Research Proposal: Association of Aflatoxin Biomarker Levels with Health Status and HIV Disease

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Geographical Location

Ghana

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Focus

Domain - Aflatoxin Region - Global

Background

Aflatoxins are a group of extremely toxic metabolites produced by the

common fungi Aspergillus flavus and A. parasiticus. Although the major aflatoxins (B1, B2, G1 and G2) occur together in various foods in different proportions, aflatoxin B1 (AFB1) is usually the predominant and most toxic form. Aflatoxin M1 (AFM1) is a major metabolic product of AFB1 that is usually excreted in milk and urine of dairy cattle and other mammals. Most rural Ghanaians depend on the food that they produce for survival. In Ghana, staples such as maize and groundnuts are contaminated with levels of aflatoxin that far exceed the 30 ppb considered tolerable in food for human consumption by the FAO/WHO/UNICEF Protein Advisory Board. These basic staples that are consumed or the excess traded in urban and rural markets are poorly handled and stored prior to marketing, and are contaminated with high levels of fungi, the most common of which are A. flavus and A. parasiticus. The most pronounced aflatoxin contamination is found in groundnuts, maize, and other grains. Aflatoxin causes carcinoma of the liver in a number of animal species and has been associated with hepatocellular carcinoma in humans, especially in humans infected with hepatitis B virus. There is also strong evidence to show that aflatoxin is an immunosuppressive agent that results in increased susceptibility to infections and reduction of antibody responses to vaccines in animals. There have been only two reports on the effect of aflatoxin on the human immune system. One study conducted with Gambian children reported that secretory immunoglobulin A in saliva may be reduced by dietary levels of aflatoxin. In the second study, we examined the effect of aflatoxin on the cellular immune status in non-HIV infected Ghanaians and found significantly lower percentages of activated T (CD3+CD69+) and B (CD19+CD69+) cells, and CD8+ T cells that contained perforin, and both perforin and granzyme A in those with high AFB1 levels (39). These data suggest that there may be immunological impairments associated with high AFB1 levels.

Human immunodeficiency virus type 1 (HIV-1) infection results in impaired immune function that can be measured by changes in immunphenotypically defined lymphocyte subsets and other in vitro functional assays. The altered expression of lymphocyte surface antigens reflects the dynamic interaction between the immune system and HIV-1. Immune dysfunction is particularly of concern in countries in sub-Saharan Africa, such as Ghana, where millions of people are infected with HIV and other conditions that cause immune dysfunction/lower immunity such as nutritional deficiencies and parasitic infections. An interaction between malaria and HIV has already been estimated to result in excess HIV infection and excess malaria episodes in Kenya. At the same time, these people are chronically exposed to aflatoxin in their diets. Since both aflatoxin and HIV are immunosuppressive agents, it is reasonable to hypothesize that aflatoxin exposure may influence the

pattern of infection and lead to less favorable outcomes in those infected with HIV. This investigation of the effect of aflatoxin in HIV positive individuals is urgently needed. Thus, we will measure AFB1 albumin adduct levels in plasma and AFM1 levels in urine of HIV positive and negative people in Ghana, and conduct analyses to examine the association between aflatoxin levels and HIV clinical status (CD4 count, viral load, occurrence of opportunistic infections) of participants and identify immune impairments associated with aflatoxin biomarker levels. We will also follow-up a sub-group of HIV positive participants over a 3 year period and examine the association between aflatoxin biomarker levels and HIV disease progression.

Technical Review

The study of the effect of aflatoxin on the immune system in humans has not been given enough importance considering the multitude of people that are affected. Although numerous studies have been conducted in animals and have shown immune impairments induced by aflatoxin, only two studies have been conducted in humans to date. Only one of these two studies investigated changes in defined lymphocyte subsets using multiparametric immunophenotyping and flow cytometry. These are sophisticated scientific techniques which can contribute tremendously in unraveling immune impairments associated with aflatoxin exposure. However, our laboratory was the first to apply these techniques to the study of this topic in humans. The studies outlined in this proposal are even more important for HIV positive people living in developing countries since they are exposed to a myriad of infectious diseases as well as aflatoxin in the diet.

