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Association between Herpes Simplex Virus Type 1 and Type 2 with Prevalent Diabetes Mellitus Among the US Adults: Findings from the National Health and Nutrition Examination Survey, 2007-2016

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Approval Page

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Author's Statement Page

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Nikita Kute

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Abstract

Background: Infectious diseases are associated with an increase in the risk of developing insulin resistance and subsequent diabetes mellitus due to a possible role of chronic inflammation. While this relationship is well established for viral infections like hepatitis C or hepatitis B, little is known about the association of herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) with diabetes mellitus.

Methods: We conducted a cross-sectional study using the data from the 2007-2016 cycle of the National Health and Nutrition Examination Survey. Participants from the age group of 18-49 years with valid diabetes mellitus and HSV-1 and HSV-2 results and who completed the interview and examination were eligible for the analysis. HSV-1 and HSV-2 were defined by type specific enzymatic immunodot assay as positive or negative. Diabetes status was defined by glycohemoglobin level (HbA1c) as euglycemic (≤5.6%), prediabetes (5.7-6.4%), and diabetes (≥6.5%); participants who self-reported being diabetic were considered as having diabetes regardless of their HBA1c levels. We conducted logistic regression analysis to estimate the crude and adjusted odds ratio and 95% confidence interval of diabetes mellitus comparing participants with positive HSV-1 and HSV-2 to those with negative HSV-1 and HSV-2.

Results: The prevalence of HSV-1 among participants with diabetes was 67.2% (95% CI 62.3,72.0), and the prevalence of HSV-2 among participants with diabetes was 26.9% (95% CI 23.2,30.7). The prevalence of diabetes among participants with HSV-1 infection was 5.7% (95% CI 5.1,6.3), and the prevalence of diabetes among HSV-2 infection was 7.9% (95% CI 6.8,8.9). After adjusting for confounding factors, the odds of diabetes versus euglycemia among participants with HSV-1 was 1.05 (95% CI 0.9,1.2), and odds of diabetes versus euglycemia among participants with HSV 2 was 1.1 (95% CI 0.9,1.2).

Conclusion: After controlling for potential confounders, HSV-1 and HSV-2 infections are not associated with diabetes mellitus in the US population aged 18-49 years, 2007-2016

CHAPTER 1

INTRODUCTION

Background

There is a growing concern about the increasing prevalence of major public health conditions like diabetes mellitus and its co-infections. Even though herpes simplex virus (HSV) is not a major public health concern yet, in 2012 herpes simplex virus type 1 (HSV-1) and herpes simplex virus type 2 (HSV-2) was prevalent in 67% and 11% respectively of the global population under the age of 50 years (1). In 2015-2016 according to National Health and Nutrition Examination Survey (NHANES), the national estimates of prevalence by age, sex, and race among persons aged 14-49 years in the US for HSV-1 were 47.8% while HSV-2 was 11.9%. (2)

HSV is one of the members of a large group of double stranded DNA viruses called the Herpesviridae family and is of two types HSV-1 and HSV-2. It frequently causes ulcerative vesicular mucocutaneous lesions in human beings. HSV 1 is most commonly transmitted through non-sexual contact like contact with sores, saliva or surfaces around the mouth. Lesions occur on lips, inside the mouth, or on the face. They are commonly found in children and young adults. Recently HSV-1 has also been associated with genital herpes due to oral-genital contact. HSV-2 is a more common type. It is characterized by lesions on the genital surfaces as it is commonly transmitted by sexual contact. Rarely, it can also be transmitted from infected mothers to neonates during the delivery (3, 4).

Previous studies reported that chronic viral infections like hepatitis B and hepatitis C are associated with the pathogenesis of diabetes mellitus, likely due to increases in pro-inflammatory cytokines like tumor necrosis factor alpha, interferon-gamma and lymphocytic cell infiltration (5). This persistent chronic inflammatory state in the body increases insulin resistance, which subsequently leads to the development of diabetes mellitus (6-8). Similarly, a study conducted by Theil, D. and colleagues have found evidences that suggest the ability of HSV-1 and HSV-2 to establish latency in sensory neuronal

ganglia was responsible for increasing the pro-inflammatory cytokines and development of chronic inflammatory response during re-activation of virus (9) . Thus, a possible biological hypothesis can be made that there is an association between HSV-1 and HSV-2 with diabetes mellitus.

Previous cross-sectional studies reported an association between HSV-1 and HSV-2 infections with diabetes mellitus. A study conducted by Sun, Y. and colleagues in 2005 found a significant association of diabetes with HSV-1 infection [adjusted odds ratio (aOR) 1.5% 95% CI 1.1-2.0] (10). Ghane, M. also investigated the frequency of HSV infection with diabetes mellitus using PCR and ELISA and found a relationship between them (11). However, a study conducted by Lutsey, P. L. and colleagues in 2009 found no association between diabetes and HSV infection (aOR 1.5% 95% CI 0.68-3.28) (12)

Research Gap and Purpose of the Study

Evidence indicates that viral infections may contribute to the pathogenesis of diabetes (8, 13, 14). However, limited studies have been conducted to explore the association between HSV-1 or HSV-2 and diabetes mellitus, and they do not show consistent results. Using the data from 2007-2016, we hypothesize that HSV-1 and HSV-2 are associated with diabetes mellitus. The objective of this study is to determine the association between HSV-1 and HSV-2 and the prevalence of diabetes mellitus and pre-diabetes in the US adult population.

CHAPTER II

REVIEW OF LITERATURE

Diabetes Mellitus

In recent years, there has been tremendous growth in the prevalence of type 1 and type 2 diabetes mellitus worldwide, and it has become the seventh leading cause of death in the United States. According to the US Centers for Disease Control and Prevention (CDC), 30.3 million Americans had diabetes in 2015, while 84 million had pre-diabetes and at increased risk of diabetes (15). Diabetes is characterized by an abnormal increase in blood glucose concentration. Several risk factors have been identified for the incidence of diabetes, such as age, sedentary lifestyle, genetic susceptibility, and environmental factors (16, 17). However, obesity is known to be a major risk factor that predisposes an individual to diabetes (18). Obesity is found to increase the levels of pro-inflammatory cytokines such as tumor necrosis factor alpha or interferon-gamma (19) These elevated levels of cytokines increase the risk of developing insulin resistance and subsequent diabetes mellitus (6).

Studies conducted by J. C. Pickup and colleagues reported that diabetes mellitus is associated with chronic inflammation that is characterized by increase in the pro-inflammatory cytokines or innate immune response (20) Other studies and reviews also suggests diabetes mellitus be associated with elevated levels of C-reactive proteins, Interferon-gamma, Interleukin-6 and tumor necrosis factor alpha (20-23) Evidence from the study conducted by Sestan, M., and colleagues in 2018 reports that elevated levels of interferon-gamma in viral infection downregulates the insulin receptors of skeletal muscles and thus virally induced inflammation develops insulin resistance and subsequent diabetes mellitus (8, 24). Choi and colleagues in 2018, Kiernan, K. in 2018 and Mason Al. and colleagues in 1999 also suggest a possible association of viral infections with chronic inflammation and subsequent pathogenesis of diabetes mellitus (7, 8, 14)

Herpes Simplex Virus

Herpes simplex virus (HSV) causes infectious diseases characterized by ulcerative and vesicular lesions, which can be complicated by life-threatening necrotizing herpes-simplex encephalitis. HSV type 1 (HSV-1) is usually non-sexually transmitted, and the lesions are commonly present on the face, around the mouth or on the neck and less frequently on genital areas due to oral-genital contact. HSV type 2 (HSV-2) is a sexually transmitted infection that causes lesions in the anal and genital areas of the body. HSV-1 and HSV-2 have an affinity towards nerve cells and are known to establish, after primary infection, lifelong latency in the sensory neuronal ganglia (4, 25). Reactivation may occur due to conditions that cause immunosuppression like AIDS, surgery or trauma, mental and physical stress (9).

According to the world health organization, in 2012, HSV-1 and HSV-2 were prevalent in 67% and 11% respectively of the global population under the age of 50 years (1). The national estimates from the National Health and Nutrition Examination Survey (NHANES) show HSV-1 to be prevalent in 48.7% and HSV-2 in 11.9% of the US population (2).

HSV-1 and HSV-2 infections are found to be associated with the development of a chronic inflammatory state in the body due to its ability to remain latent for a long time. A study conducted by Theil, D., and colleagues provided evidence for elevated levels of CD8 cells, interferon-gamma, and tumor necrosis factor-alpha in an individual latently infected by HSV infection (9). Animal studies reported that upon reactivation of HSV infection, immune response elevates the pro-inflammatory cytokines like interferon-gamma in the host (5).

Co-occurring of Herpes Simplex Virus infection and Diabetes Mellitus

Based on the evidence that associates chronic inflammation with diabetes and HSV-1 and HSV-2 infections, we can biologically hypothesize that HSV-1 and HSV-2 are associated with the pathogenesis of diabetes mellitus through the mechanism of chronic inflammation. The state of persistent chronic inflammation in the body may arise due to latent HSV infection and its frequent reactivation resulting in

elevated levels of pro-inflammatory cytokines. These cytokines may further lead to the development of insulin resistance, followed by diabetes mellitus.

