



IL-6 and IL-8 are likely associated with psychological status in treatment naïve general population

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ABSTRACT

Background: Levels of inflammatory markers are elevated in patients with psychological disorders. However, anti-psychological drugs have an effect on proinflammatory cytokine production and disturb their relationship. Limited evidence focuses on the inflammatory marker profile of psychological status before treatment. This study aimed to investigate the inflammatory biomarker profiles of psychological treatment-naïve individuals.

Methods: We included 790 psychological treatment-naïve individuals from a longitudinal cohort study of Midlife in the United States (MIDUS). Symptoms of depression, anxiety, and stress were assessed by the Center for Epidemiologic Studies Depression Scale (CES-D) subscales, the Social Anxiety Scale (STAI), and Liebowitz Social Anxiety Scale (LSAS), the Perceived Stress Scale (PSS), respectively.

Results: Spearman correlation analysis showed that a higher CESD total score was correlated with higher CRP ($p=0.009$), IL-6 ($p=0.007$), fibrinogen ($p=0.036$), E-selectin ($p=0.018$), ICAM-1 ($p=0.013$), and IL-8 ($p=0.05$) levels. Multivariate linear regression analysis showed that the CESD total score was positively associated with the levels of IL-6 ($p=0.024$) after adjustments. Moreover, the perceived stress score (PSS) was negatively associated with the levels of IL-8 ($p=0.025$). However, these associations were not significant after multiple testing ($p=0.088, 0.091$, respectively).

Limitations: The casual relationship cannot be drawn due to the cross-sectional design

Conclusion: Overall, our results suggested IL-6 and IL-8 might play a important role in the pathogenesis of psychological disorder. Larger and longitude studies are needed to confirm our results.

1. Introduction

Psychological disorders, such as depression, anxiety, and chronic stress, are common but becoming a major public health problem worldwide and are associated with adverse consequences, resulting in a considerable burden on the individual, family, society, and healthcare systems (Berto et al., 2000, Jeon and Kim, 2016). For example, depression is the leading cause of behavioral and mental disorders by the World Health Organization, affecting over 350 million people. Anxiety

is one of the most common psychiatric symptoms in the general population, with approximately 28% lifetime prevalence (Roy-Byrne, 2015), and anxiety disorders are the sixth leading cause of disability globally (Baxter et al., 2014). Psychological disorders trigger a cascade of pathways in the central nervous system (CNS) and subsequently activate stress responses in the autonomic nervous system (ANS) (Antoni et al., 2006), which has been reported to impact multiple biological processes, including inflammation (Marsland et al., 2017, Marsland et al., 2007, Glaser and Kiecolt-Glaser, 2005), metabolism, and malignant

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progression (Price et al., 2001).

Elevated levels of proinflammatory biomarkers, such as interleukin 6 (IL-6) and C-reactive protein (CRP) are known as risk factors for the development and progression of non-cardiovascular disease and cardiovascular diseases. Several studies have shown the correlation between psychological orders or status and inflammatory biomarkers. Meta-analyses (Howren et al., 2009, Dowlati et al., 2010, Liu et al., 2012) have reported proinflammatory cytokine differences between patients with depression and health controls, including IL-6, CRP, tumor necrosis factor-alpha (TNF- α), the soluble IL-2 receptor, IL-1b and the IL-1 receptor antagonist (IL-1ra), among which IL-6 and CRP are the most significant markers. Evidence from prospective studies (Valkanova et al., 2013, Giollabhui et al., 2020, Deverts et al., 2010) showed that a higher level of depressive symptoms was associated with a higher level of future IL-6/CRP. The pediatric literature (Copeland et al., 2012) also demonstrated that in children, depression predicts subsequent CRP levels. Regarding anxiety, animal studies have shown that increased cytokine expression in the periphery is associated with heightened anxiety-like behavior in mice (Sakić et al., 1994, Schrott and Cnric, 1996), and mice overexpressing IL-6 or TNF- α develop an anxiogenic phenotype (Fiore et al., 1998). These studies showed that inflammatory processes might play a part in the pathogenesis of psychological symptoms.

Although the above studies showed the potential association between different psychological scores and inflammatory factors, a study based on one and a large sample to comprehensively evaluate the relationship between depression, anxiety, stress, and various inflammatory factors is still lacking and is essential, considering the potential interaction between different psychological disorders and inflammatory processes (Reiche et al., 2004). Moreover, observational studies (Alexopoulos and Morimoto, 2011) and meta-analyses (Dowlati et al., 2010, Alexopoulos and Morimoto, 2011) have demonstrated that antidepressants could promote proinflammatory cytokine production and thus disrupt the relationship between psychological status and inflammatory factors. However, limited studies have focused on previously untreated individuals. By using a large general population-based cohort, we aimed to comprehensively assess the association between psychological status (including depression, anxiety, stress) and the levels of various inflammatory biomarkers in individuals without psychotherapeutic treatments.

