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Title: Acute phase protein and cytokine levels in serum and saliva: A comparison of detectable levels and correlations in a depressed and healthy adolescent sample

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Abstract:

Recent research has examined associations between inflammation and mental health, and has increasingly focused on utilizing younger samples to characterize the temporal relationship between inflammatory responses and the emergence of other symptoms. These studies have typically used blood to measure inflammation, although rates of detection for many inflammatory markers appear to be low. Saliva is a safe and low-cost alternative, and adult research has shown that levels of some salivary markers correlate well with those in serum. However, no research has examined this association in young people. This study examined 16 inflammatory markers in serum and saliva in 17 depressed adolescents and 18 healthy controls, aged 13-18 years. In general, detection rates were higher in saliva compared to in serum. When non-detectable levels were excluded, serum levels of C-reactive protein (CRP) correlated with salivary CRP ($r = 0.424$, $p = 0.015$), and this correlation appeared to only exist for those individuals with high levels of serum CRP ($r = 0.599$, $p = 0.014$). However, when non-detectable levels were included as zero, salivary levels of CRP, interleukin (IL)-2, IL-12p70, and interferon (IFN)- γ correlated with their serum counterparts. No significant clinical group differences in any acute phase proteins or cytokines were present. This study suggests that saliva can be used to measure inflammation in studies with adolescent participants, especially CRP, as it appears to correlate with systemic inflammation for those individuals who are expected to have high levels of inflammation. Implications for future directions in research on salivary inflammatory markers are discussed.

Keywords: C-reactive protein; Saliva; Serum; Cytokines; Acute phase proteins; Adolescence; Depression

Introduction

There is a growing number of research studies measuring secreted markers of inflammation, such as cytokines, from peripheral sites such as blood and saliva, in order to more fully understand the role of inflammation in physical and mental health (e.g., (Arsenault et al., 2009; Danesh et al., 2004; Dean, 2011; Howren et al., 2009; Ridker et al., 2000; Visser et al., 1999). Furthermore, lifespan research in this area has been increasingly interested in utilising younger samples in order to characterize the temporal relationship between inflammatory responses and the emergence of symptoms of other disorders, such as depression (Copeland et al., 2012). The need for comprehensive symptomatic evaluations and longitudinal studies in order to address contemporary questions in this area can often mean that it is necessary to test a large number of variables and research participants, which entails greater challenges in terms of cost and feasibility. Accordingly there are compelling reasons to explore whether less intrusive methods, such as collection of saliva, can be validly used to measure systemic inflammation. This study aimed to first, examine detection rates and correlations of several serum and salivary acute phase proteins and cytokines in an adolescent sample, and second, investigate differences in inflammation between depressed adolescents and healthy controls.

Advantages of examining acute phase proteins and cytokines in saliva.

Compared with blood, saliva is safer and easier to collect in research studies and with the correct protocol, can ease burden for both participants and researchers (Granger et al., 2007; Pfaffe et al., 2011). Although saliva can carry a large amount of bacteria (Sugawara et al., 2002), and must be handled carefully, infectious agents and diseases must also be safeguarded against when handling blood - especially

HIV, Hepatitis B and Hepatitis C - as concentrations of these blood-borne pathogens are higher in blood than in saliva (Shine et al., 1997; Suzuki et al., 2005). Researchers collecting and handling saliva require only minimal personal protective equipment and less training than a certified phlebotomist requires when collecting blood. Furthermore, asking study participants to give saliva will result in easier recruitment as it is less invasive and may also avoid excluding any participants with needle or blood phobias, which is especially a concern in psychiatric and behavioral research.

Second, acute phase proteins (such as CRP) and cytokines (such as IL-6) can be easier to detect in saliva than in other biological samples such as serum or plasma. Studies measuring inflammation in blood are often not able to obtain detectable levels of acute phase proteins and cytokines from all participants (e.g., (Brailo et al., 2012; Gillum, 2003; Lambert et al., 2004; Simon et al., 2008; Wu et al., 2003), although this issue has not typically been explicitly identified as a limitation of this type of analysis in blood. Bioactive cytokines found in saliva can build up over time, allowing levels of salivary proteins and cytokines to be more detectable by assays than those circulating in the blood; however, few studies of acute phase proteins and cytokines in saliva exist (Gilbertson-White et al., 2011). For example, Brailo and colleagues (2012) were able to detect salivary levels of interleukin (IL)-1 β in oral cancer patients, leukoplakia patients, and controls, and report on differences in salivary IL-1 β between these groups. However, only two out of 88 participants (one oral cancer patient and one control) had detectable levels of IL-1 β in sera. Therefore, saliva may actually be a superior medium to measure inflammation compared with blood. In this case, it may not be expected to find a strong correlation between the two, and, consistent with this, not all studies do. For example,

Fernandez-Botran and colleagues (Fernandez-Botran et al., 2011) reported a modest correlation of $r = 0.29$ ($p = 0.02$) between IL-6 in saliva and plasma in one adult sample, and in another sample, found no significant correlation. Other recent research in healthy adults (Williamson et al., 2012) has also found only modest correlations between saliva and plasma for IL-6 ($r = 0.31$; $0.01 < p < 0.05$) and interferon (IFN)- γ ($r = 0.34$; $0.01 < p < 0.05$), and no correlation for a range of other cytokines, although it should be noted that the acute phase protein C-reactive protein (CRP) was not measured in the latter study.

CRP in particular may be representative of inflammation in the entire body (Mirzaii-Dizgah et al., 2012; Ouellet-Morin et al., 2011; Out et al., 2012). One potential concern regarding salivary inflammation could be that the measurement of proteins and cytokines in this fluid may not be as indicative of systemic inflammation as it is in blood. First, there is the possibility that salivary inflammation may be more representative of local inflammation in the oral cavity. For example, in periodontitis, a chronic infection of the connective tissues that undermines the supporting tissues of the teeth, gingival cells in the mouth have been shown to produce cytokines (Huang et al., 1998; Sugiyama et al., 2002). Adolescents, however, are less likely than adults to have advanced stages of an oral disease such as periodontitis, which would contribute to substantial amounts of local oral inflammation. The current study draws on the benefit of utilising an adolescent sample for this reason.

The second concern is that, while results in blood samples represents circulating levels of these proteins (i.e., systemic levels of inflammation), there is the possibility that saliva, which comes from salivary glands, does not contain the same proportions of acute phase proteins and cytokines as in blood. Saliva also contains gingival crevicular fluid (GCF), a fluid of systemic origin containing a number of

biochemical markers, and CRP levels in the GCF of periodontal patients have been shown to be indicative of systemic inflammation rather than simply the result of local production of CRP by gingival cells (Megson et al., 2010). CRP is a liver protein that activates the complement system, which assists the immune system in killing and clearing pathogens from the body. It has been recognised as an inflammatory marker that increases rapidly after infection or tissue damage, and is part of the body's systemic inflammatory response (Black et al., 2004). CRP and other proteins can also pass through blood to saliva in other ways (Pfaffe et al., 2011), including diffusion through the porous capillaries around the salivary glands, or through a process called ultrafiltration, which is filtration through the spaces between salivary gland cells. Most importantly, recent research with adult samples has shown that salivary CRP correlates well with serum CRP in adults. A study of 61 men and women showed a strong correlation of CRP in saliva and serum ($r = .72$, $p < .001$), and high levels of salivary CRP were associated with serum IL-6, BMI and smoking (Ouellet-Morin et al., 2011). Another more recent study showed similar results: salivary CRP in 107 adult women correlated with levels of CRP in plasma both cross-sectionally at three time points ($r = .53$, $r = .38$, $r = .49$, all $p < .01$) and longitudinally across time points over two years (correlation coefficients ranging from $r = .20$, $p = .04$ and $r = .39$, $p < .01$) (Out et al., 2012). Finally, acute phase proteins in saliva can indicate non-oral diseases. Elevated salivary CRP in patients directly after acute myocardial infarction (MI) has been shown to correlate with elevated serum levels of CRP after acute MI, for both unstimulated and stimulated (i.e., after chewing gum) saliva (Mirzaii-Dizgah et al., 2012), thus, salivary CRP may be a feasible screening tool for acute MI in adults. Furthermore, research has also shown that

other cytokines, such as IL-6, correlate well between saliva and blood in adult patients with ulcerative colitis (Nielsen et al., 2005).

However, as noted above, some studies find only a modest correlation between salivary and blood cytokines. If detection rates are also higher in saliva compared to blood, then it is still possible that oral inflammation, produced locally, is contributing significantly to levels of acute phase proteins and cytokines in saliva. Therefore, levels of salivary inflammation should not always be treated as equal to the rest of the periphery without controlling for known levels of oral inflammation. Nevertheless, it should be noted that levels of local oral inflammation may be particularly salient in behavioral research as there are neural pathways connecting the inflammatory environment in the mouth directly to the brain (Navarro et al., 2006), and inflammatory markers measured in the mouth have been shown to be associated with both acute and chronic stress (Deinzer et al., 2005; Waschul et al., 2003).

Serum/saliva correlations in younger samples. The comparison of salivary and serum proteins and cytokines among young people has not yet been examined. Only one study has measured salivary CRP in young people, and none have measured both salivary and serum CRP. Azar and colleagues examined salivary CRP in 45 healthy young people in the first year of university (mean age = 18.89 years; therefore, slightly older than adolescence) and found an association between the amount of tobacco smoking and salivary CRP levels (Azar and Richard, 2011). The current study contributes to this new area of research by examining the correlation of serum and salivary acute phase proteins and cytokines, which has not yet been reported in child or adolescent samples. If these markers in saliva can be shown to correlate with circulating blood levels in adolescents, this would have

important implications for inflammatory research in youth due to the relatively non-invasive nature of saliva collection, improving sample size, and variety of diseases examinable.

The current study aims to first, describe the detectable levels of 16 separate acute phase proteins and cytokines in both serum and saliva to ascertain if either is a more useful medium to measure inflammation in adolescents, and second, examine correlations between serum and salivary acute phase proteins and cytokines. In particular, due to findings from previous adult research (Mirzaii-Dizgah et al., 2012; Ouellet-Morin et al., 2011; Out et al., 2012), CRP was predicted to show an association between its serum and saliva counterparts. Furthermore, the current study utilises a multiplex approach. In general, many studies examining inflammation and mental or physical health have had a relatively narrow focus. In the past, the reason for this narrow focus was mainly due to cost and efficiency. However, new, more modern assays, such as Multiplex Bead Array Assays (Elshal and McCoy, 2006) as opposed to ELISAs (Remick, 1997), can now look at acute phase proteins and cytokines on a much broader scale than in the past. The current study is able to examine a multitude of these markers in saliva and serum. Therefore, we conducted exploratory analyses of correlations between all serum and salivary markers, as this has not been reported on in previous research.

