Journal of Affective Disorders 225 (2018) 593–598

Contents lists available at ScienceDirect

Journal of Affective Disorders

journal homepage: www.elsevier.com/locate/jad

Research paper

# Peripheral proinflammatory cytokines in Chinese patients with generalised anxiety disorder

Zhen Tang[a,1], Gang Ye[a,1], Xinyun Chen[d], Mingzhi Pan[a], Jialin Fu[a], Tian Fu[a], Qichun Liu[a], Zhenyong Gao[a], David S. Baldwin[b,c], Ruihua Hou[b,*]

[a] Suzhou Psychiatric Hospital, Suzhou, Jiangsu, China
[b] Department of Psychiatry, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, United Kingdom
[c] University Department of Psychiatry and Mental Health, University of Cape Town, South Africa
[d] Suzhou University, Suzhou, Jiangsu, China

ARTICLE INFO

*Keywords:*
Generalised anxiety disorder
Inflammation
Cytokine
Proinflammatory cytokines

ABSTRACT

*Background:* Inflammatory responses and inflammatory cytokines have been implicated in the pathogenesis of affective disorders, particularly major depression. Given the limited evidence relating to the potential role of proinflammatory cytokines in generalised anxiety disorder (GAD), we aimed to examine peripheral proinflammatory cytokines in Chinese patients with GAD.

*Methods:* A case-controlled cross-sectional study design, with recruitment of 48 patients with first episode GAD and 48 matched healthy controls. All participants completed measures of anxiety using well-established questionnaires, and serum levels of pro-inflammatory cytokines were measured using multiplex technology.

*Results:* Serum levels of CRP, IL-1α, IL-2, IL-6, IL-8, IL-12, IFN-γ, and GM-CSF were significantly higher in the GAD group in comparison to the control group ($p < 0.05$). Pearson correlation revealed significant positive correlations between anxiety measures and serum levels of CRP, IL-1α, IL-6, IL-8, IFN-γ, and GM-CSF ($p < 0.05$).

*Limitations:* The cross-sectional study design does not permit definite conclusions on causal directions between inflammation and GAD. The study was limited to a panel of 8 cytokines and does not exclude the possibility of other important cytokines being involved.

*Conclusions:* These findings indicate an elevated peripheral proinflammatory response, and provide further support for low grade inflammation in GAD. Further research may identify an 'inflammatory signature' for diagnosis and treatment response, and guide the search for novel pharmacological interventions.

## 1. Introduction

Generalised anxiety disorder (GAD) is a common, impairing and often chronic condition characterized by excessive and uncontrollable worrying. Systematic reviews of epidemiological studies within Europe have found a 12-month prevalence of 1.7–3.4% and a lifetime prevalence of 4.3–5.9% (Wittchen and Jacobi, 2005; Wittchen et al., 2011).

Recent advances in understanding of the role of cytokines in communications between the central nervous system and the immune system have led to integrative and explanatory models for various neuropsychiatric disorders. Inflammatory responses and inflammatory cytokines have been implicated in the pathogenesis of affective disorders, particularly major depression (Leonard and Myint, 2009). Apart from the high comorbidity of GAD and major depression, similar treatment effects with antidepressants suggest possible common or similar neurobiological substrates. Camacho (2013) proposed that anxious-depression should be considered as a chronic inflammatory phenomenon, and the pronounced response of central and peripheral cytokines to stress has prompted further interest in the potential role of cytokines in the pathogenesis of anxiety disorders. A recent case-control study indicates a relatively increased pro-inflammatory response, decreased anti-inflammatory response, and an altered cytokine balance in patients with GAD (Hou et al., 2017). A large cohort study examined the association between anxiety disorders (including GAD, social phobia, panic disorder, and agoraphobia) and inflammation (Vogelzangs et al., 2013), and found elevated CRP levels in male patients with current anxiety disorders and immune dysregulation in

* Correspondence to: University Department of Psychiatry, Clinical and Experimental Sciences, University of Southampton Faculty of Medicine Academic Centre, College Keep, 4-12 Terminus Terrace, Southampton SO14 3DT, United Kingdom.
*E-mail address:* r.hou@soton.ac.uk (R. Hou).
[1] Both authors have equal contributions to the manuscript as first authors.

http://dx.doi.org/10.1016/j.jad.2017.08.082
Received 26 April 2017; Received in revised form 31 July 2017; Accepted 27 August 2017
Available online 31 August 2017

