ICCR IRB Protocol
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Title: Effect of Asymptomatic Celiac Disease in the Nurses Health Study Population

A. Study Purpose and Rationale: Celiac disease (CD) is an immune mediated disease, in which affected individuals mount an immune response to gluten, which is a term for the proteins in wheat, barley, and rye. This immune response then causes damage to the inner lining of the small intestine, resulting in malabsorption, and other systemic inflammatory responses. The diagnosis is made by the combination of serologic testing and pathologic analysis of small bowel specimens. However due to the high sensitivity and specificity of the serologic tests, some argue that small bowel biopsy is not needed.

   Celiac disease was originally thought of as a disease affecting only Europeans, but recent epidemiologic studies have found the prevalence of the disease in the general United States population to be approximately 1%.[1,2] Initially, the majority of patients who were diagnosed with the disease were symptomatic with "classical" symptoms of diarrhea, malabsorption, and weight loss. However, due to the recognition of the association between celiac disease and other conditions, and the increased amount of screening of at-risk asymptomatic individuals, approximately 40% of newly diagnosed individuals have classical GI symptoms.[3] Celiac disease has been shown to be associated with conditions affecting many organ systems, including osteoporosis, iron deficiency anemia, growth failure, and multiple types of malignancies, most notably lymphoma.[4] However, the majority of the aforementioned associations were seen in patients with "symptomatic" celiac disease.

   Because of its high prevalence, the fact that studies have shown that many of the associated conditions can be prevented or managed with proper treatment (the only current treatment for the condition involves dietary restriction of all gluten-containing products), and the recognition of the severe complications of the disease, many gastroenterologists propose mass screening for the disease.[5] The WHO guidelines for disease mass screening include: early detection of the disease can be difficult on a clinical basis, the disease must be common and cause significant morbidity, the screening tests are highly sensitive and specific, a treatment must be available, and if not recognized, the disease can result in severe complications. Using the WHO criteria as a basis for mass screening for CD, one realizes that it is not clear cut, as most of the studies performed on patients with celiac disease are in those that are symptomatic, and thus it is difficult to know what, if any, long term complications occur in those that are asymptomatic.

   The benefits of treatment, both in terms of future GI and non-GI symptoms, has not been proven in asymptomatic individuals diagnosed on mass screening. In addition, in making an argument to screen the general population for celiac disease, one must argue that early screening can prevent future complications, beyond just those of GI symptoms. While acknowledging that GI symptoms can be severe, and affect one's quality of life, it is difficult to show in a retrospective study that GI complaints are related to celiac disease without a full workup being performed. However, a strong argument can be made to perform mass screening for celiac disease if it can be shown that a non-GI condition associated with the disease, one that can be easily treated and managed and causes significant morbidity, occurs at an increased rate in those with "asymptomatic" disease…in this case, osteoporosis.

   As mentioned, osteoporosis is a known complication of celiac disease. Studies in Europe, South America, and recently the U.S. have shown that the prevalence of osteoporosis and low bone mass is significantly increased in subjects with celiac disease.[6-15] The presumed mechanisms for low bone density in patients with CD is multifactorial: reduced intestinal absorption of calcium results in

secondary hyperparathyroidism [7, 16, 17], altered levels of intestinal calcium-binding protein calbindin-D9k [18], and increased level of inflammatory cytokines. [19] Interestingly, in multiple studies, it has been shown that bone mineral density (BMD) may normalize when a gluten-free diet is instituted in childhood [20-22], but not so in adults [23-29], suggesting that the damage to bones may occur before patients become symptomatic with CD. In a study performed at CPMC, 105 women and 23 men with celiac disease were compared to age matched controls to evaluate the bone density and prevalence of osteoporosis of those with celiac disease compared to controls. The researchers found that 8% of premenopausal women with CD had osteoporosis (t-score on DEXA <2.5) and 42% had osteopenia, and 40% of postmenopausal women had osteoporosis, while an additional 37% had osteopenia. In addition, the average z-score for premenopausal women was -0.507 compared to the average for their age, and -0.605 for postmenopausal women.[6]

Given that the standard of care of patients with celiac disease entails a gluten-free diet, a randomized control trial of asymptomatic individuals, randomized to diet vs. no diet, cannot ethically be performed. Thus a retrospective study will need to be performed.