Inflammatory markers in depression. Finally, much adult research has shown a relationship between clinical depression and the circulating pro-inflammatory markers CRP (Howren et al., 2009), IL-1 α (Howren et al., 2009; Simon et al., 2008), IL-2 (Jozuka et al., 2003; Simon et al., 2008; Sutcgil et al., 2007), IL-6 (Dowlati et al., 2010; Howren et al., 2009), IL-8 (Simon et al., 2008), IL-12 (Simon et al., 1995; Sutcgil et al., 2007), IFN- α (Bonaccorso et al., 2002; Cai et al., 2005;

Capuron et al., 2000; Lotrich et al., 2007), IFN- γ (Pavón et al., 2006; Simon et al., 2008), tumor necrosis factor(TNF)- α (Dowlati et al., 2010), haptoglobin (Maes et al., 1993), and alpha-2-macroglobin (A2M) (Fujita et al., 2003; Tsiouris et al., 2000), as well as anti-inflammatory markers IL-4 (Sutcgil et al., 2007), IL-10 (Dhabhar et al., 2009), and IL-13 (Hernández et al., 2008; Pavón et al., 2006). Fewer studies have examined the pro-inflammatory cytokine IL-17A, but it has been shown to be associated with anxiety (Liu et al., 2012). Serum amyloid P (SAP) has not yet been examined in depression but it is an acute-phase protein related to CRP, haptoglobin, and A2M (Tsiouris et al., 2000).

Less work has been done with younger samples. Some studies show increased inflammation in depressed adolescents (Brambilla et al., 2004; Capuron et al., 2000; Gabbay et al., 2009), however, other studies have found no such association in this age group (e.g., (Chaiton et al., 2010)). Therefore, the current study examined differences in the serum and salivary acute phase proteins and cytokines discussed above, between a group of depressed adolescents and healthy age- and sex-matched controls recruited from the community. Pro-inflammatory markers were expected to be elevated in depressed adolescents compared to controls, while anti-inflammatory markers were expected to be decreased.

Methods

Participants. This study involved participants from the Sleep, Mood and Heart Health Study (SMHHS), a cross-sectional research project conducted at the Sleep Laboratory in the Department of Psychological Sciences at The University of Melbourne, Australia during 2010-2012. This study evaluated the sleep and cardiovascular health of adolescents with symptoms of anxiety and depression aged 12-18 years compared to adolescent peers with no lifetime history of psychopathology. The overarching aim of the project was to investigate potential relationships between disrupted sleep, depression and anxiety and the future development of cardiovascular problems during adulthood.

Three groups of participants were recruited from high schools across metropolitan Melbourne based on questionnaire scores of anxiety and depressive symptoms: High anxious (not depressed) group, a clinically depressed group, and matched controls. This study examined a subsample of participants from two groups from that study: the clinically depressed group and age- and sex-matched healthy controls.

Phase I: Screening. Phase I of the study involved researchers administering the Center of Epidemiologic Studies Depression Scale, CES-D (Radloff, 1977, 1991); a self-report questionnaire of depressive symptoms to a total of 889 high school students (352 males, 39.6%). The CES-D has been used in both adult (Morin et al., 2011) and adolescent community samples (Roberts et al., 1990), as well as in medical patient samples (Kim and Park, 2012; Thombs et al., 2008). Furthermore, it has been shown to have good reliability in the age group of the current study (Chabrol et al., 2002; Dierker et al., 2001; Radloff, 1991) and has been used before

in adolescent samples to discriminate between those that have a depressive illness and those that do not (Prescott et al., 1998). Fourteen secondary schools from metropolitan Melbourne, Australia, out of 50 randomly selected schools, consented to take part in the SMHHS. Seven of these schools were public state (government) schools, three were private Catholic schools, and four were private independent schools.

Phase II: Diagnostic interview. Two hundred and four participants (45 males, 22.1%) out of the 889 total participants (22.9%) that completed the screening CES-D had scores above the “clinical cut-off”, a score considered likely to be indicative of the presence of a DSM-IV depressive disorder. Although a clinical cut-off score of 16 has been used for adults (Radloff, 1977), we used a cut-off score of 22 and above to predict likely cases of depressive illness, which is consistent with adolescent research using this measure (Chabrol et al., 2002; Cuijpers et al., 2008). Participants with high CES-D scores were approached and invited to participate in the diagnostic interview and blood test (if eligible), and 74 possible clinical participants consented to participate in Phase II. Thirty-seven sex-and age- matched participants with CES-D scores of 12 and below were approached as possible controls and consented to participate in the diagnostic interview, the Schedule for Affective Disorder and Schizophrenia for School-age Children, Epidemiologic Version (KSADS; (Orvaschel and Puig-Antich, 1994). All interviews were conducted by three trained researchers under supervision of the principal investigator, a clinical psychologist, who met with researchers once a week to discuss symptoms and diagnoses. Approximately 20% of interviews were double-scored by another researcher, and inter-rater reliability was calculated by the author at the item level, including symptoms and diagnoses, using the kappa (κ) statistic (Cohen, 1960;

Fleiss, 1971). For this study, the average κ was 0.90, which indicates excellent inter-rater reliability at item (both diagnostic and symptom) level.

Phase III: Saliva and blood collection. Of the potential clinical participants that completed the KSADS, 31 had a current DSM-IV depressive illness and were eligible to participate in Phase III, the biological and immunological assessment, as part of the clinical group. Reasons for ineligibility for the clinical group included not having any current or past depressive illness (30 participants), having a past depressive episode but no current episode (12 participants), or having a medical illness that would affect cardiovascular or immunological functioning (1 participant; hypertension). Of these, 13 declined to give a blood sample, resulting in a total clinical sample size of 18 participants (4 males), with 16 participants having a current episode of MDD and 2 participants (both female) having a current episode of Depression Not Otherwise Specified (DNOS). Twenty-five control participants were eligible after completing the KSADS, and 18 age- and sex-matched controls, matched within eight months of age of the clinical participants, consented to giving blood and saliva samples. Exclusion criteria for the control group included any current or lifetime Axis-I psychopathology (4 participants) or medical illnesses that would affect cardiovascular or immunological functioning (1 participant; Postural orthostatic tachycardia syndrome). A total of 36 adolescents aged 13-18 years (mean age = 16.33 years; 28 females, 8 males) consented to give a blood and saliva sample, which took place at the University of Melbourne and the Royal Melbourne Hospital, Melbourne, Australia.

As a basic measure of dental health, participants were asked “Have you ever noticed bleeding from your gums when not brushing?” No participants endorsed this item.

Informed consent was obtained from the participants, as well as a legal guardian if the participant was under the age of 18 at the time of the assessment. This study was approved by the Human Research Ethics Committee at The University of Melbourne, Australia, and recruitment, which was conducted in schools, was also approved by the Department of Education and Early Childhood Development or The Catholic Education Office (depending on if the school was a state government school or a private Catholic school) in the state of Victoria, Australia. Participants were informed that they could cease participation at any time.

Saliva and blood collection. Immunological measures were examined in saliva and blood. All samples were collected between 9 am – 12 pm to control for possible diurnal variations. Saliva tubes had 300 μ L of protease inhibitor cocktail (“Complete, Mini”, Roche, Castle Hill; NSW, Australia) added before saliva was collected. Saliva was collected by the researcher by the passive drool method, in which participants are instructed to let saliva pool in the mouth before drooling into a tube. Participants were fasting the morning of the assessment and did not smoke or chew gum in the 30 minutes before saliva collection. Approximately 2 mL of whole saliva was collected and was kept on ice until processing. Blood samples were collected by a trained phlebotomist at the Royal Melbourne Hospital, Melbourne, Australia. 3.5 mL total blood was collected to analyse serum in an SST vacutainer (BD, Franklin Lakes, NJ, USA, www.bd.com) designed to produce clotting.

Data analysis

Saliva samples. Saliva samples were kept on ice until preliminary processing within two hours of collection. Saliva was centrifuged at 10,000 g at room temperature (24°C) for 10 minutes to remove cells and mucus. The collected supernatant was then aliquoted into separate eppendorf tubes of 300 μ L each and

stored at -70°C until further processing (1 – 12 months). On the day of analysis, samples were thawed to room temperature, and centrifuged again at 10,000 g for 10 minutes. Pilot testing showed that a second centrifugation resulted in much lower viscosity, with less likelihood of clogging the Bio-Plex suspension array system. Furthermore, the lower viscosity enabled us to analyse the samples without further dilution with the Bio-Plex immunoassays. Samples had a total of only one freeze/thaw cycle. Multiplex bead array assay technology such as Bio-Plex has been used to examine salivary cytokines in previous research (Arellano-Garcia et al., 2008; Thorman et al., 2010).

Serum samples. Blood samples were inverted 5 times directly after collection and allowed to clot for 30-120 minutes at room temperature. Serum was separated from the clot by centrifugation at room temperature (24°C) for 15 minutes at 1500 g and 250 μL serum aliquots were stored at -70°C until further processing. On the day of analysis, serum samples were thawed to room temperature and diluted 1:10,000 for the acute-phase protein immunoassay and 1:4 for the cytokine immunoassays, as per the Bio-Plex assay manufacturer instructions.

Determination of acute-phase proteins and cytokines using Bio-Plex assays. Cytokine (IL-1 α , IL-2, IL-4, IL-6, IL-8, IL-10, IL12p70, IL-13, IL-17, IFN- γ , TNF- α , IFN- α 2) and acute phase protein (A2M, CRP, haptoglobin, SAP) analysis was done according to manufacturer's instructions by the Bio-Plex multiplex bead array immunoassay system of human cytokine panel and plates read on Bio-Plex Array Reader (Bio-Plex 200 System and Bio-Plex Manager Version 4.0, Bio-Rad Laboratories, Inc., New South Wales, Australia), using the xMAP detection technology. Saliva samples were not diluted, whilst serum samples were diluted 1:10,000 with human serum sample diluent for acute-phase proteins and 1:4 for

other cytokines and then added in duplicate to 96-well microplates, already containing antibody-coupled beads. The beads then reacted with the samples (i.e., the unknown amount of cytokine) as well as with a standard solution containing a known amount of cytokine. The Bio-Plex cytokine standards were reconstituted with 50 μ L Bio-Plex human serum standard diluent (according to manufacturer's instruction). Samples were then incubated (30 min) and washed with Bio-Plex wash buffer (3 x 100 μ L) to remove unbound protein using vacuum filtration. A 25 μ L aliquot of 1 x concentration of Bio-Plex biotinylated detection antibody specific for a different epitope on the cytokine was added to each well, incubated (30 min), and subsequently washed with Bio-Plex wash buffer (3 x 100 μ L) using vacuum filtration. The reaction mixture was detected by streptavidin-phycoerythrin (streptavidin-PE), which binds to the biotinylated detection antibodies. The assay was developed in a 50 μ L aliquot of 1 x concentration of streptavidin-PE (10 min), followed by a Bio-Plex wash buffer (3 x 100 μ L) using vacuum filtration. Beads were resuspended in each well with 125 μ L of Bio-Plex assay buffer and shaken well on a plate shaker (1,100 RPM, 30 seconds). The components of each well were drawn up into the flow-based Bio-Plex suspension array system, which identifies each biochemical reaction based on bead colour and fluorescence. The reaction was measured using fluorescently labelled reporter molecules associated with each target protein. Intensity of fluorescence detected on the beads indicates the relative quantity of targeted molecules. Cytokine or protein concentrations in the samples were calculated by Bio-Plex software using a standard curve derived from a recombinant cytokine standard, also added to the 96-well plate.

