MARKERS OF INFECTION PREDICT COGNITIVE DECLINE OVER FOUR YEARS

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Abstract: The objectives of this study were to examine whether viral antibody levels to herpesviruses influence cognitive decline over a four year follow-up period and assess whether serum cortisol modifies the effect of viral antibody levels on cognitive decline. A randomly selected subset of participants (N=1,204/1789) from an ongoing cohort study of community dwelling Mexican Americans ages 60-100 were screened annually for cognitive functioning and baseline serum samples were assayed for antibodies to cytomegalovirus (CMV) and herpes simplex virus (HSV-1), and fasting cortisol. Higher levels of CMV antibody were associated with a faster rate of cognitive decline over the four year period (ß = -0.011, standard error=0.003; P= 0.001), controlling for age, education, and chronic health conditions. Understanding the mechanisms by which CMV influences cognitive decline may allow development of intervention strategies targeting viral reactivation over the life course.

Key words: Herpesvirus, cytomegalovirus, cognitive decline, dementia.

INTRODUCTION

Identification of herpesviruses in areas of the brain of deceased elderly individuals affected by Alzheimer's disease has led to the notion that they

may be causal agents or promoters of dementia. [1] Few studies have assessed whether circulating levels of antibody to these latent viruses are associated with cognitive decline. One of the mechanisms by which herpesviruses reactivate from a latent state includes stress related fluctuations in the hormone cortisol. [2] Increased cortisol levels have also been identified as a risk factor for cognitive deficits in the elderly. [3] Given the potential pathways between infection, cortisol, and cognitive decline, we hypothesized that latent viral infections directly influence cognition and that cortisol modulates the relationship between herpesviruses and cognitive decline. The specific aims of this study were to: 1) examine whether baseline viral antibody levels to Cytomegalovirus (CMV) and Herpes Simplex Virus (HSV-1) influence cognitive decline over a four year follow-up period, and 2) assess whether baseline serum cortisol modifies the effect of viral antibody levels on cognitive decline, among elderly individuals participating in a community based cohort study.

METHODS

The participants were from the Sacramento Area Latino Study on Aging (SALSA). SALSA is an ongoing prospective cohort study of community dwelling Mexican Americans who were aged 60-100 at baseline in 1998-99. A randomly selected sub-sample of participants (N=1,204) from the overall SALSA cohort (N=1,789) with available blood samples and who had at least two follow-up visits after baseline were included in the analyses. The details of the parent study have been discussed previously and all analyses were approved by the Institutional Review Board at the University of Michigan. [4]

The study participants were screened annually for cognitive functioning using the modified Mini Mental State Examination (3MSE) over a four year period. [4] During the annual interviews, information on education, socioeconomic status of the household, medical history, and duration and date of diagnoses for 35 health conditions were gathered. A commercially available enzyme linked immunosorbent assay (ELISA) IgG detection systems (Wampole Laboratories, Princeton, NJ) was used to analyze the level (expressed as optical density units (OD)) of IgG reactive to HSV-1 and CMV among aliquots of continuously frozen (-20° C) serum samples. Cortisol levels were measured by an automated chemiluminescence system ACS:180 (Bayer Health Care, Tarrytown, NY).

Linear mixed effects models were used to estimate the effect of baseline viral antibody levels on changes in cognitive functioning over a four year period. The measure of cognitive functioning used in the analyses was the

3MSE scores at baseline and each of a maximum of 4 follow-ups. Since the distribution of 3MSE scores was negatively skewed, we applied a natural logarithmic transformation to this outcome to meet the model assumption of normality. The unadjusted mixed models included a term for the viral antibody or cortisol levels, year of measurement (since baseline), and the interaction of the viral antibody or cortisol levels with year. The final models contained all of the variables in the unadjusted model along with all other significant covariates (age, gender, education, income, and number of baseline health conditions). To examine whether serum cortisol modified the effect of viral antibody levels on global cognitive decline (3MSE), we fitted mixed models with two and three-way interaction terms. For example, the unadjusted model for CMV included the following terms: CMV, cortisol, year, CMV*cortisol, CMV*year, cortisol*year, and CMV*cortisol*year. Adjusted models included all of the terms in the unadjusted model as well as age, gender, education, income, and number of baseline health conditions. Analyses were conducted with SAS Version 9.1 (SAS Institute, Cary, NC) using the MIXED model procedure.

RESULTS

For each one-unit increase in CMV IgG antibody level, participants experienced a significantly more rapid decrease in 3MSE scores over the four year period (see table1). This association maintained significance even after controlling for age, gender, education, income, and health conditions (CMV*year: ß = -0.011, standard error (SE) =0.003; P= 0.001). In contrast, HSV-1 antibody levels did not significantly influence rate of cognitive decline over the four year period (HSV-1*year: ß = -0.002, SE=0.004; P= 0.652). In addition, there was no significant association between serum cortisol levels and cognitive decline after controlling for covariates included in the final model (cortisol*year: ß = 0.001, SE=0.001; P= 0.361). Last, serum cortisol did not significantly modify the effect of CMV or HSV-1 antibody levels on rate of cognitive decline (see table 1).