The Nurses Health Study was established in 1976, as a means to investigate the potential long term consequences of the use of oral contraceptives. Nurses, ages 30-55 who lived in one of the 11 most populous states in the U.S. were enrolled prospectively. Data obtained from the nurses included questionnaires filled out every 2 years that queried nurses about issues concerning their own health, diet, and other demographic data. In addition, from 1989 to 1990, 33,000 blood samples were collected from nurses without known cancer, diabetes, or cardiovascular disease, and were stored to be used for future analysis. At the time, the women were ages 43-69, which currently would make them ages 61-87. Thus an overwhelming majority of the women are at the age at which they should have had a screening DEXA scan.

B. Study Design and Statistical Analysis: This study will be a nested cohort study, with the number of enrolled subjects being approximately 33,000, which is equal to the number of frozen blood samples obtained as part of the Nurses Health Study between 1989 and 1990. Subjects will not be crossed over or randomized. Those subjects who serology is positive for celiac disease will be the cohort with the risk factor of celiac disease, and the remaining subjects will be the control. Assuming a prevalence of celiac disease of approximately 1%, there will be approximately 330 subjects in the group with the risk factor of celiac disease, and greater than 32,000 controls. The primary outcome of this study will focus on the development of osteoporosis in subjects with undiagnosed celiac disease. The statistical means by which this will be analyzed will be comparing the z scores on the DEXA scans of patients with celiac disease versus the z scores for controls from this study, using an unpaired t-test to perform the statistical analysis. Based on what I see as a clinically meaningful difference, and based on prior data, the expected mean Z-score for women with celiac disease is -0.5, and assuming that the controls represent the general population, the average Z-score for that group should be 0, with a standard deviation of 1. Given this, the number of subjects in each group needed to have 80% power to detect a statistically significant difference ($p=0.05$) is 64 in each group. Given that questionnaires also contain data regarding cigarette smoking, diet, body type, and other variables which may affect the development of osteoporosis, these factors will be looked at via a multiple linear regression analysis. There will be other secondary outcomes in this study, including the development of malignancies, iron deficiency anemia, osteoporosis, and GI symptoms; however, the study may not be powered to detect significant differences. These variables will be assessed statistically by use of a chi-square test.

C. Study Procedure: The blood samples that were collected from the 33,000 nurses in 1989-1990 will be obtained. All subjects will be contacted prior to testing of blood samples, and informed consent will be obtained. Subjects will be asked if their blood can be tested for research purposes. If subjects

express a desire to have their blood tested, but not to be made aware of the result, this will be granted. The samples will be thawed and each sample will be tested for levels of IgA tissue transglutaminase (TTG) and IgA endomysial antibody (EMA). Presence of IgA EMA will be measured by an immunofluorescence method using monkey esophagus. TTG will be measured using an enzyme-linked immunosorbent assay, with results expressed as a percentage of the positive control serum. Positive results will be determined according to the manufacturers' recommendations. Given that there is a high prevalence of IgA deficiency in subjects with celiac disease, quantitative IgA levels will also be measured, and those with IgA deficiency will be tested using IgG TTG and IgG EMA. Given that these tests are nearly 100% sensitive and specific, but not perfect, and given that the gold standard for diagnosis, an EGD with biopsy will not be performed, a positive result for both tests will be considered adequate to diagnose a patient with celiac disease for research purposes. However, subjects will be contacted, and it will be recommended that they obtain an esophagogastroduodenoscopy to confirm diagnosis, to evaluate the extent of disease, and to be in contact with a gastroenterologist for management of their disease.