There is little in the literature that explores the association between HSV-1 and HSV-2 with diabetes mellitus, and the results are inconsistent. A cross-sectional study conducted by Sun and colleagues has found the significant association between HSV-1 and diabetes with an adjusted odds ratio (aOR) of 1.5% and 95% C.I. of 1.1-2.0 (10). They controlled for risk factors such as hypertension, cholesterol, age, and sex. Ghane also investigated the frequency of HSV infection with diabetes mellitus using PCR and ELISA testing and found a relationship between them (11) But, another cross-sectional study conducted by Lutsey and colleagues in 2009 found no association between HSV infection and diabetes (aOR 1.5% 95% C.I. 0.68-3.28) after controlling for demographic characteristics (12)

Summary of literature review

Highlights from the literature review of previous studies include:

1. Globally there is growing concern regarding the co-occurrence of viral infections and diabetes mellitus.

2. Immune activation due to viral infection may result in chronic systemic inflammation, which is directly linked to the pathogenesis of insulin resistance and subsequent type 2 diabetes mellitus.

3. Viral infections like hepatitis B and C are associated with diabetes mellitus.

4. Viral infections like HSV-1 and HSV-2 are responsible for the development of the chronic inflammatory state during latent and active periods and therefore, may be associated with the prevalence of diabetes mellitus.

5. Only two studies found an association between HSV-1 and HSV-2 infections and diabetes mellitus.

6. Prospective studies are required to better characterize the association between HSV-1 and HSV-2 infections and diabetes mellitus.

CHAPTER 3

MANUSCRIPT

Introduction

According to the World Health Organization, 67% of the global population under 50 years of age has Herpes Simplex virus type 1 (HSV-1) infection, and 11% has Herpes Simplex virus type 2 (HSV-2) infection. (26). In 2015-2016, the prevalence of HSV 1 in the United States was 47.8%, and the prevalence of HSV 2 was 11.9% (2). HSV-1 is transmitted non-sexually via contact with sores, saliva or surfaces around the mouth of an infected individual. Transmission of HSV-1 occurs most commonly during childhood and may cause recurrent orolabial and facial lesions. However, recently, HSV-1 infection has also cause genital lesions due to oral-genital contact (1). HSV-2 is transmitted sexually and typically includes ulcerative and vesicular lesions on genital or anal areas (1) (27). Rarely, HSV-2 can also be transferred from mother to neonate during vaginal delivery and is potentially life-threatening (28). While behavioral risk factors associated with HSV 1 and HSV 2 infections are well established, additional individual factors and comorbidities that may increase the risk of HSV are poorly understood. (1) (28)

Given the rapid global increases in non-communicable diseases (NCDs), there is a growing concern over the synergy between co-occurring infectious diseases with NCDs. Infectious diseases that persist latently in the host for long periods may contribute to chronic inflammation over the life course (9). Both HSV 1 and HSV 2 infections can establish lifelong latency in neuronal sensory ganglia (5, 29). Reactivation of HSV 1 and HSV 2 infections may trigger innate inflammatory responses in the body which in turn can promote inflammatory cytokines like interleukin 6, tumor necrosis factor alpha, interferon beta and interferon-gamma (type 1 and type 2 interferons) (5) and could contribute to chronic systemic inflammation. The role of chronic inflammation in the pathogenesis of insulin resistance and subsequent diabetes mellitus incidence is well established (8). In addition, other viral infections which contribute to the systemic inflammation are associated with diabetes mellitus.

Despite of accumulating evidence indicating viral infections may contribute to diabetes pathogenesis (7, 8, 14, 30), there is limited evidence regarding the association between HSV-1 and HSV-2 infections with the risk of developing diabetes mellitus. In this study, we aimed to determine the association between prevalence of HSV-1 and HSV-2 infections with the prevalence of diabetes mellitus and pre-diabetes using cross-sectional data from the US National Health and Nutrition Examination Survey (NHANES) study, 2007-2016.

Methods

Study Design and Overview

We performed a cross-sectional analysis using NHANES 2007-2016 data, which included five continuous cycles. Briefly, NHANES is a nationally representative health examination survey that includes in-person interviews and a health examination administered by trained medical personnel. Detailed information on NHANES methodology has been published in the literature previously (31). During 2007-2016, 50,588 NHANES individuals were selected for participation, and 48,710 participants completed the interview and physical examinations (32). The exposures for this analysis were HSV-1 and HSV-2, and the outcome for the study was Diabetes Mellitus.

Participant Eligibility Criteria and Laboratory Methods

Eligible participants included adults aged 18 to 49 years who completed the NHANES interview, and underwent a health examination, had a valid measure of diabetes and had a valid HSV-1 and HSV-2 lab result. Serum specimens for HSV-1 and HSV-2 infections were tested using Type specific Enzymatic Immunodot Assay to detect antibodies reactive to HSV-1 and HSV-2 infections. Participants with missing or indeterminate HSV-1 and HSV-2 Immunodot Assay results were excluded.

Diabetes mellitus was defined by glycated hemoglobin (HbA1c) levels in combination with self-reported diabetes status. Those with missing and indeterminate results for HbA1c were excluded from the analysis to avoid the assumption of results. Glycohemoglobin levels were measured using standardized

Glyco-analyzers. Finally, a total of n=14,638 participants were eligible for HSV-1 analyses, and n=14,618 participants were eligible for HSV-2 analyses.

Variables and Study measures

HSV-1 and HSV-2 were interpreted based on the US CDC guidelines for type-specific serologic testing. (4) Participants who self-reported to have a history of diagnosis of diabetes by a healthcare professional were considered diabetes regardless of their HbA1c level. Those with missing or no history of self-reported diabetes were then categorized using glycated hemoglobin levels (HbA1c) as euglycemic (<5.6%), pre-diabetes (5.7-6.4%), or diabetes (>=6.5%) according to the American Diabetes Association guidelines (16).

Total cholesterol, current smoking status, lifetime smoking status, sexual behavior by age when had first sex, condom use during sex, number of sex partners per year and lifetime, history of genital herpes and history of circumcision were some of the covariates included in the analysis. Total cholesterol levels were characterized as desirable (<200 mg/dl), borderline high (200-239 mg/dl) and high (>=240mg/dl) based on the guidelines developed by National Institute of Health- National Cholesterol Education Program (33). Smoking status was defined in two ways, current smoking status, and lifetime smoking status. We categorized the current smoking status of the participant's non-smokers, every day smokers, and someday, smokers based on their self-reported responses for currently smoking. Lifetime smoking status was defined as smokers if participants smoked atleast100 cigarettes in a lifetime and are currently smoking, as former smokers if participants smoked at least 100 cigarettes in a lifetime but currently not smoking and as non-smokers if participants had not smoked at least 100 cigarettes in a lifetime and they currently do not smoke. (34), (35).

We characterized sexual behavior using self-reported measures that included the age of first sex, number of sexual partners, use of condom during sex and history of genital herpes. The age when had

first sex was categorized as before the age of 15 years, between the age of 15-18 years and after the age of 18 years. Use of condom during sex was categorized as never use a condom, always use a condom, and sometimes use a condom. Sexual partners per year and sexual partners in a lifetime were categorized as no partners, single partner, two partners, three to five partners, and more than six partners. History of Genital Herpes diagnosis by a health professional was self-reported by the participants as yes or no. Similarly, circumcision history for males was self-reported as yes or no by the participant.

Statistical Analysis

The association between HSV-1 and HSV-2 with diabetes mellitus was assessed using bivariate analyses and multivariable logistic regression. In the bivariate analysis, the Rao-Scott chi-square test was used to assess for potential confounders. We reported weighted prevalence estimates and their 95% confidence intervals (CI) to examine the prevalence of HSV-1, HSV-2, and diabetes status in relation with included participant characteristics. The unadjusted association was examined using prevalence differences and crude odds ratios (cOR). Adjusted odds ratio (aOR) and 95% CI for both exposure-outcome pairs were estimated using multivariable logistic regression models and were adjusted for potential confounders (36). We used four different models for logistic regression. Model 1 as odds of diabetes and pre-diabetes versus euglycemic, model 2 as odds of diabetes versus pre-diabetes and euglycemic, model 3 as odds of diabetes versus euglycemic and model 4 as odds of pre-diabetes versus euglycemic. Covariate selection criteria included previous study findings, bivariate association analysis, and causal model theory (directed acyclic graphs) (37). Statistical Analytical Software (SAS) 9.4 version was used to conduct the analysis. To produce unbiased variance estimates, in the complex datasets of NHANES during analysis, we used the proposed weighting methodology and guidelines set forth by National Center for Health Statistics, Centers for Disease Control and Prevention (38). The weight variable used during analysis to

calculate the measures of associations, prevalence estimates, and confidence intervals was WTMEC2YR. Statistical significance for all the tests was determined by a two-sided p-value of <0.05.

Results

Study Participants

Before accounting for a corrected variance by using selection weights, from those with valid diabetes and HSV-1 results, 831 eligible participants had diabetes, and 600 of them were HSV-1 positive. Among those 831, 267 were HSV-2 positive (Figure 1).

