Pro-inflammatory cytokines IL-1α, IL-6 and TNF-α in major depressive disorder: Sex-specific associations with psychological symptoms

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Abstract

The pro-inflammatory cytokines IL-1α, IL-6 and TNF-α are associated with major depressive disorder, psychological distress, cardiovascular health, and obesity. However, there is limited research that has examined multiple associations between these variables, among individuals with major depressive disorder in comparison to a control cohort, including sex differences. In this study, data was analysed from 60 individuals with major depressive disorder and 60 controls, including plasma IL-1α, IL-6 and TNF-α, adiposity measures (body mass index; waist circumference), cardiovascular health indices (blood pressure; heart rate) and psychological symptoms (depressive severity; anxiety; hostility; stress). The cytokines were compared by group and sex, and correlated with measures of adiposity, cardiovascular health indices and psychological health. Plasma IL-1α and IL-6 were higher in major depressive disorder group versus control, but with a sex interaction for IL-6, with this group difference only among females. TNF-α did not differ between groups. IL-1α and IL-6 correlated with depressive severity, anxiety, hostility, and stress, while TNF-α correlated only with anxiety and hostility. Psychopathology was associated with IL-1α in males only, and with IL-6 and TNF-α in females only. None of the cytokines correlated with body mass index, waist circumference, blood pressure or heart rate. The result of group by sex interaction for IL-6, and sex specific associations between pro-inflammatory cytokines and psychometrics could be aetiologically important in depression interventions and treatments for females versus males, warranting further investigation.

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Acknowledgments:

TL, ST and JK contributed to the design of the study. AE completed sample, data and statistical analyses, interpreted the results, and drafted the article. TL and ST revised the article. JB completed the data collection. All authors approved the final version.

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Keywords:

Major depressive disorder; Interleukin 1 alpha, Interleukin 6; Tumor necrosis factor alpha; psychological distress; Cardiometabolic Disease

Introduction

Major depressive disorder (MDD) is a debilitating mental disorder, and a leading cause of disability globally (GBD, 2017). Individuals with MDD have a higher incidence of inflammatory illnesses such as diabetes, obesity and heart disease (Maes et al. 2011; Buckley and Abbate, 2018) and higher levels of several pro-inflammatory cytokines (Beurel et al. 2020; Osimo et al. 2020; Bhatt et al. 2022) than those without MDD. Accordingly, pro-inflammatory cytokines may contribute to the increased risk for those with MDD of cardiometabolic disease (CMD) (Celano et al. 2011). CMD is characterised by the markers of both cardiovascular disease (CVD) and metabolic syndrome (Castro et al. 2003; Srivastava, 2012), and associated with high mortality rates (WHO, 2017). In non-depressed cohorts, pro-inflammatory cytokines IL-1α, IL-6, and TNF-α have been associated with specific aspects of psychological distress, including stress, anxiety and hostility (Suarez et al. 2004; Postal et al. 2016; Barnard et al. 2019), obesity (Schmidt et al. 2014) and cardiovascular risk factors (Ridker and Rane, 2021; Urschel and Cicha, 2015). However, concurrent assessment of multiple associations between pro-inflammatory cytokines, specific psychological symptoms associated with both MDD and CVD, body mass indices associated with metabolic syndrome, measures of cardiovascular health, including sex differences, among individuals with MDD with comparison to a control cohort is lacking.

The pro-inflammatory cytokines interleukin 1 alpha (IL-1α), interleukin 6 (IL-6) and tumor necrosis factor alpha (TNF-α) are produced by mononuclear cells and macrophages and released into the blood stream in response to an immunologic challenge (Dolwati et al. 2010; Beurel et al. 2020). Each coordinates a variety of cell functions that ultimately stimulate and enhance inflammation (Bacchiega et al. 2017; Beurel et al. 2020). However, elevation of these pro-inflammatory cytokines in the absence of infection or tissue injury is considered abnormal (Dolwati et al. 2010). Cytokines can cross the blood brain barrier and affect central neural function, and increasing evidence suggests that abnormal inflammatory responses may play a role in the pathophysiology of MDD (Beurel et al. 2020). A causative role of cytokines in MDD is supported by a recent study that showed that administration of cytokines can cause depression-like behaviors and symptoms (Bhatt et al. 2022) and from evidence that inflammation-based treatments and inflammation illnesses are linked to depressed mood or depressive disorders (Dantzer et al. 2008, Capuron et al. 2009; Bhatt et al. 2022). Further, blockade of cytokine signalling is associated with mood-enhancing effects (Tyring et al. 2006; Hersey et al. 2021) and most cytokines decrease with effective anti-depressant treatment (Dahl et al. 2014; Bhatt et al. 2022). The potential mechanism underlying these effects may be due to the role of pro-inflammatory cytokines in serotonin availability. The cytokines have been shown to mediate serotonin deficiency via tryptophan degradation by indoleamine 2,3-dioxygenase, which results in decreased availability of tryptophan for cerebral serotonin synthesis (Felger and Lotrich, 2013).