Nurses enrolled in the study are sent questionnaires every 2 years. Since the inception of the study, subjects have been queried every 2 years about whether they have a number of medical conditions, including osteoporosis or cancer, as well as questions about their dietary habits and social history. In order to obtain the data needed for this study, a special questionnaire for 2008 will be sent to all of those nurses whose blood was tested. In addition to the standard questions asked, of those subjects who had a DEXA scan performed, they will be asked for all of their t- and z-score values. They will be asked about the presence of "classical" celiac disease GI symptoms or chronic diarrhea. They will be asked when they had these symptoms, specific details about the onset of symptoms, how they were treated if present, and if they were given a diagnosis as to the cause. Subjects will also be asked about the presence of iron deficiency anemia. In the section on malignancies, questions will be added concern the presence of small bowel adenocarcinoma and non-Hodgkin's lymphoma. Lastly, patients will be asked about family history of celiac disease, as a marker for having an increased risk of developing the disease.

D. Study Drugs: None
E. Medical Device: None
F. Study Questionnaires (see attached)
G. Study Subjects
    a. Inclusion
        i. Nurses enrolled in the initial nurses health study, whose blood samples from 1989-1990 are available for testing
        ii. Ability to give informed consent
    b. Exclusion
        i. Refusal to give informed consent
        ii. Subject diagnosed with celiac disease at the time blood sample taken in 1989-1990
H. Recruitment of Subjects: Potential subjects will be recruited from those whose blood samples were taken between 1989 and 1990 as part of the Nurses Health Study. Contact information will be obtained from the principal investigators of the Nurses health study, and nurses will be contacted, by telephone, mail, and/or e-mail, regarding their participation in this study. Each nurse will be given a detailed description of the study.
I. Confidentiality of Study Data: Blood samples ID numbers will be correlated with questionnaire ID numbers so as to ensure confidentiality as much as possible. Given that subjects may be diagnosed with celiac disease, coding will be deidentified if needed so as to inform a patient of the potential diagnosis. However, if a patient requests not to be made aware of the result of the testing, even if positive, this will be granted, and the data will be confidential to all parties not involved in the study, including insurance companies and the subject's employers. Data will be stored in a secure location, accessible only to investigators.

J. Potential Conflict of Interest: None
K. Location of the Study: The study will have more than one location. It will include Harvard University, where blood samples are stored, in addition to the location of all of the nurses. All blood samples will be tested at one laboratory. All of the data will be analyzed and stored at CPMC.
L. Potential Risks: The potential risk of becoming involved in this study relate to those of being diagnosed with celiac disease. While there is no direct risk of participating in the study, if a patient consents and is diagnosed with celiac disease, there are emotional and psychological risks associated with being diagnosed with celiac disease, and financial risks associated with start a gluten-free diet upon being diagnosed with the disease.
M. Potential Benefits: The benefits relate to those directly to subjects, and to society as a whole. For those patients who are diagnosed with celiac disease, there is the potential benefit of being diagnosed with the disease while asymptomatic, which carries the potential, although not definitive, benefit of starting treatment for the disease before it becomes symptomatic. This includes starting the diet prior to having GI symptoms and prior to having non-GI associated conditions. It also includes the benefit of being under the care of a physician, astute to the potential risks, notably malignant, associated with the disease. The benefits to society include helping researchers answer the questions whether it is beneficial to do widespread screening for celiac disease.
N. Alternative Therapies: None
O. Compensation to Subjects: There will be no compensation to subjects.
P. Costs to Subjects: None directly related to the study. However, there may be costs to the subjects as a result of this study, if a patient is diagnosed with celiac disease. These costs may include the cost of a gluten-free diet, and costs related to long-term referral to a gastroenterologist. Referrals, if needed, will be provided by the study investigators. However, it is expected that if needed, subjects' insurance companies will pay for the cost of endoscopies.

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