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Inflammation and internalizing disorders in adolescents

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ABSTRACT

Serum inflammatory markers have been studied in adults with anxiety and depression, but little is known about cytokine levels in young adolescents with emotional disorders. The objective of this study is to compare serum levels of interleukin-6 (IL-6) and interleukin-10 (IL-10) between adolescents with internalizing disorders and adolescents from the same community without internalizing disorders. A total of 134 non-medicated subjects (n = 76 with internalizing disorders) were recruited from a larger sample of 2457 individuals. Serum levels of IL-6 and IL-10 were quantified and psychiatric diagnosis was evaluated using structured clinical interviews. Adolescents with internalizing disorders presented significantly higher levels of IL-6 as compared to youngsters without internalizing disorders. Differences between groups in IL-10 levels were not statistically significant. This study points out that IL-6 levels may be associated with internalizing disorders in youths and suggests that inflammation might be an early biomarker of emotional distress. High levels of cytokines may adversely affect general health in the long-term, which raise broader issues in terms of public health if results are replicated.

1. Introduction

Major depressive disorder (MDD) and anxiety disorders are both characterized by varying degrees of internal emotional distress and are thus classified as internalizing disorders. They typically begin early in life or adolescence and frequently have a chronic course (Merikangas et al., 2003). Even though the literature supports a pro-inflammatory state across internalizing disorders in adult samples (Dowlati et al., 2010; Hoge et al., 2009; Howren et al., 2009), there are fewer studies in children or adolescents (Mitchell and Goldstein, 2014). Therefore, studying pro-inflammatory states in youngsters may clarify whether inflammation represents an early biomarker for the pathophysiology of emotional disorders.

Among inflammatory markers, having elevated levels of interleukin-6 (IL-6) are one of the most replicated correlates of both MDD (Dowlati et al., 2010; Howren et al., 2009) and anxiety disorders (Belem da Silva et al., 2017; Hoge et al., 2009). This interleukin has pleiotropic actions, such as proliferation and activation of T cells, differentiation of B cells and orchestration of the acute-phase inflammatory response (Hunter and Jones, 2015). More recently, IL-6 has also been found to be an important neuroendocrine factor (Hao et al., 2014; Hunter and Jones, 2015). Moreover it is constitutively produced within the central nervous system (CNS) (Anisman and Merali, 2002) and causes both depressive and anxiety-like phenotypes when injected into the brain of experimental animals (Hao et al., 2014; Sukoff Rizzo et al., 2012). In humans, however, behavioral aspects of IL-6 elevations remain to be determined.

Two meta-analyses reported increased levels of IL-6 in depressed subjects (Dowlati et al., 2010; Howren et al., 2009). Albeit with a lower number of studies, evidence suggests anxiety disorders are also associated with higher levels of IL-6 (Belem da Silva et al., 2017; Hoge et al., 2009). Nevertheless, fewer studies have reported data on cytokine levels in children and adolescents diagnosed with MDD and anxiety disorders (Mitchell and Goldstein, 2014), and available evidence is somewhat mixed (Gabbay et al., 2009a; Henje Blom et al., 2012; Pandey et al., 2012; Slopen et al., 2013). Internalizing behaviors assessed at age 8 were associated with elevated serum IL-6 levels measured at age 10 in a large community cohort (Slopen et al., 2013). Similarly, adolescents with MDD or anxiety disorders presented significantly higher levels of IL-6 than healthy controls in a smaller study (Henje Blom et al., 2012). Interestingly, higher levels of IL-6 were detected in the prefrontal cortex (PFC) of adolescents who completed suicide as compared to normal controls (Pandey et al., 2012). In contrast, another study evaluated IL-6 serum levels of 45 adolescents with a mean age of 16 years with moderate to severe MDD and found no statistically significant differences among groups (MDD with or without

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suicidal ideation and a comparison group) (Gabbay et al., 2009b).

Another interleukin potentially associated with MDD and anxiety disorders is IL-10. The production of IL-10 by T cells, B cells, macrophages, dendritic cells, neutrophils and eosinophils is considered one of the most important mechanisms to counteract damage inflicted by excessive inflammation (Lobo-Silva et al., 2016). Therefore, IL-10 plays an important role in the resolution of acute inflammatory response and immunomodulation. Congenital or acquired deficiencies in IL-10 production are associated with chronic conditions such as inflammatory bowel disease (IBD) and multiple sclerosis (MS) (Lobo-Silva et al., 2016), both of which may be associated with anxiety disorders and depression (Boeschoten et al., 2017; Neuendorf et al., 2016). Within the CNS, IL-10 is produced by microglia and participates in both innate and acquired immune responses and plays an important role in neuroprotection, which explains the association between IL-10 dysregulation and neurodegenerative conditions (Tang and Le, 2016).

Although several studies reported a positive association between MDD or anxiety disorders and elevated levels of IL-10 in adults (Belem da Silva et al., 2017; Hernández et al., 2008; Hoge et al., 2009; Simon et al., 2008), evidence in samples of children and adolescents is scarce. One report showed no statistically significant differences in IL-10 levels between female adolescents with MDD or anxiety disorders and healthy controls (Henje Blom et al., 2012). Considering that IL-10 plays a role in the anti-inflammatory response and that MDD and anxiety disorders may be associated with increased inflammation, the authors suggested that the ratio between IL-6 and IL-10 levels could be a better marker of impaired inflammatory control.