Intra-assay Coefficient of Variability (%CV) was calculated for all inflammatory markers to assess the precision of the immunoassay test results. The average %CV

was 14.56%. For salivary hormones such as cortisol, usually %CVs of <10% are recommended (Schultheiss and Stanton, 2009), and this level is generally reported in studies of cytokine analysis in blood, as well. However, the %CVs for salivary cytokines may be slightly higher, as studies have reported %CVs of “less than 20%” in duplicate (Ouellet-Morin et al., 2011). Furthermore, in this study, all acute phase proteins (salivary and serum A2M, CRP, Haptoglobin, and SAP) had %CVs of 10% or less (average approximately 9%). For all assays, the test volume was 50 μ L.

Correlations and group differences. All statistical transformations and analyses were conducted with IBM SPSS Statistics for Windows, version 19 (SPSS Inc., Chicago, IL, USA). All reported p-values are exact two-sided significance levels. Statistical significance was defined as $p < 0.05$.

Correlations were analysed in several ways. Firstly, Spearman’s correlations between continuous measures of salivary and serum in the four acute-phase proteins (A2M, haptoglobin, CRP, and SAP) and IL-8 (from which there were enough detectable levels) were calculated as per an earlier study which examined correlations between salivary and serum CRP in adults (Ouellet-Morin et al., 2011). Correlations were computed on raw (non-transformed) data. Outliers were included in this first analysis in order to determine the sensitivity of the results to deviations from the normal distribution.

Secondly, to account for outliers that may have possibly driven correlations, Pearson’s correlations were calculated on \ln transformed and winsorized (i.e., replaced with the next highest value to 0.01 of the remaining distribution) data, as in the Out and colleagues (2012) study.

To further explore significant correlations, the strength of the correlation was examined separately for participants above the median and below the median of the

protein level, in order to determine if the association was dependent on the level of systemic inflammation, as in the Ouellet-Morin and colleagues (2011) study. A dichotomous score of high serum or low serum was calculated for each person depending on if they were above or below the median serum value.

Finally, as the literature has not yet come to a conclusion regarding the inclusion of non-detectable values of acute phase proteins and cytokines, correlations were re-analysed with non-detectable values replaced as 0 (unless the values were not detectable because they were too high, in which case they remained missing values).

Group differences between depressed adolescents and controls were calculated using one-way between-group ANOVAs to assess differences in means of *ln* transformed and winsorized cytokine values. This was done separately for data with non-detectable values excluded, and again with non-detectable values replaced with 0.

Results

Medication / substance use. The sample size was too small to exclude participants that had taken medication in the 24 hours prior to testing (10 people), however one participant took medication during the 8-hour fast (a selective serotonin reuptake inhibitor) and was excluded from further analyses, resulting in a final sample size of 35 (17 depressed, 18 controls). Twenty-five participants had abstained from alcohol over the past month, three had consumed one standard drink, four had consumed two to nine drinks, and three had consumed 10 to 15 drinks. Furthermore, no participants reported taking any illicit substances in the past month. Only one participant reported being a “regular” smoker and reported smoking on average 2 cigarettes per week.

Detectable levels of acute phase proteins and cytokines. Table 1 reports the number of subjects for which the assay could detect levels of a particular acute phase protein (A2M, CRP, haptoglobin, SAP) or cytokine (IL-1 α , IL-2, IL-4, IL-6, IL-8, IL-10, IL-12p70, IL-13, IL-17, IFN- γ , TNF- α , IFN- α 2) in saliva and serum. Most undetectable values were too low for the assay to detect, with the exceptions of salivary and serum A2M and haptoglobin, for which some levels were too high. In saliva, the lowest rates of detection were 60% for IL-10 and 49% for IFN- α 2. All other markers in saliva were detected in at least 83% of samples, with six markers (SAP, IL-1 α , IL-6, IL-8, IL-13, and TNF- α) detected in 100% of samples. No participants had undetectable levels for all salivary markers, even for non-acute-phase proteins. In general, CRP, A2M, SAP and haptoglobin (acute-phase proteins) were successfully detected in both saliva and serum (>49%). However, for the remaining 12 cytokines assayed in serum the detection rate was less than 20% with the

exception of IL-8, which was detected in 49% of serum samples. Interestingly, IL-6 was detected in all saliva samples but only in 6% of serum samples, and although IFN- α 2 was not detected in any of the serum samples, 49% of saliva samples had detectable levels. No participants had undetectable levels for all serum markers, as in saliva; however, 11 out of 35 participants had undetectable levels for all cytokines in serum (i.e., all of the interleukins, interferons, and TNF- α assayed).

A Wilcoxon signed-rank test showed that levels (shown in Table 2) of the acute-phase proteins (A2M, CRP, haptoglobin, and SAP) were significantly ($p < 0.01$) higher in serum than in saliva, which was also the case for IL-10 ($p < 0.05$). However, IL-2, IL-4, IL-6, IL-12p70, IL-13, IL-17, IFN- γ , and TNF- α had comparable ($p > 0.05$) levels in serum and saliva. IL-1 α ($p < 0.05$) and IL-8 ($p < 0.01$) were found to have significantly higher levels in saliva compared to serum levels.

We also explored whether participants that had detectable levels in sera had elevated levels of the same acute phase protein or cytokine in saliva compared to participants that did not have detectable sera values of that marker. However, t-tests showed no significant differences in mean values of saliva between these two groups for any acute phase protein or cytokine.

As noted above, the literature has not yet come to a conclusion regarding inclusion of samples with non-detectable levels of acute phase proteins and cytokines, thus analyses with and without these data are presented in this study. Firstly, the mean, standard deviation and ranges in the sample (ng/mL), with non-detectable samples excluded are presented in Table 2. While some would consider samples with non-detectable levels erroneous and thus exclude them, it is also possible that non-detectable levels in a sample simply indicate that the level of that acute phase proteins or cytokine is very low or non-existent, which is plausible in

medically healthy individuals. Therefore, the second set of data, presented in Table 3, includes non-detectable values represented by 0 (except for salivary and serum A2M and haptoglobin, which were still treated as missing data as the values were too high for the assay to detect). This second set of data not only includes more samples, but the means, standard deviations, and ranges are noticeably different when including data that may not necessarily be erroneous.

Correlation between serum and salivary acute-phase proteins and cytokines. For raw data using nonparametric testing, when non-detectable levels were excluded, serum levels of CRP only correlated with those of salivary CRP, although there was a trend correlation for IL-8 (refer to Table 4). For transformed, winsorized data using parametric testing, results were similar, and the correlation between serum and salivary CRP was stronger (refer to Table 5). No other acute-phase proteins, or IL-8, showed an association between the salivary and serum versions. Cytokines other than IL-8 were not included in these analyses because rates of detection were less than 50%.

After separating participants into high or low raw serum CRP scores based on a median split (7338.66 ng/ml), nonparametric testing on raw data showed that those with high serum CRP scores had a significant association of serum CRP and salivary CRP, but those with low serum CRP scores did not have such an association (refer to Table 6 and Figures 1 and 2), suggesting that the correlation of serum and salivary CRP levels exists only at higher serum levels in adolescents, even though these levels may not be clinically significant for diseases such as cardiovascular disease. Parametric testing on *ln* transformed and winsorized data showed similar results (refer to Table 7 and Figures 3 and 4).

When non-detectable levels were included as levels of 0, nonparametric testing on raw data showed that salivary levels of CRP, IL-2, IL-12p70, and IFN- γ correlated with their serum counterparts (refer to Table 8). There were trend correlations for IL-4 and TNF- α . Parametric testing on transformed, winsorized data showed similar results; however, IFN- γ the correlation was a trend only, while CRP, IL-2, and IL-12p70 correlations remained significant (refer to Table 9).

Clinical group differences. One-way ANOVAs on data with non-detectable values excluded showed no significant differences between depressed and control adolescents for any serum or salivary acute-phase protein or cytokine. There was a trend difference for salivary TNF- α [$F(1,33) = 2.979$, $p = 0.094$] and salivary IFN- $\alpha 2$ [$F(1,15) = 3.379$, $p = 0.086$], with controls exhibiting elevated levels.

Data with non-detectable values included as 0 also showed no significant differences, and only trend differences between depressed and control adolescents for salivary IL-17A [$F(1,33) = 3.596$, $p = 0.067$], salivary TNF- α [$F(1,33) = 2.977$, $p = 0.094$], serum IL-4 [$F(1,33) = 3.283$, $p = 0.079$], and serum IFN- γ [$F(1,33) = 3.521$, $p = 0.069$]. Controls displayed higher means than depressed for all the above cytokines. It should be noted there were no depressed adolescents that had detectable levels of serum IL-4, which is anti-inflammatory.

Discussion

In a sample of adolescents aged 13-18, there were no differences in acute phase proteins or cytokines between depressed adolescents and healthy age- and sex-matched controls. However, there were trend differences for some markers depending on whether non-detectable levels were either excluded or replaced with 0. This is incongruent with adult research, which has consistently shown that levels of IL-6, TNF- α , and IL-1 α are elevated in depressed persons (for review, see (Dowlati et al., 2010; Howren et al., 2009). Therefore, these findings could suggest that inflammatory markers are not associated with depression status as early as adolescence, or that being young is protective against these effects even amongst depressed persons.

However, other child and adolescent research in this area has shown an association between depression and inflammation, including in salivary measures of inflammation (Keller et al., 2010). Nevertheless, all of the previous research with younger samples comparing inflammation in depressed and healthy controls (two-group design) has relied on patient samples (Brambilla et al., 2004; Gabbay et al., 2009; Henje Blom et al., 2011; Rojas et al., 2011), whereas the current study recruited participants from the community. Copeland and colleagues (2012) and Keller and colleagues (2010) recruited adolescents from the community, but these studies did not employ a two-group methodology of depressed adolescent and matched healthy controls. The current study is the first to do so with a community-based sample. This is important given that depressive symptoms amongst a clinical sample of people that are in treatment are more likely to be of unusual or non-representative severity and chronicity (Cohen and Cohen, 1984). In other words, a

clinical sample may have more severe depression or have more treatment-seeking characteristics compared to a community sample. The participants in the depressed group of the current study are therefore more likely to be representative of depressed adolescents in the general population, and as such we can more strongly attribute any differences in immune functioning to the presence of depression per se, rather than any confounding demographic or lifestyle factors.

The main limitation of this study for analysing these between-group differences is lack of power due to the small sample size. Although the results showed correlation of some serum and salivary acute phase proteins and cytokines, the sample may have been too small to detect differences between clinical groups, which may explain why these findings are inconsistent with previous research on inflammation and adolescent depression. Furthermore, the groups were too small to be able to explore correlations between serum and saliva within each group. Therefore, future research should examine whether or not systemic inflammation can be measured in saliva generally. In other words, it is possible that salivary inflammation is systemic only for certain groups (such as in depression or medical diseases where systemic inflammation is expected to be elevated).

