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HERPES SIMPLEX VIRUS-2 INFECTION IN BLACK AND WHITE HIV-NEGATIVE MEN WHO HAVE SEX WITH MEN

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Bachelor of Medicine, Bachelor of Surgery (MB.BS)

University of Port Harcourt

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Abstract

HERPES SIMPLEX VIRUS-2 INFECTION IN BLACK AND WHITE

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By

Netochukwu Okafor

Background: Herpes simplex virus type 2 (HSV-2) is the most common cause of genital ulcer disease in the United States, and men who have sex with men (MSM) have higher prevalence of HSV-2. HSV-2 is one of the most important biologic predictors of HIV acquisition with infected persons having a 2.1 fold increased risk of HIV. Black MSM are disproportionately affected by HIV, and individual behavioral factors fail to explain this disparity suggesting that further investigation into biologic and network-level risk is warranted. This study aimed to estimate the seroprevalence of HSV-2 and examine associations with infection in a cohort of HIV negative black and white MSM.

Methods: The InvolveMENt study is designed to examine factors that may contribute to disparities in HIV and sexually transmitted infections between black and white MSM aged 18-39 years in Atlanta, Georgia. Participants completed a survey at baseline evaluating demographic, individual, and community level risk factors and provided serum samples for HSV-2 serology. We examined the prevalence of HSV-2 and socio-demographic and behavioral associations with infection among HIV negative MSM using multivariate logistic regression.

Results: Of the 394 men in the cohort, 210 were white and 184 were black. Seventy-seven (19.5%) were seropositive for HSV-2. Being black (OR 2.7; p=0.002), increasing age (OR 1.1; p=0.01), greater number of sexual partners in the past 12 months (OR 1.04; p=0.003), and a history of child abuse (OR 1.8; p=0.047) increased the odds of HSV-2 infection in a multivariate model. There was a significant interaction between race and age with black MSM <25 years of age having the highest odds of HSV-2 infection (OR=4.7; p=0.003).

Conclusion: The odds of black MSM being infected with HSV-2 is 2.7 times more than white MSM. Our examination of socio-demographic and behavioral risk factors did not fully account for the disparity, especially among young black MSM. Further investigation into biologic/genetic and partner level factors in HSV-2 infection among MSM is warranted. As it remains an important risk factor for HIV acquisition, there is also continued need for research in the design and implementation of HSV-2 prevention programs specifically for young, black MSM.

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Introduction

Black men who have sex with men are disproportionately affected by HIV in the US.

Men who have sex with men (MSM) are at greatest risk of HIV infection in the United States with incidence rates comparable to some regions in Sub-Saharan Africa[1]. The CDC's National Center for HIV/AIDS, Viral Hepatitis, STD, and TB Prevention reported in 2010 that MSM accounted for 78% of all new HIV infections in males even though they represent only 4% of the male population [2, 3]. The data also show that estimated incidence rates of HIV among blacks were more than six times higher than that of whites (103.6 vs15.8 per 100,000) with most of these infections (72%) occurring in MSM [3-7] [7-10]. CDC's National HIV Behavioral Surveillance system estimates that non-Hispanic black MSM have the highest prevalence of HIV (28%) followed by Hispanics (18%) and then white MSM (16%) [11].

Reasons for the stark racial disparity in HIV among MSM are not clear. Black MSM are less likely than other MSM to be tested for HIV or be aware of their HIV status and are more likely to select partners of their own race than other MSM. They are also less likely to be on antiretroviral drugs (ARVs) after a positive diagnosis [12]. Black MSM are more likely than other MSM to contract sexually transmitted infections (STIs) that facilitate acquisition and transmission of HIV. Despite this, they have comparable or fewer sexual partners, lower or equal rates of unprotected anal intercourse (UAI) and comparable rates of condom use than white MSM. They are also not less likely to disclose their sexual identity/homosexual behavior or use less abuse substances that

increase risk for HIV. Other factors such as circumcision status, incarceration, and characteristics of sex partners have not been independently associated with HIV infection and thus do not explain the racial disparity of HIV among MSM [12, 13].

Socioeconomic risk factors have been implicated in the HIV epidemic. Less educated MSM (< high school) have been shown to have significantly higher prevalence of HIV [14]. The HIV seroprevalence was also found to be up to 5 times greater for indigent adults in San Francisco [15]. Incarceration has been described as a possible major determinant of the HIV epidemic among blacks in the US [16-20], however, recent studies done by the CDC show a vast majority of men already have HIV before incarceration thus suggesting the transmission rate within prisons is not as great as was earlier thought [21]. The use of other non-injection drugs is prevalent among US MSM [22] and drugs like methamphetamine are about twice as likely to engage in high risk sex, regardless of HIV serostatus [14, 23, 24]. Thus, MSM that use non-injection drugs are more likely to be seropositive for HIV. Blacks are also less likely than whites to have a usual source of health care and to have an outpatient visit in a year [25], and this decreased interaction with healthcare providers can explain why black MSM may have decreased opportunities to learn their HIV serostatus, receive recommended STD screening or start taking ARVs. Presumably, some of the impacts of these factors are mediated by changes in levels of risk behavior, in extent of concurrency, or in selection of sex partners in high-prevalence partner pools. Because of the lack of correlation of some individual level risk behaviors and the racial disparity of HIV, Millet et al. propose that it is necessary to focus research on other factors that can help to explain this stark disparity in the HIV burden of the black MSM community [12].

STIs play an important role in HIV acquisition.