2. Material and methods

2.1. Study Population

The Midlife in the United States (MIDUS) study is a longitudinal study including a national (US) sample of adults aged 34–84 years. The MIDUS II biomarkers' Projects is a subcomponent of the MIDUS study containing 1,255 participants, among which 666 were drawn from the random digit dialing respondents, 388 were from the twins' study, and additional 201 were from a supplementary survey of new participants of African Americans from Milwaukee, Wisconsin, aim at adding comprehensive biological assessments on a subsample of MIDUS respondents, thus facilitating analyses that integrate behavioral and psychosocial factors with biology. Demographic and psychosocial data were obtained through phone interviews as well as self-administered questionnaires (MIDUS II). Biomarker data were collected during 2004–2009 through follow-up (MIDUS II). More details of the study content and protocol are available elsewhere (Dienberg Love et al., 2010).

Among the valid responses, we excluded the participants who missed exposure ($n=303$) and outcome variables ($n=24$) in this study (i.e., CES-D score, PSS, SAS, STAI scores and CRP, IL-6, IL-8, IL10, Fibrinogen, E-selectin, ICAM-1), as well as those who had a history of psychotherapeutic therapy (antidepressant=182, antipsychotics=187) or anxiolytics and hypnotics medication ($n=164$). Finally, our analytical sample comprised 790 participants.

The study had gotten approval of the Institutional Review Board for each participating MIDUS center, and written informed consent was obtained from all participants.

2.2. Exposure variable of this study

Demographic and socioeconomic details, including age, sex, marital status, ethnicity, and level of education, were assessed at baseline. Healthy lifestyle factors included regular exercise, smoking, and drinking. Chronic somatic conditions/illnesses was determined by the presence of at least one or more somatic chronic conditions/illnesses consisting of a list of 20 medical conditions/illnesses, including heart disease, high blood pressure, circulation problems, blood clots, heart murmur, TIA or stroke, anemia or other blood disease, cholesterol problems, diabetes, asthma, emphysema/COPD, tuberculosis, positive TB skin test, thyroid disease, peptic ulcer disease, cancer, colon polyp, arthritis, glaucoma, cirrhosis/liver disease. History of depression was defined as ever being physician-diagnosed depression.

2.3. Center for Epidemiologic Studies Depression Scale

Depressive symptoms were assessed by using the Center for Epidemiologic Studies Depression Scale (CES-D) (Lewinsohn et al., 1997). The CES-D is a 20-item measure of depressive symptoms within the past two weeks using a 4-point Likert scale (Tsai, 2021). The Cronbach's alpha of CES-D is 0.894 in MIDUS II biomarkers' Projects, indicating excellent internal consistency. The construction of the CES-D was distinguished into four-factor solutions: somatic, negative affect, positive affect, and interpersonal symptoms. Thereinto, three subscales including negative affect, somatic features, and interpersonal disturbances, were served as indicators of depressive symptom profiles. Higher scores on each subscale suggest greater depressive symptoms. The cutoff value of CES-D were defined as previously reported (Henry et al., 2018): <16 , no depressive symptom; ≥ 16 , with depressive symptoms.

2.4. Spielberger Trait Anxiety Inventory

The Spielberger Trait Anxiety Inventory (STAI) has been considered as a tool for measuring inter- and intra-individual variability in state and trait anxiety (Spielberger and Spielberger, 1989). The inventory constitutes a state questionnaire (Form A) and a trait questionnaire (Form B). Each form is associated with a state scale, a trait scale, and a balanced scale. Each state and trait scale was measured by asking respondents to rate themselves on a total of 20 items. STAI items describe subjective phenomena associated with the presence of anxiety (e.g., "I feel worried") or without (e.g., "I feel calm"). We refer to the items tended to anxiety as the negative items and to the items that tended to the opposite site of anxiety as positive items. In the balanced scale, the reversed scores for positive items and the scores for negative items are converged to get an overall anxiety score. The Cronbach's alpha of STAI in MIDUS II biomarkers' Projects is 0.908. The cutoff value of STAI was defined as previously reported (Zingano et al., 2019): no anxiety symptoms (< 54), and with anxiety symptoms (≥ 54).