Additionally, in this study, levels of 16 different acute phase proteins and cytokines were detectable at a rate of 49% or greater in saliva (14 of the 16 had a \geq 83% detection rate). However, in serum, only acute phase proteins and IL-8 were detectable at 49% or greater, with 11 of the 16 markers having a \leq 17% detection rate. Although the percentages of detectable levels of acute phase proteins and cytokines are rarely explicitly reported, some evidence suggests that low levels of detection in blood may be common for both ELISA and multiplex bead array assay methods. For example, in a study examining levels of IFN- γ and IL-4 in the plasma of

depressed adult inpatients and controls using ELISA, Myint and colleagues (Myint et al., 2005) found that IFN- γ was detectable in 18 out of 40 patients (45%) and only four out of 80 healthy controls (5%), while IL-4 was detectable in 38 patients (95%) and 42 controls (53%). The authors only included patients and controls with detectable cytokine levels in analyses, but this resulted in a sample size of 38 patients and only three controls. Results showed that patients had higher ratios of IFN- γ /IL-4 compared to controls, but no differences in levels of IFN- γ or IL-4 compared to controls. If the full sample had been included, the study would have had greater power to detect differences between patients and controls. Another study comparing 20 cytokines in 49 adults with MDD and 49 healthy controls using a multiplex bead array assay (Simon et al., 2008) did not explicitly state the number of detectable cytokines in each group. However, in exploratory analyses examining correlations among cytokines, results showed that 13 out of 13 MDD patients with detectable levels of IL-4 had six or more elevated pro-inflammatory cytokines, which suggests that only 13 out of the 49 MDD patients (27%) had detectable levels of IL-4. Similarly, 14 out of 17 MDD patients with detectable levels of IL-10 had six or more elevated pro-inflammatory cytokines, which suggests that the detection rate was 35%.

It is commonly found that a large proportion of samples from healthy children and adolescents have undetectable serum and plasma acute phase protein levels, including high-sensitivity CRP (hs-CRP), using another type of automated protein analysis, rate nephelometry (Roberts et al., 2000), even though a higher-sensitivity CRP assay should be able to detect lower levels of CRP. For example, Lambert and colleagues (Lambert et al., 2004) found that 32-50% of healthy children and adolescents aged between 9 and 16 years had undetectable plasma CRP levels,

while Wu and colleagues (Wu et al., 2003) found similar results for healthy adolescents aged 13 years. Another study of younger children aged 6-11 years found that only 11% had detectable levels of serum CRP (Gillum, 2003). Thus it appears that currently while it is accepted that often the majority of healthy individuals will have undetectable levels of CRP, there is no clear conclusion regarding the handling of “undetectable” acute phase protein values, or if there is a threshold for the number that must be detectable in order for there to be a valid variable used in analyses. It is possible that levels of some markers are not detectable simply because they are not present, which may not be unusual, especially in healthy controls. Perhaps poor detection of CRP in healthy controls reflects the normal serum state, rather than an artefact that should be removed from analysis. On the other hand, although samples were assayed in duplicate, which is standard, there is still the possibility that non-detection is representative of an error in the assaying process. For example, our results did not show elevated saliva levels for those with detectable serum levels compared to those with non-detectable serum levels. Manufacturer’s instructions indicate that serum should be diluted 1:4, and so it may follow that this dilution could affect detection levels such that a reading of zero does not necessarily represent a zero concentration of inflammatory markers, unless the assay is repeated for confirmation. However, this dilution has been taken into account when measuring proteins and cytokines in sera and validated in the assay using standards. Nevertheless, until bio-behavioral research begins to consistently report on this information, it may be premature to draw a firm conclusion about the most effective way to handle non-detectable data. The high detection rate of all measured acute phase proteins and cytokines in saliva compared with the low

detection rate in sera in the current study, however, points to a promising direction of investigating inflammation in saliva that may circumvent these problems.

Furthermore, in the current study, salivary and serum CRP were significantly associated, although this association was driven by subjects whose levels were high, as only those with serum CRP levels above the median showed a significant association with salivary CRP, whereas those with low serum CRP levels showed no such association. This suggests that salivary CRP can be used as a valid measure of systemic inflammation in adolescents, especially amongst adolescent groups with high levels of CRP (i.e., those who are unwell or would otherwise be expected to have elevated levels). This has important implications for future bio-behavioral research. For example, high levels of salivary CRP in adolescents could be further investigated as a marker for possible depression in young people, as other studies have shown that CRP in sera is associated with depression in children (Lambert et al., 2004), adolescents (Copeland et al., 2012), and young adults (Elovainio et al., 2006). Additionally, other research with adults has found that salivary levels of IL-6 correlate with levels in sera, but only for patients with ulcerative colitis, where systemic levels of inflammation are expected to be high (Nielsen et al., 2005). Finally, when including non-detectable levels of inflammatory markers, this study found that CRP, IL-2, IL-12p70, and IFN- γ correlated with their serum counterparts, meaning that several markers in saliva may be able to be used as a measure of systemic inflammation in adolescents, not just CRP. This is important because elevated levels of these inflammatory markers in blood have been shown to be related to early stages of several diseases such as rheumatoid arthritis (Deane et al., 2010), cardiovascular disease (Correia et al., 2010), and malaria infections (Hermsen et al., 2003). Detection of these and possibly other inflammatory markers

in saliva may, therefore, be a useful screening tool for these diseases. However, not all acute phase proteins and cytokines showed an association between saliva and serum. This may be due to inflammation in saliva and serum responding to stress in different ways. Therefore, future research should examine these associations in a stress paradigm rather than just as basal levels, especially because, as discussed earlier, oral inflammation in particular has been shown to be associated with both acute and chronic stress (Deinzer et al., 2005; Waschul et al., 2003).

Additionally, this study utilised a multiplex bead array assay to measure inflammatory markers in saliva and serum samples. Other research examining these markers in blood using multiplex bead array assays have also reported low detection rates. For example, Wong and colleagues (Wong et al., 2008) used a fluorescent bead-based Luminex immunoassay kit to measure 13 cytokines in the serum of 38 adults. Of the cytokines measured, IL-1 β , IL-2, and IL-5 were not detectable in over half of the samples. Therefore, the current study shows that saliva may be a promising biological material for examining levels of cytokines using multiplex technology as well as ELISA.

Another limitation of this study is that there were substantially more females than males in the sample (28 females, 8 males). Given the higher rates of depression in females (Jorm, 1987; Piccinelli and Wilkinson, 2000), this is a predictable result of screening for depressed participants, and the sex matching of the healthy sample to that group. Therefore, it cannot yet be determined in adolescent samples if the correlation between salivary and serum CRP exists for both males and females, or only for females. While our study is female-biased, it is supported by the female-only study by Out and colleagues (2012), which showed strong associations between salivary and plasma CRP levels. Furthermore, the

sample from the Mirzaii-Dizgah and colleagues (2012) study, also showing a correlation between salivary and serum CRP levels, included more male than female adults (19 male patients, 9 female patients, 18 male controls, and 10 female controls). Nevertheless, future studies should have larger sample sizes and include enough male and female participants to be able to examine effects by sex. This is particularly important because this study was not able to control for menstrual cycle or pubertal status, either, and pubertal hormones are known to affect immune functioning (Cutolo and Wilder, 2000), as is menstrual cycle phase (Schwartz et al., 2000). Low power also prohibited this study being able to exclude participants taking oral contraceptives.

Another limitation is the window of time in which saliva and blood was collected (9 am – 12 pm). Although this was only a three-hour period, variations in acute phase proteins and cytokines could still be present. Future studies using saliva should consider collecting samples in a narrower time-window, for example, directly after waking, or at multiple time points across the day. Alternatively, sample size should be large enough to be able to include collection time as a covariate, which this study was unable to do. Although some acute phase proteins, such as CRP, do not appear to have a diurnal variation (Meier-Ewert et al., 2001; Miles et al., 2008), other cytokines, such as IL-6, do vary diurnally (Miles et al., 2008; Sothorn et al., 1995). In fact, one study showed that plasma IL-6 in depressed patients was elevated at certain times of the day compared to controls, and furthermore, the circadian rhythm of IL-6 was shifted by 12 hours compared to controls (Alesci et al., 2005). IL-6 and CRP are related; IL-6 is a determinant of production of CRP, the latter of which is released by the liver (Heinrich et al., 1990; Pepys and Hirschfiel, 2003). IL-6 also stimulates immune cells to secrete a number of other cytokines,

including IL-10, IL-13, IL-5, IL-7, and granulocyte macrophage colony stimulating factor (Fernandez-Botran et al., 2011). Therefore, due to the fact that diurnal variations appear to exist for IL-6, and IL-6 can affect levels of many other acute phase proteins and cytokines, time of day of collection is important. Additionally, there may be a time delay in levels in saliva compared to blood, and so multiple samples collected across the entire day would be beneficial. Further research, therefore, is warranted to examine changes across the day. However, collection of multiple samples would be easier to do with saliva compared to serum, especially given the difference in detection rates found in this study in saliva compared to serum.

Finally, this study only employed a very basic measure of oral health by asking participants if they had any gum bleeding, and did not include a clinician rating of oral health, which would have been more robust. This is particularly important for studies wishing to examine salivary inflammation. Although a young adolescent sample is less likely than adults to have the type of severe gum or other dental disease that would affect local inflammation in the mouth, adolescents can sometimes experience inflammatory periodontal diseases such as gingivitis and early onset periodontitis, and recent studies have suggested that gingival inflammation may be associated with salivary biomarkers (Lee et al., 2012). Due to the potential confounding association of salivary inflammatory markers with gingival inflammation, future studies, especially with adults or populations where mouth disease is likely, should incorporate a measure of dental health or include a dental examination to control for local and gingival inflammation. However, as discussed earlier, oral inflammation itself may be important to examine in behavioral research.

Conclusions. Overall, these findings suggest that some acute phase proteins and cytokines can be validly measured in saliva in adolescent samples, especially CRP, as it appears to correlate with systemic inflammation measured in serum. This study did not show inflammatory differences for depressed adolescents compared to age- and sex-matched controls, but this may have been due to low statistical power and the use of a community based depression sample. Future studies with larger samples should consider using salivary CRP as a measure of inflammation in bio-behavioral research with younger samples because it is easier and safer than blood collection, is indicative of systemic inflammation, and may avoid a participation bias in samples where comorbidity with needle or blood phobias are a concern. Finally, further research into salivary inflammation for markers other than CRP appears to be warranted as these results showed that levels are more detectable than in serum. Although these results did not show correlations between salivary and serum versions of acute phase proteins or cytokines other than CRP when non-detectable levels were excluded, when non-detectable levels were included as 0, CRP, IL-2, IL-12p70, and IFN- γ showed correlations between saliva and serum. Due to these differences, future research is advised to either analyse results in both of these ways, or to use more sensitive assays or repeat assays where many values are non-detectable. Future research could also examine if salivary inflammation correlates with other measurements of disease. Furthermore, the effects of diurnal variation and waking responses need to be examined across all acute phase proteins and cytokines in saliva.