A review of racial/ethnic disparities across the broad array of health outcomes measured by the Healthy People 2010 objectives found that HIV and STIs such as chlamydia, syphilis and gonorrhea accounted for the 5 greatest disparities for African American populations. In 2003 the rates of gonorrhea (18 times), chlamydia (8 times) and syphilis (5.4 times) were all higher in blacks than whites [26] [27, 28]. Rates of STIs are higher in the black MSM population than among other MSM regardless of their HIV serostatus [9, 29, 30]. For example, even when engaging in "low-risk" sexual and drug-related behaviors, African-American young MSM were shown to be at a higher risk for STIs [5, 31]. Little explanation has been provided for these disparities. Such disparities hold critical implications for disparities in HIV infection. Many studies have shown evidence for association between STIs and increased acquisition of HIV infection. Boilly et al. summarized data from 25 studies to estimate the risk of HIV transmission per act in heterosexuals. They found that per-act infectivity was increased five-fold if the HIV susceptible partner had current or past genital ulcers compared to no STI history[32]. Studies of MSM in the US also show a significant increase in HIV acquisition associated with STI. One cohort study found that repeated recent rectal infection with Gonorrhea or Chlamydia increased the risk for HIV more than 8-fold after controlling for known and measured confounders [33]. Among MSM, syphilis, rectal gonorrhea, and urethritis have been reported to be independent risk factors for HIV seroconversion as well [34]. There is thus evidence that black MSM are more likely than other MSM to report STIs and this likely contributes to the burden of HIV among black MSM.

HSV-2

Herpes simplex virus type 2 (HSV-2) is one of the most common STIs worldwide and the primary cause of genital ulcer disease [35, 36]. Genital herpes results from infection with herpes simplex virus type 2 (HSV-2), or less commonly, type 1 (HSV-1). Since HSV-1 is responsible for most oral outbreaks or "cold sores" and represents a minority of genital infections, testing for antibodies toHSV-2 is used in seroprevalence studies as a marker of burden of infection with genital herpes [37, 38]. Herpes simplex virus 2 is a member of a family of large double stranded DNA viruses. Humans are the only natural host of herpes simplex virus 2 (HSV-2). It has the ability to persist in an apparently inactive state for varying durations of time, and then be reactivated by proper provocative stimulus. Thus after infection, viral DNA persists in the host for the entire lifetime of the individual. HSV-2 typically replicates productively in the mucosal epithelium of the genitalia and establishes latent infection in neurons of the sacral ganglia [39].

Despite increased HSV-2 prevalence among African Americans and MSM, the majority of published data specifically addressing the clinical spectrum of genital herpes have largely described females and heterosexual men [40, 41]. Clinical features include herpetic vesicles around glans penis, prepuce shaft of penis and sometimes on the scrotum, thighs, and buttocks. Individuals having anal intercourse can also have lesions around the anus and herpetic proctitis. Herpetic urethritis may occur and may present as severe dysuria and mucoid discharge.

The diagnosis of HSV-2 can be made in several ways, including by taking a swab from the base of the genital lesion and assessing it for HSV by viral culture, HSV antigen detection, or by polymerase chain reaction (PCR) of HSV DNA [42]. In addition, serologic tests are available for HSV-1 and 2. Viral isolates can be grown in tissue culture, usually within 5 days, and typed by antibody staining. Diagnosis of HSV infection with tissue culture however has low sensitivity with HSV isolated from lesions in about 80% of primary infections but in only 25–50% of recurrent lesions, and in even fewer people whose lesions have begun to heal. Data indicate that, regardless of whether samples are obtained from HSV lesions or from genital or oral secretions during a period of subclinical shedding, detection of HSV DNA by PCR is more sensitive than virus isolation, for detection of HSV on mucosal surfaces [43].

Although PCR can detect asymptomatic shedding, viral detection tests are of little value when genital lesions are absent. Type specific serology (TSS) can be used to identify individuals who are infected with HSV-2 in asymptomatic states [44]. Currently, there are several Food and Drug Administration -cleared serological assays for type-specific HSV-2 serologic testing [45]. The serological tests approved for use in the USA have sensitivity of 97–100% and specificity of 94–98%, when measured in comparison with the gold standard western blot [42, 46]. Positive predictive values (PPV) of the serological tests vary widely based on the prevalence of HSV-2 in the sample. Values have ranged from 84% to 96% in high prevalence settings [47, 48]. Mark et al., while testing sexually active college students without a history of genital herpes, found the PPV to be much lower (37.5%), similar to other low prevalence settings [49]. Despite the accuracy of HSV serological assays, these tests are an improvement on clinical diagnosis

of genital herpes which has a 39% sensitivity and yields a false positive diagnosis about 20% of the time [50]. Of note, the multiple studies that found an association between HSV-2 and HIV-1 acquisition did so using HSV-2 serology [51-56]. Routine serologic testing might identify numerous clinically unapparent HSV-2 infections; however, the role of routine serologic screening in population-based prevention efforts has been controversial. Douglas et al in 2009 found that testing rates in demographic groups of greatest concern for both HSV-2and HIV transmission (e.g. MSM) were even lower than clinic-wide rates, and there were no data on perceived personal benefits on those tested [45].

Effects of HSV-2 infection on Health and HIV transmission

HSV-2 and HIV both have similar methods of transmission and co-morbidity is very common [42, 51, 52]. Although it is difficult to know which infection occurred first, HSV-2 has been independently found to markedly enhance the risk of acquiring HIV and is regarded as the most important biological predictor of HIV infection [13]. A meta-analysis of studies with primarily heterosexual populations reported that HSV-2 infection was associated with a 2.1-fold increased risk of HIV acquisition. This analysis in 2002 involved 9 studies documenting HSV-2 infection before HIV acquisition [53]. Another meta-analysis involving 19 studies carried out by Freeman et al. showed the risk ratio of HIV in MSM that are seropositive for HSV-2 was significant at 1.7 (95% CI, 1.2-2.4) [13].

