Systemic Inflammation in Electronic Cigarette Users versus Traditional Smokers

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ABSTRACT

The use of Electronic Cigarettes (e-cigs) has had an increase in popularity over the past several years. This can be attributed to multiple factors such as, people thinking it is less harmful than traditional cigarette use, a method to cut back on the amount of nicotine intake, and as a cessation to smoking. Cigarettes cause inflammation both in the lung and systemically; this study tested differences in pro-inflammatory cytokines between those that use e-cigs, traditional cigarettes users, and neither (control). To evaluate this, blood samples were collected from participants. Groups were selected from a suburban mountain west geographical area, mainly a university campus. Samples were centrifuged to isolate serum for testing using an Enzyme-linked Immunosorbent Assay (ELISA). 96 micro-well ELISA plates were used to test for specific pro-inflammatory cytokines: Interlukin-1 beta (IL-1 beta), Interlukin-6 (IL-6) and Tumor Necrosis Factor-alpha (TNF-alpha). In conjunction C-reactive Protein (CRP) was ran via immunoturbidimetric assay. The researchers expect e-cigs to stimulate less pro-inflammatory cytokines than traditional cigarettes, but more than the control groups. An ANOVA analysis yield no significant difference between the groups.

INTRODUCTION

The use of Electronic Cigarettes (E-cig) has had an increase in popularity over the past several years. A recent study found that 12.6% of adults have tried an E-cig and 3.7% of adults use E-Cig (Schoenborn & Gindi, 2015). This can be attributed to multiple factors such as, people thinking it is less harmful than traditional cigarette use, a method to cut back on the amount of nicotine intake, and as a cessation to smoking. These methods of behavior can be linked to the marketing of E-cigs. E-cigs have been advertised as being healthier, cheaper, and more convenient (Grana & Ling, 2014).

There have been comparisons between E-cig users that use nicotine and those that don't, and the effects on inflammation and susceptibility to Human Rhinovirus(Wu, Jiang, Minor, & Chu, 2014). This study will investigate differences in inflammation between those that use E-cigs and those that use normal cigarettes. This will help with understanding the adverse effects that E-cigs can have on the body. Studies have shown that smoking causes lung cancer and respiratory disease via inflammation; but little research comparing inflammatory markers in E-cig users versus cigarette smokers (van der Vaart et al., 2005). There have been chemical analyses done comparing E-cig to traditional smoking showing potential decrease in the inflammation between the two (Oh & Kacker, 2014).

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RESULTS

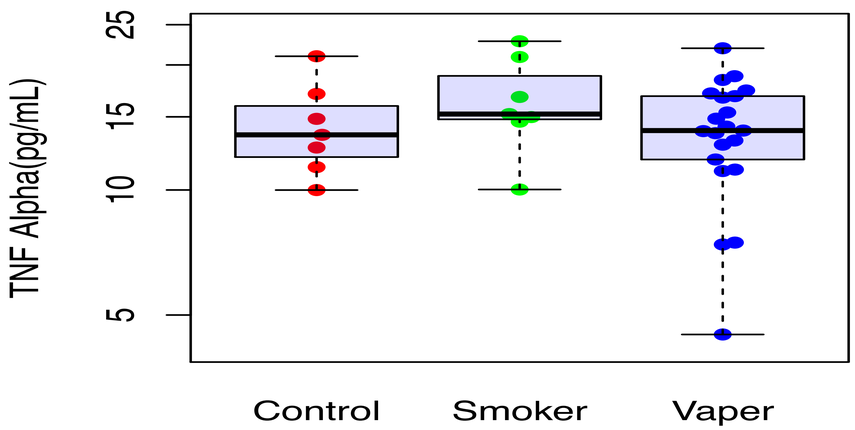

Figure 1. TNF-alpha vs. Sample Groups. TNF-alpha values measured in pg/mL for Control, Smoker, and Vaper groups. Box plot represents distribution of data. p=0.371.

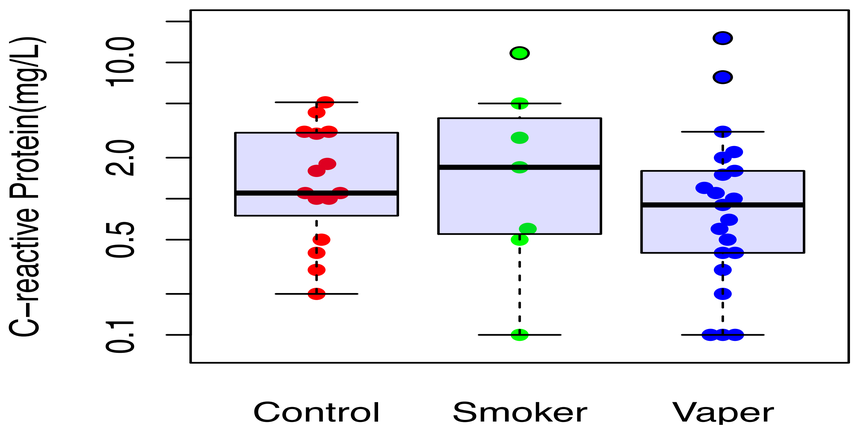

Figure 2. C-reactive Protein vs. Groups. C-Reactive Protein measured in mg/L values for Control, Smoker, and Vaper groups. Box plot represents distribution of data. p=0.59.