2.5. Liebowitz Social Anxiety scale

The Social Anxiety scale used in MIDUS was deprived from the Liebowitz social anxiety (LSAS) (Baker et al., 2002), which is a 24-item semi-structured interview that measures fear and avoidance suffered in a range of social and performance situations and has been the most popular and widely used measures of social phobia. The scale in our study is a self-report version of the LSAS and consists of 9 items, each depicting different social situations (e.g., "Talking to people in authority", "Going to a party", "Working while being observed", "Calling someone you don't know very well", "Talking with people you don't know very well", "Being the center of attention", "Expressing a

disagreement or disapproval to people you don't know very well", "Returning goods to a store", and "Resisting a high-pressure salesperson"). Responses were averaged to create a composite score of each item, with higher scores reflecting more social anxiety symptoms. The scale score was constructed by computing the mean across all items for cases. The Cronbach's alpha of LSAS in MIDUS II biomarkers' Projects is 0.852. The severity of anxiety under each situation increased from 1 "none", 2 "mild", 3 "moderate", and 4 "severe", as previously reported (Jaremka and Pacanowski, 2019).

2.6. Perceived Stress Scale

The Perceived Stress Scale (PSS) (Cohen and Williamson, 1988) is a 10-item measure that assesses the degree of pressure in participants' lives. Each item (e.g., "In the past month, how often have you been upset because of something that happened unexpectedly?") uses a 5-point scale from 1 (never) to 5 (very often), and is coded backwards as needed, so that higher scores suggest greater perceived stress ($\alpha=0.84$). The Cronbach's alpha of PSS in MIDUS II biomarkers' Projects is 0.864. The levels of stress were categorized as previously reported: low (0-13), moderate (Giollabhui et al., 2020, Deverts et al., 2010, Copeland et al., 2012, Sakić et al., 1994, Schrott and Crnic, 1996, Fiore et al., 1998, Reiche et al., 2004, Alexopoulos and Morimoto, 2011, Dienberg Love et al., 2010, Lewinsohn et al., 1997, Tsai, 2021, Henry et al., 2018, Spielberger and Spielberger, 1989), and high perceived stress (Zingano et al., 2019, Baker et al., 2002, Jaremka and Pacanowski, 2019, Cohen and Williamson, 1988, Swaminathan et al., 2016, Khandaker et al., 2014, Wium-Andersen et al., 2014, Glaser et al., 1999, Song et al., 2007, Kalin et al., 2006, Kessler et al., 1993, Meduri et al., 2013, Chu et al., 2016, Wiegner et al., 2015; Swaminathan et al., 2016).

2.7. Outcomes/measurement of inflammatory biomarkers

CRP was measured using a BNII nephelometer from Dade Behring utilizing a particle-enhanced immunonephelometric assay. IL-6, IL-8, IL10, and TNF- α were measured using the Quantikine® High-sensitivity ELISA kit #HS600B (R&D Systems, Minneapolis, MN). sICAM-1 was measured by ELISA (Parameter Human sICAM-1 Immunoassay; R&D Systems, Minneapolis, MN). sE-selectin levels were measured using a high-sensitivity ELISA. (Parameter Human sE-Selectin Immunoassay; R&D Systems, Minneapolis, MN). Fibrinogen antigen was measured using a BNII nephelometer (N Antiserum to Human Fibrinogen; Dade Behring Inc., Deerfield, IL). The amount of fibrinogen present in the sample was quantitatively determined by the immunochemical reaction. The intra-assay coefficient of variation (CV) of CRP, IL-6, IL-8 IL10, TNF- α , sE-selectin, Fibrinogen was respectively 2.2%-4.1%, 3.25%, 2.88%, 5.78%, 3.19%, 4.7%-5.0% and 2.7% (CV of ICAM1 was lacking in the MIDUS). And the inter-assay CV of CRP, IL-6, IL-8 IL10, TNF- α , sE-selectin, Fibrinogen and sICAM-1 was 4.72-5.16%, 6.78-7.34%, 6-7%, 11-14%, 7%, 5.7-8.8%, 2.6%, and 5.0%, respectively. The assay range of CRP, IL-6, IL-8 IL10, TNF- α , sE-selectin, Fibrinogen and sICAM-1 was 0.014-26 ug/mL, 0.156-10 pg/mL 1.13-375 pg/mL, 0.68-233 pg/mL, 0.69-248 pg/mL, 0.47-10.52 ng/mL, 60-1200 mg/dL, and 0.47-10.52 ng/mL, respectively. More details can be found by Weinstein M et al (Khandaker et al., 2014).