The increased susceptibility to HIV infection is thought to occur because of the ulcerations in genital skin and mucosa caused by genital herpes. Even ulcerations that

are microscopic can provide a portal of entry for HIV. Persons infected with HSV-2 are at greater risk for HIV acquisition, even in the absence of HSV-2 symptoms[13]. This may be due in part to recently documented frequent subclinical HSV-2 reactivation in genital mucosa[57] with persistent host cellular immune responses, including HIV-1 susceptible CD4+ and CCR5+ T cells and plasmacytoid dendritic cells, present in HSV-2 lesions and persisting for weeks after HSV-2 treatment. Thus, while daily antivirals in HSV-2 seropositive/HSV-1 uninfected persons reduce symptoms of genital ulcers and HSV-2 shedding, the persistence of HIV-1 susceptible cells associated with the host immune response to HSV-2 likely maintains a high risk of HIV-1 acquisition [58].

Prevalence of HSV-2 in US MSM

Infection with herpes simplex virus 2 (HSV-2) is life-long and the best estimate of its prevalence comes from serologic test results. HSV-2 is considered the most prevalent STI in the United States with approximately 16.2% of all adolescents and adults infected [35, 42]. Through the National Health and Nutrition Examination Survey (NHANES), the Centers for Disease Control and Prevention (CDC) has monitored the seroprevalence of HSV-2 among adults aged between 18 and 49 years since 1976. Between 1976 and 1980, the prevalence was estimated at 16.4% of the population. That prevalence is similar to the most recent NHANES survey (2005 – 2008) which estimates the seroprevalence at 16.2%. HIV-1 infected individuals have higher seroprevalence of HSV-2 antibodies compared to HIV-1 uninfected individuals. In these individuals, seroprevalence has ranged from 65% among MSM in San Francisco [59] to 80% among men in a US national survey [60]. Co-infection rates might be as high as 85% in some

populations in sub-Saharan Africa [61]. A study among 403 HIV negative MSM in Brazil showed a point prevalence as high as 45.7% [52]. For MSM, studies using 2001-2006 NHANES data showed that the prevalence of HSV-2 between the ages of 18 to 49 years was higher in MSM (18.4%) than in non-MSM (12.5%) but the differences were not statistically significant[62]. An international study by Barnabas et al. puts the prevalence of HSV-2 among MSM in North America to be as high as 23%; about 7% higher than the national average. This prevalence is similar to Australia, but much less than Latin America and the Caribbean (42%) [63].

Non-Hispanic blacks had the highest seroprevalence (39.2%) of HSV-2 with prevalence three times that of non-Hispanic whites (12.3%) and nearly four times that of Hispanics (10.1%) [40, 52, 64]. There is, however, limited information on the race specific prevalence of HSV-2 among MSM. Although a racial disparity is strongly suspected, there is no clear evidence of a racial/ethnic disparity in HSV-2 infection among MSM. It is possible that if a racial disparity exists, it could be a contributor to the racial disparity in HIV prevalence among MSM due to HSV-2's role in increasing acquisition of HIV. Exploring the population specific risk factors for HSV-2 infection will also shed more light on the most effective ways to prevent infection and reduce the burden of HSV-2 infection among MSM. Since HSV-2 is such a strong predictor of HIV acquisition, reduction in its prevalence could also lead to a reduction in the incidence of HIV.

Risk factors for HSV 2 infection

Several factors have been shown to increase an individual's risk for HSV-2 infection. Increased age has been shown in various studies to be strongly associated with a higher seroprevalence of HSV-2, ranging from 1.4% among those aged 14–19 years to 26.1% among those aged 40–49 years ($p<0.001$) [40, 64-66]. The increasing seroprevalence with age may be associated with an increase in the number of life-time sex partners as age increases. In the 2005-2008 NHANES, it was shown that having more sex partners was associated with an increased prevalence of HSV-2 infection in all racial groups [64]. In 2006, a cross sectional study by Sizemore et al. with non-MSM men attending STD clinics in Alabama (n=244) showed that a self-reported history of intra-nasal cocaine use was associated with increased HSV-2 seropositivity ($p<0.001$) as well. He also showed an increased association in men that have a history of incarceration ($p=0.02$) and men who have had encounters with commercial sex workers ($p=0.02$) [40]. The study however did not show a difference in seroprevalence with respect to educational level; employment status; tobacco, alcohol, or injection drug use; health insurance status or reported condom use.

A recent meta-analysis involving six studies and 5384 HSV-2–negative people found that persons who reported always using condoms had a 30% decreased risk for acquiring HSV-2 infection compared with persons who reported no condom use [67]. There have been conflicting results about an association of HSV-2 with marital/cohabitation status, as Sizemore et al. found a positive correlation with being

married in a population of heterosexual men, while Bauer, et al. found a negative correlation using the 1999-2008 NHANES [40, 65]. In addition, after controlling for multiple confounders (age, race, marital status), the 1994 NHANES showed that persons attending religious services weekly (19.8%) were less likely to be seropositive for herpes simplex type 2 (HSV-2) than those attending less frequently (23.7%) or never (25.1%) [68]. Analyses revealed the association to be partially accounted for by reduced risky sexual behavior (number of lifetime partners and age of first sexual intercourse) and illegal drug use (cocaine and marijuana) among frequent attenders.