Table 1

Relationship between CMV antibody level and change in the modified minimental state exam (3MSE) over four follow up periods

Parameter	Unadjusted Model Estimate (SE)	p-value	Final Model[a] Estimate (SE)	p-value
CMV antibody levels: [b]				
Follow-up year	0.099 (0.009)	< 0.001[d]	0.274 (0.023)	< 0.001[d]
CMV	-0.025 (0.01)	0.014[d]	0.0147 (0.010)	0.147
CMV*year	-0.017 (0.005)	< 0.001[e]	-0.011 (0.003)	0.001[e]
HSV-1 antibody levels: [b]				
Follow-up year	0.101 (0.009)	< 0.001[d]	0.283 (0.023)	< 0.001[d]
HSV-1	-0.03 (0.012)	0.012[d]	0.008 (0.011)	0.462
HSV-1*year	-0.008 (0.005)	0.140	-0.002 (0.004)	0.652
Cortisol levels: [b]				
Follow-up year	0.189 (0.020)	< 0.001[d]	0.282 (0.023)	< 0.001[d]
Cortisol	-0.006 (0.005)	0.211	0.002 (0.004)	0.638
Cortisol*year	-0.031 (0.005)	0.031[e]	0.001 (0.001)	0.361
Effect Modification: [c]				
CMV*cortisol*year	0.000 (0.001)	0.587	0.000 (0.001)	0.558
HSV-1*cortisol*year	0.002 (0.001)	0.282	0.001 (0.001)	0.134

a. Adjusted for age, gender, age*year, gender*year, education, income, and number of baseline health conditions; b. Results show the effect of a one-unit increase in viral IgG antibody levels (measured in OD units) or basal serum cortisol levels on 3MSE scores over the four year period; c. Unadjusted effect modification models included the following terms: CMV, cortisol, CMV*cortisol, CMV*year, cortisol*year, cortisol*year*CMV. Adjusted effect modification models included all of the terms in the unadjusted model as well as all covariates (age, gender, age*year, gender*year, education, income, and number of baseline health conditions) ; d. Significant at the $p < 0.05$; e. Significant at the $p \leq 0.10$ for interaction terms

DISCUSSION

This was the first study to examine the relationship between herpesvirus antibody levels and rate of cognitive decline among elderly community dwelling individuals in the US. The strengths of this study include (i) application of a rigorous, widely accepted, and standardized method for assessing cognitive functioning, (ii) use of a population-based cohort, and (iii) implementation of mixed model analyses to assess trajectories of cognitive decline. Our results suggest that increased CMV IgG antibody levels are an important predictor of cognitive decline, even after controlling for major risk factors such as age, education, gender, and chronic health conditions. In contrast, increased levels of HSV-1 antibodies were not related to cognitive decline as measured by the 3MSE over the four year period. Last, cortisol did not appear to modify the effect of viral antibody

levels on cognitive decline.

Recent findings suggest that CMV may play a role in chronic diseases such as Alzheimer's disease via immune system modulation and subsequent inflammatory cytokine cascades. [5] Specifically, peripheral cytokine concentrations may fluctuate as a consequence of immunoscenescent changes in the level of latent CMV specific T-cells in the elderly. [5] An area requiring further research is an examination of whether cytokines, such as IL-6 and TNF-α, modifies the relationship between CMV antibody levels and cognitive decline. IL-6, TNF-α, and other cytokines may increase with rises in CMV antibody levels, triggering pathological changes associated with Alzheimer's disease. [5]

In contrast to CMV, our results suggest that HSV-1 antibody levels are not a significant predictor of cognitive decline over a four year period. It is possible that HSV-1 influences cognition at a later stage of decline or that HSV-1 antibody levels increase after the onset of dementia. It was not possible to assess varying temporal trends in HSV-1 antibody levels since our serum measures were only gathered at baseline. An interaction between Apolipoprotein E-4 (ApoE-4) and the presence of HSV-1 virus in the brain has been identified as a significant risk factor for Alzheimer's Disease.1 We did not have sufficient statistical power to test for an interaction between HSV-1 and ApoE-4 since a very small percentage (5.7%) of study subjects were ApoE-4 positive.

Increased glucocorticoid levels have been shown to damage hippocampal neuronal cells.6 In our study, serum cortisol did not influence cognitive functioning nor did it modify the effect of CMV or HSV on rate of cognitive decline. It is possible that a single measure of fasting serum cortisol does not represent fluctuations in diurnal serum cortisol, which may be a more sensitive marker of circulating cortisol levels. In addition, viral antibody levels and cortisol may influence distinct cognitive domains. For example, Greendale et al. reported an association between higher levels of cortisol and decline in verbal memory, but no association with global cognitive decline as measured by the modified mini mental state exam.3 Cortisol may selectively target the hippocampus resulting in verbal memory loss, but may not directly influence global cognition as was measured in this study.

In conclusion, this is the first study to suggest that increasing levels of antibody to CMV is associated with a more rapid decline in global cognition in a population based sample of elderly individuals in the US. Our findings reinforce the need for additional large scale prospective investigations to determine the influence of CMV antibody levels on rate of development of cognitive impairment or dementia as well as a broader understanding of the potential mechanisms by which this viral immune measure may impact cognition. Confirmation of these findings will enhance our understanding

of whether latent CMV infection is an etiological factor and may impact interventions aimed at targeting infection, immunological status, or viral reactivation over the life course.

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