Psychoneuroendocrinology 86 (2017) 128–133

Contents lists available at ScienceDirect

Psychoneuroendocrinology

journal homepage: www.elsevier.com/locate/psyneuen

Depressive symptoms are associated with salivary shedding of Epstein-Barr virus in female adolescents: The role of sex differences

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ABSTRACT

Background: Adolescent females have a higher prevalence of depression in comparison to their male peers – a disparity that has been increasing over the past decade. Depression is of concern as it is associated with chronic disease and to immune dysregulation, which may be one mechanism linking depression to future pathology. This study examined the extent to which sex moderated the association between depressive symptoms and immune dysregulation during adolescence using Epstein-Barr virus (EBV) reactivation, a biomarker of cellular immune response, as a model.

Methods: A representative community sample of 259 female and 279 male adolescents aged 11–17 years who were EBV IgG positive were examined. Trained interviewers collected the data during two home visits, one week apart. Depressive symptoms were measured at the first visit using the 9 item short-form of the Center for Epidemiologic Studies-Depression scale. EBV biomarkers were collected via saliva at the second visit and included a qualitative measure of EBV viral capsid antigen immunoglobulin G to assess prior EBV infection and a quantitative measure of EBV DNA to assess the number of viral copies shed in the saliva.

Results: In multivariable logistic regression analyses, increasing depressive symptoms were significantly associated with salivary shedding of EBV DNA for adolescent females only (logit = 0.66, se = 0.30, $p < 0.05$), and the interaction between sex and depressive symptoms on salivary shedding of EBV DNA was statistically significant (logit = − 1.19, se = 0.42, $p < 0.01$). Sensitivity analyses were conducted in which sex was examined as a moderator in the relationship between depressive symptoms and salivary EBV DNA quantitative copies via Tobit regression; results were consistent with the presented findings.

Conclusions: Depressive symptoms are associated with EBV reactivation among EBV positive female adolescents, but not males. Future research is needed to examine EBV reactivation in female adolescents as a mechanism linking depression to future chronic disease and the role of sex hormones in explaining sex differences in the relationship between depressive symptoms and EBV reactivation.

1. Introduction

The prevalence of depression among U.S. adolescents has increased from 8.7% to 11.3% over the past decade. (Mojtabai et al., 2016) Adolescent females have been disproportionately affected in both the overall prevalence and the increasing trend with 17.3% of adolescent females reporting a major depressive episode in the year prior to 2014 (up from 13.1% in 2005) in comparison to 5.7% of adolescent males (up from 4.5% in 2005). (Mojtabai et al., 2016) The increasing rate of depression is of significant concern as suicide rates among adolescents have also increased over time, particularly among female adolescents aged 10–14 years in which rates have increased 200% since 1999. (Curtin et al., 2016) In addition to suicide, adolescent depression is associated prospectively with obesity, (Mannan et al., 2016; Zhu et al., 2016) substance use, (Hussong et al., 2017) accelerated atherosclerosis, and early cardiovascular disease. (Goldstein et al., 2015)

A growing body of research has found that depression, (Haeri et al., 2011; Rector et al., 2014; Zhu et al., 2013) consistent with other forms of psychosocial distress (e.g. attachment anxiety, (Fagundes et al., 2014) psychosocial stress, (Glaser et al., 1985; Glaser et al., 1994) and exposure to adverse life events(McDade et al., 2000)), is also be associated with reactivation of latent herpes viruses, including the Epstein-Barr virus (EBV) – a latent virus linked to certain autoimmune disorders, (Niller et al., 2008) cancer (Li et al., 2016) and atherosclerosis. (Espinola-Klein et al., 2002) In the U.S., primary infection with EBV is nearly ubiquitous by young adulthood, and although the virus is most

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http://dx.doi.org/10.1016/j.psyneuen.2017.09.009
Received 21 April 2017; Received in revised form 4 September 2017; Accepted 6 September 2017

well- known for causing mononucleosis during adolescence, the majority of the population experiences primary infection earlier in childhood with few to no symptoms. (Christian et al., 2009; Ford and Stowe, 2013; Glaser et al., 1991) The DNA of the virus remains latent in the B-lymphocytes after the primary infection, kept in balance through the action of cytotoxic T-cells in individuals with competent immune systems. However, physical or psychosocial stress can decrease leukocyte production and functioning enabling reactivation of the herpes virus with increased replication of the viral DNA and antiviral antibodies. (Ford and Stowe, 2013; Stowe et al., 2010) Thus, EBV reactivation may be one pathway through which depression contributes to future health outcomes.