References

- Alesci, S., Martinez, P.E., Kelkar, S., Ilias, I., Ronsaville, D.S., Listwak, S.J., Ayala, A.R., Licinio, J., Gold, H.K., Kling, M.A., Chrousos, G.P., Gold, P.W., 2005. Major depression is associated with significant diurnal elevations in plasma interleukin-6 levels, a shift of its circadian rhythm, and loss of physiological complexity in its secretion: clinical implications. *J. Clin. Endocrinol. Metab.* 90, 2522-2530.
- Arellano-Garcia, M.E., Hu, S., Wang, J., Henson, B., Zhou, H., Chia, D., Wong, D.T., 2008. Multiplexed immunobead-based assay for detection of oral cancer protein biomarkers in saliva. *Oral Dis.* 14, 705-712.
- Arsenault, B.J., Earnest, C.P., Després, J.P., Blair, S.N., Church, T.S., 2009. Obesity, coffee consumption and CRP levels in postmenopausal overweight/obese women: importance of hormone replacement therapy use. *Eur. J. Clin. Nutr.* 63, 1419-1424.
- Azar, R., Richard, A., 2011. Elevated salivary C-reactive protein levels are associated with active and passive smoking in healthy youth: A pilot study. *J. Inflammation* 8, 37.
- Black, S., Kushner, I., Samols, D., 2004. C-reactive Protein. *J. Biol. Chem.* 279, 48487-48490.
- Bonaccorso, S., Marino, V., Puzella, A., Pasquini, M., Biondi, M., Artini, M., Almerighi, C., Verkerk, R.R.T., Meltzer, H., Maes, M., 2002. Increased Depressive Ratings in Patients With Hepatitis C Receiving Interferon-[alpha]-Based Immunotherapy Are Related to Interferon-[alpha]-Induced Changes in the Serotonergic System. *J. Clin. Psychopharm.* 22, 86-90.

- Brailo, V., Vucicevic-Boras, V., Lukac, J., Biocina-Lukenda, D., Zilic-Alajbeg, I., Milenovic, A., Balijsa, M., 2012. Salivary and serum interleukin 1 beta, interleukin 6 and tumor necrosis factor alpha in patients with leukoplakia and oral cancer. *Medicina oral, patología oral y cirugía bucal* 17, e10-e15.
- Brambilla, F., Monteleone, P., Maj, M., 2004. Interleukin-1 β and tumor necrosis factor- α in children with major depressive disorder or dysthymia. *J. Affective Disord.* 78, 273-277.
- Cai, W., Khaoustov, V.I., Xie, Q., Pan, T., Le, W., Yoffe, B., 2005. Interferon- α -induced modulation of glucocorticoid and serotonin receptors as a mechanism of depression. *J. Hepatol.* 42, 880-887.
- Capuron, L., Ravaut, A., Dantzer, R., 2000. Early depressive symptoms in cancer patients receiving interleukin 2 and/or interferon alpha-2b therapy. *J. Clin. Oncol.* 18, 2143-2151.
- Chabrol, H., Montovany, A., Chouicha, K., Duconge, E., 2002. Study of the CES-D on a sample of 1,953 adolescent students. *Encephale* 28, 429-432.
- Chaiton, M., O'Loughlin, J., Karp, I., Lambert, M., 2010. Depressive symptoms and C-reactive protein are not associated in a population-based sample of adolescents. *Int. J. of Behav. Med.* 17, 216-222.
- Cohen, J., 1960. A coefficient of agreement for nominal scales. *Educ. Psychol. Meas.* 20, 37-46.
- Cohen, P., Cohen, J., 1984. The clinician's illusion. *Arch. Gen. Psychiat.* 41, 1178-1182.
- Copeland, W.E., Shanahan, L., Worthman, C., Angold, A., Costello, E.J., 2012. Cumulative depression episodes predict later C-reactive protein levels: a prospective analysis. *Biol. Psychiatry* 71, 15-21.

- Correia, L.C., Andrade, B.B., Borges, V.M., Clarêncio, J., Bittencourt, A.P., Freitas, R., Souza, A.C., Almeida, M.C., Leal, J., Esteves, J.P., Barral-Netto, M., 2010. Prognostic value of cytokines and chemokines in addition to the GRACE Score in non-ST-elevation acute coronary syndromes. *Clinica Chimica Acta* 411, 540-545.
- Cuijpers, P., Boluijt, P., van Straten, A., 2008. Screening of depression in adolescents through the Internet : sensitivity and specificity of two screening questionnaires. *Eur. Child Adolesc. Psychiatry* 17, 32-38.
- Cutolo, M., Wilder, R.L., 2000. Different roles for androgens and estrogens in the susceptibility to autoimmune rheumatic diseases. *Rheum. Dis. Clin. N. Am.* 26, 825-839.
- Danesh, J., Wheeler, J.G., Hirschfield, G.M., Eda, S., Eiriksdottir, G., Rumley, A., Lowe, G.D.O., Pepys, M.B., Gudnason, V., 2004. C-Reactive Protein and Other Circulating Markers of Inflammation in the Prediction of Coronary Heart Disease. *N. Engl. J. Med.* 350, 1387-1397.
- Dean, B., 2011. Understanding the role of inflammatory-related pathways in the pathophysiology and treatment of psychiatric disorders: evidence from human peripheral studies and CNS studies. *Int. J. Neuropsychopharmacol.* 14, 997–1012.
- Deane, K.D., O'Donnell, C.I., Hueber, W., Majka, D.S., Lazar, A.A., Derber, L.A., Gilliland, W.R., Edison, J.D., Norris, J.M., Robinson, W.H., Holers, V.M., 2010. The number of elevated cytokines and chemokines in preclinical seropositive rheumatoid arthritis predicts time to diagnosis in an age-dependent manner. *Arthritis Rheum.* 62, 3161-3172.

- Deinzer, R., Granrath, N., Spahl, M., Linz, S., Waschul, B., Herforth, A., 2005. Stress, oral health behaviour and clinical outcome. *Brit. J. Health Psych.* 10, 269-283.
- Dhabhar, F.S., Burke, H.M., Epel, E.S., Mellon, S.H., Rosser, R., Reus, V.I., Wolkowitz, O.M., 2009. Low serum IL-10 concentrations and loss of regulatory association between IL-6 and IL-10 in adults with major depression. *J. Psychiatr. Res.* 43, 962–969.
- Dierker, L.C., Albano, A.M., Clarke, G.N., Heimberg, R.G., Kendall, P.C., Merikangas, K.R., Lewinsohn, P.M., Offord, D.R., Kessler, R., Kupfer, D.J., 2001. Screening for anxiety and depression in early adolescence. *J. Am. Acad. Child Adolesc. Psychiatry* 40, 929-936.
- Dowlati, Y., Herrmann, N., Swardfager, W., Liu, H., Sham, L., Reim, E.K., Lanctôt, K.L., 2010. A Meta-Analysis of Cytokines in Major Depression. *Biol. Psychiatry* 67, 446-457.
- Elovainio, M., Keltikangas-Järvinen, L., Pulkki-Råback, L., Kivimäki, M., Puttonen, S., Viikari, L., Räsänen, L., Mansikkaniemi, K., Viikari, J., Raitakari, O.T., 2006. Depressive symptoms and C-reactive protein: the Cardiovascular Risk in Young Finns Study. *Psychol. Med.* 36, 797-805.
- Elshal, M.F., McCoy, J.P., 2006. Multiplex bead array assays: Performance evaluation and comparison of sensitivity to ELISA. *Methods* 38, 317-323.
- Fernandez-Botran, R., Miller, J.J., Burns, V.E., Newton, T.L., 2011. Correlations among inflammatory markers in plasma, saliva and oral mucosal transudate in post-menopausal women with past intimate partner violence. *Brain, Behav., Immun.* 25, 314-321.

- Fleiss, J.L., 1971. Measuring nominal scale agreement among many raters. *Psychol. Bull.* 76, 378–382.
- Fujita, T., Nagayama, A., Anazawa, S., 2003. Circulating alpha-2-macroglobulin levels and depression scores in patients who underwent abdominal cancer surgery. *J. Surg. Res.* 114, 90-94.
- Gabbay, V., Klein, R., Alonso, C., Babb, J., Nishawala, M., De Jesus, G., Hirsch, G., Hottinger-Blanc, P., Gonzalez, C., 2009. Immune system dysregulation in adolescent major depressive disorder. *J. Affective Disord.* 115, 177-182.
- Gilbertson-White, S., Aouizerat, B.E., Miaskowski, C., 2011. Methodologic issues in the measurement of cytokines to elucidate the biological basis for cancer symptoms. *Biol. Res. Nurs.* 13, 15-24.
- Gillum, R.F., 2003. Association of serum C-reactive protein and indices of body fat distribution and overweight in Mexican American children. *J. Natl. Med. Assoc.* 95, 545-552.
- Granger, D.A., Kivlighan, K.T., Fortunato, C., Harmon, A.G., Hibel, L.C., Schwartz, E.B., Whembolua, G.-L., 2007. Integration of salivary biomarkers into developmental and behaviorally-oriented research: Problems and solutions for collecting specimens. *Physiol. Behav.* 92, 583–590.
- Heinrich, P.C., Castell, J.V., Andus, T., 1990. Interleukin-6 and the acute phase response. *Biochem. J.* 265, 621-636.
- Henje Blom, E., Lekander, M., Ingvar, M., Asberg, M., Mobarrez, F., Serlachius, E., 2012. Pro-inflammatory cytokines are elevated in adolescent females with emotional disorders not treated with SSRIs. *J. Affective Disord.* 136, 716-723.
- Hermsen, C.C., Konijnenberg, Y., Mulder, L., Loé, C., van Deuren, M., van der Meer, J.W., van Mierlo, G.J., Eling, W.M., Hack, C.E., Sauerwein, R., 2003.

- Circulating concentrations of soluble granzyme A and B increase during natural and experimental *Plasmodium falciparum* infections. *Clin. Exp. Neuroimmunol.* 132, 467-472.
- Hernández, M.E., Mendieta, D., Martínez-Fong, D., Loría, F., Moreno, J., Estrada, I., Bojalil, R., Pavón, L., 2008. Variations in circulating cytokine levels during 52 week course of treatment with SSRI for major depressive disorder. *Eur. Neuropsychopharm.* 18, 917-924.
- Howren, M.B., Lamkin, D.M., Suls, J., 2009. Associations of depression with C-Reactive Protein, IL-1, and IL-6: A meta-analysis. *Psychosom. Med.* 71, 171-186.
- Huang, G.T.-J., Haake, S.K., Kim, J.-W., Park, N.-H., 1998. Differential expression of interleukin-8 and intercellular adhesion molecule-1 by human gingival epithelial cells in response to *Actinobacillus actinomycetemcomitans* or *Porphyromonas gingivalis* infection. *Oral Microbiol. Immunol.* 13, 301-309.
- Jorm, A.F., 1987. Sex and age differences in depression: a quantitative synthesis of published research. *Aust. NZ J. Psychiat.* 21, 46-53.
- Jozuka, H., Jozuka, E., Takeuchi, S., Nishikaze, O., 2003. Comparison of immunological and endocrinological markers associated with major depression. *J. Int. Med. Res.* 31, 36-41.
- Keller, P.S., El-Sheikh, M., Vaughn, B., Granger, D.A., 2010. Relations between mucosal immunity and children's mental health: The role of child sex. *Physiol. Behav.* 101, 705-712.
- Kim, J.-H., Park, E.-Y., 2012. The factor structure of the center for epidemiologic studies depression scale in stroke patients. *Top. Stroke Rehabil.* 19, 54-62.