Risk factors for HSV-2 infection among MSM

Some risk factors of HSV-2 infection are unique to MSM, while others, such as age, are not. A longitudinal study by Renzi et al. showed that case subjects infected with HSV-2 who acquired HIV during the follow up period were more likely than control subjects to be of a racial or ethnic minority [70]. Rodrigues' study among HIV negative MSM in Brazil also reflected this by showing that HSV-2 seroprevalence was higher in nonwhite MSM (PR 1.32; 95%CI: 1.06–1.64). It also associated HSV-2 seropositivity with a stable male partner in the past 6 months and unprotected anal sex with a stable female partner in the 6 months preceding baseline interview [52]. A convenience sample of 1462 MSM recruited from different settings in 3 cities in China revealed that men with a history of sexual abuse had a higher risk of HSV-2 infection [66]. The gender of sexual partners was also associated with HSV-2 infection. MSM who had bisexual behaviors had a significantly higher prevalence of HSV-2 infection. Another factor significantly associated with HSV-2 infection was the type of the setting that the participant was

recruited from. Those that were recruited from a health center (aOR 0.6; 95% CI, 0.4 – 0.9) showed a reduced odds of HSV-2 infection as compared to those recruited from other places such as bars and clubs. Age >30 years, education level lower than junior high school, not being married, involvement in commercial sex work and HIV-positive status were positively associated with HSV-2 seropositivity [66]. A randomized study with 1836 MSM participants from 34 sites in Australia, the Caribbean and the Americas was carried out by Barnabas et al. The study showed HSV-2 infection to be significantly more common among participants who were older (≥30 years), black, Hispanic, Mestizo or multi-racial, reported having more than 4 male partners, or reported unprotected insertive or receptive anal sex during the 6 months prior to study [63]. Another study among MSM in China showed that risk factors for the co-infection of HSV-2 and HIV were age <30 years, education level lower than junior high school having a few female sexual partners and history of sexual abuse. Being unmarried was a protective factor for HSV- 2/HIV co-infection [66].

While studies have identified a number of risk factors for HSV-2 infection among MSM, evidence for certain risk factors, like circumcision, is inconclusive [66]. Circumcision was associated with a borderline significant reduction (odds ratio 0.7; p value = 0.07) in HSV-2 infection ; the association was stronger (odds ratio 0.63; p = 0.003) when region was not included in the multivariate model due to the high correlation between circumcision status and region in the study population [63]. In a cohort of 4745 MSM in Seattle, HSV-2 seroprevalence did not have significant statistical association with circumcision [69]. A meta-analysis done in 2008 by Miller et al. actually showed a

reduced risk of HSV-2 infection in circumcised men, but it was also not statistically significant [70]. Other studies have been similarly equivocal [69, 71].

Prevention and Treatment considerations of HSV-2

HSV-2 infection is also an important public health concern because of the morbidity associated with symptomatic infection and the potential for high rates of clinical, subclinical recurrences and the strong association between HSV-2 and HIV infections. As many as 95% of HSV-2 seropositive individuals shed HSV-2 asymptomatically, facilitating the transmission of genital herpes [37, 38]. Despite all of this, HSV-2 infection is not a nationally reportable disease, although reporting of certain HSV-2 infections is legally mandated in some states [72]. Research has shown that the public health significance of curbing HSV-2 infection rates would be tremendous. In 2001, Szucs estimated that in the United States alone, the direct medical cost of the infection is $984 million per year. This estimate was based off of a cross-sectional study that also estimated 3.1 million symptomatic episodes per year in the US. Of these costs, 49.7% went to drug expenditures, 47.7% to outpatient medical care and 2.6% to hospital costs. Indirect costs relating to infection accounted for an additional $214 million [73]. It is thus obvious that HSV-2 is not just a burden because of the morbidity it causes, but the salient yet significant financial impact it has on the healthcare industry.

Approaches to the prevention of HSV-2 include education, counseling, and medical treatment. Primary prevention of HSV-2 infection might be the only available strategy to reduce the increased risk for HIV infection associated with HSV-2. Increasing awareness of the high HSV-2 prevalence among MSM in the United States and the link

between HSV-2 and HIV infections are important first steps in addressing the increased risk for HIV infection, especially among persons at greatest risk for HSV-2 and HIV infections [64]. Currently, three antiviral agents are effective for treatment and suppression of genital herpes: acyclovir, valaciclovir, and famciclovir [74]. In randomized placebo-controlled trials, antiviral therapy resulted in faster symptom resolution and lesion healing, decreased viral shedding and prevention of new lesions[75]. Treatment can be episodic or continuous however, antiviral agents cannot eradicate latent HSV. Episodic administration of oral or topical acyclovir for the treatment of recurrent genital HSV lesions provides only a modest benefit, with duration of lesions being shortened, at most, by 1 to 2 days. However, daily administration of oral acyclovir can effectively suppress recurrences of genital herpes in 60 to 90 percent of patients [76, 77].

Unfortunately, despite good evidence for biologic plausibility, reductions in HSV-2 shedding with antiviral treatment have not been shown to decrease acquisition of HIV. A large double-blind, randomized, placebo-controlled phase III trial involving HIV negative and HSV-2 seropositive women in Africa and MSM from sites in Peru and the USA found that twice daily antiviral (acyclovir 400mg) therapy to suppress HSV-2 infection did not decrease the risk for HIV acquisition in MSM (hazard ratio 1·16 [95% CI 0·83–1·62]) [78]. Thus, more unique strategies are needed to interrupt the relationship between HSV-2 and HIV 1.

Specific aim

The **aim** of this thesis is to estimate the seroprevalence of HSV-2 in black and white MSM and examine socio-demographic and behavioral risk associations with HSV-2 serostatus in a cohort of black and white MSM in Atlanta.

We **hypothesize** that the seroprevalence of HSV-2 in black MSM in Atlanta will be at least 2 times higher than white MSM. In addition, traditional individual level behavior risk factors such as age, number of sex partners, etc. will not fully account for this disparity.