However, despite the higher prevalence of depression reported in females than males, (Mojtabai et al., 2016) research examining the potential for sex differences in the effect of depression on latent herpes virus reactivation has been limited as prior studies have focused primarily on pregnant women. (Christian et al., 2012; Haeri et al., 2011; Zhu et al., 2013) or they controlled for sex in analyses. (Rector et al., 2014) Evidence from animal and human models that sex differences in the stress response exists is strong, (Bale and Epperson, 2015; Bekhbat and Neigh, 2017) and that it may vary across developmental stage with females more susceptible to stress and stress-associated affective disorders during peripubertal and pubertal development than males. (Bale and Epperson, 2015) For example, in one pilot study, exposure to a greater number of adverse life events was significantly associated with higher EBV IgG antibody levels in EBV seropositive female youth aged 9–13 years, but not in male youth. (McDade et al., 2000) Similarly, in an investigation of youth aged 7–13 years, higher levels of depressive symptoms were associated with increased natural killer cell cytotoxicity, but only among the stratified sample of older female youth with no significant associations found for younger females or for male youth regardless of age. (Caserta et al., 2011) Thus, to advance our understanding of potential sex differences in the linkages between depression and EBV reactivation we build on the prior research through an examination of the relationships between depressive symptoms and salivary shedding of EBV DNA among a representative community sample of EBV IgG positive adolescent females and males.

2. Methods

2.1. Study design

The current study examines data from two linked studies: (1) *The Adolescent Health and Development in Context* study– a two wave representative study on the impact of activity space exposures on the health and well-being of urban and suburban youth aged 11–17 years and (2) the *Linking Biological and Social Pathways to Adolescent Health and Wellbeing* study, which added the collection of immune function biomarkers (saliva for EBV VCA IgG and viral DNA) on a representative subsample of youth participating in the *first wave* of the AHDC study (N = 674).

The study takes place in Franklin County, OH – a large, metropolitan area that is representative of the average U.S. metropolitan area in terms of social and economic characteristics. The study area incorporates Columbus and the suburban municipalities that border, or are contained within, the boundaries of Columbus for comparisons between youth residing in low income urban neighborhoods and those residing in the highest income suburban locations. The sampling procedures for the study have been previously described elsewhere. (Ford et al., 2016) Briefly, households were mailed a flyer describing the study with instructions to call if they were interested in participating. Trained interviewers also called to determine interest and eligibility (youth aged 11–17 years, 1 primary caregiver and English speaking). If the household had more than one youth eligible for the study, one focal youth was randomly selected for participation in the AHDC study. Youth were then eligible for the biomarker collection if they had not taken corticosteroids in the previous month. Both studies were approved by the university institutional review board and parental consent and youth assent were obtained prior to data collection.

As the main objective of this study was to examine the potential for EBV *reactivation* through salivary EBV DNA shedding, the sample for this analysis included only those youth who were EBV VCA IgG positive (had evidence of past or primary EBV infection) determined through an adapted ELISA method using saliva. (Stowe et al., 2014) To date, the measurement of EBV VCA IgG has been most commonly assessed using serum, but researchers have successfully employed salivary ELISA methods in a large sample of children. (Crowcroft et al., 1998) Furthermore, our pilot study (N = 50 young adults, unpublished) found a 0.92 correlation coefficient between serum and salivary EBV VCA IgG antibodies in which an antibody titer greater than 0.02 was suggestive of prior EBV infection. Thus, youth in this study who had a salivary EBV VCA IgG antibody level greater than 0.02 were considered EBV positive (N = 538 or 81.0% of the youth who had saliva collected for EBV).

2.2. Data collection

All data were collected by trained interviewers over a weeklong period. A face-to-face interview and self-administered survey with both the focal youth and his or her primary caregiver were conducted at the beginning of the week during which items specific to mental and physical health and health behaviors were queried via the self-administered portion of the survey. A seven day smartphone-based Global Positioning System tracking and Ecological Momentary Assessment collection followed the entrance survey in which real-time data were collected on the youth's physical and social environmental exposures and health behaviors. At the end of the week, the interviewer returned to the home for a final face-to-face interview with the youth and an additional self-administered survey for the parent that asked about their perceptions of their physical and social environments. The interviewer also collected the saliva for immune function biomarkers at this second visit via passive drool. Specimens were then transported from the home to the survey research center where they were stored at −20C and then transferred on dry ice to the −80C freezers at the university laboratory until assay.

2.3. Measures

2.3.1. Dependent variable

Salivary shedding of EBV DNA is a dichotomous measure created to compare youth who had 10 or more copies (the lower bound detectable level) of EBV DNA in their saliva (yes = 1) to those youth who were below the detectable level. A quantitative measure of the logged EBV DNA copy number was also created and examined in sensitivity analyses. Assessment of the EBV DNA viral load was accomplished using PCR methodology as performed in numerous studies from Stowe's laboratory. (Mehta et al., 2013; Stowe et al., 2007) DNA was isolated from saliva using the QiaAmp blood kit (Qiagen, Valencia, CA). EBV copy numbers were measured in samples using real-time PCR using PCR primers that amplify a portion of the BALF5 gene. Real-time fluorescence measurements were taken over 40 cycles using an Mx3005P real-time PCR instrument, and unknowns were compared to a standard curve (serially-diluted plasmids containing single copy viral genes). Copy numbers were then calculated automatically using the Strategene software.

2.3.2. Primary independent variables

Depressive symptoms is the primary independent variable of interest, which was measured with a short form of the original 20 item Center for Epidemiologic Studies-Depression scale (CES-D) (Cole et al., 2004) in an effort to reduce respondent burden. The 10 item CES-D short form scale was developed and validated by Cole and colleagues through Rasch modeling and confirmatory factor analysis. In the 10 item scale,