Prevalence of HSV 1&2

The overall prevalence estimates of HSV-1 among adults in the total US population between 2007-2016 was 55.2% (95%CI 53.2,57.3%) and the overall prevalence estimate of HSV-2 was 15.9% (95%CI 14.9,16.9) (Table 1). The prevalence for HSV-1 was highest among Hispanics (74.3% 95%CI 68.3,72.4) followed by A1c level >6.5% (69.1% 95% CI 63.4,74.7), males with circumcision (68.2% 95%CI 65.1,71.3) and participants with diabetes (67.2% 95%CI 62.3,72.0). The prevalence estimates of HSV-2 among adults in the US population for the year 2007-2016 was 15.9% (95% CI 14.9,16.9). They were highest for Non-Hispanic Blacks 40.9% (95%CI 39.1,42.8) followed by participants with diabetes (26.9% 95%CI 23.2,30.7) and current smokers (23.9% 95% CI 22.1,25.8) (Table 1)

Prevalence of Diabetes Mellitus

The estimated prevalence of diabetes among US adult population between 2007-2016 was 4.7% (95% CI 4.2,5.1). The prevalence estimate for diabetes was highest among participants with the diagnosis of hypertension (13.3% 95% CI 11.8,14.9) followed by Body Mass Index > 30kg/sq. m (obese) (9.7% 95% CI 8.7,10.7), age group 41-50 years (9.1% 95% CI 7.9,10.4), HSV-2 positive (7.9% 95% CI 6.8,8.9), HSV-1 positive (5.7% 95% CI 5.1,6.3) and Non-Hispanic Blacks (7.5% 95% CI 8.7, 10.7). (Table 2)

Prevalence Difference in diabetes mellitus

The prevalence estimate of diabetes was higher among participants with positive HSV-1 infection by 2.3 percentage points (95% CI 1.4,3.1) as compared to participants with negative HSV-1 infection. The

prevalence estimate of diabetes was higher among participants with positive HSV-2 infection by 3.8 percentage points (95% CI 2.7,5.0) as compared to participants with negative HSV-2 infection. (Table 1, Table 2)

Logistic regression model results

We conducted logistic regression analysis to examine the crude and the adjusted association between adults with HSV-1 and HSV-2 and diabetes. Four different models for odds of diabetes, prediabetes, and euglycemia were examined to assess the association for different outcome variables with HSV-1 and HSV-2 (Table 3). Before adjusting for potential confounders, the odds of diabetes and pre-diabetes versus euglycemia among those with HSV-1 infection was 1.8 times (95% CI 1.6,1.9) the odds of diabetes and pre-diabetes among those with no HSV-1 infection. While odds of diabetes and pre-diabetes among those with HSV-2 infection was two times (95% CI 1.8,2.3), the odds of diabetes and pre-diabetes among those with no HSV-2 infection. Similar results were observed for other models as well. (Table 3)

After adjusting for covariates in the multivariable models, the odds of diabetes and pre-diabetes among those with HSV-1 infection was equal to odds of diabetes and pre-diabetes among those with no HSV-1 infection (aOR 1.1 95% CI 0.9,1.2). Similarly, the odds of diabetes and pre-diabetes among those with HSV-2 infection was equal to odds of diabetes and pre-diabetes among those with no HSV-2 infection (aOR 1.1 95% CI 0.9,1.2). (Table 3)

Sensitivity Analysis

In our sensitivity analysis, we reported the range of odds ratios after adjusting for multiple combinations of covariates in the models. The adjusted odds ratios for diabetes and prediabetes vs. euglycemia ranged from 1.03 (95% CI 0.8,1.3) to 1.4 (95% CI 0.9,1.5) among adults with HSV-1 (Table 4). For adults with HSV-2, the adjusted odds of diabetes versus prediabetes and euglycemia ranged from 1.1 (95% CI 0.9,1.4) to 1.5 (95% CI 1.04,1.6) (Table 4). Model adjusted for age, sex, BMI, total cholesterol and

hypertension showed odds of diabetes and pre-diabetes to be statistically significant for both HSV-1 (aOR 1.4% 95% CI 1.3, 1.5) and HSV-2 infections (aOR 1.5% 95% CI 1.3, 1.7) (Table 4).

Discussion

We attempted to examine the association between HSV-1 and HSV-2 infections and diabetes mellitus. We found a strong crude association of HSV-1 and HSV-2 with diabetes. The prevalence of HSV-1 among adults with diabetes was 14.4% points more than the adults with euglycemia and prevalence of HSV-2 among adults with diabetes was 12.9% points more than those with euglycemia. The prevalence of diabetes was also significant among adults with HSV-1 positive and HSV-2 positive as compared to negative. After adjusting for confounders, there was no association between HSV-1 and HSV-2 and diabetes mellitus. In the model that adjusted age, sex, hypertension, total cholesterol, and BMI, the association was present (aOR 1.4% for HSV-1 and aOR 1.5% for HSV-2). These covariates are a part of metabolic syndrome, and thus, this finding gives an insight into possible confounding by metabolic syndrome and a possible association of HSV-1 and HSV-2 infections with metabolic syndrome. Further studies are required to explore this association. Our study includes data of 10 years (2007-2016) from a nationally representative sample of US from NHANES and thus to our knowledge, this is the largest and most generalizable study comparing HSV-1 and HSV-2 infections with diabetes mellitus among US adults.

The results of our study are inconsistent with the findings of previous studies. A randomized cross-sectional study conducted by Sun, Y. Et al. in 2005 on patients of Beijing Fu Wai heart hospital found a significant association with an adjusted odds ratio of diabetes as 1.5 (95% CI 1.1-2.0) for HSV-1 infection (10). However, the study was conducted in a clinical setting and thus did not have a large sample size. Also, the study was not generalizable to the US population. Another randomized cross-sectional study conducted by Lutsey and colleagues in 2009 reported an adjusted odds ratio of 1.5% (95% CI 0.68,3.28) and concluded as no association between HSV infection and diabetes in a sample of multiethnic study of

atherosclerosis participants. The study, however, controlled for only demographic variables and not the potential confounding risk factors like sexual behavior, total cholesterol, hypertension, and BMI (12).

Although only two studies were conducted to investigate the direct association between HSV-1 and HSV-2 with diabetes, there are many studies and reviews that have established an association between viral infection and insulin resistance with subsequent diabetes mellitus (8, 13, 14). A cross-sectional study conducted by Mason, A. et al. in 2003 on patients from St. Louis and New Orleans have found that Hepatitis C virus (HCV) infection was an independent predictor of diabetes mellitus (p=.02) and was suggested as an additional risk factor for the development of diabetes (14). Hepatitis B virus (HBV) and Hepatitis C virus were also found to be associated with diabetes in a cohort study conducted by H. Y. Choi et al. in 2017 on the population of Korea with the adjusted Hazard Ratio for diabetes as 1.4 and 1.68 in HBV and HCV groups, respectively (7). These hypotheses of viral infections increasing the risk for diabetes mellitus may explain how HSV-1 and HSV-2 infections may increase the risk of diabetes and vice-versa.

Our study was subjected to several limitations. First, this is a cross-sectional study. We could only find an association between HSV-1 and HSV-2 infections and diabetes. But because we had no data that could indicate the time of HSV infection in reference to the diagnosis of diabetes, we were not able to determine if the observed association was due to increased risk of diabetes from HSV-1 and HSV-2 infections or the risk of developing HSV-1 and HSV-2 infections due to diabetes mellitus. Another limitation is that there may have been misclassification of outcome variable diabetes. We did not define diabetes based on oral glucose tolerance test (OGTT), which is the gold standard test according to ADA guidelines (16). HbA1c test and possibility of inaccurate self-reported diabetes status could have distorted our estimated association between HSV-1 and HSV-2 and diabetes mellitus. There may have also been misclassification of patient characteristics. For example, variables such as smoking or sexual behavior have a social stigma attached to it. Therefore, the self-reported responses from participants

might not be accurate. Information on whether the HSV infection was new or active, latent, or dormant was not available. There may have been possible confounding by other factors involved in metabolic syndrome, which were not included in the analysis due to lack of data. Our study cannot be generalized to the older population of the US as the older age group (>50 years of age) was not included in the analysis. Lastly, the Hepatitis C virus has been found to increase the risk of developing type 2 diabetes (39). We did not consider the history of hepatitis C virus antibody seropositivity, which may have confounded the relationship between HSV-1 and HSV-2 and diabetes. To understand the relationship in detail, prospective studies exploring the association and direction of the association between HSV-1 and HSV-2 and diabetes mellitus are needed.

Conclusion

The results from our study reported that before controlling for potential confounders, the prevalence of HSV-1 and HSV-2 infections was significantly associated with an increase in the odds of diabetes prevalence in US adults aged 18-49 years. After controlling for confounders, there was no association between HSV-1 and HSV-2 infections with diabetes mellitus. Overall, the prevalence of HSV-1 and HSV-2 was higher among diabetes and prediabetes patients as compared to patients with euglycemia. And the prevalence of diabetes was also higher among patients with HSV-1 and HSV-2 infections as compared to patients without HSV-1 and HSV-2 infections. This study gives an insight into a possible association of non-communicable chronic diseases like diabetes mellitus with viral infections like HSV-1 and HSV-2. With increasing public health concern for diabetes and HSV infections worldwide, further research may be needed to identify the associations of potential risk factors.