Problem Statement

Aflatoxin has been shown to suppress the immune system in numerous animal studies yet very little work has been conducted on the effect of aflatoxin on the immune system in humans. Our study in Ghanaians has shown alterations in immunological parameters in participants with high aflatoxin B1 albumin adduct (AF-ALB) levels compared to those with low AF-ALB. These alterations could result in impairments in cellular immunity in these individuals that could decrease their resistance to infections and response to vaccinations. The human immunodeficiency virus (HIV) leads to immune suppression and increase in opportunistic infections in infected individuals. This results in disease progression especially in those not receiving antiretroviral therapy. Since aflatoxin is immune suppressive it is reasonable to hypothesize that HIV infected individuals who are exposed to aflatoxin in the diet will experience faster progression to AIDS and more severe disease than those unexposed to aflatoxin. Therefore, we would like

to examine the association between AF-ALB and aflatoxin M1 biomarker levels and disease status in HIV positive individuals. This project is highly significant for Africa since approximately 25 million (70%) of people infected with HIV live on this continent and the majority are chronically exposed to aflatoxin in the diet. Eighty percent of deaths from HIV/AIDS occur in sub-Saharan Africa.

Vision and Approach

Goals

Although large proportions of people worldwide are affected by HIV infection and aflatoxin exposure, there has been no investigation/documentation of the role of aflatoxin in immune impairment and disease status in HIV infected individuals. Our goal for this project is to conduct a well-designed epidemiological and clinical study that provides information on the effects of aflatoxin on health and immune status especially in HIV-infected people. By providing the information to the scientific community and the public at large it is expected that serious attention will be paid to this matter so that further research can be conducted and appropriate interventions taken to reduce aflatoxin exposure. The overall health impact of these activities should be tremendous considering that approximately 4.5 billion people living in developing countries are chronically exposed to aflatoxin in the diet and most of these are already exposed to a myriad of infectious diseases and have poor nutritional status.

Objectives

1. Determine the association between aflatoxin biomarker levels and socio- demographic/economic characteristics of HIV- and HIV+ people in Ghana.
2. Determine the association between aflatoxin biomarker levels and health and clinical status (hepatitis B and C infections, malaria Ag, liver function, CD4 count, HIV viral load, occurrence of opportunistic infections) of HIV- and HIV+ people in Ghana.
3. Determine the association between aflatoxin biomarker levels and micronutrient status (vitamin A & E, zinc, and selenium levels) of HIV- and HIV+ people in Ghana
4. Conduct cellular immune analyses using PBMCs from participants at recruitment and analyze data to identify immune impairments associated with aflatoxin biomarker levels in HIV+ individuals (will compare HIV- with HIV+ participants). Examine interaction between HIV and aflatoxin on immune status.
5. Conduct a follow-up study and examine the association between

aflatoxin biomarker levels and progression of HIV disease to AIDS (CD4 count, HIV viral load, types of opportunistic infections, and HIV stage).

6. Determine the association between aflatoxin biomarker levels and the occurrence of active tuberculosis infection in HIV- and HIV+ people in Ghana.
7. Determine the association between aflatoxin biomarker levels and health status (malaria, HBV, HCV, intestinal helminth infections, Hb, folate, vitamin A, vitamin E) in pregnant women and on birth outcomes (low birth weight, pre-term delivery, small for gestational age, stillbirth) in Ghana.

Research Approach

This study will be conducted in Kumasi Ghana among HIV positive and negative people attending two of the major hospitals in the area. Hospital directors, physicians, hospital counselors/social workers and nurses will review the protocol that will be developed and will have significant input in the methods outlined. Local personnel identified by the Co-PI and hospital staff will be recruited to serve as recruiters, interviewers and data entry personnel. Phlebotomists will also be local hospital personnel experienced in working with HIV positive people. The research group will be in constant communication with hospital personnel and will take seriously their concerns and suggestions. The research team will meet weekly to review progress of the study and any concerns expressed by patients or hospital staff. The PI will closely monitor progress of the study. She will oversee all administrative and scientific aspects of the project. She will review achievement of the objectives of the study in a timely manner and will see that patient test results are communicated to their physicians for consideration in their care and treatment. She will be in constant communications with collaborators, monitor all experimental designs and data generated, participate in interpreting the data, write the manuscripts and progress reports and see to the overall running of the project. She will manage pitfalls and implement new strategies as needed to maintain progress and success of the study.

Training & Capacity Development Approach

The PI with significant input from Co-PI Ellis and Ghanaian health professionals will identify, employ and train Ghanaian recruiters, interviewers and data entry personnel for the study. Special attention will be paid to technical ability of these persons to carry out their assigned duties as expected. The study staff will receive training in design of the epidemiological study, in patient recruitment and in conducting patient

interviews while adhering to the highest ethical standards. They will be carefully supervised and so will develop technical ability in carrying out these duties. It is expected that by participating in this study these personnel will develop the appropriate knowledge and skills to participate in future clinical and epidemiological research studies. Because of the follow-up nature of the study these personnel will be trained and supervised over the 5-year period and should be quite proficient at the end of this time. Recruiters/interviewers will also develop technical ability in abstracting clinical data from medical records. Hospital collaborators will also participate in discussions on study design and in the conduct of the study and so will gain considerable knowledge and ability to conduct studies of their own. Thus, the institutions will be able to continue to use the research methods and data system established during the study to conduct other clinical/epidemiological research studies.

