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Serologic Evidence of Infections and Type 2 Diabetes: The MultiEthnic Study of Atherosclerosis

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Abstract

Aims—Prospective studies have identified chronic inflammation as a risk factor for type 2 diabetes. However, it is not known whether infection by specific pathogens or having a greater “pathogen burden” is associated with diabetes. The aim of this study was to examine the cross-sectional relation of seropositivity to five pathogens (*C. pneumoniae*, cytomegalovirus, *H. pylori*, hepatitis A virus, herpes simplex virus) and prevalent diabetes.

Methods—Baseline data from a random sample of MultiEthnic Study of Atherosclerosis (MESA) participants (n=1,000; age: 45–84) were used. Diabetes was defined by ADA 2003 criteria, and “pathogen burden” by the number of pathogens (0–5) for which an individual was seropositive. Logistic regression was used to test differences in diabetes prevalence by seropositivity. Linear regression was used to explore associations between pathogen seropositivity and the inflammation markers CRP, IL-6, and fibrinogen.

Results—Diabetes prevalence was 12.7%, while seropositivity for *C. pneumoniae* was 76%, cytomegalovirus 77%, *H. pylori* 45%, hepatitis A 58%, and herpes simplex virus 85%. 72% were seropositive for ≥3 pathogens. In crude analyses, the prevalence of diabetes was higher among those with a pathogen burden ≥3, and with seropositivity to cytomegalovirus, *H. pylori*, hepatitis A, and herpes simplex virus. After adjustment for demographic covariates (particularly race) all associations became nonsignificant. Pathogen seropositivity was also not related to inflammation marker levels.

Conclusions—Following demographic adjustments, no associations were observed between infection by several pathogens and diabetes status, suggesting no etiologic role for them in the occurrence of diabetes.

Keywords

diabetes; infection; pathogen; seropositivity

Introduction

Prospective studies have demonstrated a positive association between incident type 2 diabetes and markers of systemic, low-grade inflammation, such as C-reactive protein (CRP), interleukin-6 (IL-6), and fibrinogen [1–9]. In most instances, these associations have not been explained by adjustment for established diabetes risk factors. As a potential mechanism, it has

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been suggested that systemic inflammation may interfere with insulin action by suppressing insulin signal transduction [10]. Specifically, there is some evidence that tumor necrosis factor- α suppresses expression of insulin receptors, and that IL-6 inhibits insulin signal transduction in hepatocytes.

Despite the accumulating evidence that chronic inflammation is a risk factor for type 2 diabetes, knowledge is limited regarding whether infection by specific pathogens contributes to the subsequent occurrence of type 2 diabetes. In the present study we hypothesized that diabetes prevalence would be higher among those with seropositivity to individual pathogens, and those with evidence of a greater “pathogen burden.”

Methods

Baseline data from MESA (2000-2002) were used in this cross-sectional analysis [11]. Briefly, 6,814 men and women between the ages of 45 and 84, all of whom were free of clinical CVD, were recruited in 6 U.S. communities. Race/ethnicity was self-reported as Hispanic, African-American, white, or Chinese. Local institutional review committees approved the MESA protocol, and all subjects gave informed consent.

Serum glucose was measured (following ≥ 8 hour fast) at a central lab by rate reflectance spectrophotometry using thin film adaptation of the glucose oxidase method on the Vitros analyzer (Johnson & Johnson Clinical Diagnostics, Inc., Rochester, NY 14650). The laboratory coefficient of variation (CV) was 1.1%. Prevalent diabetes was defined by fasting glucose values ≥ 126 mg/dL or use of glucose-lowering medications. Serum insulin was measured by the Linco Human Insulin Specific RIA Kit (Linco Research, Inc., St. Charles, MO 63304).

