5, 6 to 9, and adults over 18 years, the EDI was calculated at 22, 5.2, and 1.8, respectively (Table III).

3.3.2. Risk Characterization

3.3.2.1. Margin of Exposure (MOE), Cancer Risk and the Hazard Index (HI)

As per EFBDG recommendations, individuals who meet their daily milk intake requirements have corresponding MOE values. For children aged 2 to 5, this MOE is 22.6, while for those aged 6 to 9 years, it is 54.3. Adults over 18 years old exhibited an MOE of 150.9. In addition, the cancer risk levels for these age groups were 0.2, 0.084, and 0.03, respectively (Table IV).

Based on our survey data regarding pasteurized milk consumption across various age groups, we recalcuated the MOE values, and it resulted in 0.18 for children aged 2 to 5 years, 0.04 for those of children aged 6 to 9 years, and 0.01 for adults over 18 years of age. The cancer risk values for these age groups were 0.18, 0.04, and 0.01, respectively. Moreover, the Hazard Index values, with mean values of 110, 26, and 7.5, were determined for children aged 2 to 5, 6 to 9, and adults over the age of 18 (Table IV).

4. Discussion

The AFM1 contamination in this study was quite prevalent, with a rate of 56.6%, which is higher when compared to similar research on dairy products. When we measure it against the Ethiopian standard, the AFM1 concentration in pasteurized milk (0.5%) exceeds it by a significant 247.4%. Other studies conducted in Ethiopia have reported varying prevalence rates, such as 41% in Gondar [34], 68% in the Gurage zone [35], and 67.20% across three regions of Ethiopia [36]. Furthermore, the percentage of AFM1 found in milk from the milksheds in Addis Ababa was even higher than our findings, reaching 100% in all samples tested. The highest recorded mean concentration of AFM1 in Ethiopia is 60 μg/L, with a standard deviation of 847 μg/L.

### Table I: Statistical Summary of the Occurrence and Prevalence of AFM1 in Pasteurized Milk Samples

<table>
<thead>
<tr>
<th>No.</th>
<th>Standard AFM1 μg/L, CES 279</th>
<th>Lower bound (LB) μg/L</th>
<th>Mean μg/L</th>
<th>Median</th>
<th>Upper bound (UB) μg/L</th>
<th>StDev</th>
<th>SE mean</th>
<th>Prevalence</th>
</tr>
</thead>
<tbody>
<tr>
<td>60</td>
<td>0.5</td>
<td>0.867</td>
<td>1.237</td>
<td>0.847</td>
<td>1.608</td>
<td>1.317</td>
<td>0.184</td>
<td>56.6%</td>
</tr>
</tbody>
</table>

Note: μg/L: microgram per liter, StDev: Standard deviation, and SE: Standard Error.
Aflatoxin M1 Exposure and Health Risk Assessment in Children and Adults

TABLE II:\hspace{0.5em} Consumption of Pasteurized Milk and Dairy Products with Different Age Groups in Addis Ababa

<table>
<thead>
<tr>
<th>Age category</th>
<th>Number of participants</th>
<th>Consumption of dairy products</th>
<th>Consumption of pasteurized milk</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>L/day</td>
<td>L/year</td>
</tr>
<tr>
<td>2–5 years</td>
<td>40</td>
<td>0.23</td>
<td>84.1</td>
</tr>
<tr>
<td>6–9 years</td>
<td>40</td>
<td>0.143</td>
<td>52.1</td>
</tr>
<tr>
<td>≥18 years</td>
<td>80</td>
<td>0.083</td>
<td>30.4</td>
</tr>
<tr>
<td>Total population</td>
<td>160</td>
<td>0.144</td>
<td>52.5</td>
</tr>
</tbody>
</table>

TABLE III: Estimated Daily Intake of AFM1 in the Children and Adult Population Due to Consumption of Pasteurized Milk in Addis Ababa, Ethiopia

<table>
<thead>
<tr>
<th>Age category</th>
<th>AFM1 conc. (μg/L)</th>
<th>Average body weight (kg)</th>
<th>Consumption of pasteurized milk (kg/day)</th>
<th>EDI (ng/kg bw/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>2–5 years</td>
<td>1.237</td>
<td>0.847</td>
<td>9.8</td>
<td>0.25</td>
</tr>
<tr>
<td>6–9 years</td>
<td>23.5</td>
<td>0.2</td>
<td>10.5</td>
<td>0.1</td>
</tr>
<tr>
<td>≥18 years</td>
<td>65.5</td>
<td>0.2</td>
<td>3.8</td>
<td>0.082</td>
</tr>
</tbody>
</table>

