ARIC Manuscript Proposal #891 **This proposal was reviewed by the ARIC Publications Committee back in August of 1999 (08/01/99 was entered in to the Manuscript Tracking System as an approximate date of receipt of the proposal by the ARIC Publications Committee.) This proposal needed a manuscript number assigned to it, which is #891.

Title: Association of Hepatitis C Virus Infection and Diabetes Mellitus

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Specific Aims:

1. To determine whether there is an independent association between hepatitis C virus (HCV) infection and incident type 2 diabetes in a large cohort of community-based men and women in the United States.
2. To examine the causal relationship between HCV infection and type 2 diabetes by examining the order of occurrence of the conditions.

Hypothesis:
HCV infection exposure predicts the subsequent development of type 2 diabetes.

Rationale:

The purpose of the proposed study is to examine the putative association between hepatitis C virus (HCV) infection and the development of type 2 diabetes. HCV infection is principally a disease of the liver. However, extrahepatic manifestations have been reported including essential mixed cryoglobulinemia, sporadic porphyria cutanea tarda and thyroid disease (Strassburg CP et al, 1996). In addition, an association between HCV infection and diabetes mellitus has been proposed. However, the association has not been proven and the mechanism remains unknown.

Specific Aim #1

In the past several years, a number of reports have suggested an association between HCV infection and diabetes based on studies conducted in patients with liver disease or diabetes. In 1994, Allison et al demonstrated that 50% of 34 patients with HCV-related cirrhosis had diabetes as compared to only 9% of those with cirrhosis due to other causes (Allison MED et al, 1994). In 1995, Simo et al estimated the risk of HCV infection to be greater than 4 times higher in diabetics as compared to a control group of volunteer blood donors (OR-4.31; 95% CI- 2.61-7.24) (Simo et al, 1996). Most recently, Mason et al conducted a case-control study to determine the seroprevalence of HCV infection in a cohort of 594 diabetics and 377 clinic patients being assessed for thyroid disease. 4.2% of diabetic patients were found to be HCV-infected as compared to 1.6% of the control group (p=.02) (Mason AL et al, 1999).

These case control studies are supported by a handful of additional cross-sectional studies as well as case reports, all of which collectively suggest an independent association between HCV infection and diabetes (Knobler H et al, 1998; Gray H et al, 1994; Marson P et al, 1998; Grimbert S et al, 1996; Fraser GM et al, 1996; Ozyilkan E et al, 1996; Ozyilkan E et al, 1994). However, most of these studies fail to distinguish between type 1 and type 2 diabetes, which has important implications for causality. In addition, these studies suffer from an inherent selection bias due to the settings in which they were conducted; All involve clinic-based patients with either prevalent diabetes or liver disease secondary to HCV infection. Finally, these reports could not definitively demonstrate the independence of this relationship because of unmeasured confounding variables including body mass index and socioeconomic status. *Therefore, there is a need to assess the independence of this association in a community-based setting where these additional factors have been evaluated.*

Specific Aim #2

In addition, the existing case-control and cross-sectional literature is insufficient to explain the causal relationship between HCV infection and type 2 diabetes. Understanding this temporal relationship should elucidate the mechanism of the association. There are two main possibilities to consider. On one hand, HCV infection could cause diabetes through non-specific effects on liver cell function (e.g. cirrhosis), direct effects on pancreatic islet cells or virus-associated autoimmunity (Hadziyannis S et al, 1999). The mechanism involving liver disease is supported by the liver's role in glucose metabolism and many previous reports suggesting an association between

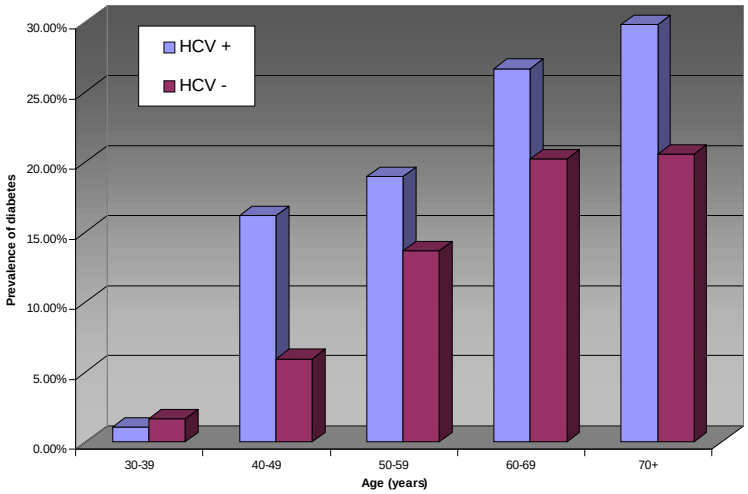
liver cirrhosis and impaired glucose tolerance (Muting D et al, 1969; Allison MED et al, 1994). If this hypothesis is true, one would expect for diabetes to occur in subjects with the most advanced liver disease. Alternatively, the mechanism involving the destruction of pancreatic islet cells or autoimmunity is supported by the handful of other autoimmune conditions (e.g. pophyria cutanea tarda) that have been recently linked to HCV infection (Strassburg CP et al, 1996). However, this mechanism involving autoimmune destruction of pancreatic islet cells typically describes type 1 diabetes. If any of these hypotheses are true, one would expect HCV infection to precede the occurrence of diabetes.