Figure 1

Flow chart describing the process of selection for NHANES 2007-2016 participants eligible for the study, including the categorization of eligible participants by diabetes status, raw numbers not weighted for NHANES methodology

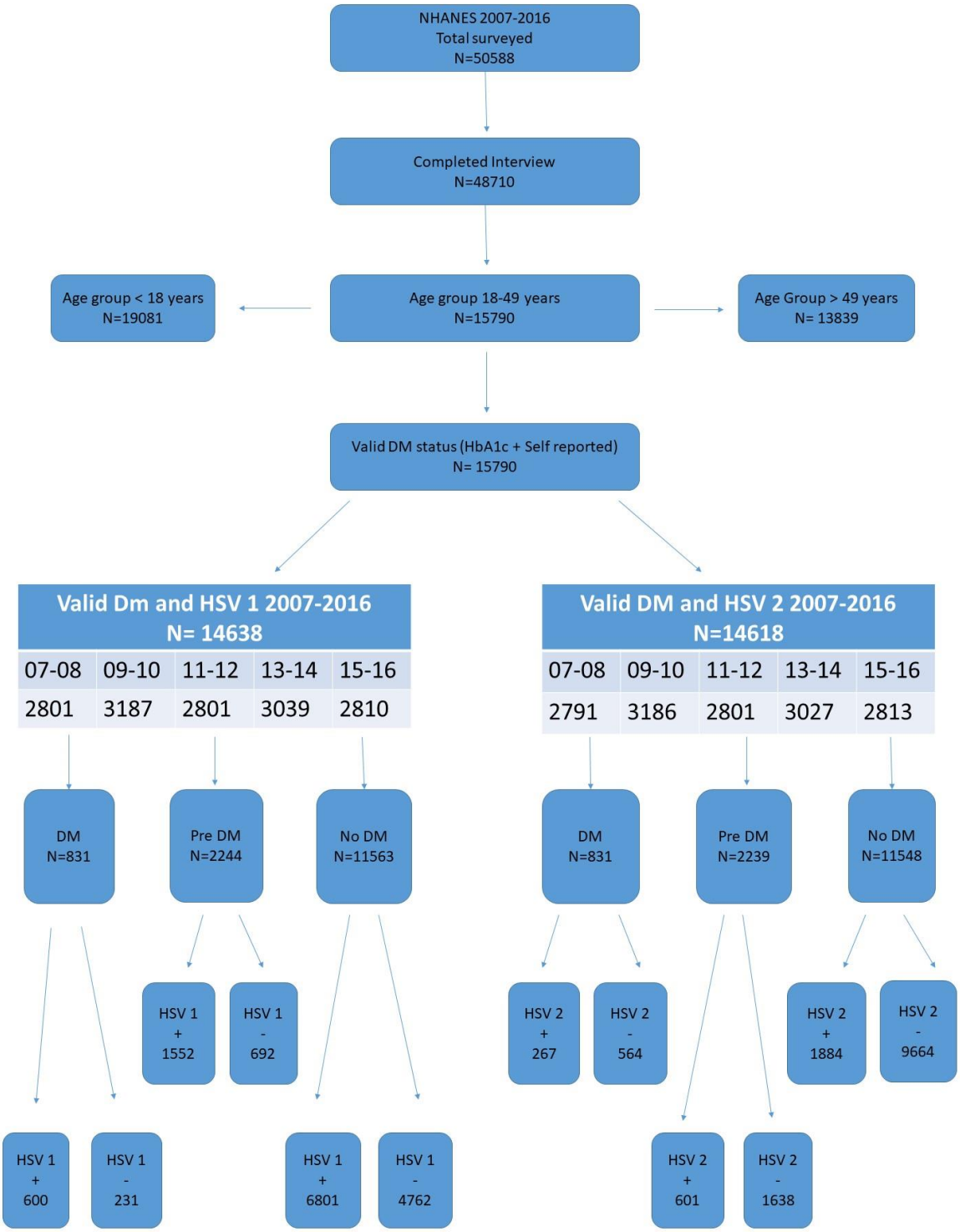
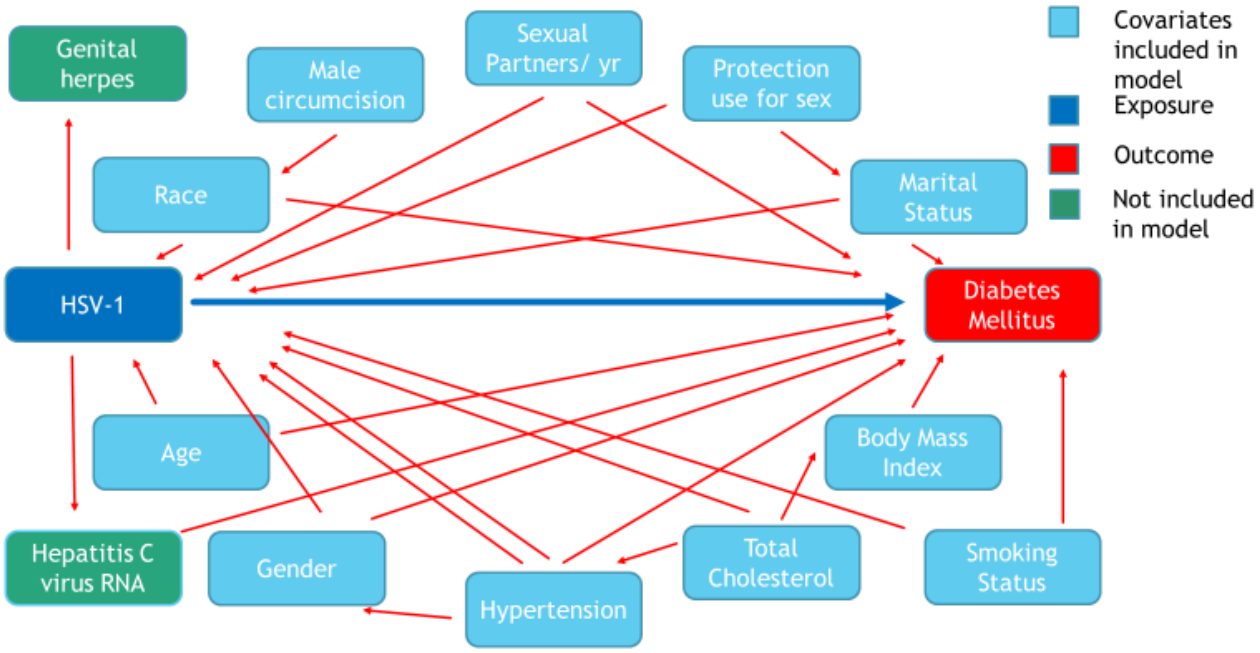


Figure 2

Directed Acyclic Graph for the covariates associated with HSV-1 and diabetes mellitus



Directed Acyclic Graph for the covariates associated with HSV-1 and diabetes mellitus

Figure 3

Directed Acyclic Graph for the covariates associated with HSV-2 and diabetes mellitus

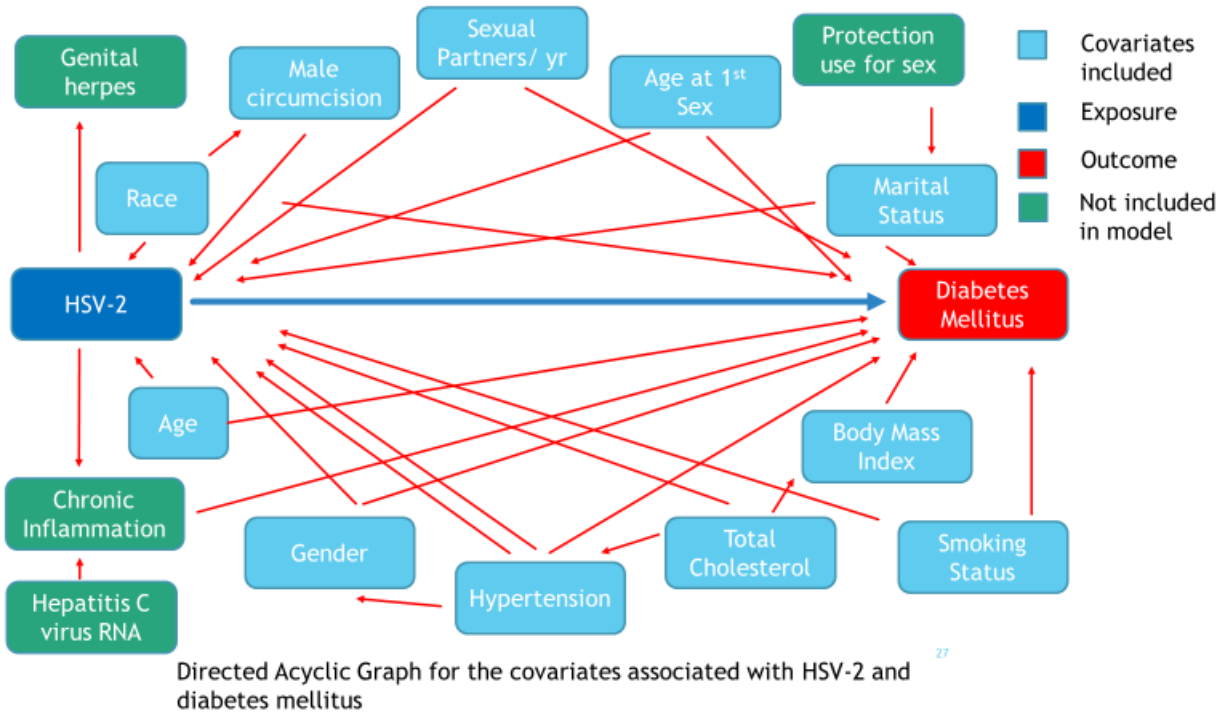


Table 1:

Distribution of Exposure 'Herpes Simplex Virus 1 ' by Characteristics for the sample with valid DM results in NHANES 2007-2016