Although synthesized in distinct pathways, some authors consider IL-6 and IL-10 markers of the two extremes of microglial activation. On the extreme represented by IL-6 elevations, there is neuronal injury or infection and cascade production of TNF-α, IL-1β and prostaglandin. On the other side, represented by increased levels of IL-10, tissue repair and neuroprotection prevail, associated with higher production of IL-4, IL-13 and heparin-binding lectin (Lobo-Silva et al., 2016; Tang and Le, 2016). In other words, levels of IL-6 and IL-10 might be considered a proxy of immunomodulatory balance within the brain (Dhabhar et al., 2009; Henje Blom et al., 2012).

No interleukins other than IL-6 and IL-10 have been consistently associated with internalizing disorders. However, previous evidence investigating the role of inflammatory markers in internalizing disorders is limited in a number of important ways. *First*, compared to studies in children, studies in adults fail to distinguish between the effects of internalizing disorders per se and the long-term health consequences of being chronically ill. *Second*, studies in adults are more prone to confounding by other factors that are known to affect interleukin levels, such as exposure to drug abuse, smoking, etc. (Singh and Newman, 2011). *Third*, studies including individuals with clinical conditions often recruit individuals on use of medications that may potentially attenuate the effects of the studied exposure on the outcomes (Gabbay et al., 2009a).

Here we aim to address those limitations using data from a community sample of adolescents, which was oversampled for individuals with internalizing disorders. The aim of the present study is to compare serum levels of IL-6 and IL-10 between non-medicated adolescents with internalizing disorders and a comparison group of adolescents without internalizing disorders from the same community. Our main hypothesis is that the former group will present significantly higher levels of pro-inflammatory cytokine IL-6 and significantly lower levels of anti-inflammatory cytokine IL-10 than controls.

2. Methods

2.1. Sample and procedures

Students aged between 10 and 17 years old from the six schools belonging to the catchment area of the primary care unit of Hospital de Clínicas de Porto Alegre (HCPA), southern Brazil, were evaluated. The detailed procedure can be found elsewhere (Salum et al., 2011). Briefly, 2457 individuals were screened for anxiety disorders with the Screen for Child Anxiety Related Disorders (SCARED) (Birmaher et al., 1997) in their schools. All individuals with a score above the 75th percentile on this scale and a random group from the other 3 quartiles were invited to have a detailed clinical evaluation at the hospital (n = 842). Among those who attended the diagnostic interview (n = 240), we identified 138 individuals with at least one anxiety disorder (apart from specific phobia) and 102 individuals without anxiety disorders. Individuals were excluded if they: (1) had significant clinical illness; (2) were using psychiatric medication; (3) had a history of bipolar disorder, pervasive developmental disorder or any psychotic disorder; and (4) had a clinical suspicion of intellectual disability. A total of 134 subjects (n = 76 with internalizing disorders) who were not excluded on this basis had their blood collected for biomarkers analysis. The sample that attended screening did not differ significantly from the one that attended detailed clinical evaluation at the hospital regarding symptoms or risk factors, except that the former was slightly older than the latter (13.9 vs 12.8 years-old, $p < 0.001$). Parents provided written informed consent for participating in the study, and the adolescents provided written assent. This study was approved by the ethical committee of Hospital de Clínicas de Porto Alegre (number 08-017).

2.2. Measures

2.2.1. Psychiatric diagnosis

Psychiatric diagnoses were based on the Schedule for Affective Disorder and Schizophrenia for School-Age Children-Present and Lifetime Version (K-SADS-PL), a DSM-IV-TR (APA, 2002) structured interview validated in many settings (Kaufman et al., 1997). Several psychometric proprieties of K-SADS have already been tested in similar populations and it is suggested that it can be a useful cross-cultural diagnostic measure (Brasil and Bordin, 2010; Polanczyk et al., 2003). Children/adolescents and their parents were interviewed by trained psychiatrists (total number of interviewers = 6; Kappa = 0.93 across anxiety disorders). Two comparison groups were constituted based on the K-SADS-PL diagnoses: those with internalizing disorders (MDD, generalized anxiety disorder, separation anxiety disorder, social anxiety disorder or panic disorder) and those without internalizing disorders. Both groups included subjects with externalizing disorders: attention deficit hyperactivity disorder (ADHD), oppositional defiant disorder (ODD) and conduct disorder (CD).

2.2.2. Cytokine measurement

The concentration of serum cytokines was determined by flow cytometry using the BD™ Cytometric Bead Array (CBA) Th1/Th2/Th17 Human Cytokine kit (BD Biosciences, San Diego, CA). Only data on IL-6 and IL-10 were analyzed for the purposes of this study. Sample processing and data analysis were performed following the manufacturer's guidelines. Data were acquired using a FACSCalibur flow cytometer (BD Biosciences, San Diego, CA) and results were generated using the BD CBA Analysis Software FCAP Array™ (BD Biosciences, San Diego, CA). The results are expressed in pg/mL.

Detectable IL-6 serum levels ranged between 0.007 and 6.07 pg/mL. Six adolescents (11.5%) within the group without internalizing disorders and 3 (4.4%) of those with an internalizing disorder presented undetectable serum IL-6 levels according to the quantifying methods described above, generating a truncated distribution.

2.2.3. Measurement of potential confounders

Socioeconomic data was obtained according to Brazilian Association of Research Companies (ABEP) Guidelines (ABEP, 2010).

Standard techniques and calibrated equipment were used to assess anthropometric measures (WHO, 1995). Body weight was measured with portable digital electronic balance scales (Marte®, SR Sapucaí, MG,