TABLE IV: Risk Characterization Values (Margin of Exposure, Cancer Risk, and Hazard Index due to the Consumption of Pasteurized Milk in Children and Adult Population of Addis Ababa)

<table>
<thead>
<tr>
<th>Population group</th>
<th>Margin of exposure</th>
<th>Cancer risk (cases/105 person/year)</th>
<th>Hazard index</th>
<th>Margin of exposure</th>
<th>Cancer risk (cases/105 person/year)</th>
<th>Hazard index</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>Median</td>
<td>Mean</td>
<td>Median</td>
<td>Mean</td>
<td>Median</td>
</tr>
<tr>
<td>2–5 years</td>
<td>22.6</td>
<td>33.0</td>
<td>0.20</td>
<td>0.14</td>
<td>126.2</td>
<td>86.4</td>
</tr>
<tr>
<td>6–9 years</td>
<td>54.3</td>
<td>79.9</td>
<td>0.084</td>
<td>0.057</td>
<td>52.5</td>
<td>36</td>
</tr>
<tr>
<td>≥18 years</td>
<td>150.9</td>
<td>537.5</td>
<td>0.03</td>
<td>0.008</td>
<td>18.9</td>
<td>5.3</td>
</tr>
</tbody>
</table>

AFM1, which was 5.58 μg/L, was from a study conducted in Bishoftu, Ethiopia [37], whereas, in this study, it was measured at 1.237 μg/L, showing a significant difference.

The findings in this research revealed a lower prevalence of AFM1 contamination compared to most studies conducted in various regions of Ethiopia. This difference could be attributed to pasteurized milk in our study being produced under more stringent manufacturing practices. In countries like India, which boasts a robust dairy production industry, a contamination prevalence of 54% was reported [38], a figure closer to our own results. Meanwhile, China reported a higher prevalence of 68% [39], and 45% of traditional and industrial milk samples were contaminated with AFM1 more than the standard level in Tiran (EU and Codex) [40]. Furthermore, in different regions around the world, notably Tanzania at 96% [41], Kenya at 99% [42], and Sudan at 98.6% [43], even higher prevalence rates were observed. This significant variation in AFM1 prevalence in dairy products can likely be attributed to several factors, with variations in feed hygiene being a key contributor.

The findings of our consumption survey indicated that children exhibit a notably higher consumption rate of pasteurized milk in comparison to adults, with an intake of 0.19 liters per day as opposed to adults, who consume 0.082 liters per day (Table II). This discrepancy in consumption is primarily attributed to economic considerations, as many adults mentioned that they face budget constraints and, therefore, prioritize providing pasteurized milk to children under the age of five. As other findings from [18] used 0.09 kg/day as the consumption of raw milk which has closer value with our dairy consumption finding.

Interestingly, pasteurized milk is widely regarded as a preferred choice due to its perceived safety [44], [45]. Furthermore, among children, those in the age group of 6 to 9 years tend to consume more pasteurized milk than adults but slightly less than children aged 2 to 5 years.

We assessed exposure and characterized the risk by comparing the recommended daily milk intake in different age groups as per EFBDG recommendations to the results of our pasteurized milk consumption study. The Estimated Daily Intake (EDI) value, considering our consumption survey results, was 5.2 ng/kg bw/day for older children aged 6–9 years and 3.8 ng/kg bw/day for adults. When we factored in the EFBDG recommended daily intake, these values doubled for children aged 6–9 years and adults, reaching 10.5 and 7.5 ng/kg bw/day, respectively.

Interestingly, the EDI value for children aged 2 to 5 years showed a comparable result of 25.2 ng/kg bw/day according to EFBDG, slightly higher than the 22.0 ng/kg bw/day recorded in our consumption survey. Overall, the EDI values ranged from 1.5 to 22.0 ng/kg bw/day. It is worth noting that if the EDI value exceeded 1 ng/kg bw/day, it signified a significant risk of AFM1 exposure from consuming pasteurized milk [46], especially for children...
aged 2–5 years. These young children, due to their lower body weight and higher milk intake, faced the highest health risk from AFM1 in the research area, followed by older children aged 6–9 years, with similar results in Argentina [46] and Italy [47].