Much research has reported that individuals with depression have higher pro-inflammatory cytokines than healthy controls including IL-1α, IL-1β, IL-6 and TNF- α (Simon et al. 2008; Dowlati et al. 2010; Al-Hakeim et al. 2015; Munjiza et al. 2018; Osimo et al., 2020; Bürhan-Çavuşoğlu et al. 2021). However, there are inconsistent results, with some studies showing no difference in plasma IL-6 or TNF-α between individuals with versus without MDD (Brambilla et al. 2004; Cilan et al. 2012; Cassano et al. 2017; Obermanns et al. 2021). Notably, IL-1 exists in two isoforms: IL-1α, the main intracellular isoform, and IL-1β, the main released isoform (Dinarello, 2018); these are equally potent in activating the inflammatory process (Voronov et al. 2013). Most research to-date with respect to MDD has focussed on IL-1β· however, since IL-1α is found constitutively as opposed to IL-1β being the induced form released in an inflammatory disease state (Voronov et al. 2013), it may be more relevant in MDD.

The pro-inflammatory cytokines are also associated with other psychological symptoms associated with depression including stress (Huesten and Deak, 2014; Barnard et al. 2019), hostility (Suarez et al. 2004) and anxiety (Postal et al. 2016; Bhatt et al. 2017). This is significant since these are in turn associated with CMD risk factors (Goldbacher and Matthews, 2007; Nabi et al. 2010, Ojike et al. 2016; Gowey et al. 2019), which may contribute to people with MDD being more vulnerable to developing CMD. Stress and depression are associated with higher waist circumference and poorer metabolic health outcomes generally (Gowey et al. 2019); anxiety is linked to cardiovascular disease and elevated heart rate (Olafiranye et al. 2011; pittig et al. 2013); hostility is associated with increased blood pressure (Spicer and Chamberlain, 1996), lipid dysregulation (Suarez et al. 1998), and the development of metabolic syndrome (Matthews and Salomon, 2003). Further, in relation to increased risk of CMD, pro-inflammatory cytokines are secreted by adipose tissue and higher in obese individuals (Schmidt et al. 2014), and have pro-hypertension effects (Dorrance, 2007; Finnel and Wood, 2016). Given the multiple associations between the pro-inflammatory cytokines IL-1α, IL-6 and TNF-α, and psychopathologies and measures of adiposity and cardiovascular health, elucidating these complex associations in a cohort of MDD, including comparison to healthy controls is needed.

Of interest is that inflammatory responses differ between sexes, with females showing more pronounced inflammatory responses during bacterial and viral infections than males (O'Connor et al. 2007; Klein and Flanagan, 2016; Wegner et al. 2017). A study by Wegner et al. (2017) among health individuals found that when bacterial endotoxin was administered, females had a higher increase in plasma TNF-α and IL-6 concentrations compared with males (Wegner et al. 2017). However, little research has examined sex differences in inflammatory markers in association with depressive symptoms, with inconsistent results. Serum levels of IL-6 and TNF-α were higher in depressed females than depressed males in one study (Birur et al. 2017), but there were no sex differences in IL-6 among depressed participants in another study (Davidson et al. 2009). In addition to females being at a greater risk for depression (Kessler et al. 2005) and showing greater inflammatory reactivity (O'Connor et al. 2007; Klein and Flanagan, 2016; Wegner et al. 2017), there are sex differences in antidepressant treatment response. It was reported that monoamine oxidase inhibitors and selective serotonin reuptake inhibitors are more effective in female patients (Quitkin et al. 2002), while male patients respond more favourably to tricyclic antidepressants (Kornstein et al. 2000). Accordingly, it is important to examine sex differences in pro-inflammatory cytokines and their associations with psychological symptoms, since these may be important to the aetiology of MDD and CMD risk.