2.8. Statistical Analysis

The normality of the data was analyzed by the Kolmogorov–Smirnov test and Q-Q plots. Non-normally distributed data are expressed as median (interquartile range [IQR]) and normally distributed data as mean \pm standard deviation ($M \pm SD$). In this study, Spearman correlation analysis was first used to calculate the correlation between psychological scores and inflammatory factors. Then, the associations between psychological scores and serum inflammatory markers were further evaluated by multivariate linear regression analysis. The selection of

adjusted covariates in the multivariable models was based on the backward stepwise method with a significance level of $P < 0.10$, including all the variables at baseline. All of the analyses were performed by using SPSS Statistics version 26.0 (IBM). To examine the robustness of our findings, we further conducted sensitivity analyses of multiple testing for the main results, using Permutation tests by statistical software package EmpowerStats (<http://www.empowerstats.com>, X&Y Solutions, Inc., Boston, MA). $P \leq 0.05$ was considered as statistically significant.

3. Result

3.1. The characteristics of the study subjects

Of the 1,255 participants, we excluded participants who lacked related psychological scales, and biomarker blood samples ($n=327$), 928 individuals remained. Our objective was to investigate the relationship between psychological scores and inflammation markers in naive treatment general population. Therefore, we further excluded those who had a history of psychotherapeutic therapy (antidepressant=182, anti-psychotics=187) or anxiolytics and hypnotics medication ($n=164$), among which 126 participants had a history of depression or other psychological disorders. Finally, 790 participants were included in the study.

The demographic and socioeconomic characteristics are shown in **Table 1**. Overall, the mean age was 56 years older, with a mean BMI of 29.0; 428 participants (53.9%) were females, 761 participants (95.8%) were Hispanic, 587 participants (73.9%) were married, 152 participants (19.1%) had bachelor's degrees or higher, 319 participants (40.1%) were current smokers, 552 participants (69.5%) were current drinkers and 636 participants (80.1%) engaged in regular exercise or activity of any type for 20 minutes or more at least 3 times/week. Additionally, there were 744 participants (94.2%) with the presence of at least one or more somatic conditions/illnesses, the most six prevalent comorbidities were diabetes (42%), hypertension (37.8%), heart diseases (9.4%), TIA or stroke (14.9%), emphysema/COPD (13.9%), and cancers (12.7%). As for psychiatric conditions, 82 participants (10.4%) had a history of depression diagnosis, and no individuals had a history of other mental disorders. The total score of CES-D in our study was 7.35 ± 7.01 , and all had no depressive symptoms (≥ 16). The score of STAI in our participants was 33.04 ± 8.42 , and 18(2.3%) participants had anxiety symptoms (≥ 54). The average items of LSAS were 1.82 ± 0.52 , and 28(3.6%) had an average score higher than 3, suggesting mild levels of social anxiety on average and subclinical social anxiety symptoms. Notably, the score of PSS in our study achieved 21.24 ± 5.84 , and 75.5% (588/790) participants have high perceived stress symptoms ($PSS > 27$).

3.2. Relationships between psychological scores and inflammation markers by Spearman correlation analysis

As shown in **Table 2**, the CES-D total score was positively associated with the levels of CRP ($r=0.092$, $p=0.009$), IL-6 ($r=0.095$, $p=0.007$), fibrinogen ($r=0.074$, $p=0.036$), E-selectin ($r=0.084$, $p=0.018$), and ICAM-1 ($r=0.089$, $p=0.013$) and negatively associated with IL-8 ($r=-0.069$, $p=0.05$), while the STAI score did not show any association with inflammatory biomarkers, and the LSAS only showed a negative association with IL-6 ($r=-0.076$, $p=0.033$). The PSS was positively correlated with E-selectin ($r=0.079$, $p=0.027$) and negatively correlated with IL-8 ($r=-0.085$, $p=0.016$) and TNF- α ($r=-0.08$, $p=0.023$).

3.3. Associations between psychological scores and inflammation markers by regression analysis

Univariate linear regression analysis (**Supplemental Table S1**) showed that the CESD score ($\beta=-0.087$, $p=0.015$), STAI score ($\beta=-0.075$, $p=0.035$), SAS score ($\beta=-0.085$, $p=0.017$) and PSS score

Table 1
Participant characteristics(N=790)