Methods

Study Population and Design

Data for this analysis of HSV-2 seroprevalence in MSM were generated from the InvolveMENt study which is an ongoing, cohort study at Emory University. The InvolveMENt study was designed to examine the individual, dyadic, and community level factors that may contribute to the disparities in HIV and sexually transmitted infection incidence between black and white MSM in Atlanta, Georgia. MSM aged 18–39 years were recruited, regardless of HIV status, from the Atlanta community primarily using time-space venue sampling, with a sampling frame built upon that used in the Atlanta site for the second MSM cycle of the National HIV Behavioral Surveillance System (NHBS) [16]. Facebook was included as a virtual ‘‘venue’’ in the venue sampling frame. Eligible participants self-identified as black or white MSM, reported sex with another man in the previous 3 months, not being in a mutually monogamous relationship, could complete survey instruments in English, lived in the Atlanta metropolitan area, were not enrolled in another HIV prevention study, and had no plans to relocate in the subsequent 2 years. Men who self-identified as Hispanic or of other/mixed race were not enrolled. All participants were tested for HIV using a rapid test with confirmatory ELISA and western-blot and completed a detailed computer-assisted self-interview (CASI) questionnaire to evaluate demographic, individual (e.g. number of sexual partners, number of unprotected anal intercourse (UAI) partners, condom use, drug/alcohol use etc.), dyadic (e.g. partner demographics such as age and

race, partnership characteristics, etc.), and community level (e.g. poverty, neighborhood violence, etc.) HIV risk. Men who were HIV negative are prospectively followed for up to 24 months and undergo HIV antibody testing at 3–6 month intervals. A large peripheral blood specimen repository was established in conjunction with the InvolveMENt study enabling retrospective analyses of biologic specimens.

Ethics statement

The Institutional Review Board of Emory University approved this study. All participants provided written informed consent prior to enrollment.

HSV-2 Analysis

For this analysis, HSV-2 serostatus was determined for 394 randomly selected HIV-negative MSM enrolled in the InvoleMENt study between July 2010 and September 2012. For 74% of MSM included in this analysis, specimens were analyzed from the baseline study visit. However, for 26%, a baseline study visit serum specimen was not available and specimens from a later study date were utilized.

Laboratory methods

HSV-2 serostatus was evaluated using stored serum specimens. Assays for HSV-2 type specific IgG antibody (HerpesSelect) were performed by technologists at Emory Medical Laboratories. Results were obtained and entered into an excel spreadsheet. Data were later merged with the InvolveMENt study database for analysis.

Variable definition

The primary outcome in this study was HSV-2 seropositivity defined as a positive result for HSV-2 antibodies by the HerpesSelect assay. Participant's race was self-reported as either white or African American/black. Age was defined by the participants' age in years at their last birthday before baseline visit. The age variable was further dichotomized as 25 years and less (<=25) and more than 25 years (>25) in order to examine possible interaction. Participant's insurance status was dichotomized as insured if they had any form of insurance (HMO/private, Medicaid, Medicare or TRICARE) or uninsured. We classified individuals with combined income <100% of the 2011 federal poverty level as "poor". Education was categorized as: college, post graduate, professional school; some college, associate's degree, and/or technical school; high school or GED; some high school; less than high school; and never attended school. The "unemployed" variable was defined as whether or not participant had any form of employment at their baseline visit. Participants were also dichotomized as those that identified their sexual orientation as homosexual or gay and those who identified as being heterosexual, bisexual or other classifications.

Other covariates used included "child abuse" reflecting if the participant reported to have experienced any form of physical or sexual abuse as a child; "arrested" if participant was arrested in the past 12 months; and "alcohol" if participant has had at least one drink of any alcoholic beverage such as beer, wine, a malt beverage or liquor in the past 12 months. "Drug use" was defined as present if the participant used any non-

injection drugs other than those prescribed including crystal meth, any form of cocaine, downers (valium, Ativan or Xanax), pain killers (Oxycontin, Vicodin or Percocet), hallucinogens (LSD or mushrooms), ecstasy, special K (ketamine), GHB, heroin, marijuana, poppers (amyl nitrate). "Viagra use" was defined by participant's use of Viagra, Levitra, or Cialis in the past 12 months.

Circumcision status was determined by participants' self-report. "Condom use" was defined as whether or not the participant had used a condom in the past six months. "Condom error" was present if the participant had experienced condom breakage or done any of the following in the last 6 months: re-used a condom; started having sex, then put the condom on during intercourse; completely unrolled the condom before putting it on; put condom on inside out, then flipped it over to use. Participant was also said to have a condom error if they failed to do any of the following: squeeze air from the tip of condom before putting it on; leave space at the tip of condom; hold the base of the condom during withdrawal. Participants were queried about the number of sexual partners in the last 12 months and whether they participated in any unprotected receptive or insertive anal or rectal intercourse in the past 6 months (UAI).

Statistical analysis

For the first part of the primary analysis, descriptive statistics and frequencies were calculated. A univariate procedure was used to analyze continuous variables (age and number of sex partners) and a frequency procedure used for the other categorical variables. To check for racial differences in variables, each of the variables were then compared between races (black vs. white) using t-test and chi-square statistical tests for numerical and categorical variables respectively. Each of the variables was then entered

into univariate logistic regression to evaluate crude associations with HSV-2 infection. Statistically significant odds ratios (OR) were those that had $p<0.05$. Variables that had significant differences in both groups were regarded as potential confounders. A multivariate associative model was then created using plausible and possible confounders. The variance inflation factors were checked and values greater than 10 were used to identify collinear variables. An interaction term was introduced in the model to check for interaction between age and race. The associative model was then calibrated. The racial difference in HSV-2 status among MSM $\leq$25 years and >25 years was also checked using estimate statement in the final multivariable model. Statistical analysis was performed using SAS 9.3.