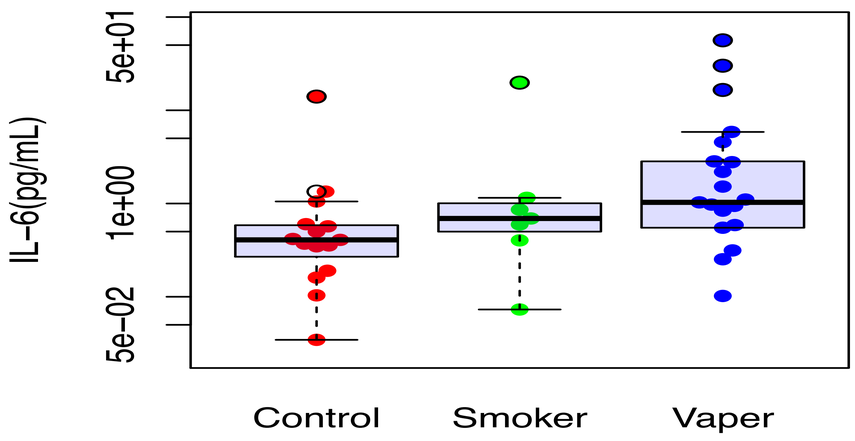

Figure 3. IL-6 vs. Groups. IL-6 values measured in pg/mL for Control, Smoker, and Vaper groups. Box plot represents distribution of data. p=0.38.

METHODS

Blood samples were collected from participants that either currently smoke traditional cigarettes (n=7), vape (n=21), or neither (control [n=15]). 43 participants came from a suburban mountain west geographical area, mainly a university campus, with a mixed gender, and an age range of 18 to 56 years old. Samples were centrifuged to isolate serum for testing, then aliquoted and placed into a -80º C freezer to preserve cytokines until further testing. Testing was done using an Enzyme-linked Immunosorbent Assay (ELISA). 96 micro-well ELISA plates were used to test for specific pro-inflammatory cytokines: Interlukin-1 beta (IL-1 beta), Interlukin-6 (IL-6), and Tumor Necrosis Factor- alpha (TNF-alpha). Controls, samples, and standards were all ran in duplicates. A standard curve was formed and absorption levels were quantitated at a wavelength of 450 nm on the Biotek Epoch 2. In conjunction, C-reactive Protein (CRP) was ran via immunoturbidimetric assay on the Mindray BS-200 with the same procedure as above. The mean pro-inflammatory cytokine levels were compared between groups using an ANOVA ,as well as two sample t-tests.

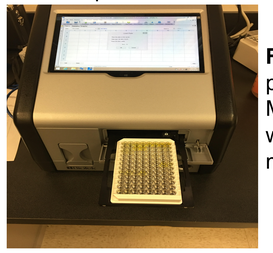

Figure 4. 96 well plate being placed into the Biotek Epoch 2 Microplate Reader. The ELISA was setup and ran according to manufacture specifications.

STATISTICAL ANALYSIS

Groups	TNF-alpha (ANOVA p=0.37)	IL-6 (ANOVA p=0.38)	CRP (ANOVA p=0.59)
Control vs. Smoker	C=7, S=7 95% CI: -6.779 to 2.528 p=0.339	C=15, S=7 95% CI:-5.375 to 1.705 p=0.293	C=15, S=7 95% CI: -3.834 to 1.114 p=0.265
Control vs. Vaper	C=7, V=21 95% CI: -3.215 to 4.053 p=0.814	C=15, V=21 95% CI: -2.794 to 1.809 p=0.624	C=15, V=21 95% CI: -2.048 to 1.833 p=0.911
Smoker vs. Vaper	S=7, V=21 95% CI: -1.197 to 6.268 p=0.174	S=7, V=21 95% CI: -2.722 to 5.557 p=0.488	S=7, V=21 95% CI: -1.992 to 4.497 p=0.435

Table 1. ANOVA and Two Sample t-test of data receive from groups. Two sample t-test analysis done between each of the groups**.** Control (C), Smoker (S), Vaper (V). Alpha of 0.05.

DISCUSSION

ANOVA analysis yield no significant difference between any of the groups. A few limitations that could be the cause for this is: the sample sizes were too small, the smoke/vape time prior to venipuncture was not accounted for, and a few of the samples were not placed into the freezer within 15 minutes. IL-1 beta did not produce any results.