AFLATOXICOSIS

MINI ROUND
DEPARTMENT OF CLINICAL MEDICINE AND
THERAPEUTICS
DR E. SAYO
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OUTLINE

• Introduction
• Predisposing factors for Aflatoxicosis (AF)
• Outbreaks of Aflatoxicosis
• Aflatoxin effects on humans
• Pathogenesis
• Clinical Manifestations of Aflatoxicosis
• Diagnostic methods
• Control of Mycotoxins in Africa
• Conclusion
Historical view

• The AF problem was first recognized in 1960, when there was a severe outbreak of a disease referred to as "Turkey 'X' Disease" in UK, in which over 100,000 turkeys died.

• The cause of the disease was due to toxins in peanuts infected with Aspergillus flavus and the toxins were named as Aflatoxins.

(Asplin and Carnaghan, 1961; Blount, 1961; Lancaster et al, 1961)
Distribution of Mycotoxins in Africa

- Aflatoxins: 43.75%
- Ochratoxins: 21.87%
- Fumonisín: 12.5%
- Zearalenone: 9.375%
- Deoxynivalenol: 6.25%
- Beauvericin: 6.25%
Aflatoxins (AF)

- AF are a group of mycotoxins produced by a large number of Aspergillus species.
- Main producers are *A. flavus*, *A. parasiticus*.
Aflatoxins cont..

• Aspergillus grows optimally at 25 °C with a minimum necessary water activity of 0.75.
• Produces secondary metabolites at 10-12°C, but the most toxic ones are produced at 25°C with a water activity of 0.95 (Hesseltine 1976)
• AF decompose at their melting points, which are between 237°C (G1) and 299°C (M1).
Types of Aflatoxins

• 18 different types of Aflatoxins have been identified.

• Major members are Aflatoxin B1, B2, G1 and G2.

• Aflatoxin M1 and M2 are major metabolites of Aflatoxin B1 and B2 respectively.

• AF display potency of toxicity and carcinogenicity in the order of AFB1 > AFG1 > AFB2 > AFG2.
Types cont.

• AF M1, M2 may be found in the absence of other Aflatoxins.
• Found in milk of animals that have consumed feeds contaminated with Aflatoxins.
• Aflatoxin B1 (AFB1) is normally predominant in food products.
Natural Occurrence

- Agricultural products contaminated with AF include:
- Cereals (maize, rice, wheat)
- Oil seeds (groundnut, soybean, sunflower, cotton)
- Spices (black pepper, coriander, turmeric, ginger)
- Tree nuts (almonds, pistachio, walnuts, coconut)
- Milk
- Derivative products made from these primary feedstuffs in low-income countries.

(Rizzi et al., 2003; Saleemullah et al., 2006; Strosnider et. al., 2006; Masoero et. al., 2007; Caloni, 2010).
# FDA-Allowable Aflatoxin Levels in Human Food

15 countries in Africa have specific mycotoxin regulation (2003).

<table>
<thead>
<tr>
<th>Amount</th>
<th>Food type</th>
</tr>
</thead>
<tbody>
<tr>
<td>20 ppb</td>
<td>Foods in general</td>
</tr>
<tr>
<td>0.5 ppb (aflatoxin M1)</td>
<td>Milk</td>
</tr>
<tr>
<td>20 ppb</td>
<td>Peanuts and peanut products</td>
</tr>
<tr>
<td>20 ppb</td>
<td>Pistachio nuts</td>
</tr>
<tr>
<td>20 ppb</td>
<td>Brazil nuts</td>
</tr>
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</table>
# Developing Country Exposure

