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Cannabis smoking and serum C-reactive protein: A quantile regressions approach based on NHANES 2005–2010

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ABSTRACT

Background: Pre-clinical studies link cannabinoid-1 receptor activation to inflammation and atherosclerotic effects; anti-inflammation and immunosuppression seem to be mediated by cannabinoid-2 receptor activation. In this epidemiological study, we aim to present estimates on suspected cannabis-attributable immunomodulation as manifest in serum C-reactive protein (CRP) levels as non-specific inflammatory markers with interpretable clinical values. With strength of data from recent large nationally representative community sample surveys, the research approach illustrates value of a quantile regressions approach in lieu of the commonly used but relatively arbitrary cutpoints for CRP values.
Methods: The study population encompasses 20–59 year old participants from the National Health and Nutrition Examination Surveys, 2005–2010 ($n = 1115$ recently active cannabis smokers and 8041 non-smokers, identified via confidential Audio Computer Assisted Self-Interviews). Age, sex, race, education, income–poverty ratio, alcohol consumption, and tobacco smoking also were measured, together with body mass index (BMI), which actually might be on a mediational path. Quantile regressions, with bootstrapping for variance estimation, made it possible to hold these covariates constant while estimating cannabis-CRP associations.
Results: Evidence suggesting possible cannabis-attributable immunomodulation emerges at CRP levels below the median ($p < 0.05$). Whereas BMI might help explain a cannabis link with serum CRP, but BMI-stratified analyses disclosed no appreciable variation of the cannabis–CRP relationship across BMI subgroups.
Conclusions: Extending pre-clinical research on cannabis-attributable immunomodulation, this study's CRP evidence points toward possible anti-inflammatory effects of cannabis smoking. More definitive evidence can be derived by combining pre-clinical research, studies of patients, and epidemiological research approaches.

1. Introduction

Several studies provide evidence that inflammation plays a key role in the development and the progression of chronic diseases such as cardiovascular disease and diabetes mellitus (CVD; DM; Calle and Fernandez, 2012; Pearson et al., 2003). Some inflammatory markers now qualify suspected causes of cardiometabolic illnesses (Goff et al., 2014; Pradhan et al., 2001). It is in this respect that the acute phase reactant C-reactive protein (CRP) is of special interest. Acute phase responses are induced by cytokines released from jeopardized tissue, which stimulate liver synthesis of acute phase proteins including CRP (Steel and Whitehead, 1994). Circulating levels of CRP are clinically useful in prediction of the occurrence of cardiovascular events as well as for therapeutic purposes. For example, individuals with CRP levels >3 mg/L represent a high-risk group for CVD deserving special attention (Hage, 2014).

The first evidence that cannabinoids might modulate cytokine production was found in the mid-1980s (Blanchard et al., 1986). Consequences of such cannabinoid immunomodulation are not fully understood (Klein, 2005). On one hand, pre-clinical studies link cannabinoid-1 receptor activation to inflammation and atherosclerotic effects (Dol-Gleizes et al., 2009). On the other hand, activation of cannabinoid-2 receptors primarily is found to mediate anti-inflammation and immunosuppression (Klein and Cabral, 2006; Pacher, 2009; Ribeiro et al., 2012). Much of this work is pre-clinical, but there has been a steady increase in evidence from human studies.

Abbreviations: NHANES, National Health and Nutrition Examination Surveys; CRP, C-reactive protein; BMI, body mass index.

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The aim of the current study is to present new epidemiological estimates on suspected cannabis smoking effects on circulating levels of CRP. There has been little epidemiological research on this interesting topic (Costello et al., 2013; Keen et al., 2014; Muniyappa et al., 2013). Due to the large nationally representative sampling and strong CRP assays, of most importance might be recent work based on the United States National Health and Nutrition Examination Survey data gathered between 1988 and 1994 (US NHANES, 1988–1994), completed by Rajavashisth et al. (2012). Even so, the main findings from NHANES data were somewhat perplexing in that past but not current cannabis smoking (CS) was associated with having serum CRP levels below 0.5 mg/dL (never smokers served as the referent group for both former and current cannabis smokers). The interpretation is that lower inflammation values are seen among former cannabis smokers, not among active smokers.

Seeking to re-visit these issues, in the present study we turn to independent samples from more recently completed NHANES cycles (2005–2010), and we replace the somewhat arbitrary CRP cutpoint approach used in prior research, substituting a quantile regressions approach that makes more complete use of the full range of CRP values. Quantile regression (QR) can be used to model any percentile, not just the mean or median. By launching a QR line of CPR research, we hope that QR might replace or possibly complement the use of arbitrary cutpoints that have clear utility when making judgments about individual patients, but that might less useful in epidemiological workup of suspected exposure-effect relationships. Nonetheless, in order to help confirm or disconfirm what Rajavashisth and colleagues found with a cutpoint, after completing the quantile regressions approach, we imposed their CRP cutpoint (0.5 mg/dL; CRP 85th percentile in the current study), and tried to complete a more or less exact replication of that work, using the newer NHANES data.

Two methodological issues should be noted. First, CRP levels have skewed distributions, with an interquartile spacing of CRP values that increases across the CRP range. The third quartile range of CRP levels tends to be twice as wide as lower quartiles; the fourth quartile concentration range is larger (Campbell et al., 2003; Ockene et al., 2001). Second, to the extent that body mass index (BMI) is responsive to cannabinoids, and BMI might fall on a causal pathway that leads from CS toward immunomodulation, in the current study we study BMI as a stratification variable to detect subgroup variations. Formal mediational analysis is not possible, given uncertainty about temporal sequencing and feedback loops in the cross-sectional NHANES data. We return to both of these issues in Section 4.