Results

Demographic and behavioral characteristics of the 394 MSM included in this analysis are presented in Table 1. Forty-seven percent (184/394) self-identified as black or African American. The mean age of the cohort was 27; white MSM were significantly older than black MSM (28 vs. 25 years; p=0.0003). White MSM reported higher levels of education (54% vs. 32% for college; p<0.001), higher income, and were more likely to have health insurance (75% vs. 50%; p<0.001) than black MSM. Overall, most of the men identified themselves as homosexual or gay (88%); however, white MSM were significantly more likely than black MSM to identify as homosexual or gay (91% vs. 79%; p<.001). Almost all (95.2%) of the participants reported consuming at least one alcohol containing drink in the past 12 months, and this was similar for both black and white MSM. White MSM were more likely to report using non-prescription drugs in the last 12 months than black MSM (30% vs. 53%; p<.001). Viagra use was more common among white MSM (3% vs. 26%; p<.001). There were no significant differences between the proportion of white and black MSM who reported a history of abuse (42% vs. 36%; p=0.2) or being circumcised (85% vs. 90%; p=0.1). White MSM reported a greater number of male sexual partners (oral and anal) in the past 12 months than black MSM (11 vs. 9; p=0.02) and were also more likely to engage in unprotected sexual intercourse in the previous 6 months (42.6% vs.32.1%; p=0.03).

The overall seroprevalence of HSV-2 was 20%; black MSM were significantly more likely than white MSM to have HSV-2 infection (25% vs. 15%; p=0.02) (Table 2). When stratifying by age, this significant difference persisted for MSM under 25 years of

age (23% vs. 10%; p=0.02) but was not significant for MSM greater than 25 years (27% vs. 19%p=0.2). In the unadjusted univariate model, black MSM had greater odds of being seropositive for HSV-2 than white MSM (OR 1.8; p=0.02) (Table 3). For every 1 year increase in age, the odds of being infected with HSV-2 increased by 5% (p=0.03). The odds of HSV-2 infection was increased with higher numbers of male sex partners (OR 1.02; p=0.02) and in participants with a history of child abuse (OR 1.9; p=0.02). There were no significant association between HSV-2 status and higher education (OR 1.1; p=0.3), unemployment (OR 0.8, p=0.5), poverty (OR 1.5, p=0.2), or insurance status (OR 1.2, p=0.2). Drug (OR 1.1, p=0.7) or alcohol use (OR 0.7, p=0.5) were also not significantly associated with HSV-2 status. Circumcision was not significantly associated with HSV-2 infection. Unprotected anal intercourse in the last 6 months (OR 1.3; 95% CI 0.79, 3.55), condom use in the last year (OR 1.7; 95% CI 0.79, 3.55) and reported condom errors in the past year (OR 0.8; 95% CI 0.42, 1.43) were not found to significantly increase the odds of infection in this cohort of MSM.

Results of multivariable logistic regression modeling are presented in Table 4. In the adjusted model, race (aOR 2.5; p=0.002, age (aOR 1.1; p=0.01), the number of male sexual partners in the past 12 months (aOR 1.04; p=0.003), and reported history of child abuse were independently associated with HSV-2 infection (aOR 1.8; p=0.047). Reported history of UAI in the past 6 months was not independently associated with HSV-2 infection.

Results of multivariable logistic regression modeling assessing for interaction are presented in Table 5. There was a significant interaction between race and age (β=-0.11; p=0.04). In order to further characterize this interaction, age was stratified into $\leq$25 years

and >25 years and examined in the final multivariable model with interaction term (Table 6). Among the younger MSM (≤25 years), black race was significantly associated with HSV-2 infection (aOR=4.7; p=0.003) when adjusting for number of sexual partners, child abuse and unprotected anal intercourse in the past 6 months. However, among the older MSM (>25 years) there was no significant association between race and HSV-2 infection (aOR 1.6; p=0.3).

Discussion

HSV-2 is a common sexually transmitted infection that is an important risk factor for HIV acquisition. The prevalence of HSV-2 continues to be higher in the MSM population than it is in the general population. The results of this study show the prevalence of HSV-2 is 19.5% which is higher than the national average [42] as expected and even higher than the prevalence in MSM calculated from the 2001 -2006 NHANES (18.4%) [59, 62]. It is lower than what was proposed in MSM by Barnabas et al. (23%) in 2011 [63]. This however might be because their sample also comprised of HIV negative participants in sites in both Australia and US. This indicates the continued need for the design and implementation of HSV-2 prevention programs which are specifically aimed at young, black MSM as it remains the most common cause of genital ulcer disease in the US and an important risk factor for HIV acquisition [35].

In this analysis, we have shown that Black MSM, particularly young black MSM, are disproportionately affected by HSV-2. Our study presents one of the first indicators of a racial disparity of HSV-2 among MSM in the US. In the multivariate model, race was strongly associated with HSV-2 infection more so than sexual risk behaviors and age with the odds of black MSM being infected 2.7 times more likely than white MSM. Satisfactory explanations for this disparity are yet to be made. Some of the hypotheses proposed by Millet et al. for the racial disparity of HIV, when applied to HSV-2 also fail to be supported by our results. For instance, even though this study reiterated the preexisting knowledge that the number of sexual partners was a determinant of infection, it could not explain the disparity since black MSM had significantly lower numbers of

sex partners than whites. Although a meta-analysis by Martin et al. showed decreased risk among individuals that consistently use condoms compared to those that never used condoms, we did not find any association with lack of condom use or the presence of errors involving condom use and infection. This is likely because the lesions of herpes infection are not limited to penile or rectal skin, so condom use might not be fully protective. Also, Martin's study was not limited to MSM and included HIV positive individuals. Other indicators of possibly increased sex risk such as alcohol use, non-injection drug use and use of Viagra were not associated with a positive HSV-2 serostatus. The finding that circumcision state was not a significant predictor of HSV-2 infection is similar to most of the studies that have compared HSV-2 infection with circumcision [69-71] but in contrast to the findings of Barnabas et al. who found HSV-2 prevalence higher in the uncircumcised MSM [63].