<table>
<thead>
<tr>
<th>Country</th>
<th>Food Stuff</th>
<th>Concentration (ppb)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nigeria</td>
<td>Rice</td>
<td>28–372</td>
<td>Makun et al., 2011</td>
</tr>
<tr>
<td>Ghana</td>
<td>Maize</td>
<td>0.7–355</td>
<td>Kpodo, 1996</td>
</tr>
<tr>
<td>Kenya</td>
<td>Animal feed and milk</td>
<td>&gt;5</td>
<td>Kang’ethe and Lang’a, 2009</td>
</tr>
<tr>
<td></td>
<td>Maize</td>
<td>&gt; 20</td>
<td>Daniel et al., 2011</td>
</tr>
<tr>
<td>Zambia</td>
<td>Maize</td>
<td>20,000</td>
<td>Mukanya et al., 2010</td>
</tr>
</tbody>
</table>
Aflatoxin-prone food Consumption Patterns
Predisposing Factors for AF

- Climate of the region
- Genotype of the crop planted
- Soil type
- Minimum and maximum daily temperatures
- Daily net evaporation
- Stress or damage to the crop due to drought before harvest
- Insect activity
- Poor timing of harvest
- Heavy rains during and after harvest
- Inadequate drying of the crop before storage
Aflatoxins: Human, Animal, and Environmental Interactions

Fungal growth / aflatoxin production

Contamination of human & animal food

Environment: extreme drought, moisture, heat, compromised plants

Human consumption
  - Breast milk
  - In utero

Animal consumption
  - Milk
  - Eggs

Infants
The Fatal Decades (80’s & 90’s)

• In India, at least 400 people were affected by eating infected corn, and 104 of them died (1981).

• In Southeast Asia, 21 patients became jaundiced and sick within hours after eating rice and pasta; 14 died because of liver failure and 7 because of renal failure.

• In biopsies, high concentrations of Aflatoxins were found in liver, lungs, kidneys and other organs (Hendrickse 1999).

• In Kenya, 12 people died following high consumption of Aflatoxins (Mehan & Mc Donald, 1991).
July 20, a total of 317 cases had been reported, with 125 deaths (CFR = 39%)

During the outbreak  51% of maize samples, had AF levels above the Kenyan regulatory limit of 20 ppb in grains for human consumption
KENYA 2009

• In Kenya, a total of 830 animal feeds and 613 milk samples from 4 urban centres were analysed for AFB1 and M1.

• 86% of the feed samples were positive for AFB1, and 67% of these exceeded the FDA/FAO/WHO level of 20 ppb.

• 72% of the milk samples was positive for AFM1
• Nandi and Makueni Counties in Kenya were selected to compare the distribution in maize of Aspergillus spp. and their toxigenicity.

• 255 households were sampled in Nandi and 258 in Makueni

• Aspergillus flavus was the most common contaminant, and the incidence of occurrence in Nandi and Makueni was not significantly different (82.33% and 73.26%, respectively).

• Toxigenic strains were more prevalent than non-toxigenic strains. All the toxigenic strains from Makueni were of the S-type while those from Nandi belonged to the L-type.
Frequency of Toxigenic Strains of *Aspergillus Flavus* in Two Zones in Kenya
Toxicity

- Acute toxicity
- Chronic toxicity
- Carcinogenicity
- Teratogenicity
- Immune suppression
Acute Aflatoxicosis

• Occurs when moderate to high levels of AF are consumed.

• Acute high-level exposure can progress to potentially lethal hepatitis.

• Acute episodes of disease include haemorrhage, acute liver damage and diarrhoea.

• A case fatality rate of approximately 25% (Walderhaug, 1992; Cullen & Newberne, 1994; Strosnider et. al., 2006).
Chronic Aflatoxicosis

- It results from ingestion of low to moderate levels of AF.
- Symptoms are subclinical and causes slower rates of growth with or without the production of an overt AF syndrome (Walderhaug, 1992)
- It is estimated that > 5 billion people in developing countries worldwide are at risk of chronic exposure to AF through contaminated foods (Shephard, 2003; Williams et. al., 2004)
- Gambia, Benin, Guinea, China, Ghana & Kenya > 90% chronic exposure
• Modify cytokine synthesis
• Decrease cell mediated immune response
• Decrease T/B cell activity
• Suppress NK cell

CYP 3A4

AFB1 8,9 exo epoxide

Mixed function oxidase

Teratogenicity

MFO

Placenta, Lungs, Liver, Kidney

ROS

P53
• When metabolized slowly, the untransformed toxin activates the molecular species that cause chronic liver damage as the most probable result.