2. Methods

This study's estimates are based on cross-sectional survey data gathered in the 2005–2010 NHANES cycles. By design, NHANES seeks nationally representative sample survey estimates for the United States non-institutionalized civilian population, with multistage area probability sampling prior to recruitment of designated respondents. NHANES sampling works downward from its primary sampling units through US counties, blocks, households, and individuals within households, with an oversampling of certain subgroups to increase statistical precision of estimates for these subgroups (United States Centers for Disease Control and Prevention, 2010). In analysis steps, the use of analysis weights takes into account this over-sampling, with post-stratification adjustments that bring study estimates into balance with known US Census Bureau population distributions.

For this study, we specified a sample that includes all NHANES designated respondents aged 20–59 years on the assessment date. Some did not consent to participate; others had missing or invalid responses to key variables under study. For this reason, the effective unweighted sample size for the present study is 9156. Supplemental Fig. S1 provides flow chart that leads from all NHANES participants to those who contribute information for this study's estimates.

The key response variable in this study is the level of serum CRP (mg/L). In NHANES, CRP has been quantified by latex-enhanced nephelometry, with no apparent changes in equipment, lab methods, or lab sites across the years from 2005–2010 (United States Center for Disease Control and Prevention, 2006).

The explanatory covariate of central interest in this study is recently active cannabis smoking, assessed via a confidential Audio Computer Assisted Self-Interviews (ACASI) approach on the day of a physical examination at the NHANES mobile examination center. The ACASI approach is intended to promote accuracy and completeness of reporting on sensitive topics, including age of first cannabis smoking, and how many days cannabis was used in the 30 days just prior to assessment. On this basis, NHANES respondents can be classified as never smokers; past CS (smoked cannabis at least once in lifetime but not in the past 30 days); and recently active CS (smoked cannabis at least once in the past 30 days).

The initial guiding conceptual model was one in which the serum CRP levels are expressed as a function of recent and past cannabis smoking, with 'never CS' as a reference subgroup, and with statistical adjustment for covariates: age (years), sex (male/female), ethnic self-identification (ESI: coded for non-Hispanic White/non-Hispanic Black/Hispanics/all others), educational attainment (coded for less than high school/high school/above high school), income–poverty ratio (coded for less than 1 versus 1 or more), tobacco cigarette smoking (coded for never/past/current), and past-year alcohol consumption (coded for yes/no).

The plan for data analysis was organized in relation to standard "explore, analyze, explore" cycles. First, in the "explore" step, we examine univariate distributions and the first five moments of each variable's distributions; there is no exploration of the CS–CRP relationships under study. In the subsequent main "analysis/estimation" step and due to the skewed nature of the CRP levels, we turned to the quantile regressions, estimating the degree to which serum CRP level might depend upon presence of recently active cannabis smoking (with never smokers as the referent group and a covariate term for former CS). Final post-estimation "exploratory" steps include stratified QR analyses that addressed sex, ESI, tobacco smoking, alcohol consumption and BMI, one by one.

As mentioned before, quantile regression can be used to model any percentile. Here, for illustrative purposes, the manuscript reports quantile estimates based on the 10th, 25th (Q1), 50th (median), 75th (Q3) and the 90th percentiles. In the QR context, the best estimation approach for variances, standard errors, and 95% confidence intervals (CI) is based on bootstrap re-sampling, according to statistical theory worked out and published by Efron (1979) and subsequently refined. In brief, for this study, via SAS SURVEYSELECT software, the observed sample was re-sampled with replacement 1000 times, and the QR model was fit iteratively to these samples (Lohr, 2012; Suhr, 2009). The resulting distribution of 1000 QR estimates was produced, from which lower and upper CI bounds are the 2.5th and 97.5th percentiles, respectively (Efron, 1979; Feng et al., 2011; Rust and Rao, 1996). An online appendix provides additional details, including citations to general approaches that might be useful to other readers of this article (Koenker, 2005; Koenker and Hallock, 2001). Implementing software has been described by Chen et al. (2010). All analyses were performed in SAS 9.3 (SAS Institute, Cary, NC).

3. Results

Table 1 describes the study sample, showing 50:50 male–female ratio for NHANES study participants. In the sample, about one in eight (12.2%) qualified as recently active cannabis users; a majority had tried cannabis; a minority qualified as tobacco smokers. Normal weight, overweight, and obese status values were equally distributed among study participants. The arithmetic mean of serum CRP levels was 3.9 mg/L with median of 1.7 mg/L. The weighted distribution of serum CRP levels is displayed in Supplemental Fig. S2.

Fig. 1 is set up as a plot of the QR-estimated difference of serum CRP levels in the contrast of recently active CS values minus never CS values, derived as covariate-adjusted regression slope estimates from analysis-weighted quantile regressions modeling, with inclusion of a covariate term for past CS (with no product-terms included). The solid line depicts point estimates for the estimated difference between recent CS and never CS, all uniformly below the null difference value of 0.0, with a shaded 95% confidence interval that fails to entrap the null value of 0.0 at lower serum CRP values until just below the CRP median. From the median CRP value to the highest CRP value, the association remains inverse. Nevertheless, because the number of participants at these higher serum CRP levels become smaller and smaller, the width of bootstrapped confidence intervals becomes larger and larger. It should be noted that there is no appreciable variation in the size of the QR regressions slope estimate above the median. Instead, the width of the confidence interval depends strictly upon the NHANES effective sample size across these higher serum CRP levels. Studied one by one, product-terms to index subgroup variation in the CS–CRP