	Mean (% or IQR)
Demographic factors	
Age, years	69 (34, 84)
Ethnicity, %	
Hispanic	761(95.8)
Latino	33(4.2)
BMI	29.0 ± 5.9
Socioeconomic factors	
Education, % with bachelor's degree or higher	152(19.1)
Marital status, % married	587(73.9)
Health lifestyle	
Regular exercise, %	636(80.1)
Smoking, %	319(40.1)
Drinking, %	552(69.5)
Medical	
Presence of somatic conditions/illnesses, %	94.2%
Heart disease, n (%)	94(9.4%)
High blood pressure, n (%)	300(37.9%)
TIA or stroke, n (%)	118(14.9%)
Diabetes, n (%)	332(42.0%)
Emphysema/COPD, n (%)	110(13.9%)
Cancers	100(12.7%)
Systolic blood pressure, mmHg	131.7 ± 17.8
Diastolic blood pressure, mmHg	75.2 ± 10.3
Blood hemoglobin A1c %	6.0 ± 0.9
Triglycerides, mg/dL	106.5(78, 154)
Total cholesterol, mg/dL	185.6 ± 39.9
LDL-C, mg/dL	104.8 ± 34.8
HDL-C, mg/dL	54.9 ± 17.6
Creatinine, mg/dL	0.87 ± 0.27
Serum inflammatory markers	
C-reactive protein, ug/mL	1.23(0.62, 2.98)
IL-6, pg/mL	0.78(0.55, 1.12)
IL8, pg/mL	12.3(9.1, 15.4)
IL-10, pg/mL	0.23(0.17, 0.33)
TNF-α, pg/mL	2.23 ± 0.80
Fibrinogen, mg/dL	340.2 ± 82.4
Soluble E-Selectin, ng/mL	41.6 ± 20.9
sICAM-1, ng/mL	285.6 ± 98.9
History of depression, n(%)	82 (10.4%)
Psychosocial scores	
Depression	
CESD total score	7.35±7.01
No depressive symptoms (<16), n (%)	790 (100.0%)
With depressive symptoms (≥16), n (%)	0 (0.0%)
Anxiety	
STAI score	33.04±8.42
No anxiety symptoms (<54), n (%)	772 (97.7%)
With anxiety symptoms (≥54), n (%)	18 (2.3%)
LSAS	1.82±0.52
Moderate to severe(3-4)	28(0.35%)
Stress	
PSS score	21.24±5.84
Low stress(0-13), n (%)	58 (7.3%)
Moderate stress(14-26), n (%)	144 (18.2%)
Severe stress(>27), n (%)	588 (75.5%)

Notes: Notes: M(IQR) for nonnormally distributed data, M ± SD for normally distributed data, and n (%) for categorical variables.

Abbreviations: BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein; IL, Interleukin; CRP, C-reactive protein, TNF-α, tumor necrosis factor-α; ICAM-1, intercellular adhesion molecule-1; CESD, Center for Epidemiologic Studies Depression Scale; STAI, Spielberger Trait Anxiety Inventory; LSAS: Liebowitz social anxiety; PSS, Perceived Stress Scale; M (IQR), median interquartile range; M ± SD, mean ± standard deviation;

($S\beta=-0.121$, $p=0.001$) were all negatively associated with IL-8. And the CESD score also demonstrated a positive association with IL-6 ($S\beta=0.093$, $p=0.008$) and Fibrinogen ($S\beta=0.070$, $p=0.047$). To further elucidate the association between psychological scores and inflammation biomarkers, we performed multivariate linear regression analysis. As shown in **Table 3**, the CES-D score was positively associated with IL-6 ($S\beta=0.113$, $p<0.001$) and fibrinogen ($S\beta=0.085$, $p=0.016$) after adjusting for age and sex (**Model 1**). After adjusting for more

confounding factors (model 2), the CESD total score showed a significant association with IL-6 ($S\beta=0.073$, $p=0.024$). There was no significant association between the CESD score and IL-8, fibrinogen TNF-α E-selectin, or ICAM-1.

Regarding the anxiety score, which was represented by the LSAS and STAI score, the anxiety degree showed a positive relationship with IL-6 ($S\beta=0.092$, $p=0.009$) and ICAM-1 ($S\beta=0.856$, $p=0.041$) and a negative relationship with IL-8 ($S\beta=-0.073$, $p=0.041$) after adjusting for age and gender (model 1). However, these associations were nonsignificant after adding other confounding factors (model 2). There was no association between the STAI score and serum levels of CRP, IL-8, IL-10, fibrinogen, TNF-α, and E-selectin in any of the models.

The PSS, which represents stress degree, also showed a positive relationship with IL-6 ($S\beta=0.068$, $p=0.054$) and a negative relationship with IL-8 ($S\beta=-0.075$, $p=0.028$) after adjusting for age and gender (model 1). In model 2, PSS only showed a strong negative relationship with IL-8 ($S\beta=-0.076$, $p=0.025$).

These above results did not significantly change when further adjusting for comorbidities (heart disease, high blood pressure, TIA or stroke, diabetes, COPD, cancers) (data not shown).