• When metabolized rapidly, acute liver damage may be caused by the intracellular formation of Aflatoxin hemiacetal in many species. (Patterson, 1973)
Clinical Features

• Following ingestion: 5-24 h (average 15h) nausea, vomiting, abdominal pain, and diarrhea begins (regardless of dose above 20 ppb).

• These initial symptoms are followed by a brief period of apparent improvement.

• Without supportive treatment, severe liver damage and kidney failure often result in coma and death.
Differential Diagnosis

• Acute food poisoning
• Hepatitis A
• Leptospirosis
• Sepsis
• Haemorrhagic fever
Aflatoxins and Chronic diseases

- Hepatocellular carcinoma
- Lung cancers
- Oesophageal cancer
- Neural tube defects
- ? Infertility in men
- Anemia
- Failure to thrive
- ? Chronic Tubulointerstitial Nephritis

- Fungal Sinusitis
- Keratitis
- Otomycosis
- Pul Aspergillosis
- Osteomyelitis
- Endocarditis
- Craniocerebral aspergillosis.
Mycotoxin-related public health problems in African countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Health Problem</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Egypt</td>
<td>Primary hepatocellular carcinoma</td>
<td>Tumer et al., 2008</td>
</tr>
<tr>
<td>Ghana</td>
<td>Anemia</td>
<td>Shuaib et al., 2010</td>
</tr>
<tr>
<td>Kenya</td>
<td>Aflatoxicosis</td>
<td>Azziz-Baumgartner et al., 2005</td>
</tr>
<tr>
<td>Benin</td>
<td>Stunting and being underweight</td>
<td>Gong et al., 2003</td>
</tr>
<tr>
<td>Tunisia</td>
<td>Chronic Tubulointerstitial Nephritis</td>
<td>Hmaissia et al., 2012</td>
</tr>
</tbody>
</table>
Carcinogenicity

- The International Agency for Research on Cancer (IARC) recognized AF as carcinogenic in 1976 - and classed as group 1 carcinogen in 1993.

(Chen et. al., 2001; Henry et. al., 2002; Omer et. al., 2004; Qian et. al., 1994; Wang et. al., 1996).

- HCC is the 6\textsuperscript{th} most prevalent cancer worldwide.

- HCC generally is association with hepatitis B virus (HBV) and hepatitis C virus (HCV) infections.
• AFB1 is associated with a specific AGG to AGT transversion mutation at codon 249 of the p53 gene in humans, providing mechanistic support to a causal link between exposure and disease (Goldman & Shields, 2003; Sugimura, 2000; Wild & Montesano, 2009).

• Aflatoxins may play a causative role in 5%-28% of all global HCC cases (Liu & Wu, 2010)

• Developing countries have a higher incidence rate, with approximately 82% of the 600,000 new HCC cases each year. (Parkin et. al., 2005)
AF and Hepatitis B Virus

- AF consumption raises the risk of liver cancer by more than 10 fold compared to either exposure to hepatitis B and C viral infection alone.

- Hepatitis B infection may exacerbate the effects of AF exposure.

- Vaccination against Hepatitis B reduces liver cancer rates by 45-50%.
Prevalence of Exposure to Aflatoxin and Hepatitis B and C Viruses in Guinea, West Africa.

• A total of 75 sera were collected from men living in the Kindia region of Lower Guinea.
• Over 90% of the sera contained detectable adduct levels, the highest level being 385 ppb.
• 14.7% were positive for HBsAg and these subjects had a higher AF-alb adduct levels than the other subjects.
• 8 subjects were positive for antibodies to HCV antigens.