Overall, despite much previous research that has investigated pro-inflammatory cytokines in people with depression (Tuglu et al. 2003; Simon et al. 2008; Al-Hakeim et al. 2015), there are limited reports on associations with both psychological symptoms (Suarez et al. 2004; Postal et al. 2016; Barnard et al. 2019) and measures relevant to increased risk of CVD and metabolic syndrome (Ridker et al. 2000; Urschel and Cicha, 2015), particularly in an MDD cohort. The present study aimed to compare the plasma concentration of pro-inflammatory cytokines (IL-1α, IL-6, TNF-α) in participants with MDD versus healthy controls and by sex, and to determine associations between these and measures of adiposity (waist circumference and BMI) and cardiovascular health (blood pressure and heart rate), and psychological symptoms (stress, depression, anxiety, hostility and global severity index of psychopathology), relevant to MDD and CMD risk. It was hypothesized that plasma concentration of these pro-inflammatory cytokines would be: 1) higher in the MDD group than healthy controls, and in females than males; 2) positively associated with waist circumference,

BMI, blood pressure and heart rate, and 3) positively associated with psychological symptoms of stress, depression, anxiety and hostility.

Method:

2.1 Participants:

The method and participants of the current study have been previously published (Elgellaie et al. 2021). Participants with MDD and healthy controls were recruited by media and university advertisements. Exclusion criteria across both diagnostic groups included any use of corticosteroids, neurological illness, and substance use disorders. Depressed participants were thoroughly pre-screened to confirm that they met DSM-5 criteria for a current major depressive episode, and had not been receiving any psychological, pharmacological or somatic treatment for MDD within the last two months. All control participants had no significant history of mental health problems or diagnosed mental disorders and they were age and sex matched to MDD participants.

2.2 Measures and procedure:

The design of this study was cross-sectional. All participants attended the university clinical trials research unit. Participants were given information about the study and provided written informed consent prior to participating. Participants in the MDD group were interviewed using the Mini Neuropsychiatric Interview, version 7.0.2 for Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) (MINI; Sheehan, 2015). All participants had their height, weight, waist circumference measured and their BMI (kg/m^2) was calculated, and their blood pressure and heart rate recorded. A phlebotomist then collected a non-fasted morning (between 9:00 – 11:00 a.m.) sample of blood (10 mL) into an EDTA tube. Within 5 minutes of blood collection, 200 μL of aprotinin was added to each blood sample, which was then immediately centrifuged at 4°C and 3000 rpm for 10 minutes. Plasma aliquots were stored at -80 degC and thawed prior to hormone analysis and measurements. Plasma concentrations of inflammatory cytokines (IL-1α, IL-6, TNF-α) were measured using standard ELISA methods.

For measures of psychopathology, all participants completed the Brief Symptom Inventory (BSI) questionnaire, which consists of 53 items covering nine symptom dimensions. For the current study, the scores for the symptom dimensions of depression (e.g. feeling no interest in things), anxiety (e.g. feeling tense or keyed up), and hostility (e.g. having urges to break or smash things) were used, along with the Global Severity Index (GSI) as a measure of overall symptomatology during the past seven days (Derogatis, 1975). Good internal consistency reliability is reported for all dimensions of the BSI (Derogatis, 1975). Participants also completed the depression, anxiety and Stress Scale (DASS), which a 21 item self-report questionnaire assessing depression, anxiety and stress severity over the past week (Lovibond and Lovibond, 1995), and with high internal consistency (Brown et al. 1997). Subscale scores were summed and then doubled for comparison with the DASS 42. For the current study, each participant's score for stress was included in analyses. Severity descriptors were calculated for both groups according to the established cut off scores for each DASS subscale (Lovibond and Lovibond, 1995).

2.3 Data statistical analysis:

Statistical analyses were conducted using 'Statistical Package for the Social Sciences' (SPSS, Version 26). Shapiro-Wilk Test of Normality was used to examine the normality distribution of the data. Logarithmic transformations were used if the data were not normally distributed, and outliers as identified on boxplots were removed. Two-way factorial analyses of variance (ANOVAs) were used to compare each of the plasma inflammatory cytokines levels (IL-1α, IL-6, TNF-α), and measures of adiposity, cardiac health and psychopathology symptoms between the MDD versus control groups, by sex. Spearman's correlations were performed to determine associations between each of the plasma inflammatory cytokines (IL-1α, IL-6, TNF-α) and the measures of adiposity, cardiac health and psychopathology symptoms across both groups. Due to previously reported differences in inflammatory cytokines level by MDD diagnosis and sex, correlations were also conducted separately by diagnostic group and sex. Multiple regression analyses were conducted to

determine the unique contribution of each of the pro-inflammatory cytokines and sex to the psychopathology symptoms. For all statistical tests $\alpha < .05$ was considered statistically significant.