	HSV TYPE 1 N= 14638			
Characteristics	HSV 1 positive Prevalence estimates % (95% CI)	HSV 1 negative Prevalence estimates % (95% CI)	Prevalence Difference and 95% CI	P value Rao-Scott Chi test
Total population %	55.2 (53.2,57.3)	44.8 (42.7,46.8)		
Age (Years)				<0.0001
Median (Q3 - Q1)	36 (43 - 27)	29 (38 - 22)		
18-30	44.1 (41.7,46.4)	55.9 (53.6,58.3)	Reference	
31-40	60.3 (57.7,62.8)	39.7 (37.2,42.3)	16.2 (13.6,18.9)	
41-50	65.2 (62.4,68.0)	34.8 (31.9,37.6)	21.2 (18.7,23.7)	
Gender				<0.0001
Male	52.3 (49.9,54.5)	47.7 (45.5,50.0)	-5.9 (-7.9,-3.9)	
Female	58.2 (55.9,60.5)	41.8 (39.5,44.1)	Reference	
Missing				
Race/Ethnicity				<0.0001
Non-Hispanic White	46.9 (44.6,49.2)	53.1 (50.8,55.4)	Reference	
Non-Hispanic Black	62.5 (59.7,65.2)	37.5 (34.8,40.3)	15.6 (12.8,18.3)	
Other	70.4 (68.3,72.4)	29.7 (27.6,31.7)	23.5 (20.8,26.1)	
Missing				
HbA1c (%)[2]				<0.0001
Median (Q3 - Q1)	5.4 (5.1 - 5.6)	5.3 (5.5 , 5.0)		
<5.7	52.8 (50.7,54.9)	47.2 (45.1,49.3)	Reference	
5.7-6.5	66.2 (63.4,68.9)	33.8 (31.1,36.6)	13.3 (10.9,15.8)	
>6.5	69.1 (63.4,74.7)	30.9 (25.3,36.6)	16.2 (10.6,21.8)	
Missing				
DM status (A1c+Self report)[3]				<0.0001
Euglycemia				
Prediabetes	52.8 (50.7,54.9)	47.2 (45.1,49.3)	Reference	
Diabetes	66.4 (63.7,69.1)	33.6 (30.9,36.3)	13.6 (11.1,16.1)	
	67.2 (62.3,72.0)	32.9 (27.9,37.7)	14.4 (9.7,18.9)	
BMI[4]				<0.0001
<18.5	48.4 (41.2,55.4)	51.6 (44.6,58.7)	Reference	
18.5-24.9	49.5 (46.9,52.2)	50.5 (47.8,53.1)	1.2 (-5.5,7.8)	
25.0-29.9	56.0 (53.6,58.5)	43.9 (41.5,46.4)	7.7 (0.5,14.9)	
>=30.0	60.2 (57.6,62.8)	39.8 (37.2,42.4)	11.9 (4.5,19.2)	
Missing	65.3 (53.2,77.3)	34.7 (22.7,46.8)	16.9 (4.4,29.4)	

Total cholesterol (mg/dl)[5]				<0.0001
<200				
200-239	53.1 (50.9,55.3)	46.9 (44.7,49.1)	Reference	
>=240	58.9 (56.4,61.4)	41.1 (38.6,43.6)	5.8 (3.2,8.4)	
Missing	59.4 (55.3,63.6)	40.6 (36.4,44.7)	6.3 (2.6,10.1)	
	100.0 (100,100)	----------	46.9 (44.7,49.1)	
Marital Status				<0.0001
Never married	47.2 (44.8,49.5)	52.8 (50.5,55.2)	Reference	
Married	59.7 (57.1,62.2)	40.3 (37.8,42.9)	12.5 (9.7,15.2)	
Missing	52.3 (49.2,55.5)	47.7 (44.5,50.9)	5.1 (1.7,8.6)	
Current Smoking status[6]				<0.0001
No Smoking	57.4 (53.9,60.9)	42.6 (39.1,46.0)	Reference	
Smoking	60.9 (58.4,63.4)	30.1 (36.6,41.6)	3.5 (-0.3,7.3)	
Missing	52.6 (50.2,54.9)	47.4 (45.1,49.7)	-4.8 (-8.0,-1.6)	
Smoking status-Lifetime[7]				<0.0001
Never Smoker				
Current Smoker	53.6 (51.2,56.1)	46.4 (43.9,48.8)	Reference	
Former Smoker	60.9 (58.4,63.4)	39.1 (36.6,41.6)	7.3 (4.6,10.0)	
Missing	57.4 (53.9,60.9)	42.6 (39.1,46.0)	3.8 (0.5,7.1)	
	35.1 (30.3,39.8)	64.9 (60.1,69.7	-18.6 (-23.9,-13.2)	
Hypertension[8]				<0.0001
No Hypertension	53.8 (51.6,55.9)	46.2 (44.0,48.4)	Reference	
Yes Hypertension	62.6 (58.8,65.5)	37.4 (34.5,40.2)	8.8 (5.9,11.8)	
Missing	79.7 (60.8,98.5)	20.3 (1.5,39.2)	25.9 (6.8,44.9)	
SB - Age of 1st sex[9]				0.7692
18-27 yrs	55.2 (53.1,57.2)	44.8 (42.8,46.9)	Reference	
28-37 yrs	50.9 (41.5,60.5)	49.0 (39.5,58.5)	-4.2 (-13.5,5.1)	
38-49 yrs	61.3 (30.9,91.7)	38.7 (8.3,69.1)	6.2 (-24.8,37.1)	
Missing	55.8 (52.4,59.2)	44.8 (42.8,46.9)	0.7 (-2.2,3.5)	
SB- Protection Use[10]				<0.0001
Always	61.5 (58.3,64.8)	38.5 (35.2,41.7)	Reference	
Never	55.1 (52.7,57.5)	44.9 (42.5,47.3)	-6.4 (-9.2,-3.6)	
Sometimes	50.3 (47.7,52.9)	49.7 (47.1,52.3)	-11.2 (-14.9,-7.6)	
Missing	55.7 (52.9,58.6)	44.3 (41.4,47.1)	-5.8 (-9.2,-2.4)	
SB - Sex partners / Year[11]				<0.0001
No partner				
One partner	49.8 (46.9,52.7)	50.2 (47.3,53.1)	Reference	
Two partners	56.1 (53.8,58.4)	43.9 (41.6,46.2)	6.3 (3.4,9.2)	
3-5 partners	56.1 (51.9,60.3)	43.9 (39.7,48.1)	6.3 (1.5,10.9)	
>6 partners	47.0 (42.3,51.7)	52.9 (48.3,57.7)	-2.8 (-7.6,2.0)	
Missing	54.6 (46.7,62.4)	45.4 (37.6,53.3)	4.8 (-2.9,12.5)	
	61.5 (57.8,65.2)	38.5 (34.8,42.2)	11.7 (8.4,14.9)	
SB - Sex Partners in Lifetime[14]				<0.0001
No partner				
One partner	54.3 (50.3,58.2)	45.7 (41.8,49.7)	Reference	
Two partners	45.4 (41.6,49.2)	54.6 (50.8,58.4)	-8.8 (-13.9,-3.8)	

3-5 partners	53.6 (49.6,57.7)	46.4 (42.3,50.4)	-0.6 (-6.0,4.8)	
>6 partners	54.8 (51.4,58.1)	45.2 (41.9,48.5)	0.5 (-4.4,5.4)	
Missing	57.9 (55.5,60.2)	42.1 (39.8,44.5)	3.6 (-0.9,8.1)	
	61.5 (57.8,65.2)	38.5 (34.8,42.2)	7.3 (2.8,11.7)	
SB- Doctor told to have Genital Herpes[15]				0.8100
No	55.1 (53.1,57.2)	44.9 (42.8,46.9)	Reference	
Yes	54.1 (47.5,60.7)	45.9 (39.3,52.5)	-1.0 (-7.8,5.7)	
Missing	55.9 (52.6,59.3)	44.0 (40.7,47.4)	0.8 (-2.1,3.7)	
SB- Male circumcision status[16]				<0.0001
No				
Yes	68.2 (65.1,71.3)	31.8 (28.7,34.9)	Reference	
Missing	47.7 (45.2,50.3)	52.3 (49.7,54.8)	-20.5 (-24.4,-16.5)	
	57.6 (55.2,59.9)	42.4 (40.1,44.8)	-10.6 (-14.1,-7.2)	
Hepatitis C virus RNA[17]				0.0007
Negative	76.1 (65.6,86.5)	23.9 (13.5,34.4)	Reference	
Positive	66.1 (54.9,77.3)	33.9 (22.7,45.1)	-9.9 (-1.7,21.6)	
Missing	55.0 (52.9,57.1)	44.9 (42.9,47.0)	-21.0 (10.3,31.8)	

Table 2:

Distribution of Exposure 'Herpes Simplex Virus 2 ' by Characteristics for the sample with valid DM results in NHANES 2007-2016