(Diallo MS1, Sylla A, Sidibé K, Sylla BS, Trepo CR, Wild CP-Prevalence of exposure to aflatoxin and hepatitis B and C viruses in Guinea, West Africa)
Biomarkers of Aflatoxin exposure

- In Egypt AF–alb was detected in 7/22 samples from HCC-positive cases.
- AF-alb was detected in 35% samples, AFM1 in 48% samples
- AFs were observed in 41% of the subjects concurrently.

(A cross-sectional study assessed serum AF-alb, urinary AFM1 and urinary DON in 98 pregnant women from Egypt, in relation to diet and socioeconomic status, during the 3rd trimester.)
Progression of HIV and Aflatoxin

Only cross sectional studies have been done to date.

![Graph showing percentages of CD4+CD25+CD45RO+ regulatory T cells](image)
Detection and Estimation of Aflatoxins

• Analytical methods (TLC & HPLC).

• Immunological methods.
Analytic methods

• Analytical methods for detection and quantification of AF have to be specific, sensitive, and simple to carry out:
  – Thin-layer chromatography (TLC)
  – High-performance liquid chromatography (HPLC)
  – Mass spectroscopy
Immunological methods

• Biochemical technique performed to evaluate either the presence of antigen or the presence of antibody.
  – Radio Immuno Assay (RIA)
  – Immunoaffinity Column Assay (ICA).
  – Enzyme-Linked Immuno Sorbent Assay (ELISA)
Treatment

In cases where early diagnosis is accomplished effective therapies include:

- Amphotericin B, Itraconazole, Voriconazole, Posaconazole and Caspofungin
- The use of milk-thistle extract inhibits the toxins from effecting their most severe liver damage and promote regrowth of damaged cells
- Albumin dialysis.

In more severe cases, especially those in which diagnosis is delayed

- Liver transplant often becomes the only therapy.
Control of Mycotoxin Problems in Africa

• In primary prevention- the goal is to reduce exposure to AF in the diet.
• A range of interventions includes:
  - planting pest-resistant varieties of staple crops, attempting to lower mould growth in harvested crops
  - improving storage methods following harvest
Farming and Storage Practices that Prevent *Aspergillus* Growth

- **Pre-harvest**
  - Pest management for insects (particularly soil insects), weeds, and nematodes
  - Planting date
  - Irrigation
  - Crop rotation or fertilization
  - Use of drought tolerant and locally adapted varieties

- **Harvest**
  - Prevent compromise to the crop by harvesting when mature
  - For maize, harvest early to prevent completion of the *Aspergillus* life cycle

- **Post-harvest**
  - Proper drying
  - Storage in a dry place
Use of Biocontrol Agents

• Biopesticides consisting of a non-Aflatoxigenic strain of *Aspergillus* (AF-36) may competitively exclude toxic strains from infecting crops

• But the allergenic and human health aspects of the Atoxigenic strain need still to be evaluated.
Secondary Prevention

• One goal is to modulate the metabolism of ingested AF to enhance detoxification processes, thereby reducing internal dose and subsequent risk

  (Groopman 2008)

  – Chemoprotection e.g Boric acid

  – Enterosorption to limit biologically effective exposure E.g NovaSil clay (NS)
Health Sector

• Refers basically to those kinds of food we can eat and how hygienically food is prepared.
• The dietary intervention maybe the easiest way to prevent cancer
• However, for many communities in developing countries a change in diet is simply not feasible because of various reasons.
• It is important that simple food preparation methods such as sorting, washing, crushing, and grain duelling may reduce AF levels

(Fandohan et. al. 2005)
Conclusion

• Aflatoxins are not only a big problem at crop production level, but also is a global health issue because of the human consequences.
• Acute Aflatoxicosis is preventable and chronic exposure can be reduced.
Efforts to reduce AF exposure requires commitment of sufficient resources and collaboration between the agriculture and public health communities and the local, regional, national, and international governments.
The Benefits of an Interdisciplinary One Health Approach

• Educating stakeholders on the inter-relationship of humans, animals and the environment is the first step in preventing Aflatoxin-related health issues
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• Dr. Maritim
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• Colleague Registrars