On the other hand, it has also been postulated that diabetes may be associated with HCV infection because individuals with diabetes are subject to an increased number of hospital interventions. It can also be argued that people with diabetes who require insulin are at higher risk for acquiring HCV infection due to frequent use of syringes (Simo et al, 1996). If the latter were true, then diabetes would be expected to antedate HCV infection. *In order for these issues to be resolved, the temporal association between HCV infection and diabetes needs to be defined.* The availability of a substantial number of incident diabetes cases as well as repository of frozen serum samples from each visit in ARIC will allow us to elucidate this causal relationship.

Preliminary Analysis:

A preliminary cross-sectional analysis was performed using data from the third National Health and Nutrition Examination Survey (NHANES III) (NCHS, 1994; NCHS, 1996). Information on diabetes and HCV infection was available for a subset of people (7576) who were examined between the years 1988 and 1994. In age-adjusted analysis, individuals with type 2 diabetes (1139) were 2 fold more likely to have HCV infection than those without diabetes (OR- 1.91; 95% CI- .91-4.01). The prevalence of diabetes in HCV-positive persons was increased in each age group except those 30-39 years of age (Figure 1). The magnitude of the association between HCV infection and type 2 diabetes was greatest between 40-49 years of age (OR-3.1; 95% CI- 1.0-9.9), the strata that also had the highest HCV prevalence. The association between HCV infection and type 2 diabetes was strengthened when ethnicity, gender, body mass index, socioeconomic status and previous drug and alcohol use were included in the model (OR- 4.31; 95% CI- 1.31-14.08).

Figure 1: Prevalence of diabetes in individuals with and without hepatitis C



Methods:

Overview:

In order to address the specific aims, we are proposing a case-cohort analysis on a subgroup of the ARIC study. We will use the same study sample that has been proposed for Dr. Pankow's study. The study sample will consist of a simple random sample of 600 incident diabetes cases (from a total of 1252) and a stratified random sample of the total cohort (n=700). The prevalence of hepatitis C virus infection among individuals with diabetes will be compared to that in the cohort itself.

Timeline:

We anticipate that it will take 6 months to perform the laboratory work and data analysis.

Sample Selection:

The sampling frame for this study will consist of 10,826 individuals who were free of diabetes at baseline, have available stored ARIC visit 1 plasma specimens and complete follow-up information. Cases will include participants who at any one of the three follow-up visits meet any one of the following criteria: (1)self-reported use of hypoglycemic medications, (2) causal serum glucose of >200mg/dL, (3) fasting (>8h) serum glucose > 126 mg/dL, or (4) a self-report of physician diagnosis. A total of 1252 incident cases of type 2 diabetes were identified using this criteria. A simple random sample of 600 incident diabetes cases will be selected for this study. Since 33% (418) of the total number of diabetes cases are African-Americans, 200 (33%) of the sample are expected to be African-American. A representative sample of all eligible cohort members (n=10,826) will be selected to serve as a comparison group for this study. This sample (n=700) will be random and stratified by gender/ethnicity group. This method will permit oversampling of African-Americans to ensure greater balance between the case and cohort samples with respect to ethnicity. According to cumulative diabetes incidence reports in this cohort, it is anticipated that approximately 97 individuals of the 700 cohort members will be diagnosed with diabetes during follow-up. Thus, 603 individuals should be free of type 2 diabetes during the entire follow-up period.

Exposure Assessment:

All cases and cohort members will be assessed for HCV antibody. A small fraction of the stored visit 1 plasma samples will be tested with a third-generation Ortho HCV enzyme immunoassay (EIA) (Ortho Diagnostics, Raritan, NJ). Specimens positive by EIA will be tested using a confirmatory assay, the second-generation Chiron Recombinant Immunoblot Assay (RIBA) HCV Test System (Chiron Corporation, Emeryville, CA and Ortho Diagnostics). The EIA test is highly sensitive (97%) and the predictive value is good in high prevalence settings. However, in low prevalence settings (e.g. blood banks), approximately 40-50% of specimens positive by EIA test negative by RIBA (false-positives) (Gretch DR, 1997; Lok ASF et al, 1997). Thus, the confirmatory RIBA, with a higher specificity, will be used since the ARIC cohort is likely to be a low HCV prevalence setting.