Results:

3.1 Participants' characteristics

A total of 60 MDD participants and 60 healthy control participants aged between 18 and 54 years (M = 25.05, SD = 6.61 years) were included in data analyses. There were 34 females and 26 males in each group. Participant characteristics including anthropometric and mental health symptoms are reported in Table 1, as reported previously in the context of prolactin (Elgellaie et al. 2021). There were no significant differences between diagnostic groups for age or any of the measures of adiposity or cardiac health. Weight, systolic blood pressure and heart rate were higher in males than females (Elgellaie et al. 2021). There were no further sex differences or group by sex interactions for any of the adiposity or cardiac health measures. The psychometric measures (depression, anxiety, hostility, stress, GSI) were all significantly higher in the MDD than the control group, and females scored significantly higher than males on GSI and marginally higher in anxiety (p = 0.051).

Table 1 : Biometric and psychometric data (mean ± standard deviation) overall, by group (MDD and control) and sex

		Group	Group			Sex	Sex			Interaction (Group by Sex)	
	Overall Cohort	MDD	Control	Between groups comparison ^	Effect size partial η^2	Female	Male	Between sex comparison ^	Effect size partial η^2		
Number	120	60	60	-	-	68	52	-	-	-	-
Age (Years)	25.1 ± 6.60	24.7 ± 6.03	25.4 ± 7.20	F = 0.65 p = 0.42	0.006	24.9 ± 7.40	25.3 ± 5.40	F = 0.14 p = 0.71	0.001	F = 2.98 p = 0.09	0
Weight (Kg)	73.8 ± 16.3	74.6 ± 16.1	73.3 ± 16.7	F = 0.13 p = 0.72	0.001	68.8 ± 16.2	80.8 ± 13.9	F = 18.2 p < 0.001	0.135	F =0.87 p = 0.35	0
BMI (Kg/m^2)	25.5 ± 5.40	25.8 ± 5.40	25.1 ± 5.40	F = 0.31 p = 0.58	0.003	25.4 ± 6.10	25.5 ± 4.40	F = 0.01 p = 0.91	0.001	F =1.36 p =0.25	0
Waist (Cm)	87.0 ± 0.14	89.0 ± 0.14	85.0 ± 0.13	F = 2.10 p = 0.15	0.018	82.0 ± 0.13	90.0 ± 0.12	F = 3.33 p = 0.07	0.028	F =2.95 p =0.09	0
HR (Beats/min)	73.7 ± 12.9	74.5 ± 12.1	72.9 ± 13.8	F = 0.33 p = 0.57	0.003	69.2 ± 12.2	77.2 ± 12.5	F = 12.1 p = 0.001	0.095	F = 0.97 p =0.33	0

		Group	Group			Sex	Sex			Interaction (Group by Sex)	
SBP (mmHg)	120 ± 13.9	119 ± 12.5	120 ± 15.2	F = 1.28 p = 0.26	0.011	112 ± 9.20	129 ± 12.9	F = 74.3 p < 0.001	0.390	F = 1.95 p = 0.17	0
DBP (mmHg)	73.1 ± 8.80	73.9 ± 8.04	72.2 ± 9.58	F = 0.77 p = 0.38	0.007	72.3 ± 8.70	74.1 ± 9.01	F = 1.14 p = 0.29	0.010	F = 1.19 p = 0.28	0
Global Severity Index	1.04 ± 0.91	1.79 ± 0.67	0.28 ± 0.24	F = 275 p < 0.001	0.703	1.13 ± 0.99	0.91 ± 0.79	F = 6.02 p = 0.016	0.049	F = 3.08 p = 0.08	0
Depression	1.34 ±1.25	2.43 ± 0.79	0.24 ± 0.32	F = 386 p < 0.001	0.769	1.41 ± 1.31	1.24 ± 1.18	F = 2.38 p = 0.13	0.020	F = 0.43 p = 0.51	0
Anxiety	0.95 ± 0.94	1.67 ± 0.82	0.23 ± 0.25	F = 164 **p < 0.001**	0.586	1.04 ± 0.99	0.82 ± 0.87	F = 3.89 p = 0.051	0.032	F = 2.27 p = 0.13	0
Hostility	0.89 ± 0.92	1.52 ± 0.91	0.27 ± 0.27	F = 100 p < 0.001	0.464	0.99 ± 1.01	0.77 ± 0.78	F = 2.99 p = 0.08	0.025	F = 1.75 p = 0.19	0
Stress	13.9 ± 11.3	22.4 ± 9.28	5.37 ± 4.86	F = 155 p < 0.001	0.573	15.0 ± 11.4	12.4 ± 11.0	F = 3.55 p = 0.06	0.030	F = 0.65 p = 0.42	0