Intended Benefits & Impact Responsiveness

Development Benefits

The study will contribute immensely to the scientific knowledge on the health and immune effects of aflatoxin in humans. By identifying the association between aflatoxin biomarker levels and HIV clinical and immune status the scientific and managerial groups can become involved in designing appropriate interventions to prevent the adverse health effects of aflatoxin exposure in humans, especially in HIV+ people. All test results (aflatoxin levels, viral load status, vitamins A and E concentrations, liver function profile) obtained in the study will be made available to the Ghanaian physicians for intervention and care of patients. Since the overwhelming majority of people affected by aflatoxin exposure live in developing countries the study should have tremendous impact in improving the health of these people. These health benefits are expected to translate into economic and other social benefits.

US Benefits

Although dietary aflatoxin exposure in humans occurs predominantly in developing countries aflatoxin contamination in food is of concern to the USA and is a matter that the US Centers for Disease Control and Prevention fervently addresses especially in Kenya. The scientific knowledge obtained on the health and immune effects of aflatoxin in humans will be a significant scientific contribution and will bring recognition to the humanitarian interest of the USA in the health and economic development of people worldwide.

Potential Impacts

This will be the first project to provide information on the association between aflatoxin biomarker levels and clinical and disease status in HIV positive people. Approximately 40 million people are infected with HIV worldwide and the overwhelming majority live in developing countries where they are exposed to a variety of infectious diseases and micronutrient and macronutrient deficiencies. Providing information on the effects of aflatoxin on health status and HIV disease progression should significantly impact the scientific community to conduct further research in this area. The health, agricultural and educational communities should also be stimulated to find solutions to the problem. Since the toxic effects of aflatoxin exposure on nutritional status and immunity negatively impact health (including HIV infection) reduction in aflatoxin exposure should significantly improve health status and survival and lead to improved economic and social status of those currently affected. Our strategy to realize these impacts will include timely publication of our research findings and dissemination of our grouped findings to local health, educational and agricultural officials. We will also provide the physicians with test results of the participants and conduct educational intervention programs among the general public. The educational intervention programs will be conducted with funds from the Minority Health International Research Training Program (NCMHD/NIH) that Dr. Jolly directs. The impact of the findings from this project will be evaluated from the interest displayed by the scientific community through dialogue, increase in research in this area and funding of new research. The impact of the findings from this project to the health, agricultural and educational departments in Ghana and other countries with similar aflatoxin-health problems will be evaluated from the policies and measures implemented to address chronic dietary exposure to aflatoxin and to ameliorate existing health effects. A better understanding of the health and economic costs of aflatoxin contamination could potentially lead to changes in policy and attitudes. Overall, it is expected that the Ghanaian (and other) people will become acutely aware of the harmful effects of aflatoxin and take steps themselves to prevent exposure. This can be evaluated through surveys of knowledge, attitudes and practices of the population regarding aflatoxin exposure.

Equipment

No equipment will be purchased.

Project Timeline

Objective 1 will be conducted in months 1-3
Objective 2 will be conducted in months 4-9
Objective 3 will be conducted in months 10-18
Objective 4 will be conducted in months 10-18
Objective 5 will be conducted in months 10-18
Objective 6 will be conducted in months 10-13
Objective 7 will be conducted in months 14-18
Objective 8 will be conducted in months 19-21
Objective 9 will be conducted in months 22-24
Objective 10 will be conducted in months 15-24
Objective 11 will be conducted in months 22-27
Objective 12 will be conducted in months 10-17
Objective 13 will be conducted in months 17-19
Objective 14 will be conducted in months17-19
Objective 15 will be conducted in months 20-21
Objective 16 will be conducted in months 16-51
Objective 17 will be conducted in months 52-57
Objective 18 will be conducted in months 58-60

USAID Mandate Responsiveness:

MDGs

Poverty/Hunger: Improved Health: Raised Rural Incomes: Sustainable Development

Foreign Assistance Framework

Governance: Human Capacity: Economic Structure: Persistent Dire Poverty: Global Issues (HIV and Infectious Diseases, climate change, biodiversity)

IEHA

Science and Tech Applications: Increased demand for peanuts: Market Access: Increased Trade

USAID Focal Areas

Greater incomes: Greater value and market demand: Public Health: Food

Security: Sustainable Value Chain: Improved Human Capacity