The roles of cortisol and pro-inflammatory cytokines in assisting the diagnosis of autism spectrum disorder

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ABSTRACT

Autism spectrum disorders (ASD) is a severe neurodevelopmental disorder characterized by impairments in social interaction and repetitive behaviors. Diagnosis of ASD is currently phenotype based with no reliable laboratory test available to assist clinicians. The desire for clinically useful and reliable biomarkers is strong. Researches have shown that individuals with autism often exhibit dysfunction of hypothalamic–pituitary–adrenal (HPA) axis and cytokines. The purpose of this study was to evaluate diurnal variation of cortisol (cortisol VAR), interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) as potential biomarkers for ASD. The present results demonstrated that in comparison to the healthy controls, the individuals with autism showed a lower level of cortisol VAR, higher level of IL-6 and TNF-α. The levels of cortisol VAR, IL-6 and TNF-α have significantly correlations with the severity of ASD measured by CARS scores. The results of ROC analysis indicated the cortisol VAR, IL-6 and TNF-α were potential biomarkers in diagnosis of ASD. The combination of three factors performed the best sensitivity and specificity for diagnosis of ASD. Therefore, the present study may reveal a simple clinical approach with great potential for assisting the diagnosis of ASD.

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1. Introduction

Autism spectrum disorders (ASD) is characterized by substantial impairment in reciprocal social interaction and a markedly restricted repertoire of activities and interests in the early developmental period (American Psychiatric Association, 2013). In addition to these features, children with autism have been described as experiencing difficulty tolerating novelty and environmental stressors (Kanner, 1943). It is estimated that 1 autism case could arise in 80–240 children born, and it has been noted that the incidence showed significant increase in recent years (Yang, Tan, & Du, 2014). Despite this relatively high prevalence, our understanding of the neurodevelopmental biology and pathophysiology of this disorder remains limited. The disorder is currently diagnosed solely using core behavioral criteria selected to define ASD. However, there is presently no trusted laboratory test available to aid the clinicians.

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It is shown that the individuals with autism may be easily stressed in previous studies (Bellini, 2006; Corbett, Schupp, Simon, Ryan, & Mendoza, 2010). The hypothalamic–pituitary–adrenal (HPA) axis is intimately involved in the stress response. It has been shown that dysfunction of HPA involved in the ASD (Ćurin et al., 2003; Lakshmi Priya, Geetha, Suganya, & Sujatha, 2013). The HPA axis, like most biological systems, is highly regulated and dependent on the ability of the system to maintain, respond and reset itself (homeostasis). Cortisol is the primary glucocorticoid in humans. It has been well studied in many populations as it is an important measure of the biologic reactivity to stress. Both excessive and deficient cortisol responses have been associated with deregulations of the HPA axis (Gillespie, Phifer, Bradley, & Ressler, 2009). Cortisol exhibits diurnal variations with peaking in the early morning hours (about 30 min after waking), declining rapidly in the morning, with a slower decrease in the afternoon, and reaching its lowest level in the evening. This pattern is already well developed in the third month of infancy (Price, Close, & Fielding, 1983; Vermes, Dohanics, Toth, & Pongracz, 1980). Many reports suggested that children with autism showed alterations in the normal circadian patterns of cortisol (Hill, Wagner, Shedlarski, & Sears, 1977; Hoshino et al., 1987; Richdale & Prior, 1992; Tordjman et al., 1997). Therefore, the role of the relative diurnal variation of cortisol (cortisol VAR) deserves further study.

Researches over the past few decades have shown immunological disturbances in ASD and a lot of studies have reported cytokines abnormalities in the peripheral blood of autistic individuals (Ashwood et al., 2011; El-Ansary & Al-Ayadhi, 2012; Goines & Ashwood, 2013; Ricci et al., 2013). In the case of developmental diseases such as ASD, the neuroimmune system could affect not only function, but also development, resulting in long-term alterations and disease (Patterson, 2002). Observations indicated significant increases in plasma level of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in the individuals with autism compared with typically developing controls (Ashwood et al., 2011; Emanuele et al., 2010; Malik et al., 2011). Increase of IL-6 and TNF-α was also found in postmortem brain specimens from individuals with autism (Li et al., 2009; Wei et al., 2011). What's more, it is reported that IL-6 and TNF-α increased in the anterior cingulated gyrus of autistic brains and also in the cerebrospinal fluid of autistic children (Vargas, Nascimbene, Krishnan, Zimmerman, & Pardo, 2005). Levels of IL-6 and TNF-α were associated with core deficits of ASD or impairments in associated behaviors (Ashwood et al., 2008; Okada et al., 2007). Recent findings suggested that proinflammatory cytokines production increased in response to psychological stress in humans (Maes et al., 1998; Steptoe, Willemsen, Owen, Flower, & Mohamed-Ali, 2001).

It is known that there is an important feedback loop between cytokines and glucocorticoids: proinflammatory cytokines, such as IL-6 are potent activators of the HPA axis (Turnbull & Rivier, 1999). Glucocorticoids in turn negatively control cytokine production and by this mechanism are able to shut down inflammatory processes to prevent host destruction due to prolonged immune activity (Besedovsky & del Rey, 2000; Sapolsky, Romero, & Munck, 2000). The separate role of cortisol and proinflammatory cytokines in ASD, coupled with the evidence for interaction between them led us to examine the role of cortisol and proinflammatory cytokines as biomarkers in ASD.

Therefore, the present study aimed to evaluate the levels of cortisol VAR, IL-6 and TNF-α in individuals with autism compared with typically developing controls. We also examine the connections between levels of cortisol VAR, IL-6, TNF-α in autistic individuals and severity of ASD respectively. Importantly, the present study assessed roles of cortisol VAR, IL-6 and TNF-α as potential biomarkers in assisting the diagnosis of ASD.

2. Method

2.1. Participants

The participants were recruited from area schools or autism outreach groups. The study was made of thirty-eight autistic individuals and thirty-two healthy individuals in control after clinical evaluations. Participants were placed in one of two groups: (1) diagnosed with ASD or (2) confirmed as typically developing controls. For the ASD group, all of the individuals met the DSM-IV-TR diagnostic criteria for ASD (American Psychiatric Association, 2000). Participants were excluded from the study if they had a diagnosis of fragile X syndrome, epileptic seizures, obsessive–compulsive disorder, affective disorders, or any additional psychiatric. Also excluded were those with inflammation, known endocrine, cardiovascular, pulmonary, liver, kidney or neurological diseases. The control individuals were normally developing, healthy individuals, unrelated to the autistic participants and without any of the exclusion criteria. Two groups of individuals were matched on age and gender, and they were not taking any medication that could interfere with endocrine or inflammation four weeks prior to the screening and in good health at time of blood draw. The intelligence quotient (IQ) was based on the previous recording in the hospital. Three autistic individuals were drop from the study due to refuse to phlebotomize. Thus, the participants consisted of thirty-five autistic individuals and thirty-two healthy control individuals. An informed consent was obtained from the parents of each individual case prior to inclusion in the study. The ethical committee of East China Normal University approved this study.

2.2. Laboratory assessment

2.2.1. Sample collection

For cortisol collection, we used saliva sampling, a noninvasive method, to avoid stressors (Kirschbaum & Hellhammer, 1994). For the ASD group, it was deemed particularly important to minimize novelty in the collection procedure, so saliva samples were collected at home on weekend. A total of eight salivary samples were collected from each research participant to obtain the cortisol diurnal rhythm. Salivary cortisol were collected immediately upon waking (around 06:00), 30 min post