MDD: Major Depressive Disorder. BMI: Body Mass Index. HR: Heart Rate. SBP: Systolic Blood Pressure. DBP: Diastolic Blood Pressure. Global Severity Index, depression, anxiety and hostility scores measured by Brief Symptom Inventory scale. Stress score measured by Depression, Anxiety, Stress Scale. ˆ Two-Way ANOVA. Significance at $p < 0.05$

3.2 Plasma cytokine concentrations:

Each cytokine showed positive skewed distribution (IL1-α skewness = 5.684, IL6 skewness = 8.562, TNF-α skewness= 9.047). Shapiro-Wilk tests for all the cytokines data were significant ($p < 0.001$) indicating that the data did not follow normal distribution. Two outliers were removed from the IL-1α data (one MDD and one control) with concentrations of 8.31 pg/mL and 8.06 pg/mL, respectively; one outlier from the control group was removed from each of IL-6 and TNF-α data with concentrations of 165.8 pg/mL and 42.3 pg/mL, respectively. The ranges of IL-1α, IL-6 and TNF-α were 0.36 - 4.69 pg/mL, 2.67 - 42.3 pg/mL and 0.39 - 9.32 pg/mL respectively. Mean cytokine values (IL-1α, IL-6 and TNF-α) are shown in Table 2. All values were logarithmic-transformed for all analyses to reduce skewness.

As shown in Table 2, both the means of IL-1α and IL-6 concentrations were higher in MDD than controls, with no effect of sex. However, there was a significant group by sex interaction for IL-6, whereby depressed females had significantly higher plasma IL-6 than females in the control group (0.95 ± 0.25 versus 0.77 ± 0.21; $p = 0.002$), while males in the MDD and control groups had similar plasma IL-6 (0.84 ± 0.16 versus

0.83 ± 0.21, $p = 0.862$). There were no significant effects or interaction for TNF-α. When age and BMI were considered as covariates, they were not significant cofounders for any of the cytokines. Among the cytokines, IL-6 and TNF-α were strongly correlated ($r = 0.651$, $p < 0.001$), however IL-1α did not correlate with IL-6 ($r = 0.058$, $p = 0.365$) nor with TNF-α ($r = 0.130$, $p = 0.162$).

Table 2 : Plasma concentrations of IL-1α, IL-6 and TNF-α per group and sex. Mean values with standard deviation in parentheses

	Group	Group			Sex	Sex			
	MDD	Control	Between groups comparison^	Effect size partial η^2	Female	Male	Between sex comparison^	Effect size partial η^2	Interaction (Group by Sex)
IL-1α (pg/ml)	1.01 (0.61)	0.82 (0.32)	F = 5.211 p = 0.024	0.044	0.95 (0.37)	0.88 (0.61)	F = 0.616 p = 0.434	0.005	F = 2.122 p = 0.148
Log IL-1α	2.97 (0.17)	2.88 (0.17)	F = 8.060 p = 0.005	0.066	2.95 (0.16)	2.89 (0.19)	F = 3.215 p = 0.076	0.027	F = 3.005 p = 0.086
IL-6 (pg/ml)	9.49 (7.75)	7.11 (4.34)	F = 3.234 p = 0.075	0.027	8.83 (7.60)	7.61 (4.22)	F = 1.133 p = 0.289	0.010	F = 3.905 p = 0.051
Log IL-6	0.90 (0.22)	0.80 (0.21)	F = 5.595 p = 0.020	0.046	0.86 (0.25)	0.84 (0.19)	F = 0.380 p = 0.539	0.003	F = 4.552 p = ***0.035***
TNF-α (pg/ml)	1.95 (1.79)	1.48 (0.86)	F = 2.614 p = 0.109	0.022	1.72 (1.54)	1.71 (1.26)	F = 0.001 p = 0.970	0.000	F = 1.571 p = 0.213
Log TNF-α	3.19 (0.27)	3.12 (0.20)	F = 1.684 p = 0.197	0.014	3.14 (0.25)	3.16 (0.23)	F = 0.177 p = 0.675	0.002	F = 2.254 p = 0.136