In addition to the EIA testing, we would like to perform HCV RNA quantitative testing using a quantitative reverse transcriptase polymerase chain reaction (RT-PCR) assay (AMPLICOR HCV MONITOR, Roche Diagnostic Systems, Branchburg, NJ) as well as HCV genotypic testing through restriction length polymorphism of nested polymerase chain reaction products. These tests would only be performed on those individuals determined to have HCV infection by the EIA and RIBA assays.

Depending on the availability of serum, we would like to test for hepatitis B infection. Since hepatitis B is also a cause of liver disease, it is a potential confounder of the association between HCV infection and diabetes. A sandwich radioimmunoassay (Abbot Laboratories, North Chicago, IL) will be used to obtain a semiquantitative determination of hepatitis B surface antigen (HbsAg) in human serum.

Finally, a subsample (n=50) of the non-HCV infected diabetics will be tested for incident HCV infection. In these individuals, serum from visit 4 will need to be tested to determine if anyone developed HCV infection after diabetes. We do not expect any incident HCV cases in this population; however, if there is a high rate of HCV infection following diabetes, more samples will need to be tested.

Power of the Study:

The prevalence of Hepatitis C virus in the general population has been estimated to be approximately 1.8% (Alter MJ, 1997; Harris MI et al, 1998). Previous studies have determined the prevalence of HCV in diabetics to range between 4.2% and 11.5% (Mason AL et al, 1999; Simo R et al, 1996). Power calculations were based on the assumption of a prevalence of 1.8% in people without diabetes. Power was calculated for a range of values corresponding to HCV prevalence (the exposure). With a sample size of 600 cases and 700 cohort members and an α level of .05, the power to detect a difference between cases and controls in the proportion exposed (with HCV infection) is as follows:

Proportion of Diabetics with HCV	Relative Risk	Power
4.0%	2.2	.58
4.5%	2.5	.74
5.0%	2.8	.85
5.5%	3.0	.92

6.0%	3.3	.96
6.5%	3.6	.98

Material Requested:
We will need a total of 400 μL of plasma (or serum) to perform the following tests:

- HCV EIA test - 20μL
- Confirmatory RIBA - 20μL
- HCV RNA RT-PCR assay - 40μL
- HCV genotyping - 40μL
- Hepatitis B Surface Antigen assay- 200μL

(Depending upon the amount of plasma/serum available to us, we are willing to forego the Hepatitis B testing.)

Data Requested:
age, gender, race, education, income, diabetes status, body mass index, family history of diabetes, insulin use

Funding:
All testing will be performed at the Viral Hepatitis Laboratory of the Johns Hopkins Medical School under the supervision of Dr. David Thomas. Existing funds will be used.

Human Subjects:
The burden to the ARIC participants will be minimal because no contact will be made with the study participants except in the case where a participant is determined to have HCV infection. The investigators are willing to work with the Steering Committee to decide which of the following two mechanisms will be employed to manage the data generated from the study. If the committee decides that subjects should be notified of their HCV infection, the investigators have experience with counseling messages and protocols that should be employed to disseminate information to the participants. We will provide written materials, counseling by phone, and medical referrals based on the American Association of the Society for Liver Disease.

Alternatively, the testing could be done after all identifiers have been stripped thus relieving the ARIC investigators of the responsibility of notification. Since all laboratory testing is being conducted in a research laboratory, the results should be considered preliminary and; therefore, the latter option may be preferable. However, we would envision that the Steering Committee could choose either option and we would be willing to work with them.

Public Health Significance:
It is estimated that nearly 4 million Americans are currently infected with hepatitis C virus (HCV). Each year, approximately 30,000 new cases occur and an estimated 8,000 to 10,000 deaths result from HCV-associated chronic liver disease. Due to the high rate of chronicicty (85% of infected individuals progress to chronic disease), there is a large reservoir of chronically infected individuals who are at risk for transmitting the disease to others and for developing any of the chronic complications associated with the disease (Alter MJ, 1997; Di Bisceglie AM, 1998; Marwick C, 1997). Data from the NHANES III (1988-1994) estimate the prevalence of diabetes in the U.S. to be 7.8% for adults over the age of 20 and 18.8% for adults over the age of 60 such that approximately 16 million individuals in the U.S. are living with diabetes (Harris MI et al, 1998). Diabetes, in addition to be the seventh leading cause of death, is a strong independent risk factor for atherosclerotic cardiovascular disease (Alberti KGMM et al, 1998).

This study has the potential to, for the first time, establish whether a causal relationship between HCV infection and type 2 diabetes exists. If a causal relationship is established, further epidemiologic and physiological research into the role of the liver in diabetes pathogenesis would be stimulated. These findings will also have tremendous implications for populations with a high prevalence of HCV infection (e.g. injection drug users), instigating a potential need for diabetes prevention efforts in these settings.

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