Across diverse international studies, a range of Estimated Daily Intake (EDI) values surfaces, creating a multifaceted portrait of AFM1 exposure risks, particularly among children. In Kenya, a notable EDI of 3.5 suggests increased vulnerability among youngsters [48], while Ghana’s results reveal substantial exposure disparities, with EDI values spanning from 0.06 to 2.03, signifying pronounced risks for younger children [1]. Conversely, China’s notably low EDI values, ranging from 0.021 to 0.023, indicate a relatively limited health threat associated with AFM1 in milk, possibly attributed to rigorous monitoring measures [49]. Serbia’s exploration of milk and milk-based foods unveils variable EDI values, with toddlers (aged 1–3) exhibiting higher EDIs of 0.164 and 0.193 compared to older children (3–9 years) [9]. Meanwhile, Italy’s diverse population groups present EDIs spanning from 0.025 to 0.328 ng/kg body weight per day [47]. In Serbia, the fluctuation in seasonal EDI values, ranging from 0.022 to 0.330 across genders, suggests a reduced risk [50]. Brazil’s investigation reveals EDIs of 0.468 for adolescents, 0.384 for adults, and 0.559 for the elderly, shedding light on no potential toxicological concerns within Lon-drina’s population [51]. These global variations in AFM1 exposure assessments underscore the intricate interplay of factors contributing to health risks associated with milk consumption.

The MOE serves as a crucial indicator of potential public health risks, particularly when it falls below the threshold of 10,000, as noted in previous studies [1], [38]. In our current investigation, the MOE values based on the EFBDG recommendations stood at 22.6, 54.3, and 150.9 for children aged 2–5 years, 6–9 years, and adults over the age of 18, respectively. Meanwhile, when considering the results from our consumption survey, the MOE values were slightly higher at 25.9, 109.6, and 380 for the respective age groups. These findings undeniably highlight the elevated risk faced by children aged 2–5 years, followed by children aged 6–9 years and adults. A parallel study conducted in Ghana, examining MOE values resulting from the consumption of raw cow milk, reported a range of 197.04–6666.67, which translated to 0–0.0323 ng AFM1/kg bw/day [1]. These values, falling below the critical threshold of 10,000, reflect risks like our own findings.

The cancer risk associated with consumption, based on EFBDG recommendations, was calculated to be 0.20, 0.084, and 0.03 for children aged 2–5 years, 6–9 years, and adults over the age of 18, respectively. Conversely, according to the results obtained from our consumption survey, the cancer risk values were slightly lower, measured at 0.18, 0.04, and 0.01 cases/105 person/year for the same age groups. These findings indicate a comparatively higher cancer risk in our study compared to others, such as a study in Kenya, which reported a rate of 0.004 cases per 100,000 [48].

Comparing Hazard Index (HI) values across various studies reveals notable differences in the health risks associated with AFM1 consumption. In the study, HI values for pasteurized milk consumption, based on EFBDG recommendations, were 126.2, 52.5, and 18.9 for children aged 2–5 years, 6–9 years, and adults, respectively. In contrast, the HI values derived from our consumption survey were slightly lower at 110, 26, and 7.5 for the corresponding age groups, indicating potential risks across all groups. A study in Italy among different population segments, including infants and toddlers, reported HI calculations of 1.64 and 1.4, signifying a risk level consistent with our results [47]. Conversely, in India, health risk assessments for AFM1 in raw milk revealed a significantly higher HI of 3.57, especially impacting adults, suggesting a potentially substantial health risk associated with such consumption [52]. Meanwhile, in Iran, the HI values for cheese consumers, both adult females and males, were notably lower at 0.38 and 0.32, respectively, indicating no significant health concerns associated with cheese consumption [43]. These variations underscore the diverse health risks posed by AFM1 in dairy products across different regions and consumption patterns.

5. Conclusions

In our investigation, we analyzed pasteurized milk samples collected in Addis Ababa. These analyses revealed a notably higher prevalence of AFM1 contamination. Our health risk assessment, which utilized parameters such as MOE, HI values, and cancer risk through a deterministic approach, highlights the potential public health implications linked to average AFM1 exposure from the consumption of pasteurized milk. Notably, our findings indicate an elevated risk of cancer linked to AFM1 exposure via pasteurized milk consumption. When we delve into specific population groups, children emerge as particularly vulnerable due to their substantial consumption of pasteurized milk, raising concerns about potential health complications. It is imperative to underscore the significance of detecting aflatoxin contamination at its source, particularly in animal feed, and the essential need for stringent regulations to mitigate AFM1 contamination in pasteurized milk. However, it is important to be aware of certain constraints within our study. One notable limitation is the absence of precise data on actual pasteurized milk consumption, which, if available, would have allowed for more accurate estimations. Additionally, our study’s scope was limited to the child and adult populations of Addis Ababa, which may not fully represent the broader situation in other regions of the country. Given these constraints, we recommend conducting further risk characterization studies related to pasteurized milk consumption in diverse geographical areas to enhance our understanding of this issue.

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**AUTHORS’ CONTRIBUTIONS**

HA conceived the study, collected, and analyzed the data, interpreted the results, and wrote the manuscript. GD and AT contributed to the interpretation of the main findings and provided comments and corrections on the manuscript. All authors read and approved the final version of the manuscript.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest.

**REFERENCES**