Chronological age was found to be an independent predictor of HSV-2 infection. Other published reports on HSV-2 have also established an increase in prevalence of infection with age [36, 40, 64, 65, 79-81]. This reflects the fact that HSV-2 infection is life-long and that increasing age increases cumulative exposure and is not because there is an increased susceptibility to infection or alterations in immunity as a result of aging [52]. In addition, significant statistical interaction was observed between race and HSV-2 infection, with the racial disparity increased in the younger age group. Although the prevalence of HSV-2 was higher in black MSM at both age categories, younger black are disproportionately affected as compared to young white MSM. The odds of black MSM 25 years and younger being infected was almost 5 times that of white MSM, whereas in those older than 25 years, the odds were of infection was not significantly different.

There is no clear explanation to this but it could be as a result of some genetic or biological factors that have not yet been explored. For instance, genetic factors related to MHC might influence the risk of HSV-2 infection and expression because HLA-Cw4 was found to be significantly associated with HSV-2 infection [82, 83].It could also be as a result of partner factors. If the prevalence of HSV-2 is higher among the partners of young black MSM than white MSM, they are more likely to be exposed and become infected.

Some social determinants of health such as poverty, education, insurance status, etc. were less favorable for black MSM in our study. Other socioeconomic factors like incarceration were not significantly different between black and white MSM. However, none of these factors were significantly associated with HSV-2 serostatus in our analysis. It has previously been suggested that low adverse socioeconomic factors might delay access to STI services and thus increase disease prevalence in the network[84]. It is possible that our study was not adequately powered to show this association. Nonetheless, our data argue against a strong association with low socioeconomic status.

A reported history of child abuse, which included physical and/or sexual abuse, was also an independent predictor of HSV-2 infection. This was consistent with the study performed by Yin et al. on MSM in China that also found that a history sexual abuse ("sexual mistreatment") was an independent predictor of HSV-2 infection [66]. Reasons for this association are unclear. It is possible that perpetuators of the abuse are more likely to be HSV-2 positive leading to direct transmission of HSV-2. Or the abuse may operate in a more indirect manor if victims of abuse are more likely to engage in future high risk behavior than non-victims. There was no correlation between child abuse

and the number of male sexual partners the individual had in the past 12 months in our data. Rodrigues et al. in their study among HIV negative MSM in Brazil also found that when there was no adjustment for other factors, younger age at first sexual intercourse with a man was significantly associated with HSV-2 infection [52]. The young age at sexual debut may be as a result of child sexual abuse [85]. Future studies could improve upon the definition of child abuse / sex abuse to collect information on the nature and timing of the abuse so as to help explore its implications in HSV-2 infection.

Because the effectiveness of standard acyclovir therapy is beneficial for reducing recurrence of lesions and viral shedding but is not effective in reducing HIV transmission in HSV-2 seropositive MSM, other preventive measures such as vaccines for HSV-2 need to be further explored [52]. Few HSV-2 vaccine trails have been tested in clinical trials and those that have been tested on humans have not been consistently effective [86]. Although not statistically significant, some sexual behavior especially relating to condoms were associated with increased odds of HSV-2 infection. Most of the cohort used condoms in sexual activity, however, almost 3 quarters of them reported having at least one condom error. These findings suggest that although their influence might be limited, behavioral intervention and improved access and training on consistent and proper condom use might be of some benefit in the reduction of HSV-2 infection as it has done with HIV[87, 88]. The high prevalence rates of infection suggest that increased HSV-2 testing among high risk populations may have some clinical utility. Healthcare providers of MSM in hospitals and STI clinics could offer testing to their clients especially as infection is frequently subclinical and knowledge of serostatus might

encourage less risky sexual behavior and result in treatment that can reduce the morbidity associated with HSV-2 infection.

This analysis is limited by the fact that our sample size is moderate and metrics generated from the InvolveMENt cohort study are based on MSM in Atlanta, so results may not be generalizable to the broader MSM populations. The fact that the sampling methodology excluded MSM in mutually monogamous relationships also limits the generalizability of our results and might have resulted in an overestimation of the prevalence of HSV-2. On the other hand, MSM in mutually monogamous relationships might be more likely to have unprotected anal intercourse because of mutual trust. This might increase transmission of HSV-2. If this is the case, then our prevalence rate would have been underestimated. White MSM were significantly older than black MSM in this analysis. However, because older white MSM had a greater prevalence of HSV-2, this could have resulted in an underestimation of the racial disparity. We did not gather any information on the participants' knowledge of their HSV-2 status and this might have been helpful in planning enlightenment and educative interventions.

This study was cross-sectional therefore we were only able to examine prevalent and not incident cases of HSV-2 infection. These results are based on the assumption that current behavior is associated with behavior prior to HSV-2 exposure; however this cannot be determined from this data. More funding would be required to do longitudinal monitoring of HSV-2 serostatus in order to calculate time to infection and model predictors. Approximately one quarter of the HSV-2 tests were performed on blood samples drawn from a later time-point study visit because baseline samples were unavailable. This could have biased the results if these were truly incident and not

prevalent HSV-2 infections. It is also possible that the prevalence of high risk sexual behaviors like UAI and drug use might have been underestimated because individuals may be less likely to report activities that are known to be obvious high risk behaviors due to social acceptability and psychological factors. If there was underreporting, it may have obscure the effect of these high risk behaviors on HSV-2 infection.