Immunoglobulin G (IgG) antibodies to *C. pneumoniae* were measured in the entire cohort at baseline, while IgG antibodies to cytomegalovirus, *H. pylori*, hepatitis A virus, and herpes simplex virus (type 1 and type 2) were assessed in a random sample of 1,000 participants. Serum IgG antibodies to *C. pneumoniae* were measured with a microimmunofluorescent antibody assay (Focus Technologies, Cypress, CA), while antibodies to cytomegalovirus, *H. pylori*, and herpes simplex virus were measured with indirect enzyme immunoassays (CMV IgG Test Kit, *H. pylori* IgG Test Kit, HSV 1 & 2 Test Kit; Diamedix Corporation; Miami, Florida), and to hepatitis A virus with the IM[®]x HAVAB qualitative microparticle enzyme immunoassay (Abbott Laboratories; Abbott Park, IL). Standard cut-points were utilized to define seropositivity: fluorescence of ≥ 1 for *C. pneumoniae*, concentration ≥ 10 EU/mL for cytomegalovirus, index value ≥ 1.10 for *H. pylori*, concentration ≥ 20 EU/mL for herpes simplex virus, and as compared to a calibrator for hepatitis A. All IgG antibody assay CVs were less than 8%. A “pathogen burden” score was computed by summing the number of pathogens for which an individual was seropositive (range 0 – 5).

Sex, age, race/ethnicity, education and smoking status were self-reported. Body mass index (BMI) was calculated as weight over height squared (kg)/(m²). Leisure physical activity (MET-min/wk) was computed via responses to a questionnaire. CRP and fibrinogen were measured using the BNII nephelometer (N High Sensitivity CRP, N Antiserum to Human Fibrinogen; Dade Behring Inc., Deerfield, IL), while IL-6 was measured by ultra-sensitive ELISA (Quantikine HS Human IL-6 Immunoassay; R&D Systems, Minneapolis, MN).

Statistical Analysis

Race/ethnicity-stratified means and frequencies of unadjusted dependent variables, primary independent variables, and covariates were calculated. Prevalence ratios (PRs) and 95% confidence intervals (CIs) were estimated using relative risk regression (binomial regression with a log link) to assess the relationship between diabetes and pathogen seropositivity. Linear

regression was used to estimate, by seropositivity status, relative differences in inflammation marker levels, and in glucose and insulin concentrations among non-diabetic participants. Demographic-adjusted models controlled for age, sex, race/ethnicity, education, and site, while models adjusting for traditional diabetes risk factors additionally controlled for smoking status, BMI, and leisure physical activity. Analyses were performed with SAS (version 9.1; Cary, NC: SAS Institute Inc.).

Results

The prevalence of diabetes was 12.7%, while the prevalence of seropositivity for *C. pneumoniae* was 75.5%, cytomegalovirus 76.7%, *H. pylori* 45.4%, hepatitis A 57.5%, and herpes simplex virus 84.9%. 72.5% were seropositive for ≥ 3 pathogens. Racial/ethnic differences were present in demographic and lifestyle factors, the prevalence of pathogen seropositivity, inflammation marker levels, and the prevalence of diabetes (Online Supplement 1). After demographic adjustments, pathogen burden was higher among participants who were older, and those who were current smokers, while no association was observed with sex, leisure physical activity, and BMI (data not shown).

Following demographic adjustments, pathogen seropositivity was largely unrelated to inflammation marker levels (Online Supplement 2). However, as compared to their nondiabetic counterparts, diabetic participants had higher mean levels of CRP (4.42 vs. 3.68 mg/L; $p = 0.0006$), IL-6 (1.81 vs. 1.52 pg/mL; $p < 0.0001$), and fibrinogen (363 vs. 345 mg/dL; $p < 0.0001$).

In crude analyses, the prevalence of diabetes was higher among those with a pathogen burden ≥ 3 , and among those with seropositivity to cytomegalovirus, *H. pylori*, hepatitis A, and herpes simplex virus, while no association was observed with *C. pneumoniae* (Table 1). Upon adjustment for race/ethnicity, however, all associations became non-significant.

Associations remained non-significant following further adjustment for additional demographic variables, and for traditional diabetes risk factors (data not shown). Similar results were observed for both the crude and adjusted analyses when, among non-diabetic participants, insulin and glucose concentrations were modeled as dependent variables (data not shown).

Discussion

In this multi-ethnic sample of 1,000 men and women, a greater prevalence of diabetes was found among those with a pathogen burden ≥ 3 , and those with seropositivity to cytomegalovirus, *H. pylori*, hepatitis A, and herpes simplex virus. These associations were, however, highly confounded; after adjustment for demographic factors, particularly race/ethnicity, the prevalence of diabetes was not related to pathogen burden or to seropositivity to individual pathogens.