	HSV TYPE 2 N=14618			
Characteristics	HSV 2 positive Prevalence estimates % (95% CI)	HSV 2 negative Prevalence estimates % (95% CI)	Prevalence Difference and 95% CI	P value Rao-Scott Chi test
Total population %	15.9 (14.9,16.9)	84.1 (83.1,85.1)		
Age (Years)				<0.0001
Median (Q3 - Q1)	39 (44 - 32)	32 (40 - 24)		
18-30	8.1 (7.1,9.1)	91.9 (90.9,92.9)	Reference	
31-40	17.8 (16.2,19.4)	82.2 (80.6,83.8)	9.6 (7.9,11.4)	
41-50	24.6 (22.4,26.9)	75.4 (73.1,77.6)	16.6 (14.1,18.9)	
Gender				<0.0001
Male	10.6 (9.7,11.5)	89.4 (88.5,90.3)	-10.5 (-11.7,-9.3)	
Female	21.1 (19.7,22.5)	78.9 (77.5,80.2)	Reference	
Race/Ethnicity				<0.0001
Non-Hispanic White	11.9 (10.8,13.2)	88.0 (86.8,89.2)	Reference	
Non-Hispanic Black	40.9 (39.1,42.8)	59.0 (57.2,60.9)	28.9 (26.7,31.1)	
Other	13.6 (12.4,14.9)	86.4 (85.2,87.6)	1.6 (-0.2,3.5)	
Missing				
HbA1c (%)[2]				<0.0001
Median (Q3 - Q1)	5.4 (5.7 - 5.2)	5.3 (5.6 - 5.1)		
<5.7	14.1 (13.0,15.1)	85.9 (84.9,86.9)	Reference	
5.7-6.5	24.1 (21.7,26.4)	75.9 (73.6,78.3)	10.0 (7.6,12.4)	
>6.5	26.9 (22.6,31.3)	73.1 (68.8,77.4)	12.8 (8.5,17.1)	
Missing				
DM status (A1c+Self report)[3]				<0.0001
Euglycemia	14.0 (12.9,15.1)	85.9 (84.9,87.0)	Reference	
Prediabetes	23.9 (21.6,26.3)	76.1 (73.8,78.4)	9.9 (7.5,12.3)	
Diabetes	26.9 (23.2,30.7)	73.1 (69.3,76.8)	12.9 (9.1,16.7)	
BMI[4]				<0.0001
<18.5	11.2 (6.7,15.7)	88.9 (84.4,93.4)	Reference	
18.5-24.9	11.7 (10.4,12.9)	88.3 (87.0,89.6)	0.5 (-4.0,5.0)	
25.0-29.9	16.6 (15.2,17.9)	83.4 (82.1,84.8)	5.4 (0.6,10.2)	
>=30.0	19.8 (17.9,21.6)	80.2 (78.4,82.1)	8.6 (3.7,13.6)	
Missing	13.1 (7.6,18.6)	86.9 (81.4,92.4)	1.9 (-4.5,8.4)	

Total cholesterol (mg/dl)[5]				<0.0001
<200	14.6 (13.4,15.7)	85.5 (84.3,86.6)	Reference	
200-239	18.6 (16.9,20.3)	81.4 (79.9,84.4)	4.0 (2.2,5.8)	
>=240	17.9 (15.6,20.1)	82.1 (79.9,84.4)	3.3 (0.9,5.7)	
Missing	30.2 (0.00,89.5)	69.8 (10.5,100.0)	15.6 (-44.0,-75.3)	
Marital Status				0.0023
Never married	14.4 (12.8,16.1)	85.6 (83.9,87.2)	Reference	
Married	17.1 (15.9,18.4)	82.9 (81.6,84.2)	2.7 (0.8,4.6)	
Missing	14.1 (12.2,15.9)	85.9 (84.1,87.8)	-0.4 (-2.3, 1.5)	
Current Smoking status[6]				<0.0001
No Smoking	17.4 (15.2,19.6)	82.6 (80.4,84.8)	Reference	
Smoking	23.9 (22.1,25.8)	76.1 (74.2,77.9)	6.6 (4.1,9.0)	
Missing	12.6 (11.6,13.6)	87.4 (86.4,88.4)	-4.8 (-6.9,-2.7)	
Smoking status-Lifetime[7]				<0.0001
Never Smoker	13.2 (12.1,14.3)	86.8 (85.7,87.9)	Reference	
Current Smoker	23.9 (22.0,25.8)	76.1 (74.2,77.9)	10.7 (8.9,12.6)	
Former Smoker	17.4 (15.2,19.6)	82.6 (80.4,84.8)	4.2 (2.0,6.3)	
Missing	2.4 (1.5,3.3)	97.6 (96.7,98.5)	-10.8 (-12.2,-9.4)	
Hypertension[8]				<0.0001
No Hypertension	14.5 (13.5,15.4)	85.5 (84.6,86.5)	Reference	
Yes Hypertension	23.4 (20.9,25.9)	76.6 (74.1,79.1)	8.9 (6.6,11.2)	
Missing	19.4 (0.00,40.7)	80.6 (59.3,100.0)	4.9 (-16.5,26.3)	
SB - Age of 1st sex[9]				<0.0001
18-27 yrs	16.6 (15.4,17.7)	83.5 (82.3,84.6)	Reference	
28-37 yrs	4.9 (1.7,8.2)	95.0 (91.8,98.3)	-11.6 (-14.9,-8.3)	
38-49 yrs	22.1 (2.3,41.9)	77.9 (58.1,97.7)	5.6 (-14.2,25.3)	
Missing	13.2 (11.7,14.8)	86.8 (85.2,88.3)	-3.4 (-5.0,-1.7)	
SB- Protection Use[10]				0.0514
Always	17.3 (15.3,19.3)	82.7 (80.7,84.7)	Reference	
Never	16.6 (14.9,18.3)	83.4 (81.7,85.0)	-0.7 (-2.9,1.5)	
Sometimes	14.9 (13.4,16.5)	85.0(83.5,86.6)	-2.4 (-4.8,0.01)	
Missing	14.8 (13.4,16.2)	85.2 (83.8,86.6)	-2.5 (-4.8,-0.4)	
SB - Sex partners / Year[11]				0.0061
No partner	16.6 (14.5,18.7)	83.4 (81.3,85.5)	Reference	
One partner	14.9 (13.7,16.1)	85.1 (83.9,86.3)	-1.7 (-4.1,0.7)	
Two partners	19.9 (16.5,23.2)	80.1 (76.8,83.5)	3.3 (-0.4,6.9)	
3-5 partners	18.1 (15.2,20.9)	81.9 (79.1,84.8)	1.5 (-1.9,4.8)	
>6 partners	16.4 (11.7,21.1)	83.6 (78.9,88.3)	-0.02 (-5.2,4.8)	
Missing	16.6 (14.7,18.6)	83.4 (81.4,85.3)	0.00 (-2.5,2.5)	
SB - Sex Partners in Lifetime[14]				<0.0001
No partner	15.4 (13.1,17.7)	84.6 (82.4,86.9)	Reference	
One partner	7.9 (6.3,9.6)	92.1 (90.5,93.7)	-7.5 (-10.3,-4.6)	
Two partners	10.4 (8.3,12.5)	89.6 (87.5,91.8)	-4.9 (-8.4,-1.6)	
3-5 partners	14.8 (12.9,16.6)	85.2 (83.4,87.0)	-0.6 (-3.5,2.4)	
>6 partners	20.9 (19.4,22.5)	79.1 (77.5,80.6)	5.6 (2.8,8.4)	
Missing	16.8 (14.8,18.9)	83.2 (81.2,85.2)	1.5 (-1.4,4.3)	

SB- Doctor told to have Genital Herpes[15]				<0.0001
No	14.3 (13.3,15.4)	85.7 (84.7,86.7)	Reference	
Yes	68.3 (61.9,74.7)	31.7 (25.3,38.0)	54.0 (47.8,60.3)	
Missing	13.2 (11.7,14.8)	86.8 (85.2,99.3)	-1.1 (-2.8,0.6)	
SB- Male circumcision status[16]				<0.0001
No	11.2 (9.6,12.8)	88.8 (87.2,90.4)	Reference	
Yes	10.8 (9.6,11.9)	89.2 (88.1,90.4)	-0.5 (-2.5,1.6)	
Missing	19.9 (18.5,21.2)	80.1 (78.8,81.5)	8.7 (6.8,10.6)	
Hepatitis C virus RNA[17]				<0.0001
Negative	24.5 (12.1,36.9)	75.5 (63.1,87.9)	Reference	
Positive	49.8 (37.2,62.4)	50.2 (37.6,62.4)	25.3 (10.3,40.3)	
Missing	15.6 (14.6,16.7)	84.4 (83.3,85.4)	-8.9 (-21.5,3.6)	

Table 1 Abbreviations: HbA1c-Glycated Hemoglobin; DM status- Diabetes Mellitus Status; BMI-Body Mass Index; SB- Sexual behavior, HSV1-Herpes Simplex Virus type 1, HSV 2- Herpes Simplex Virus type 2

1: Ratio of family income to poverty guidelines; poverty guidelines are determined by the Department of Health, and Human Services used as poverty measure to calculate the ratio of family income to poverty.

2: HbA1c categories as determined by the American Diabetes Association.

3: Diabetes Mellitus Status as determined by American Diabetes Association guidelines for HbA1c level and self-reported (those who answered 'yes' to having been told by a doctor or health professional that he/she had diabetes). Participants who self-reported as diabetes were classified as having diabetes regardless of HbA1c and participants with HbA1c level as diabetes were classified as diabetes regardless of self-reporting.

4: BMI categories as determined by CDC guidelines.

5: Total Cholesterol categorized by National Institute of Health guidelines.

6: Current Smoking Status defined by self-reporting if now smoking cigarettes.

7: Smoking status of a lifetime defined by self-reporting of having smoked at least 100 cigarettes in their lifetime. Currently smoking at least one cigarette every day or some days and have smoked at least 100 cigarettes in the past; formerly smoked at least 10 cigarettes in their lifetime but are not smoking currently at all; Never smoked at least 100 cigarettes in their lifetime.

8: Hypertension self-reported by participants as 'yes' to having been told by a doctor or a health professional that he/she had hypertension/high blood pressure.

9: Sexual behavior defined by self-reporting of the age of having 1st sex in a lifetime.

10: Sexual behavior defined by self-reporting of having used protection (Condom) while having sex; Participants who always used protection; participants who never use any protection and participants who sometimes use protection.

11: Sexual Behavior defined by self-reporting of a number of sex partners per year. Participants categorized as No partner, one partner per year, two partners per year, three to five partners per year, and more than six partners per year.