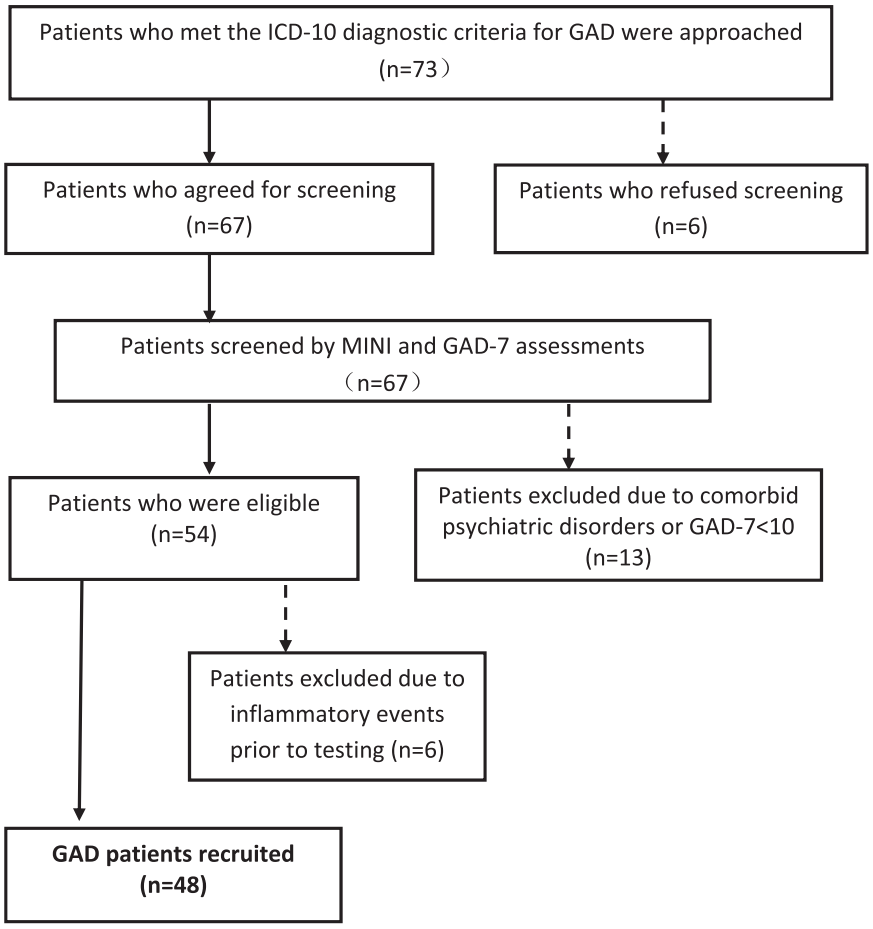


**Fig. 1.** GAD patient recruitment flow chart.

patients with a late-onset anxiety disorder. Evidence for adopting a neuroimmunological perspective on anxiety disorders has been extensively reviewed by Hou and Baldwin (2012). Due to a reliance on single cytokine measures, small sample sizes, the lack of standardized measurements, and high co-morbidity with other psychiatric conditions, findings in anxiety disorders are not consistently reported.

The aim of the current study was to investigate peripheral proinflammatory cytokine expression in Chinese patients with first episode GAD in comparison to healthy controls, and to explore possible associations with clinical characteristics, with the goal of identifying both state and trait inflammatory markers. The main predictions were as follows: Hypothesis 1: There will be an elevated serum proinflammatory cytokine levels in the GAD group in comparison to the control group; Hypothesis 2: The elevated cytokine levels will be associated with measures of anxiety.

## 2. Methods

### 2.1. Participants

#### 2.1.1. GAD patient group

Following referrals from consultant psychiatrists in the outpatient clinic at Suzhou Psychiatric Hospital, 73 patients, aged 18–60 years, with a BMI between 18–30, with 6 or more years of education, and a primary diagnosis of first episode GAD based on the International Classification of Diseases 10th Revision (ICD-10), were initially approached by researchers. 67 patients completed a pre-test screening interview comprising a structured diagnostic Mini International Neuropsychiatric Interview - MINI (Sheehan et al., 1998) and the 7-item Generalised Anxiety Disorder Questionnaire (GAD-7) with a threshold score of 10 points (Spitzer et al., 2006). Patients were excluded if they had comorbid psychiatric disorders. All GAD patients were medication naive and had no history of any antidepressant or anxiolytic intake. Participants were excluded if they reported any inflammatory events or had any intake of any medication with known immune-modulating effects, such as glucocorticoids, within 2 weeks prior to their testing session. 48 GAD patients were recruited (see GAD patient recruitment flow chart in Fig. 1).

#### 2.1.2. Healthy control group

48 age-, gender-, and BMI-matched controls were recruited through advertising in local communities in Suzhou: they were healthy volunteers aged between 18 and 60 years, had a BMI between 18–30, had no physical illness or mental disorder, had 6 or more years of education and were not taking any medication. Participants who experienced any inflammatory event or taking any medication with known immune regulating effects within two weeks before the testing were excluded.

The study was approved by the Clinical Research Ethics Committee in Suzhou Psychiatric Hospital.

### 2.2. Measures

#### 2.2.1. Measure of inflammatory cytokines

A sample of 10 ml venous blood was taken from all participants at approximately the same time of day (9:00–10:00 a.m.) and centrifuged for 15 min at 2500 rpm. The cell free-serum was pipetted and aliquoted in 2 ml standard freezer vials which were then stored within 2 h at −80 °C until further analysis. The following inflammatory cytokines were