In conclusion, the exact role of HSV-2 in the transmission and acquisition of HIV is yet to be clarified, however it has been shown to increase HIV acquisition. The health disparity of this infection which is similar to the disparity seen in HIV has so far been unexplained by socioeconomic factors or sexual behaviors. Further studies, including genetic and biologic factors of HSV-2 and HIV and partnership-level analyses, demand more attention in explaining the increased prevalence of HSV-2 in young, black MSM. Also, the high seroprevalence among HSV-2 should be a cause of concern in the healthcare system. More attention needs to be given to the control and prevention of HSV-2 infection as a method of HIV prevention.

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Tables

Table 1: Demographic and behavioral characteristics of black and white HIV negative MSM in the InvolveMENt cohort

Variables	Black n (%) N=184	White n (%) N=210	p value
Age (mean,sd)	25.3 (5.3)	27.9 (5.7)	0.0003
Uninsured	89/179 (50)	51/208 (25)	<.0001
Poverty	44/137 (32)	26/194 (13)	<.0001
Education			
College, post graduate, or professional school	59 (32)	114 (54)	
Some college, Associate's degree, and/or technical school	77 (42)	75 (36)	<.0001
High school or GED	42 (23)	20 (10)	
Some high school	6 (3)	1 (1)	
Unemployed (now)	46/183 (25)	37/210 (18)	0.07
Alcohol (12mos)	172/183 (94)	202/210 (96)	0.3
Uncircumcised	26/173 (15)	19/200 (10)	0.1
Child abuse	77/184 (42)	75/210 (36)	0.2
Gay identity	145/184 (79)	192/210 (91)	0.0004
Number of sexual partners (12mos) (mean,sd)	9 (11)	11 (12)	0.02
Viagra use(12mos)	6/177 (3)	33/202 (16)	<.0001
Arrested (12mos)	21/184 (11)	20/210 (10)	0.5
Condom use (12mos)	157/182 (86)	168/210 (80)	0.1
Condom errors (12mos)	112/151 (74)	123/167 (74)	0.9
Unprotected anal intercourse (6mos)	59/184 (32)	89/209 (43)	0.03
Drug use (12mos)	51/183 (28)	111/210 (53)	<.0001

Table 2: Seroprevalence of HSV-2 stratified by age among HIV negative black and white MSM in the InvolveMENt cohort

	Black n (%)	White n (%)	p value
HSV-2	45/184 (25)	32/210 (15)	0.02
Age group			
Age <=25	27/116 (23)	8/81 (10)	0.02
Age >25	18/68 (27)	24/129 (19)	0.2

Table 3: Univariate associations between HSV-2 serostatus and demographic and behavioral characteristics of HIV negative MSM in the InvolveMENt cohort

Variables	OR	95% Confidence Interval	p-value
Black	1.8	1.1 – 3.0	0.02
Age	1.1	1.004 – 1.095	0.03
Uninsured	1.2	0.7 – 2.0	0.5
Poverty	1.5	0.8 – 2.9	0.2
Education			
College or more vs. some high school	1.1	0.1 - 9.6	
Some college, Associate's degree, and/or technical school vs. some high school	1.6	0.2 - 14.4	0.3
High school or GED vs. some high school	1.9	0.2 – 16.0	
Unemployed (now)	0.8	0.4 - 1.5	0.5
Alcohol	0.7	0.2 - 1.9	0.5
Uncircumcised	1.4	0.7 – 2.8	0.4
Child abuse	1.9	1.1 - 3.1	0.02
Gay identity	1.2	0.6 - 2.4	0.7
Number of sexual partners (12mos)	1.02	1.003 - 1.04	0.02
Viagra (12mos)	1.5	0.7 - 3.2	0.3
Arrested (12mos)	0.7	0.3 - 1.7	0.4
Condom use (12mos)	1.7	0.8 - 3.6	0.2
Condom errors (12mos)	0.8	0.4 - 1.4	0.4
Unprotected anal intercourse (6mos)	1.3	0.8 - 2.2	0.3
Drug use (12mos)	1.1	0.7 - 1.8	0.7

Table 4: Multivariate model examining associations with HSV-2 serostatus in the InvolveMENt cohort

Variable	β	aOR	95% CI	p-value
Race	0.98	2.7	1.4 - 4.9	0.002
Age	0.064	1.1	1.01 - 1.12	0.01
Number of sexual partners (12mos)	0.034	1.04	1.01 - 1.06	0.003
Unprotected anal intercourse (6mos)	0.14	1.2	0.6 - 2.1	0.6
Child abuse	0.59	1.8	1.01 - 3.26	0.047

Table 5: Multivariate model assessing interaction in associative model of HSV-2 serostatus in the InvolveMENt cohort

Variable	β	Standard Error	Wald Chi-Square	Pr > ChiSq
Intercept	-5.96	1.23	23.34	<.0001
Race	4.028	1.55	6.71	0.0096
Age	0.12	0.03	9.48	0.002
Child abuse	0.60	0.30	3.94	0.047
Number of sexual partners (12mos)	0.035	0.01	8.63	0.003
Unprotected anal intercourse (6mos)	0.11	0.31	0.14	0.7
Age*Race	-0.11	0.051	4.08	0.04

Table 6: Adjusted association of race/ethnicity with HSV-2 serostatus, within age strata, in a multivariate logistic regression model of 394 MSM in the Involvement cohort

Black vs. White MSM	aOR	95% CI	p-value
Age <= 25 years	4.7	1.69 – 13.28	0.003
Age > 25 years	1.6	0.69 – 3.67	0.3