- Lambert, M., Delvin, E.E., Paradis, G., O'Loughlin, J., Hanley, J.A., Levy, E., 2004. C-reactive protein and features of the metabolic syndrome in a population-based sample of children and adolescents. *Clin. Chem.* 50, 1762-1768.
- Lee, A., Ghaname, C.B., Braun, T.M., Sugai, J.V., Teles, R.P., Loesche, W.J., Kornman, K.S., Giannobile, W.V., Kinney, J.S., 2012. Bacterial and Salivary Biomarkers Predict the Gingival Inflammatory Profile. *J. Periodontol.* 83, 79-89.
- Liu, Y., Ho, R.C.-M., Mak, A., 2012. The role of interleukin (IL)-17 in anxiety and depression of patients with rheumatoid arthritis. *Int. J. Rheum. Dis.* 15, 183-187.
- Lotrich, F.E., Rabinovitz, M., Gironde, P., Pollock, B.G., 2007. Depression following pegylated interferon-alpha: Characteristics and vulnerability. *J. Psychosom. Res.* 63, 131-135.
- Maes, M., Scharpé, S., Meltzer, H.Y., Cosyns, P., 1993. Relationships between increased haptoglobin plasma levels and activation of cell-mediated immunity in depression. *Biol. Psychiatry* 34, 690-701.
- Megson, E., Fitzsimmons, T., Charmapatni, K., Bartold, P.M., 2010. C-Reactive Protein in Gingival Crevicular Fluid may be Indicative of Systemic Inflammation. *J. Clin. Periodontol.* 37, 787-804.
- Meier-Ewert, H.K., Ridker, P.M., Rifai, N., Price, N., Dinges, D.F., Mullington, J.M., 2001. Absence of diurnal variation of C-Reactive protein concentrations in healthy human subjects. *Clin. Chem.* 47, 426-430.
- Miles, M.P., Andring, J.M., Pearson, S.D., Gordon, L.K., Kasper, C., Depner, C.M., Kidd, J.R., 2008. Diurnal variation, response to eccentric exercise, and

- association of inflammatory mediators with muscle damage variables. *J. Appl. Physiol.* 104, 451-458.
- Mirzaii-Dizgah, I., Riahi, E., Miri, R., 2012. Serum and saliva levels of high-sensitivity C-reactive Protein in acute myocardial infarction. *J. Mol. Biomarkers Diagn.* 3.
- Morin, A.J., Moullec, G., Maïano, C., Layet, L., Just, J.L., Ninot, G., 2011. Psychometric properties of the Center for Epidemiologic Studies Depression Scale (CES-D) in French clinical and nonclinical adults. *Revue d'épidémiologie et de santé publique* 59, 327-340.
- Myint, A.-M., Leonard, B.E., Steinbusch, H.W.M., Kim, Y.-K., 2005. Th1, Th2, and Th3 cytokine alterations in major depression. *J. Affective Disord.* 88, 167-173.
- Navarro, V.P., Iyomasa, M.M., Leite-Panissi, C.R., Almeida, M.C., Branco, L.G., 2006. New role of the trigeminal nerve as a neuronal pathway signaling brain in acute periodontitis: participation of local prostaglandins. *Pflugers Arch.* 453, 73–82.
- Nielsen, A.A., Nielsen, J.N., Schmedes, A., Brandslund, I., Hey, H., 2005. Saliva Interleukin-6 in patients with inflammatory bowel disease. *Scand. J. Gastroenterol.* 40, 1444-1448.
- Orvaschel, H., Puig-Antich, J., 1994. Schedule for Affective Disorder and Schizophrenia for School-age Children, Epidemiologic Version. Nova University, Center for Psychological Study, Ft. Lauderdale, FL.
- Ouellet-Morin, I., Danese, A., Williams, B., Arseneault, L., 2011. Validation of a high-sensitivity assay for C-reactive protein in human saliva. *Brain, Behav., Immun.* 25, 640-646.
- Out, D., Hall, R.J., Granger, D.A., Page, G.G., Woods, S.J., 2012. Assessing salivary C-reactive protein: Longitudinal associations with systemic inflammation and

- cardiovascular disease risk in women exposed to intimate partner violence. *Brain, Behav., Immun.* 26, 543-551.
- Pavón, L., Sandoval-López, G., Eugenia Hernández, M., Loría, F., Estrada, I., Pérez, M., Moreno, J., Ávila, U., Leff, P., Antón, B., Heinze, G., 2006. Th2 cytokine response in Major Depressive Disorder patients before treatment. *J. Neuroimmunol.* 172, 156-165.
- Pepys, M.B., Hirschfiel, G.M., 2003. C-reactive protein: a critical update. *J. Clin. Invest.* 111, 1805-1812.
- Pfaffe, T., Cooper-White, J., Beyerlein, P., Kostner, K., Punyadeera, C., 2011. Diagnostic potential of saliva. Current state and future applications. *Clin. Chem.* 57, 675-687.
- Piccinelli, M., Wilkinson, G., 2000. Gender differences in depression: critical review. *Brit. J. Psychiat.* 177, 486-492.
- Prescott, C.A., McArdle, J.J., Hishinuma, E.S., Johnson, R.C., Miyamoto, R.H., Andrade, N.N., Edman, J.L., Makini, G.K., Nahulu, L.B., Yuen, N.Y.C., Carlton, B.S., 1998. Prediction of major depression and dysthymia from CES-D scores among ethnic minority adolescents. *J. Am. Acad. Child. Adolesc. Psychiatry* 37, 495–503.
- Radloff, L.S., 1977. The CES-D scale: a self-report depression scale for research in the general population. *J. Applied Psychological Measurement* 1, 385–401.
- Radloff, L.S., 1991. The use of the Center of Epidemiologic Studies Depression Scale in adolescents and young adults. *J. Youth Adolesc.* 20, 149-166.
- Remick, D.G., 1997. Quantitation of Cytokines. In: Remick, D.G., Friedland, J.S. (Eds.), *Cytokines in Health and Disease*. Marcel Dekker, Inc., New York, NY, USA, pp. 281-297.

- Ridker, P.M., Rifai, N., Stampfer, M.J., Hennekens, C.H., 2000. Plasma concentration of interleukin-6 and the risk of future myocardial infarction among apparently healthy men. *Circulation* 101, 1767-1772.
- Roberts, R.E., Andrews, J.A., Lewinsohn, P.M., Hops, H., 1990. Assessment of depression in adolescents using the Center for Epidemiologic Studies Depression Scale. *Psychol. Assess.* 2, 122–128.
- Roberts, W.L., Sedrick, R., Moulton, L., Spencer, A., Rifai, N., 2000. Evaluation of four automated high-sensitivity C-reactive protein methods: Implications for clinical and epidemiological applications. *Clin. Chem.* 46, 461-468.
- Rojas, P., Villar, M., Gonzalez, A., Poblete, C., Funez, F., Tong, A., Liberman, C., 2011. Increase in C-reactive protein and lipids in adolescents with psychiatric disease. *Psychiat. Res.* 190, 372–374.
- Schultheiss, O.C., Stanton, S.J., 2009. Assessment of salivary hormones. In: Harmon-Jones, E., Beer, J.S. (Eds.), *Methods in Social Neuroscience*. Guilford Press, New York.
- Schwartz, E., Schafer, C., Bode, J.C., Bode, C., 2000. Influence of the menstrual cycle on the LPS-induced cytokine response of monocytes. *Cytokine* 12, 413–416.
- Shine, N., Konopka, K., Düzgüneş, N., 1997. The anti-HIV-1 activity associated with saliva. *J. Dent. Res.* 76, 634-640.
- Simon, G., Ormel, J., VonKorff, M., Barlow, W., 1995. Health care costs associated with depressive and anxiety disorders in primary care. *Am. J. Psychiatry* 152, 352-357.
- Simon, N.M., McNamara, K., Chow, C.W., Maser, R.S., Papakostas, G.I., Pollack, M.H., Nierenberg, A.A., Fava, M., Wong, K.K., 2008. A detailed examination

- of cytokine abnormalities in Major Depressive Disorder. *Eur. Neuropsychopharm.* 18, 230-233.
- Sothorn, R.B., Roitman-Johnson, B., Kanabrocki, E.L., Yager, J.G., Roodell, M.M., Weatherbee, J.A., Young, M.R.I., Nemchausky, B.M., Scheving, L.E., 1995. Circadian characteristics of circulating interleukin-6 in men. *J. Allergy Clin. Immun.* 95, 1029-1035.
- Sugawara, S., Uehara, A., Tamai, R., Takada, H., 2002. Innate immune responses in oral mucosa. *Innate Immunity* 8, 465-468.
- Sugiyama, A., Uehara, A., Iki, K., MAatsushita, K., Nakamura, R., Ogawa, T., Sugawara, S., Takada, H., 2002. Activation of human gingival epithelial cells by cell-surface components of black-pigmented bacteria: augmentation of production of interleukin-8, granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor and expression of intercellular adhesion molecule 1. *J. Med. Microbiol.* 51, 27-33.
- Sutcgil, L., Oktenli, C., Musabak, U., Bozkurt, A., Cansever, A., Uzun, O., Sanisoglu, S.Y., Yesilova, Z., Ozmenler, N., Ozsahin, A., Sengul, A., 2007. Pro- and Anti-Inflammatory Cytokine Balance in Major Depression: Effect of Sertraline Therapy. *Clin. Dev. Immunol.* 2007, 7639-7646.
- Suzuki, T., Omata, K., Satoh, T., Miyasaka, T., Arai, C., Maeda, M., Matsuno, T., Miyamura, T., 2005. Quantitative detection of Hepatitis C virus (HCV) RNA in saliva and gingival crevicular fluid of HCV-infected patients. *J. Clin. Microbiol.* 43, 4413-4417.
- Thombs, B.D., Hudson, M., Schieir, O., Taillefer, S.S., Baron, M., 2008. Reliability and validity of the center for epidemiologic studies depression scale in patients with systemic sclerosis. *Arthritis Care Res.* 59, 438-443.