IL-1α: Interleukin-1α, IL-6: Interleukin-6, TNF-α: Tumor Necrosis Factor-α. MDD: Major Depressive Disorder. ^Two-Way ANOVA. Significance $p < 0.05$

Associations between cytokines, measures of adiposity, cardiac health and psychological symptoms:

In the total cohort, IL-1α and IL-6 were significantly correlated with all psychometric measures, while TNF-α was correlated significantly with anxiety and hostility but not with depression, stress or GSI (Table 3). In contrast, none of the pro-inflammatory cytokines (IL-1α, IL-6, TNF-α) correlated with adiposity or cardiac health measures. When analyses were performed separately per diagnostic group and per sex, there were differential correlations. In the MDD group, IL-6 was correlated with anxiety, hostility, GSI, and stress, and TNF-α with hostility, while IL-1α did not correlate with any psychological symptoms. In the control group, IL-1α was correlated with hostility, IL-6 negatively with depression and stress, and TNF-α negatively with depression. Among females, IL-6 correlated with all of the psychometric measures, TNF-α correlated with anxiety, hostility, GSI and stress and IL-1α did not show any significant correlations. In contrast, among males, IL-1α correlated with depression, hostility, GSI and stress, while IL-6 and TNF-α did not correlate with any psychological symptoms.

Table 3 : Spearman's correlations of pro-inflammatory cytokines (IL-1α, IL-6, TNF-α) with psychometric measures per cohort, group (MDD and control) and sex (females and males)

	Measure	Total cohort	Total cohort	MDD	MDD	Control	Control	Females	Females
		r	p	r	p	r	p	r	p
IL-1α	Depression	0.215	0.019	0.038	0.772	-0.024	0.376	0.092	0.462
	Anxiety	0.190	0.040	-0.032	0.807	0.180	0.173	0.145	0.245
	Hostility	0.214	0.020	0.035	0.791	0.259	**0.047**	0.040	0.748
	Global Severity Index	0.243	0.008	0.048	0.717	0.226	0.085	0.128	0.305
	Stress	0.230	0.012	0.070	0.597	0.116	0.381	0.153	0.220
IL-6	Depression	0.207	0.024	0.124	0.344	-0.349	0.007	0.320	0.008
	Anxiety	0.291	0.001	0.307	0.017	-0.218	0.098	0.425	0.000
	Hostility	0.362	0.000	0.435	0.001	-0.141	0.286	0.487	0.000
	Global Severity Index	0.273	0.003	0.281	0.029	-0.248	0.059	0.390	0.001
	Stress	0.233	0.011	0.266	0.040	-0.287	0.027	0.324	0.007
TNF-α	Depression	0.088	0.344	-0.009	0.943	-0.297	**0.022**	0.199	0.104
	Anxiety	0.200	0.029	0.196	0.134	-0.031	0.817	0.376	0.002
	Hostility	0.261	0.004	0.299	**0.020**	-0.012	0.929	0.427	0.000
	Global Severity Index	0.153	0.429	0.128	0.329	-0.158	0.232	0.296	0.014
	Stress	0.168	0.068	0.186	0.154	-0.136	0.306	0.305	0.012

IL-1α: Interleukin-1α, IL-6: Interleukin-6, TNF-α: Tumor Necrosis Factor-α. GSI: Global Severity Index of the Brief Symptom Inventory. Significance $p < 0.05$

Multiple linear regression analyses revealed that both IL-1α and IL-6 were associated with depression, hostility, and GSI, and that IL-1α was also associated with Stress, and IL-6 was also associated with anxiety. TNF-α and sex were not significant independent variables of psychological symptoms in any of the regression models (Table 4).