12: Herpes Simplex Virus Type 1 test results determined by testing participant serum for antibodies to herpes simplex type 1. The test was conducted by the National Health and Nutrition Examination Survey, US.

13: Herpes Simplex Virus Type 2 test results determined by testing participant serum for antibodies to herpes simplex type 2. The test was conducted by the National Health and Nutrition Examination Survey, US.

14. Sexual behavior defined by self-reporting of a number of sexual partners in a lifetime. Participants categorized as No partner, one partner, two partners, three to five partners, and more than six partners in a lifetime.

15. Sexual behavior defined by self-reporting of doctor ever told to have Genital herpes.
16. Sexual behavior defined by self-reporting of male circumcision status.
17. Hepatitis C virus RNA defined by the results of in vitro nucleic acid amplification test conducted by National Health and Nutrition Examination Survey, US.

Table 3:

Distribution of outcome diabetes mellitus, prediabetes and no diabetes by characteristics for sample with valid DM results in NHANES 2007-2016

Characteristics	Diabetes (2) Prevalence Estimates % (95% CI)	Prediabetes (1) Prevalence Estimates % (95% CI)	Euglycemia (0) Prevalence Estimates % (95% CI)	Prevalence Difference for Diabetes (2) 5 (95% CI)	P value Rao-Scott Chi test
Total population %	4.7 (4.2,5.1)	13.0 (12.3,13.8)	82.3 (81.4,83.2)		
Age Median (Q3 - Q1) 18-30 31-40 41-50	 42 (46 - 42) 1.4 (1.1,1.7) 4.6 (3.9,5.2) 9.1 (7.9,10.4)	 39 (45 - 31) 6.8 (6.1,7.4) 13.8 (12.6,14.9) 20.8 (19.3,22.4)	 31 (40 - 24) 91.8 (91.1,92.5) 81.7 (80.3,83.1) 70.0 (67.9,72.1)	 Reference 3.1 (2.5,3.8) 7.7 (6.5,8.9)	<0.0001
Gender Male Female	 4.9 (4.3,5.6) 4.4 (3.9,4.9)	 14.0 (13.2,14.9) 12.1 (11.1,13.1)	 81.3 (79.9,82.2) 83.5 (82.4,84.7)	 0.6 (-0.2,1.3) Reference	0.0016
Race/Ethnicity Non-Hispanic White Non-Hispanic Black Other	 3.5 (2.9,4.1) 7.5 (6.5,8.6) 5.9 (5.0,6.8)	 9.6 (8.6,10.6) 24.9 (23.2,26.7) 15.5 (14.6,16.4)	 86.9 (85.7,88.1) 67.5 (65.6,69.5) 78.6 (77.3,79.9)	 Reference 4.0 (2.8,5,3) 2.4 (1.3,3.4)	<0.0001
BMI[4] <18.5 18.5-24.9 25.0-29.9 >=30.0 Missing	 0.8 (0.05,1.51) 1.3 (1.9,1.6) 3.0 (2.5,3.6) 9.7 (8.7,10.7) 7.4 (2.9,11.9)	 6.2 (3.2,9.2) 6.3 (5.5,7.2) 11.6 (10.5,12.6) 21.2 (19.7,22.7) 18.3 (10.1,24.4)	 93.1 (89.8,96.3) 92.4 (91.5,93.3) 85.4 (84.2,86.7) 69.1 (67.2,70.9) 74.3 (65.9,82.9)	 Reference 0.5 (-0.3,1.3) 2.2 (1.3,3.2) 8.9 (7.6,10.2) 6.7 (2.3,11.0)	<0.0001
Total cholesterol (mg/dl)[5] <200 200-239 >=240 Missing	 4.2 (3.7,4.7) 4.8 (3.9,5.7) 7.1 (5.8,8.5) ----------	 10.7 (9.8,11.6) 16.3 (15.1,17.6) 19.9 (17.5,22.4) 30.2 (0.00,89.5)	 85.1 (84.0,86.2) 78.8 (77.2,80.5) 72.9 (70.3,75.6) 69.8 (10.5,100)	 Reference 0.6 (-0.4,1.5) 2.9 (1.6,4.2)	<0.0001
HSV 1 [12] Negative Positive	 3.4 (2.8,3.9) 5.7 (5.1,6.3)	 9.8 (8.9,10.7) 15.7 (14.8,16.6)	 86.8 (85.7,87.9) 78.6 (77.6,79.7)	 Reference 2.3 (1.4,3.1)	<0.0001
HSV 2[13] Negative Positive	 4.1 (3.6,4.5) 7.9 (6.8,8.9)	 11.8 (11.2,12.5) 19.6 (17.5,21.8)	 84.2 (83.3,85.0) 72.5 (69.8,75.1)	 Reference 3.8 (2.7,5.0)	<0.0001

Marital Status					<0.0001
Never married	3.6 (2.9,4.3)	10.5 (9.4,11.7)	85.9 (84.6,87.1)	Reference	
Married	5.7 (4.9,6.4)	14.7 (13.8,15.7)	79.6 (78.3,80.8)	2.1 (1.1,3.1)	
Missing	2.7 (2.1,3.4)	11.1 (9.5,12.6)	86.2 (84.4,87.9)	-0.9 (-1.8,0.05)	
Current Smoking status[6]					<0.0001
No Smoking	6.3 (4.9,7.7)	13.1 (11.2,14.9)	80.7 (78.4,82.9)	Reference	
Smoking	5.0 (4.1,5.9)	16.1 (14.6,17.7)	78.8 (77.1,80.6)	-1.3 (-2.9,0.4)	
Missing	4.1 (3.6,4.6)	11.9 (11.1,12.8)	83.9 (82.9,85.0)	-2.2 (-3.6,-0.7)	
Smoking status-Lifetime[7]					<0.0001
Never Smoker	4.3 (3.8,4.8)	12.2 (11.3,13.2)	83.5 (82.3,84.6)	Reference	
Current Smoker	5.0 (4.1,5.9)	16.1 (14.6,17.7)	78.8 (77.1,80.6)	0.7 (-0.4,1.8)	
Former Smoker	6.3 (4.9,7.7)	13.1 (11.2,14.9)	80.7 (78.4,82.9)	1.9 (0.6,3.4)	
Missing	1.2 (0.4,1.9)	6.4 (4.7,8.2)	92.4 (90.6,94.2)	-3.1 (-4.0,-2.2)	
Hypertension[8]					<0.0001
No Hypertension	2.9 (2.6,3.4)	11.6 (10.9,12.2)	85.4 (84.6,86.9)	Reference	
Yes Hypertension	13.3 (11.8,14.9)	20.7 (18.6,22.8)	65.9 (63.5,68.4)	10.3 (8.8,11.8)	
Missing	6.1 (0.00,17.9)	13.6 (0.00,29.6)	80.3 (60.3,100)	3.1 (-8.7,14.9)	
SB - Age of 1st sex[9]					0.402
18-27 yrs	4.7 (4.2,5.2)	12.8 (11.9,13.7)	82.5 (81.4,83.6)	Reference	
28-37 yrs	5.4 (2.3,8.5)	19.2 (12.5,25.9)	75.3 (68.3,82.4)	0.7 (-2.3,3.8)	
38-49 yrs	2.9 (0.00,9.1)	33.9 (5.4,62.4)	63.1 (34.5,91.8)	-1.7 (7.9,4.5)	
Missing	4.5 (3.7,5.3)	13.7 (12.2,15.2)	81.8 (80.8,83.4)	-0.2 (-1.2,0.8)	
SB- Protection Use[10]					<0.0001
Always use condom	4.7 (3.7,5.7)	15.8 (14.4,17.2)	79.5 (77.9,81.2)	Reference	
Never use condom	4.5 (3.7,5.2)	12.2 (11.1,13.4)	83.3 (81.9,84.6)	-0.2 (-1.4,1.0)	
Sometimes use condom	3.6 (2.9,4.4)	10.3 (8.9,11.5)	86.1 (84.5,87.7)	-1.0 (-2.2,0.2)	
Missing	5.8 (5.0,6.6)	15.0 (13.8,16.2)	79.2 (77.8,80.6)	1.1 (-0.1,2.4)	
SB - Sex partners / Year[11]					<0.0001
No partner	5.8 (4.8,6.8)	12.9 (11.2,14.6)	81.4 (79.6,83.1)	1.1 (0.01,2.2)	
One partner	4.7 (4.1,5.3)	13.1 (12.2,14.0)	82.2 (81.0,83.4)	Reference	
Two partners	3.4 (2.4,4.4)	11.1 (8.6,13.5)	85.6 (82.8,88.3)	-1.3 (-2.5,-0.1)	
3-5 partners	3.0 (1.9,4.1)	11.9 (9.3,14.5)	85.1 (82.2,87.9)	-1.7 (-2.7,-0.6)	
>6 partners	3.8 (0.9,6.8)	11.2 (6.9,15.6)	84.9 (79.8,90.1)	-0.9 (-3.9,2.1)	
Missing	4.7 (3.6,5.7)	14.9 (13.1,16.8)	80.4 (78.4,82.5)	0.0 (-1.3,1.2)	
SB - Sex Partners in Lifetime[14]					0.0029
No partner	4.2 (3.3,5.1)	11.0 (9.3,12.8)	84.8 (82.8,86.8)	-0.1 (-1.4,1.3)	
One partner	4.3 (3.2,5.3)	11.8 (10.2,13.4)	83.9 (82.2,85.6)	Reference	
Two partners	3.8 (2.5,5.2)	12.1 (9.7,14.6)	84.1 (81.1,87.0)	-0.5 (-2.0,1.1)	
3-5 partners	4.4 (3.5,5.3)	13.1 (11.3,14.9)	82.5 (80.3,84.6)	0.1 (-1.1,13.7)	
>6 partners	5.5 (4.6,6.4)	14.2 (12.9,15.4)	80.3 (78.8,81.9)	1.2 (-0.1,2.5)	