- Thorman, R., Lundahl, J., Yucel-Lindberg, T., Hylander, B., 2010. Inflammatory cytokines in saliva: early signs of metabolic disorders in chronic kidney disease. A controlled cross-sectional study. *Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endod.* 110, 597-604.
- Tsiouris, J.A., Mehta, P.D., Patti, P.J., Madrid, R.E., Raguthu, S., Barshatzky, M.R., Cohen, I.L., Sersen, E., 2000. Alpha2 macroglobulin elevation without an acute phase response in depressed adults with Down's syndrome: implications. *J. Intellectual Disabil. Res.* 44, 644-653.
- Visser, M., Bouter, L.M., McQuillan, G.M., Wener, M.H., Harris, T.B., 1999. Elevated C-reactive protein levels in overweight and obese adults. *JAMA* 282, 2131 – 2135.
- Waschul, B., Herforth, A., Stiller-Winkler, R., Idel, H., Granrath, N., Deinzer, R., 2003. Effects of plaque, psychological stress and gender on crevicular Il-1beta and Il-1ra secretion. *J. Clin. Periodontol.* 30, 238-248.
- Williamson, S., Munro, C., Pickler, R., Grap, M.J., Elswick, R.K., 2012. Comparison of biomarkers in blood and saliva in healthy adults. *Nurs. Res. Pract.* 2012, 246178.
- Wong, H.-L., Pfeiffer, R.M., Fears, T.R., Vermeulen, R., Ji, S., Rabkin, C.S., 2008. Reproducibility and correlations of multiplex cytokine levels in asymptomatic persons. *Cancer Epidemiol., Biomarkers Prev.* 17, 3450 - 3456.
- Wu, D.M., Chu, N.F., Shen, M.H., J.B., C., 2003. Plasma C-reactive protein levels and their relationship to anthropometric and lipid characteristics among children. *J. Clin. Epidemiol.* 56, 94–100.

Figures

Figure 1 – Nonparametric correlations of salivary and serum CRP for those with low serum CRP

Figure 2 - Nonparametric correlations of salivary and serum CRP for those with high serum CRP

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Figure 4 - Parametric correlations of salivary and serum CRP for those with high serum CRP

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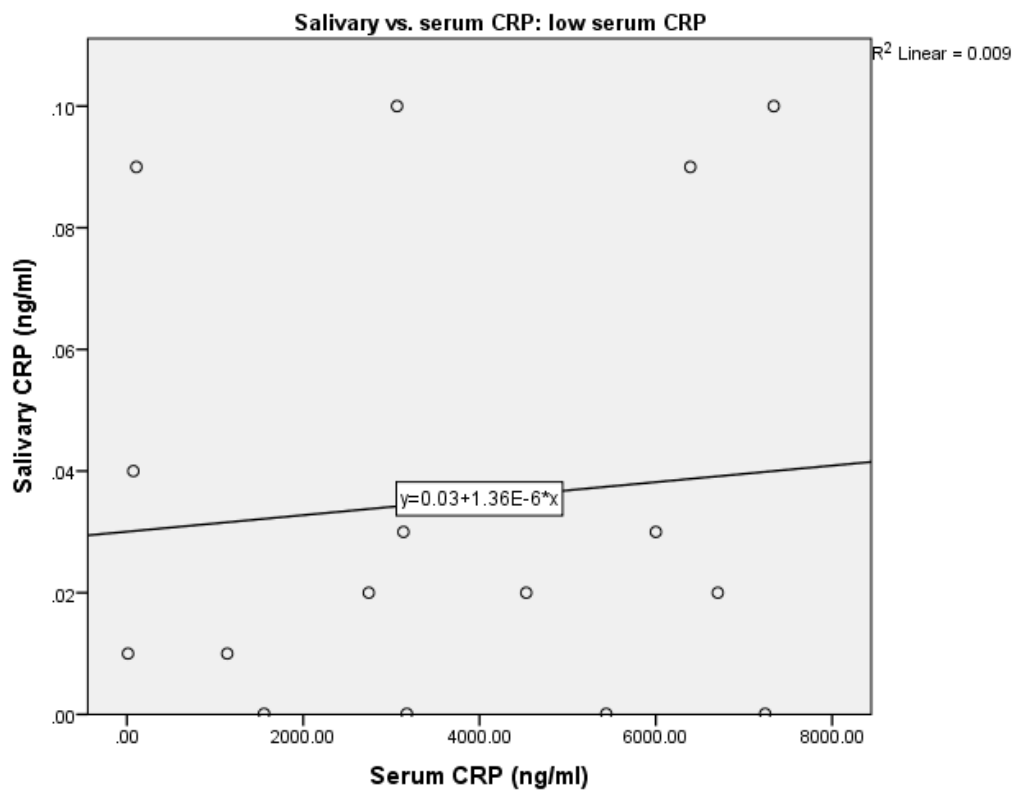
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Figures

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Inflammatory markers in serum and saliva

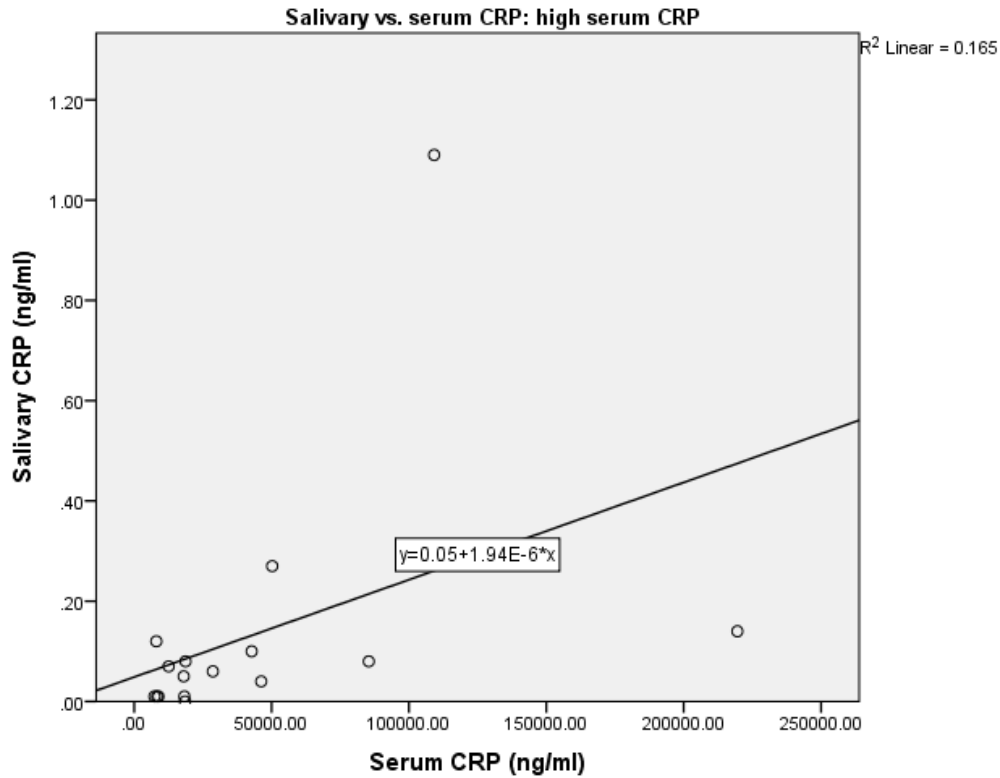
Figure 1 – Nonparametric correlations of salivary and serum CRP for those with low serum CRP



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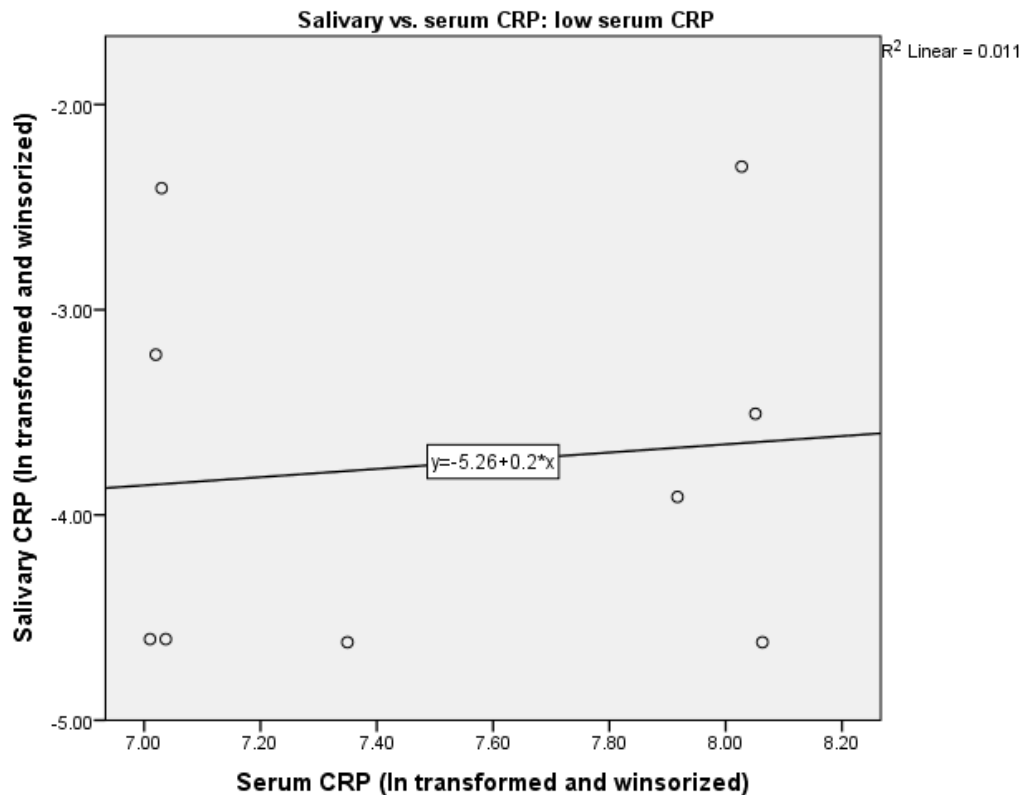
Figure 2 - Nonparametric correlations of salivary and serum CRP for those with high serum CRP