3.4. Subgroup analysis of the associations between psychological scores and inflammation markers in the multiple-variance analysis

We further performed subgroup analysis stratified by age and sex. In the sex-subgroup, there was no association between IL-6 and CES-D, either in females ($n=425$, $S\beta=0.068$, $p=0.130$) or in males ($n=325$, $S\beta=0.061$, $p=0.212$), with no significant interaction for gender ($p=0.920$). The negative association between IL-8 and stress only observed in females ($S\beta=-0.013$, $p=0.022$), but not in males ($S\beta=-0.103$, $p=0.484$), with a significant interaction for gender ($p=0.011$). Notably, the association between IL-8 and LSAS was more marked among females ($S\beta=-0.087$, $p=0.058$) than males ($S\beta=-0.021$, $p=0.680$), with a significant interaction for sex ($p=0.011$). (**Supplemental Table S2**)

As for age-subgroup analysis **Supplemental Table S3**. The positive association between IL-6 and CES-D scores persisted in elders (≥ 65 years, $n=201$) ($S\beta=0.141$, $p=0.034$) but not in mid-adults (<65 years, $n=589$) ($S\beta=0.051$, $p=0.181$) after fully adjustments (model 2). Conversely, the negative association between IL-8 and stress only showed in mid-adults ($S\beta=-0.096$, $p=0.018$) but not in elders ($S\beta=0.038$, $p=0.588$), without interactions (all $p>0.05$).

3.5. Sensitivity analysis of multiple testing

To control the type I error and the robustness of our main findings, we further conducted sensitivity analyses of multiple testing. As shown in **Table 3**, the positive association between IL6 and CESD score and the negative association between IL8 and PSS score in multivariate linear regression analysis (**Table 3**) were not significant (p adjusted value=0.088, 0.091, respectively).

4. Discussion

In this comprehensive assessment of inflammatory markers in the general population without psychotherapeutic medication, we assessed the association between psychological status, including depression, anxiety, stress, and serum levels of inflammation biomarkers. We found that psychological status was positively associated with IL-6 levels and inverse IL-8 levels after adjusting for confounding factors, however, the results were not significant in the sensitivity analysis of multiple testing. No significant association was found between psychological status and CRP or other inflammation levels.

Although several studies have investigated the association between psychological disorders and inflammatory factors, the results were inconsistent. A meta-analysis (Valkanova et al., 2013, Giollabhui et al., 2020) showed a weak association between high CRP levels at baseline

Table 2
Spearman correlation analysis between depression, anxiety, stress and inflammatory markers

Variables	CESD total score	CESD Depressive Affect	CESD Positive Affect	CESD Somatic Complaints	CESD Interpersonal Subscale	STAI score	LSAS score	PSS score
CRP	0.092*	0.062	-0.034	0.121*	0.071*	0.042	-0.40	-0.002
IL-6	0.095#	0.033	-0.009	0.154#	0.085*	0.026	-0.076*	-0.012
IL-8	-0.069*	-0.065	0.053	-0.046	-0.108*	-0.048	-0.067	-0.085*
IL-10	0.039	-0.022	-0.023	0.065	0.056	0.034	-0.044	0.003
TNF- α	0.020	-0.0306	-0.034	0.035	-0.013	-0.012	-0.041	-0.08*
Fibrinogen	0.074*	0.066	-0.066	-0.039	0.032	0.012	-0.028	0.017
E-Selectin	0.084*	0.074*	-0.037	0.076*	0.139#	0.037	0.016	0.079*
ICAM-1	0.089*	0.004	-0.60	0.134#	0.008	0.055	0.003	0

Notes: * $P < 0.05$, # $P < 0.001$ Spearman correlation analysis was used.

Abbreviations: IL, Interleukin; CRP, C-reactive protein, TNF- α , tumor necrosis factor- α ; ICAM-1, intercellular adhesion molecule-1; CESD, Center for Epidemiologic Studies Depression Scale; STAI, Spielberger Trait Anxiety Inventory; PSS, Perceived Stress Scale; LSAS, Liebowitz Social Anxiety scale

and future depressive symptoms and no association between baseline IL-6 and future depressive symptoms at follow-up. A recent meta-analysis³⁶ found that higher CRP/IL-6 was associated with depressive symptoms in the future, and higher depressive symptoms were in turn associated with higher future CRP/IL-6 in both unadjusted and adjusted analyses. However, almost all of the previous studies did not exclude patients who were exposed to psychotherapeutic therapy. Prior exposure to various regimens of antidepressants might affect cytokine production in some depressive patients. In our present study, after excluding antidepressants or other medications for psychiatric conditions, we focused only on individuals without psychotherapeutic medication. We found that depression was positively associated with levels of IL-6 but not CRP or any other inflammatory factors after adjusting for age, sex, BMI, smoking history, exercise lifestyle physician, and biological parameters. The results did not significantly change after chronic diseases were further adjusted (data not shown). The association between depressive symptoms and CRP or IL-6 is still under debate. Notably, our results were in line with a Mendelian genetic research study (Wium-Andersen et al., 2014). Their results showed that elevated CRP was associated with an increased risk of depression in the general population, but genetically elevated CRP was not (Wium-Andersen et al., 2014). Collectively, it suggested that IL-6, but not CRP, might be a causal risk factor for depression before treatments.