Table 4 : Multiple regression analyses for psychometrics (dependents) by the predictors (Sex, IL-1α, IL-6, TNF-α)

				Dependent variables	Dependent variables	Dependent variables	Dependent variables	Depend variable
				Depression	*Anxiety*	*Hostility*	*GSI*	*Stress*
Model summary	Model summary		R R^2	0.284 0.081	0.308 0.062	0.386 0.149	0.337 0.113	0.289 0.
ANOVA	ANOVA		F P	2.460 0.049	2.930 0.024	4.889 0.001	3.578 0.009	2.559 0.
Standardised coefficients	*Sex*	*Sex*	β P	-0.004 0.968	-0.064 0.488	-0.060 0.501	-0.055 0.545	-0.058 0.533
	IΛ-1a	*IΛ-1a*	β P	0.210 0.025	0.161 0.083	0.179 0.048	0.223 0.016	0.208 0.
	IL-6	*IL-6*	β P	0.247 0.037	0.254 0.030	0.314 0.006	0.280 0.016	0.190 0.
	TNΦ-a	*TNΦ-a*	β P	-0.133 0.261	-0.028 0.810	0.007 0.954	-0.099 0.392	-0.032 0.787

IL-1α: Interleukin-1α, IL-6: Interleukin-6, TNF-α: Tumor Necrosis Factor-α. GSI: Global Severity Index of the Brief Symptom Inventory. Significance $p < 0.05$

4.0 Discussion:

Overall, the current results suggest that despite their common actions in the inflammatory response, the pro-inflammatory cytokines are differentially associated with psychological symptoms related to MDD, with some interesting sex-specific effects. The pro-inflammatory cytokines IL-1α and IL-6 were significantly higher

in those with MDD than healthy controls; however, a group by sex interaction for IL-6 and post-hoc analysis revealed that IL-6 was significantly higher in MDD versus control only for females. Both IL-1α and IL-6 were associated with all psychological symptoms measured. In contrast, TNF-α did not differ between those with versus without MDD and was correlated with anxiety and hostility only. Despite none of the inflammatory cytokines being different between sexes, there was a sex difference in their associations with psychological symptoms; these were associated with IL-6 and TNF-α, but not IL-1α in females, and with IL-1α but neither IL-6 nor TNF-1α in males. In contrast to hypotheses, the pro-inflammatory cytokines were not significantly associated with adiposity or cardiac health measures.

The current finding of significantly higher plasma IL-1α with MDD is consistent with a previous study (Simon et al. 2008) and add to the limited reports on examination of IL-1α in MDD. Interestingly, in the current study, although IL-6 was similarly higher in the MDD group, this was with a sex interaction and IL-6 was only higher in females with MDD versus without MDD with no difference between MDD males and control males. While previous studies also reported that individuals with MDD had higher IL-6 than controls, they did not examine any sex differences (Al-Hakeim et al. 2015; Munjiza et al. 2018). This gives the assumption of a difference between groups for both males and females, which may be incorrect. The current results highlight how important it is to test for the sex effect. Given females are more prone to MDD (Kessler et al. 2005), show more pronounced inflammatory responses (Wegner et al. 2017) and have higher CVD-related mortality rate than males (Gao et al. 2019), and the female-specific association of IL-6 with depression here, it is worth further investigating a potential role for IL-6 in mediating these relationships.

Both IL1-α and IL-6 were significantly correlated with the GSI score as well as scores of depression, anxiety, hostility and stress, and accounted for unique variance in each of these psychometric measures, when included alongside TNF-α and sex in multiple linear regression analyses. Interestingly, they both were independent predictors of depression and hostility, while only IL1-α was predictive of stress and only IL-6 was predictive of anxiety. Similarly, previous studies in non-depressed cohorts reported associations of IL-1α with depressive symptom severity (Suarez et al. 2003) and with hostility (Suarez et al. 2004), and associations of IL-6 with depressive symptoms, anxiety (Reichenberg et al. 2001) and hostility (Suarez et al. 2003; Suarez et al. 2004). The positive associations of IL-1α and IL-6 with stress in the current study is expected, since stress stimulates the release of glucocorticoids and catecholamines which themselves promote inflammation, and the pro-inflammatory cytokines are also involved in the adaptation to stressors (McEwen, 2008). The current results are important since they extend beyond the previous studies in non-depressed individuals to report on the association of pro-inflammatory cytokines with specific psychopathology symptoms in participants with MDD.

