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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 utilising younger samples to characterise 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.

1. 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 characterise 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.

1.1. 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

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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 behavioural 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)-1β in oral cancer patients, leukoplakia patients, and controls, and report on differences in salivary IL-1β between these groups. However, only two out of 88 participants (one oral cancer patient and one control) had detectable levels of IL-1β 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 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 behavioural 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).

1.2. Serum/saliva correlations in younger samples

The comparison of salivary and serum proteins and cytokines amongst 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

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