We originally hypothesized that the presence of prior infection would influence diabetes prevalence through increasing systemic inflammation [10]. In this dataset, however, seropositivity to the five pathogens studied was unrelated to several markers of systemic inflammation. Thus, our null results for diabetes are not surprising. As expected, inflammation marker levels were higher among those with diabetes.

Our null findings are contrary to those of several prior studies, which have reported diabetes prevalence to be elevated among participants with antibodies to cytomegalovirus [12] and herpes simplex virus [13]. *H. pylori* infection has been positively related to diabetes prevalence in most [14-17], but not all [18], prior studies. No literature was identified which assessed the relation between diabetes and infection with *C. pneumoniae*, hepatitis A virus, or total pathogen

burden. Though not measured in MESA, infection with hepatitis B [19], hepatitis C [19-23], and periodontal pathogens [24] have generally been adversely associated with type 2 diabetes.

Prior studies exploring associations of infection by the pathogens assessed in MESA to prevalent diabetes are, however, limited. Most had extremely small sample sizes ($n < 150$) [12,14-16], the majority had case-control rather than population-based designs [12,14-16,18], and some were conducted in clinical populations [12,13,17]. Further, as evidenced by our data, the relation between pathogen seropositivity and diabetes may be greatly confounded by sociodemographic factors; it is possible that confounding was not adequately controlled in some of these studies. Finally, publication bias may also provide an explanation for the discrepancy between our results and those of previously published studies.

There are also significant limitations of our study. Foremost, pathogen infection was determined based on seropositivity to IgG antibodies. IgG antibodies reflect prior infection, but are not sensitive indicators of current infection or the chronicity of prior infections. Though our results were null, it is possible that active pathogen infection, or chronic active infection, is associated with systemic inflammation and elevated diabetes risk. Unfortunately, our data are unable to address this issue. Notably, IgG antibodies were used to define infection in most [12,13,17,18], but not all [14-16], prior studies assessing the relation between diabetes and the pathogens studied here.

Regardless of the means by which pathogen infection was assessed, inferences from cross-sectional data exploring the relation between pathogens and diabetes are tenuous, as the temporal direction of the relationship is unclear. While, as proposed in this manuscript, pathogen infection may lead to inflammation and diabetes, an alternate theory suggests that hyperglycemia may impair host defenses and predispose to infection [25]. Prospective data are clearly needed. This study's null findings, however, do not provide support for either hypothesis. Another limitation of our study is that the prevalence of seropositivity was high for some pathogens, resulting in relatively low exposure variability.

While acknowledging several limitations, strengths of our study include the relatively large sample size, population-based ascertainment, and the large number of pathogens assessed. In this dataset, pathogen seropositivity was not related to prevalent diabetes or to systemic inflammation. Elevated levels of systemic inflammation markers have, however, emerged as a strong risk factor for diabetes [1-9], and rational mechanisms have been identified [10]. Elucidating factors that contribute to chronic systemic inflammation is an important goal in understanding, and potentially intervening on, type 2 diabetes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CRP	C-reactive protein
IL-6	interleukin-6
BMI	body mass index
MESA	MultiEthnic Study of Atherosclerosis
CV	coefficient of variation
PR	prevalence ratio

Table 1

Prevalence ratio of diabetes by pathogen seropositivity; the MESA study, 2000-2002.

Pathogen Seropositivity	Crude	Race/Ethnicity-Adjusted	Demographic-Adjusted ^a
Pathogen Burden ≥ 3	1.68 (1.07, 2.64)	0.98 (0.59, 1.62)	0.81 (0.48, 1.37)
<i>C. pneumoniae</i>	1.12 (0.97, 1.30)	1.01 (0.87, 1.17)	0.90 (0.78, 1.05)
Cytomegalovirus	1.73 (1.06, 2.84)	1.16 (0.69, 1.95)	1.02 (0.60, 1.72)
<i>H. pylori</i>	1.65 (1.16, 2.34)	1.21 (0.84, 1.74)	1.12 (0.78, 1.62)
Hepatitis A	1.74 (1.19, 2.54)	1.30 (0.84, 2.00)	1.20 (0.77, 1.87)
Herpes simplex	2.73 (1.30, 5.75)	1.73 (0.80, 3.75)	1.50 (0.68, 3.28)

^a Adjusted for age (years), sex, race/ethnicity, education (< high school, high school degree or some college, college degree), and site.