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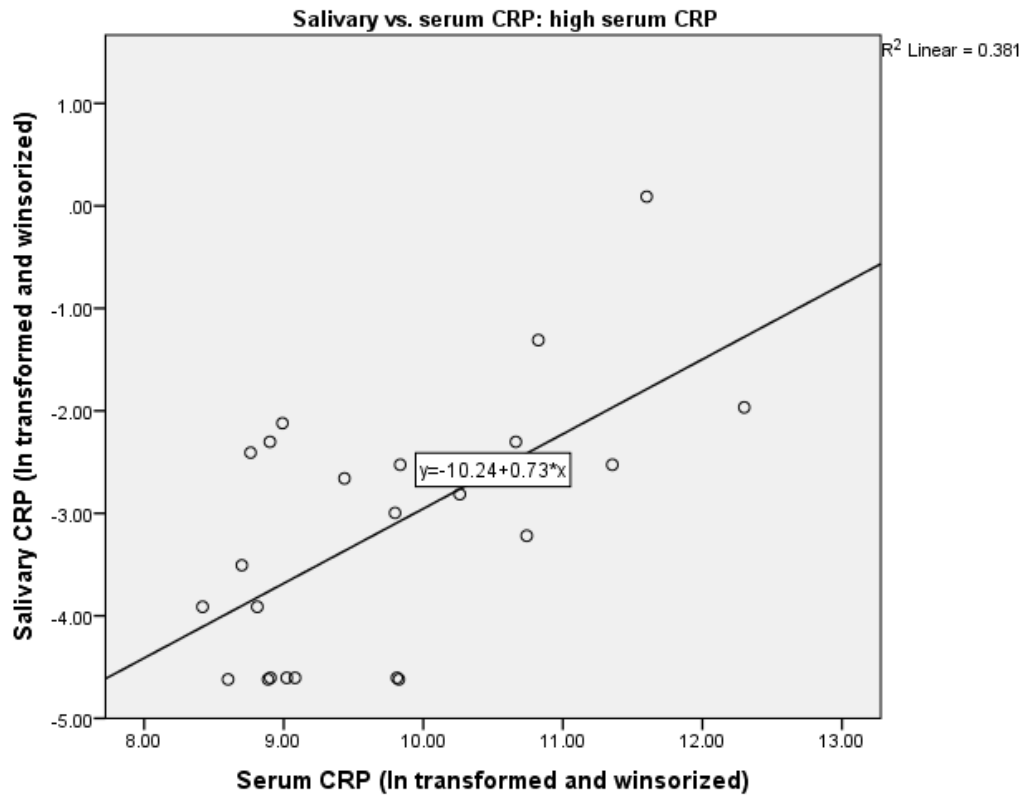
Figure 3 – Parametric correlations of salivary and serum CRP for those with low serum CRP



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Figure 4 - Parametric correlations of salivary and serum CRP for those with high serum CRP



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Tables

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Acute phase protein and cytokine levels in serum and saliva

Table 1 – Rates of detection of acute phase proteins and cytokines in saliva and serum

Acute phase protein	Detection rate in saliva	Detection rate in serum
A2M	34/35 (97%†)**	33/35 (94%)**
CRP	32/35 (91%)**	35/35 (100%)**
Haptoglobin	32/35 (91%)**	17/35 (49%)**
SAP	35/35 (100%)**	35/35 (100%)**
Cytokine		
IL-1 α	35/35 (100%)**	05/35 (14%)
IL-2	29/35 (83%)**	04/35 (11%)
IL-4	33/35 (94%)**	04/35 (11%)
IL-6	35/35 (100%)**	02/35 (06%)
IL-8	35/35 (100%)**	17/35 (49%)**
IL-10	21/35 (60%)**	06/35 (17%)
IL-12(p70)	30/35 (86%)**	06/35 (17%)
IL-13	35/35 (100%)**	02/35 (06%)
IL-17	33/35 (94%)**	07/35 (20%)
IFN- γ	34/35 (97%)**	05/35 (14%)
TNF- α	35/35 (100%)**	03/35 (09%)
IFN- α 2	17/35 (49%)**	00/35 (00%)

† Percent of participants with detectable levels of cytokine

** 49% or more were detectable

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Acute phase protein and cytokine levels in serum and saliva

Table 2 – Descriptive statistics of acute phase protein and cytokine levels in saliva and serum – excluding non-detectable samples

APP	Levels of detectable cytokines in saliva (ng/ml)				Levels of detectable cytokines in serum (ng/ml)			
	N*	Mean	Std Dev	Range	N*	Mean	Std Dev	Range
A2M	34	123.02	468.85	0.71 – 2740.25	33	4.54 x 10 ⁷	8.29 x 10 ⁷	5.58 x 10 ⁵ – 3.84 x 10 ⁸
CRP	32	0.08	0.19	0.00 – 1.09	35	2.24 x 10 ⁴	4.18 x 10 ⁴	11.42 – 2.20 x 10 ⁵
Haptoglobin	32	8.20	13.02	0.33 – 69.37	17	1.45 x 10 ⁶	1.61 x 10 ⁶	2.20 x 10 ⁵ – 6.73 x 10 ⁶
SAP	35	1.49	3.95	0.03 – 22.32	35	1.07 x 10 ⁶	6.38 x 10 ⁵	6482.16 – 2.42 x 10 ⁶
Cytokine								
IL-1 α	35	1874.25	7606.96	13.76 – 44868.51	5	2.95	5.80	0.20 – 13.32
IL-2	29	2.76	2.94	0.22 – 10.44	4	15.95	7.02	8.36 – 24.72
IL-4	33	0.82	0.96	0.00 – 3.53	4	4.13	2.77	1.99 – 7.92
IL-6	35	6.13	10.19	0.84 – 61.02	2	18.83	8.49	12.83 – 24.83
IL-8	35	249.13	588.59	8.06 – 3131.57	17	2.35	1.93	0.07 – 5.69
IL-10	21	1.77	1.78	0.02 – 5.94	6	33.81	26.80	8.57 – 75.07
IL-12(p70)	30	5.84	6.41	0.07 – 22.04	6	38.72	45.82	1.91 – 123.46
IL-13	35	1.42	1.47	0.20 – 5.43	2	10.40	6.33	5.92 – 14.87
IL-17	33	6.91	7.81	0.18 – 26.62	7	15.87	12.57	0.58 – 37.53
IFN- γ	34	38.39	39.08	3.08 – 136.33	5	354.82	389.38	13.29 – 1000.45
TNF- α	35	11.39	12.39	0.22 – 52.40	3	141.66	128.47	20.09 – 276.07

IFN- α 2	17	45.23	12.07	23.20 – 61.47	0	N/A	N/A	N/A
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Note: This set of data excludes samples for which the cytokine was not detectable

APP = acute phase protein

** Out of 35 samples*

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Acute phase protein and cytokine levels in serum and saliva

Table 3 – Descriptive statistics of acute phase protein and cytokine levels in saliva and serum – including non-detectable samples

APP	Levels in saliva (ng/ml)				Levels in serum (ng/ml)			
	N*	Mean	Std Dev	Range	N*	Mean	Std Dev	Range
A2M	34	123.02	468.85	0.71 – 2740.25	33	4.54×10^7	8.29×10^7	5.58×10^5 – 3.84×10^8
CRP	35	0.08	0.18	0 – 1.09	35	2.24×10^4	4.18×10^4	11.42 – 2.20×10^5
Haptoglobin	32	8.20	13.02	0.33 – 69.37	17	1.45×10^6	1.61×10^6	2.20×10^5 – 6.73×10^6
SAP	35	1.49	3.95	0.03 – 22.32	35	1.07×10^6	6.38×10^5	6482.16 – 2.42×10^6
Cytokine								
IL-1 α	35	1874.25	7606.96	13.76 – 44868.51	35	0.42	2.25	0 – 13.32
IL-2	35	2.29	2.87	0 – 10.44	35	1.82	5.55	0 – 24.72
IL-4	35	0.77	0.95	0 – 3.53	35	0.47	1.57	0 – 7.92
IL-6	35	6.13	10.19	0.84 – 61.02	35	1.08	4.67	0 – 24.83
IL-8	35	249.13	588.59	8.06 – 3131.57	35	1.14	1.78	0 – 5.69
IL-10	35	1.06	1.63	0 – 5.94	35	5.80	16.51	0 – 75.07
IL-12(p70)	35	5.01	6.27	0 – 22.04	35	6.64	22.98	0 – 123.46
IL-13	35	1.42	1.47	0.20 – 5.43	35	0.59	2.68	0 – 14.87
IL-17	35	6.51	7.75	0 – 26.62	35	3.17	8.33	0 – 37.53
IFN- γ	35	37.30	39.04	0 – 136.33	35	50.69	183.59	0 – 1000.45
TNF- α	35	11.39	12.39	0.22 – 52.40	35	12.14	50.89	0 – 276.07
IFN- α 2	35	21.97	24.38	0 – 61.47	35	0	0	0

Note: This set of data includes samples with non-detectable cytokines and assumes the levels are 0.

APP = acute phase protein

** Out of 35 samples*

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Acute phase protein and cytokine levels in serum and saliva

Table 4 – Nonparametric correlations of raw salivary and serum acute phase proteins and cytokines (does not include markers with detection rates < 49%). Non-detectable values excluded.

	A2M	CRP	Haptoglobin	SAP	IL-8
Correlation coefficient	0.069	0.424*	-0.275	0.086	0.478
Significance (2-tailed)	0.709	0.015	0.286	0.623	0.052
N	32	32	17	35	17

* $p < 0.05$; Results showed an association between salivary and serum CRP only.

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Acute phase protein and cytokine levels in serum and saliva

Table 5 – Parametric correlations of transformed and winsorized salivary and serum acute phase proteins and cytokines (does not include markers with detection rates < 49%). Non-detectable values excluded.

	A2M	CRP	Haptoglobin	SAP	IL-8
Correlation coefficient	0.128	0.532**	-0.285	0.076	0.435
Significance (2-tailed)	0.486	0.002	0.268	0.665	0.081
N	32	32	17	35	17

** $p < 0.01$; Results showed an association between salivary and serum CRP only.

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Acute phase protein and cytokine levels in serum and saliva

Table 6 – Nonparametric correlations of raw salivary and serum CRP for high vs. low serum CRP (non-detectable levels excluded)

		Salivary vs. Serum CRP
Low serum CRP	Correlation coefficient	0.054
	Significance (2-tailed)	0.844
	N	16
High serum CRP	Correlation coefficient	0.599*
	Significance (2-tailed)	0.014
	N	16

* $p < 0.05$; 3 participants had undetectable salivary or serum CRP values.

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Acute phase protein and cytokine levels in serum and saliva

Table 7 – Parametric correlations of transformed and winsorized salivary and serum CRP for high vs. low serum CRP (non-detectable levels excluded)

		Salivary vs. Serum CRP
Low serum CRP	Correlation coefficient	0.105
	Significance (2-tailed)	0.788

	N	9
High serum CRP	Correlation coefficient	0.617**
	Significance (2-tailed)	0.002
	N	23

** $p < 0.01$; 3 participants had undetectable salivary or serum CRP values.

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Acute phase protein and cytokine levels in serum and saliva

Table 8 – Nonparametric correlations of raw salivary and serum acute phase proteins and cytokines (non-detectable levels replaced with 0)

	CRP	IL-2	IL-4	IL-12p70	IFN- γ	TNF- α
Correlation coefficient	0.393*	0.410*	0.284	0.353*	0.415*	0.324
Significance (2-tailed)	0.019	0.014	0.098	0.038	0.013	0.058
N	35	36	35	35	35	35

* $p < 0.05$; Results showed an association between salivary and serum CRP, IL-2, IL-12p70, and IFN- γ only.

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Acute phase protein and cytokine levels in serum and saliva

Table 9 – Parametric correlations of transformed and winsorized salivary and serum acute phase proteins and cytokines (non-detectable levels replaced with 0)

	CRP	IL-2	IL-12p70	IFN- γ
Correlation coefficient	0.470**	0.380*	0.337*	0.328
Significance (2-tailed)	0.004	0.025	0.048	0.055
N	35	35	35	35

** $p < 0.01$, * $p < 0.05$; Results showed an association between salivary and serum CRP, IL-2, and IL-12p70 only.

Findings show that salivary markers correlate with levels in serum in an adolescent sample, and detection rates in saliva are higher than in serum.

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