Missing	4.6 (3.6,5.6)	14.9 (13.1,16.8)	80.5 (78.4,82.5)	0.3 (=1.2,1.9)	
SB- Doctor told to have Genital Herpes[15]					0.9195
No	4.7 (4.2,5.2)	12.9 (12.1,13.8)	82.4 (81.3,83.4)	Reference	
Yes	4.3 (2.2,6.4)	12.8 (8.7,16.8)	82.9 (78.4,87.4)	-0.4 (-2.5,1.7)	
Missing	4.5 (3.7,5.3)	13.7 (12.2,15.2)	81.8 (80.2,83.4)	-0.2 (-1.2,0.8)	
SB- Male Circumcision status[16]					0.0003
No	5.6 (4.3,7.0)	17.0 (15.0,18.9)	77.4 (74.9,79.8)	Reference	
Yes	4.6 (3.8,5.5)	13.0 (12.0,14.1)	82.3 (80.9,83.7)	-1.0 (-2.7,0.7)	
Missing	4.5 (4.1,4.9)	12.4 (11.4,13.3)	83.1 (81.9,84.2)	-1.1 (-2.5,0.4)	
Hepatitis C virus RNA[17]					0.0834
Negative	8.1 (0.0,16.7)	16.8 (7.4,26.1)	75.1 (63.6,86.6)	Reference	
Positive	6.9 (1.5,12.5)	21.6 (12.8,30.3)	71.5 (62.7,80.3)	-1.2 (-8.0,5.6)	
Missing	4.6 (4.2,5.1)	12.9 (12.3,13.7)	82.4 (81.5,83.3)	-3.5 (-12.0,4.9)	

Table 2 Abbreviations: HbA1c-Glycated Hemoglobin; DM status- Diabetes Mellitus Status; BMI-Body Mass Index; SB- Sexual behavior, HSV1-Herpes Simplex Virus type 1, HSV 2- Herpes Simplex Virus type 2

1: Ratio of family income to poverty guidelines; poverty guidelines are determined by the Department of Health, and Human Services used as poverty measure to calculate the ratio of family income to poverty.

2: HbA1c categories as determined by the American Diabetes Association.

3: Diabetes Mellitus Status as determined by American Diabetes Association guidelines for HbA1c level and self-reported (those who answered 'yes' to having been told by a doctor or health professional that he/she had diabetes). Participants who self-reported as diabetes were classified as having diabetes regardless of HbA1c and participants with HbA1c level as diabetes were classified as diabetes regardless of self-reporting.

4: BMI categories as determined by CDC guidelines.

5: Total Cholesterol categorized by National Institute of Health guidelines.

6: Current Smoking Status defined by self-reporting if now smoking cigarettes.

7: Smoking status of a lifetime defined by self-reporting of having smoked at least 100 cigarettes in their lifetime. Currently smoking at least one cigarette every day or some days and have smoked at least 100 cigarettes in the past; formerly smoked at least 10 cigarettes in their lifetime but are not smoking currently at all; Never smoked at least 100 cigarettes in their lifetime.

8: Hypertension self-reported by participants as 'yes' to having been told by a doctor or a health professional that he/she had hypertension/high blood pressure.

9: Sexual behavior defined by self-reporting of the age of having 1st sex in a lifetime.

10: Sexual behavior defined by self-reporting of having used protection (Condom) while having sex; Participants who always used protection; participants who never use any protection and participants who sometimes use protection.

11: Sexual Behavior defined by self-reporting of a number of sex partners per year. Participants categorized as No partner, one partner per year, two partners per year, three to five partners per year, and more than six partners per year.

12: Herpes Simplex Virus Type 1 test results determined by testing participant serum for antibodies to herpes simplex type 1. The test was conducted by the National Health and Nutrition Examination Survey, US.

13: Herpes Simplex Virus Type 2 test results determined by testing participant serum for antibodies to herpes simplex type 2. The test was conducted by the National Health and Nutrition Examination Survey, US.

14. Sexual behavior defined by self-reporting of a number of sexual partners in a lifetime. Participants categorized as No partner, one partner, two partners, three to five partners, and more than six partners in a lifetime.

15. Sexual behavior defined by self-reporting of doctor ever told to have Genital herpes.

16. Sexual behavior defined by self-reporting of male circumcision status.

17. Hepatitis C virus RNA defined by the results of in vitro nucleic acid amplification test conducted by National Health and Nutrition Examination Survey, US

Table 4:

Multivariable model for the odds of diabetes mellitus in US population aged 18-49 years, 2007-2016

Models	Crude Odds Ratio (95% CI) for Diabetes	Adjusted Odds Ratio (95% CI)[1] for Diabetes	Adjusted Odds Ratio (95% CI) for diabetes controlling for age, race, and gender
Model 1	Odds of Diabetes and Prediabetes vs. Odds of Euglycemia		
HSV 1[2] (N=14638) Positive Negative	1.8 (1.6,1.9) Reference	1.1 (0.9,1.2) Reference	1.1 (1.1,1.4) Reference
HSV 2[3] (N=14618) Positive Negative	2.0 (1.8,2.3) Reference	1.05 (0.9,1.2) Reference	1.2 (1.1,1.4) Reference
Model 2	Odds of Diabetes vs. Odds of Prediabetes and Euglycemia		
HSV 1[2] (N=14638) Positive Negative	1.70 (1.4, 2.1) Reference	1.02 (0.8,1.3) Reference	1.15 (0.9,1.4) Reference
HSV 2[3] (N=14618) Positive Negative	2.03 (1.7,2.5) Reference	1.1 (0.9,1.4) Reference	1.2 (1.1,1.6) Reference
Model 3	Odds of Diabetes vs. Odds of Euglycemia		
HSV 1[2] (N=12394) Positive Negative	1.83 (1.5,2.3) Reference	1.05 (0.8,1.3) Reference	1.2 (0.9,1.5) Reference
HSV 2[3] (N=12379) Positive Negative	2.3 (1.9,2.8) Reference	1.12 (0.9,1.4) Reference	1.3 (1.1,1.6) Reference
Model 4	Odds of Prediabetes vs. Odds of Euglycemia		
HSV 1[2] (N=13807) Positive Negative	1.8 (1.6,1.9) Reference	1.12 (0.9,1.3) Reference	1.3 (1.1,1.4) Reference
HSV 2[3] (N=13787) Positive Negative	1.9 (1.7,2.2) Reference	1.03 (0.9,1.2) Reference	1.2 (1.0,1.4) Reference

1: Models adjusted for Age, Gender, Race, Poverty ratio, BMI, Total Cholesterol, Current Smoking status, Hypertension, Protection use, Sex partners in a lifetime, Sex partners in a year and male circumcision status.

2: Herpes Simplex Virus Type 1 status determined by testing participant serum for antibodies to herpes simplex type 1. The test was conducted by the National Health and Nutrition Examination Survey, US.

3: Herpes Simplex Virus Type 2 test results determined by testing participant serum for antibodies to herpes simplex type 2. The test was conducted by the National Health and Nutrition Examination Survey, US.

Table 5:

Supplementary Multivariable models for odds of HSV 1 by diabetes mellitus status in United States population aged 18-49 years, 2007-2016:

Models	Adjusted Odds ratio (95% CI) for Diabetes[1] vs. Prediabetes and No Diabetes	Covariates[3]
Model 1		
HSV 1[2] (N=14599) Positive Negative	1.03 (0.8,1.3)	Age, Sex, Race, Poverty ratio, BMI, Total Cholesterol, HSV 2, Current Smoking status, Hypertension, marital status, Sex partners in a lifetime, Sex partners in a year and male circumcision status.
HSV 2[2] (N=14599) Positive Negative	1.11 (0.9,1.4)	
Model 2		
HSV 1[2] (N=14638) Positive Negative	1.2 (0.9,1.5) Reference	Age, Sex, Race, Marital Status, Sex partners in lifetime, Sex partners per year, Male circumcision
HSV 2[2] (N=14618) Positive Negative	1.3 (1.04,1.6) Reference	
Model 3		
HSV 1[2] (N=14638) Positive Negative	1.1 (0.8,1.3) Reference	Age, Sex, Race, BMI, Total Cholesterol, Lifetime Smoking status
HSV 2[2] (N=14618) Positive Negative	1.2 (0.9,1.4) Reference	
Model 4		
HSV 1[2] (N=14638) Positive Negative	1.1 (0.9,1.4) Reference	Age, Sex, Total cholesterol, Current smoking status, Hypertension
HSV 2[2] (N=14618) Positive Negative	1.2 (1.0,1.5) Reference	
Model 5		
HSV 1[2] (N=14638) Positive Negative	1.4 (1.3,1.5) Reference	Age, Sex, Total Cholesterol, Hypertension, BMI
HSV 2[2] (N=14618) Positive Negative	1.5 (1.3,1.7) Reference	

1: Individuals defined as prediabetes and no diabetes based on American Diabetes Association guidelines (16) were classified as No diabetes for models

2: Herpes Simplex Virus Type 1 status determined by testing participant serum for antibodies to herpes simplex type 1. The test was conducted by the National Health and Nutrition Examination Survey, US.

3: Covariates controlled for in the multivariable logistic model.

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