IL-8, released by macrophages, endothelial cells, and T cells, is a well-characterized member of the chemokine superfamily. There were limited studies investigating the association between IL-8 and stress, and thus a firm conclusion cannot be drawn. Our present study found that there was an inverse association between stress level and IL-8. In consist with our research, a human cross-sectional study (Glaser et al., 1999) reported that individuals with higher perceived stress scales had significantly lower IL-8 levels than the general population. Post-traumatic stress disorder (PTSD), a common stress-related mental disorder, was also associated with a reduced level of serum IL-8 (Song et al., 2007). Additionally, in an experimental study (Kalin et al., 2006), acute stress significantly decreased IL-8 gene expression. The possible mechanism for the reduced IL-8 level in individuals under high stress level was uncertain. Furthermore, our sex-subgroup analyses showed that a significant interaction in the association between IL-8 and anxiety, and stress. These results might be consistent with an article that reported females almost 1.7 times as likely as males to suffer from psychological disorders (Kessler et al., 1993). However, considering the limited sample size, this result still needs to be assessed by further researchers.

Further, it is notably that there are certainly interactions between depression, anxiety and stress. For insistence, experimental studies reported that mice under chronic stress experienced increased anxiety and depressive-like behaviors (Meduri et al., 2013). Similarly, a mouse model of 24h-restraint stress exhibited anxiety and depressive-like behaviors (Chu et al., 2016). As for human data, a cross-sectional study that included 587 patients under chronic stress reported 64% of patients had anxiety and 33% had depressive-like symptoms (Wiegner et al.,

2015). Interestingly, the interactions between depression, anxiety, and stress might be correlated with proinflammatory processes (Beurel et al., 2020). Therefore, it would be better to consider this interaction between various psychological disorders when we study their association between inflammatory factors. However, our present study did not elucidate these interactions due to the data restriction, which was a limitation and deserved to be further studied.

Notably, the positive association between IL6 and CESD score and the negative association between IL8 and PSS score were not significant anymore in the sensitive analysis of multiple testing. We should explain these results with caution. First, this negative results after multiple testing may due to the insufficient sample size of our present study. Second, it is worth noting that there were indeed some studies supporting our results. For instance, two longitude studies found expression IL-6 was positive associated with depression (Gimeno et al., 2009, Stewart et al., 2009). Experiment studies found IL-6 knockout mice exhibit resistance to stress-induced development of depression-like behaviors (Chourbaji et al., 2006). Regarding IL-8, Chourbaji et al. (Jar-emka and Pacanowski, 2019) found low IL-8 is associated with anxiety in suicidal patients, suicide attempters carrying IL-8-251T allele showed more severe anxiety. Vitro experiment also showed expression of IL-8 in response to vitro stimulation of blood by lipopolysaccharide was associated with anxiety disorder (Vogelzangs et al., 2016). Overall, although the results were not significant after multiple testing, our results suggested IL-6 and IL-8 might play a role in the pathogenesis of psychological disorder. Larger and longitude studies are needed to confirm our results.

4.1. Limitations

There are several limitations. First, this is a cross-sectional study, which precluded us from investigating and cannot prove causation relationships between psychological status and inflammatory biomarkers.

Second, although some confounding factors were adjusted, unmeasured and insufficiently measured variables (e.g., subclinical disease state and cardiovascular medication) would result in the possibility of residual confounding factors. Third, there is an interaction between depression, anxiety, and stress. These psychological disorders can act synergistically to potentiate larger inflammatory responses that could in turn further fuel depression and other mental problems. However, due to the data restriction, our present study did not elucidate the interactions between those psychological status. Further research is needed to clarify these interactions.

5. Conclusion

Overall, our results suggested IL-6 and IL-8 might play a role in the pathogenesis of psychological disorder. Larger and longitude studies are needed to confirm our results.

Table 3
Multivariate Linear Regression analysis of association between psychological scales and inflammatory markers.

