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Correlations among inflammatory markers in plasma, saliva and oral mucosal transudate in post-menopausal women with past intimate partner violence

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ABSTRACT

The relationship between psychosocial factors and an increased risk for disease has been related to a heightened pro-inflammatory status reflected in increased circulating levels of pro-inflammatory cytokines and/or C-reactive protein (CRP). Routinely, epidemiological studies rely on measurements of inflammatory markers in serum or plasma, but the use of biological fluids such as saliva or oral mucosal transudate (OMT) may offer potential advantages. This study investigated correlations among plasma CRP and levels of IL-6 and soluble IL-6 receptor (sIL-6R) in plasma, saliva and OMT in a population of middle aged women with histories of past intimate partner violence (IPV). A total of 67 women without existing chronic diseases participated in the study, which included two visits each in which psychological tests were administered, and blood, saliva and OMT samples were collected. Although significantly higher plasma CRP levels were found in past IPV sufferers compared to controls, there were no significant differences in IL-6 or sIL-6R levels in plasma, saliva or OMT between the two groups. There were only relatively modest correlations between IL-6 levels in plasma and those in saliva or OMT and between plasma IL-6 and CRP levels. A significant correlation between IL-6 and sIL-6R levels in both saliva and OMT, but not in plasma, was also detected. No significant correlations were found between levels of IL-6 in saliva or OMT and periodontal health measures. Results indicate that IL-6 and sIL-6R levels in saliva or OMT do not closely reflect those in plasma, and therefore are not a good surrogate for systemic levels.

1. Introduction

The existence of a relationship between psychosocial factors and an increased risk for disease and death has been well established (Hemingway and Marmot, 1999; Krause et al., 2004). This risk has been shown to be associated with increased circulating levels of a variety of inflammatory markers, including pro-inflammatory cytokines such as tumor necrosis factor-α (TNFα), interleukin-1 (IL-1) and interleukin-6 (IL-6); soluble forms of several cytokine receptors such as soluble IL-6 receptors (sIL-6R) and soluble TNF receptors I and II ; and acute phase proteins such as C-reactive protein (CRP) (Papanicolaou et al., 1998; Harris et al., 1999; Maes, 1999; Appels et al., 2000; Ridker et al., 2002; Grassi-Oliveira et al., 2009). The activation of the hypothalamic–pituitary–adrenal (HPA) axis by these pro-inflammatory mediators is thought to be a contributing factor in the development of a variety of chronic inflammatory diseases (Lyson and McCann, 1991; Mastorakos et al., 1993; Kiecolt-Glaser et al., 2003; Danese et al., 2007; Davis et al., 2008).

IL-6 is a pleiotropic cytokine involved not only in inflammatory responses but also in immune regulation, promoting antibody production and the differentiation of Th17 CD4+ T lymphocytes, a subset that has been associated with autoimmune disorders (Betelli et al., 2006). Its importance in studies relating psychosocial factors and inflammatory mechanisms is based on the fact that IL-6 is considered a key cytokine in inflammatory responses and the major regulator of acute phase protein responses by the liver (Hirano et al., 1990; Ohzato et al., 1992; Kishimoto, 2005). In addition, its levels in serum and plasma are more consistently detected and measured compared to other pro-inflammatory cytokines such as TNFα and IL-1. The levels of IL-6 in serum or plasma are increased in an array of pathologic conditions including both acute and chronic inflammatory diseases (Hirano et al., 1990). Elevated systemic IL-6 levels have also been reported in association with a variety of health-related psychosocial factors such as chronic stress conditions, depression and low socioeconomic level, suggesting a potential association with a heightened inflammatory status

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(Maes et al., 2001; Kiecolt-Glaser et al., 2003; Brydon et al., 2004; Danese et al., 2007; Davis et al., 2008).

IL-6 is produced by a wide variety of cells, including T lymphocytes, macrophages, glial cells, fibroblasts, keratinocytes, endothelial cells and adipocytes (Hirano et al., 1990; Kishimoto, 2005). Consistent with its role in inflammatory responses, the expression of IL-6 is normally induced by a variety of pro-inflammatory stimuli such as the cytokines TNFα and IL-1 and bacterial lipopolysaccharide (LPS) (Hirano et al., 1990; Kishimoto, 2005). The activity of IL-6 is mediated by a heterodimeric membrane receptor formed by an IL-6-binding chain (gp80 or IL-6R) and a signal-transducing subunit (gp130 or CD130) (Ward et al., 1994; Kishimoto, 2005). Both of these receptor subunits are also released as soluble proteins (sIL-6R and sgp130) in biological fluids (Fernandez-Botran et al., 2002). Unlike most soluble cytokine receptors, the sIL-6R acts as an agonist, binding IL-6 and allowing it to signal on cells that do not express membrane IL-6R but only the gp130 protein, such as hemopoietic cells, osteoclasts and neuronal cells, by a process known as "trans-signaling" (Mackiewicz et al., 1992; Jones and Rose-John, 2002). Thus, the sIL-6R is considered as an important regulator of IL-6 activity (Jones and Rose-John, 2002). The levels of sIL-6R in serum have been reported to be elevated in conditions such as autoimmune diseases, multiple myeloma and sepsis (Gaillard et al., 1999; Jones and Rose-John, 2002; Kallen, 2002; Peake et al., 2006). Although potential alterations related to psychosocial factors have not been widely investigated, there is evidence that increased sIL-6R levels occur in psychotic disorders and patients with post-traumatic stress disorder (PTSD) with concurrent major depression (Kallen, 2002) and that negative correlations exist between sIL-6R levels and scores on a purpose of life scale (Friedman et al., 2007).