In the current study, TNF-α concentration did not differ between diagnostic groups or between females and males and was only correlated with the psychological measures of anxiety and hostility, and not with the GSI, depression severity or stress. Although this result agrees with previous research (Cilan et al. 2012; Karlović et al. 2012; Cassano et al. 2017; Obermanns et al. 2021; Bürhan-Çavuşoğlu et al. 2021), others have reported that TNF-α was significantly higher in MDD than healthy controls (Tulgu et al. 2003; Liu et al. 2012; Al-Hakeim et al. 2015; Köhler et al. 2017). However, two of these latter studies had small sample size (N = 43) (Tulgu et al. 2003) and (N = 60) (Al-Hakeim et al. 2015), included depressed patients who were using anti-depressed treatment (Al-Hakeim et al. 2015), and they were not age and sex matched with the control participants (Tulgu et al. 2003); all these factors may have confounded the findings.

TNF-α had a more discriminatory profile than IL-1α and IL-6 in terms of associations with psychological symptoms, being correlated only with anxiety and hostility. This agrees with previous research that reported an association of TNF-α with anxiety (Reichenberg et al. 2001; Postal et al. 2016) and hostility (Suarez et al. 2004; Takahashi et al. 2018), albeit among healthy participants. Of note is that there is a well-established relationship between hostility and CMD risk: hostility is linked with high blood pressure (Spicer and Chamberlain, 1996), elevated levels of low-density lipoproteins (Suarez et al. 1998) and greater risk of coronary heart disease (Wong et al., 2013), all associated with inflammatory processes (Goldbacher and Matthews, 2007; Nabi et al. 2010). Given these pieces of evidence, TNF-α could be associated with the risk of

CMD directly by its pro-inflammatory nature and indirectly through its link with hostility. Although TNF-α correlated significantly with hostility and anxiety, when it was entered in regression analyses alongside the other pro-inflammatory cytokines in the current study, it did not significantly account for unique variance in psychological symptoms. This is perhaps due to the positive correlation between IL-6 and TNF-α, and since IL-6 was a stronger predictor of psychological symptoms than TNF-α, IL-6 accounted for unique variance in most types of psychological symptoms.

Investigating the sex effect on the association of pro-inflammatory cytokines with psychological symptoms showed compelling results in the current study. IL-1α was correlated with all psychological symptoms in males but none in females, while IL-6 and TNF-α were correlated with all psychological symptoms, except not TNF-α with depression severity, in females, but none in males. IL-1α may be a risk factor for depression, hostility and stress in males, and IL-6 and TNF-α could be risk factors to being anxious, hostile and stressed in females. These novel findings of sex-specific relationships between pro-inflammatory cytokines and psychopathology symptoms in a clinical cohort with MDD may be related to sex differences in MDD prevalence, symptoms and treatment response (Seney et al. 2021).

In the current study, none of the pro-inflammatory cytokines were correlated with CMD risk indices of waist circumference, BMI, blood pressure or heart rate. Although one study presented an association between pro-inflammatory cytokines (IL-1α) and obesity (Um et al. 2011), this was in non-depressed participants. A previous study among people with MDD reported that IL-6 and TNF-α were both correlated with BMI (Shelton et al. 2015); however, these individuals were obese or severely obese with BMI greater than 30 Kg/m^2. The lower mean BMI of participants in the current study, in the overweight rather than the obese range, may have contributed to the lack of significant association between the inflammatory cytokines and BMI or waist circumference. Similarly, participants in the current study were in the normotensive range on average, and the lack of correlation between any of the pro-inflammatory cytokines and blood pressure likely indicates that pro-inflammatory cytokines are more relevant to high blood pressure.

There were several limitations to the current study. Firstly, the cohort was normotensive, and most participants were non-obese. This likely affected the non-significant association between pro-inflammatory cytokines with blood pressure and BMI. Being a cross-sectional study allowed only for associations to be assessed rather than examination of any causative effects as per a longitudinal study design. Further, since research supports a role of IL-1β in the development of adipose tissue inflammation, insulin resistance and metabolic syndrome (Ballak et al. 2015), it would be important to assess IL-1β alongside IL-1α in a cohort with MDD who have physical and physiological CMD risk indices such as high blood pressure and BMI.

In conclusion, of the plasma pro-inflammatory cytokines, only IL-1α was elevated in MDD versus control, IL-6 was higher in MDD versus control among females, and TNF-α did not differ between groups. The latter finding and the lack of a main effect of sex on IL-1-α and TNF-α were contrary to our hypothesis. The group by sex interaction for IL-6 and sex specific associations between pro-inflammatory cytokines and psychometrics suggests potentially differential roles of these cytokines and mental health between males and females. These results of sex differences in inflammatory processes linked to depressive symptoms are compelling and could be aetiologically important in depression interventions and treatments.

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Declaration of interest:

None.

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