Model 1	CESD score				STAI score				LSAS score				PSS score			
	R ²	Sβ	P value	P †	R ²	Sβ	P value	P †	R ²	Sβ	P value	P †	R ²	Sβ	P value	P †
Ln(CRP)	0.017	0.069	0.055	0.264	0.014	0.030	0.397	0.394	0.015	-0.048	0.176	0.948	0.013	0.00	0.990	0.948
Ln(IL6)	0.067	0.133	<0.001#	0.002	0.058	0.092	0.009*	0.048	0.050	-0.024	0.494	0.811	0.054	0.068	0.054	0.218
Ln(IL8)	0.102	-0.043	0.210	0.532	0.102	-0.044	0.195	0.486	0.105	-0.070	0.041*	0.120	0.106	-0.075	0.028*	0.090
Ln(IL10)	0.009	0.029	0.418	0.774	0.011	0.051	0.152	0.344	0.010	-0.031	0.391	0.732	0.009	-0.004	0.913	1.000
TNF-α	0.093	0.056	0.102	0.341	0.093	0.056	0.104	0.316	0.090	0.022	0.518	0.932	0.090	-0.011	0.775	0.976
Fibrinogen	0.030	0.085	0.016*	0.070	0.024	0.028	0.433	0.838	0.023	-0.022	0.534	0.894	0.024	0.032	0.366	0.784
E-Selectin	0.017	0.050	0.159	0.356	0.015	0.001	0.974	1.000	0.015	-0.014	0.692	0.965	0.018	0.060	0.096	0.263
ICAM-1	0.019	0.080	0.025*	0.970	0.018	0.073	0.041*	0.120	0.014	0.037	0.295	0.686	0.014	0.029	0.421	0.884
Model 2																
Ln(CRP)	0.232	0.006	0.849	1.000	0.232	-0.021	0.507	0.865	0.235	-0.056	0.077	0.169	0.235	-0.055	0.082	0.160
Ln(IL6)	0.219	0.073	0.024*	0.088	0.216	0.042	0.190	0.501	0.215	-0.037	0.254	0.546	0.214	0.014	0.672	0.980
Ln(IL8)	0.110	-0.046	0.179	0.453	0.110	-0.046	0.177	0.438	0.112	-0.062	0.067	0.204	0.114	-0.076	0.025*	0.091
Ln(IL10)	0.038	0.026	0.461	0.839	0.040	0.051	0.145	0.361	0.038	-0.020	0.577	0.933	0.038	-0.003	0.924	1.000
TNF-α	0.029	0.025	0.437	0.283	0.209	0.023	0.470	0.999	0.208	0.003	0.932	0.940	0.210	-0.043	0.181	0.999
Fibrinogen	0.107	0.049	0.153	0.275	0.104	-0.000	0.990	1.000	0.105	-0.023	0.505	0.937	0.104	0.001	0.980	1.000
E-Selectin	0.081	0.019	0.576	0.906	0.081	-0.026	0.444	0.903	0.080	-0.015	0.663	0.983	0.081	0.033	0.347	0.695
ICAM-1	0.048	0.054	0.128	0.355	0.049	0.060	0.090	0.700	0.047	0.036	0.297	0.235	0.0454	0.007	0.833	1.000

Notes: Sβ for standardized beta. Multivariate Linear analysis was used. * $p < 0.05$, # $p < 0.001$,

Model 1 was adjusted for age and sex.

Model 2 was adjusted for age, sex and other related factors in univariate regression analysis. CRP was adjusted for gender, BMI, exercise, HDL, total cholesterol, Hb1c% and creatine. IL-6 was adjusted for age, gender, BMI, smoke, exercise, HDL and creatine. IL-8 was adjusted for age, gender and Hb1c%. IL-10 was adjusted for age, total cholesterol, HDL and Hb1c%. TNF-α was adjusted for age, gender, BMI, creatine and HDL. Fibrinogen was adjusted for age, gender, ethnic, BMI, DBP, Hb1c% and total cholesterol. E-selection was adjusted for age, BMI, HDL and Hb1c%. ICAM-1 was adjusted for age, smoke, total cholesterol and HDL

† Adjusted for multiple testing

Abbreviations: IL, Interleukin; CRP, C-reactive protein, TNF-α, tumor necrosis factor-α; ICAM-1, intercellular adhesion molecule-1; CESD, Center for Epidemiologic Studies Depression Scale; STAI, Spielberger Trait Anxiety Inventory; PSS, Perceived Stress Scale; LSAS, Liebowitz Social Anxiety scale

CRedit authorship contribution statement

J.F-W and Y.L-Z were responsible for the entire project and revised the draft. X-L, J-Y, and J.J-H performed the data extraction, statistical analysis, and interpreting the data. J.J-H and X-L drafted the first version of the manuscript. All authors participated in the interpretation of the results and prepared the final version of the manuscript.

Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

Ethical Approval and Consent to participate

Not applicable.

Consent for publication

Not applicable.

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Availability of supporting data

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

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Supplementary materials

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