In most cases, the measurements of cytokine levels related to psychosocial factors are performed in serum or plasma. However, the ease and lower costs of collection without the need for trained personnel have generated interest in the use of other biological fluids such as saliva or oral mucosal transudate (OMT) for epidemiological studies involving a variety of markers (Nishanian et al., 1998). For example, cortisol analyses in saliva have been used by many investigators, while OMT testing is routinely done for antibodies against HIV-1 (Gallo et al., 1997; Preussner et al., 1997). These samples, however, have not been extensively evaluated for their suitability for measuring cytokines and soluble cytokine receptors in psychobiological studies. The purpose of the present study was to investigate the correlations among measurements of IL-6 and sIL-6R in plasma, saliva and OMT as part of a study of inflammatory markers in a population of healthy middle aged women with a history of past intimate partner violence (IPV), a stressor that is often chronic and that has been positively associated with the presence of chronic medical conditions (Tjaden and Thoennes, 2000; Breiding et al., 2008). Results of the parent study, including associations of plasma IL-6 and CRP levels with different IPV dimensions (i.e., cumulative IPV history, and the specific IPV types physical assault, sexual coercion, and stalking), are reported elsewhere (Newton et al., 2010).

2. Materials and methods

2.1. Overall study design

The current study was part of a larger project investigating pro-inflammatory markers in post-menopausal women with past histories of IPV. A detailed description of the overall study design has been recently reported (Newton et al., 2010). Briefly, the study included a group of healthy post-menopausal women with divorce histories who were recruited after a phone interview and psychological and biomedical evaluation. The subjects completed two research visits during which they had blood drawn, provided saliva and OMT samples, and completed questionnaires about past intimate relationships. Based on their responses to the Revised Conflict Tactics Scale (described below), 46 women were included in the "IPV+" group and 21 in the "IPV−" (control) group. Results of the measurements for IL-6 and sIL-6R in plasma, saliva and OMT samples, as well as plasma CRP, were then used for this study.

2.2. Participant recruitment and selection

Mailings and community advertisements recruited women ever divorced or separated from extremely stressful relationships. Eligibility assessment included: (a) a phone interview; (b) mental status interviews at research visits; and (c) laboratory tests conducted on blood and urine sampled at the first research visit. Initial eligibility required English language skills, no ongoing divorce-related legal issues, no recent psychiatric hospitalization, and no current IPV (i.e., IPV involving an ex-partner in the preceding year, or any IPV history with a current partner) defined as a score of 1 or greater on the 3-item STaT, a screening tool for IPV with a sensitivity and specificity of 96% and 76% for lifetime IPV (Paranjape and Liebschutz, 2003), and 95% and 37% for ongoing IPV (Paranjape et al., 2006). Biomedical eligibility criteria were age 45–60; 12-month cessation of menses (except women ages 45–54 who reported hysterectomy without bilateral ovariectomy) (Johnson et al., 2004); free of chronic disease other than unmedicated hypertension; no use of street drugs, prescription or over-the-counter medications (including botanicals) with potential anti-inflammatory effects; no blood or needle phobia and no current alcohol use disorder defined as an AUDIT-C score of 5 or greater, which yields sensitivities $\geqslant$72% and specificities $\geqslant$88% (Dawson et al., 2005)..

2.3. Procedure

Research visits began between 8 a.m. and 1 p.m. First, in order to ensure that women were not too psychologically vulnerable to complete the study protocol, mental status interviews were conducted to screen for suicidal ideation (visits 1 and 2), and for psychosis and cognitive impairment (visit 1 only). Afterwards, a female nurse assessed acute medical conditions, evaluated signs of illness or infection, took blood pressure, pulse, and body measurements, and obtained a urine sample for a toxicology screen and urinalysis (visit 1 only). Blood, drawn once at each visit via antecubital venipuncture, was collected next with sodium citrate (for IL-6 and sIL-6R) or lithium heparin (for high-sensitivity CRP, comprehensive metabolic profile, thyroid stimulating hormone [TSH], ethanol, and follicle-stimulating hormone [FSH]), or EDTA (for complete blood count [CBC] and HbA1c). Women then completed computer-administered questionnaires at both visits. Previous to visit 2, women were reminded that the focus of that visit would be past abuse and violence and given opportunity to decline participation if they wished to do so. Those choosing to participate provided blood, saliva, and OMT samples and then completed IPV measures and interviews about anxiety symptoms related to IPV or other stressors. Saliva and OMT samples were collected during visits 1 and 2, both immediately before and immediately after the completion of the questionnaires (samples A and B, respectively). Times elapsed between collection of samples A and B were normally 1.5–2 h for visit 1 and 2.5–3 h for visit 2. Interleukin-6, sIL-6R and CRP were assayed at both visits. All other assays were conducted at visit 1 to evaluate eligibility.

2.4. IPV classification

The Revised Conflict Tactics Scale (Straus et al., 2003) was used to assess frequency (0 = *never*, 6 = *more than 20 times*) of minor and severe forms of physical assault, sexual coercion, injury and