Plasma Inflammatory Cytokines and Depressed Patients With Comorbid Pain: Improvement by Ketamine

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Research

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Abstract

Background: Depression and pain frequently coexist clinically. Ketamine has analgesic and antidepressant effects, but few studies have evaluated individual differences in antidepressant outcomes to repeated ketamine in depressed patients with comorbid pain. Our aims were to determine the difference in ketamine's antidepressant effects in depressed patients with or without pain and then to examine whether inflammatory cytokines might contribute to ketamine's effect.

Methods: Seventy-eight patients with major depressive disorder received six infusions of ketamine. Plasma levels of 19 inflammatory cytokines were assessed at baseline and post-infusion (day 13 and day 26) using the Luminex assay. Plasma inflammatory cytokines of sixty healthy controls (HCs) were also examined.

Results: At baseline, the levels of GM-CSF, IL-1β and IL-6 were higher in pain group than in non-pain and HC groups. Pain group had better antidepressant outcomes than non-pain group. Pain group showed a greater decrease in IL-6 at day 13 and a greater decrease in IL-10, MIP-3α, IL-1β, IL-5 and IL-6 at day 26 than non-pain group. In the pain group, the changes in IL-6 levels were associated with improvement in pain intensity (β=0.347, t=2.159, P=0.038) and depressive symptoms (β=0.590, t=4.201, P<0.001) at day 13. The Sobel test showed indirect effects between decreases in IL-6 levels and improvement in depressive symptoms (Z=2.026, P=0.043).

Conclusion: This study suggested that an elevated inflammatory response plays a key role in individual differences in depressed patients with or without pain. Ketamine showed great antidepressant and analgesic effects in depressed patients with pain, which may be related to its anti-inflammatory effect.

Background

Depression and pain frequently coexist clinically [1–4]. The presence of comorbid depression and pain, compared to depression or pain alone, has been associated with greater treatment difficulties, poor functional outcomes and increasing economic burdens on patients, families, and societies [5].

Although patients with comorbid depression and pain are common in clinics, the pathogenesis remains unclear. The mechanism of the interaction between depression and pain is likely more complex than that of a single disorder and suggests that the overlapping pathogenesis might have a role in the development of these two common disorders, including abnormal glutamate signaling, excessive activation of inflammation and decreased brain-derived neurotrophic factor (BDNF) [6–8]. An abundance of evidence supports that chronic neuroinflammation independently plays a critical role in the pathophysiology of depression and pain and that alterations in inflammatory mediators have also been observed in this comorbid condition in recent years [9, 10]. Increased proinflammatory cytokine expression in the brain, especially tumor necrosis factor (TNF)-α, interleukin (IL)-6 and IL-1β in the hippocampus, amygdala, anterior cingulate and frontal cortex, contributes to the development of depression-pain comorbidity, which has been repeatedly shown in rodent models of neuropathic and inflammatory pain that also

exhibit depressive-like behavior and, conversely, in rodent models of depression that also display altered nociceptive responses [11–13]. Imbalanced peripheral proinflammatory cytokine levels have also been independently observed in patients with depression and pain and in comorbid patients compared to healthy controls. For example, increased IL-6 levels can be found in patients with chronic pain and comorbid depressed mood, and IL-6 levels and depressive symptoms were positively associated with pain intensity [9]. TNF-α is another general inflammatory mediator reported to be increased in patients with depression and comorbid with pain, and augmented peripheral levels of TNF-α were associated with reduced pain thresholds in a correlative analysis [14]. Elevated peripheral C-reactive protein levels and depression symptoms were observed in patients with fibromyalgia or sciatica, while higher C-reactive protein levels correlated with greater depressive symptoms [15, 16]. Thus, taken together, these data suggest that a persistent inflammatory response may underlie comorbid depression and pain, or at least partly contribute to the development of this comorbidity.

Currently, some analgesics and antidepressants are being used to treat this comorbidity; however, sometimes, there is limited clinical remission [17, 18]. Ketamine is an N-methyl-D-aspartic acid receptor antagonist with anesthetic and analgesic effects and has been used for acute pain for several decades. Recently, an increasing number of clinical studies have identified that a single subanesthetic dose of ketamine has a fast and robust antidepressant effect in patients with treatment-resistant depression [19, 20]. Our previous results also showed greater effectiveness and longer remission periods with six infusions of intravenous ketamine for patients with major depressive disorder (MDD) [21], consistent with other research [22]. Ketamine infusions also significantly reduced pain and depression in patients experiencing refractory neuropathic pain syndromes and depressive comorbidities [23, 24] and even successfully relieved depressive symptoms, suicidal ideation and neuropathic pain in an adolescent with severe depression, suicidality, and neuropathic leg pain who failed multiple antidepressant and analgesic modalities [25]. In addition, oral ketamine thrice daily for six weeks was proven to have superior antidepressant effects compared to diclofenac for the treatment of depressed patients suffering from chronic pain [26].

Based on evidence from previous studies, ketamine may be ideal for the treatment of comorbid pain and depression, however, few clinical studies have evaluated individual differences in antidepressant outcomes to repeated ketamine infusions in patients with MDD and comorbid pain. Moreover, although plasma levels of inflammatory cytokines decreased after six infusions of ketamine administration based on our previous results [27], the roles of cytokines in ketamine's effect on concurrent pain and depressive symptoms have not been explored. In the present study, we aimed to first determine differences in ketamine's antidepressant effects in depressed patients with and without the presence of painful symptoms and then to examine whether cytokines might contribute to ketamine's effect in depressive patients with the presence of painful symptoms.

Materials And Methods

Participants

We present a post hoc analysis of an original study designed to assess the antidepressant response of six adjunctive ketamine infusions in patients with MDD [21, 28]. This study was approved by the Clinical Research Ethics Committee of the Affiliated Brain Hospital of Guangzhou Medical University. All participants provided informed consent prior to participation.

This study included 78 patients with MDD who received six doses of ketamine, along with 60 healthy controls (HCs) matched with patients for age and sex. The main inclusion criteria for MDD patients were as follows: diagnosis of MDD established using the Diagnostic and Statistical Manual of Mental Disorders (DSM-V) criteria; age between 18 and 65 years; 17-item Hamilton Depression Rating Scale (HAMD-17) score ≥ 17 at screening without hallucination or delusion; and treatment resistance defined as the failure of two adequate antidepressant trials or a suicidal tendency confirmed by a Beck Scale for Suicide Ideation-part I score ≥ 2 at screening. The exclusion criteria included the presence of alcohol or substance dependence or any serious or unstable medical conditions, including neurological, endocrine, rheumatic and infective diseases. Patients taking anti-inflammatory agents were also excluded. Current psychotropic medication had to be stable for ≥ 4 weeks, and the same dose was maintained during the six-infusion period. Additional detailed information regarding these participants has been described in our previous studies [21, 28, 29].

Study design

The patients received ketamine three times weekly for two weeks. The detailed study design and methods have been previously published [21, 28, 29]. Intravenous ketamine (0.5 mg/kg) was administered over 40 minutes following an overnight fast via IV intravenous pump continuous infusion.

Depressive symptoms were assessed using the Montgomery-Asberg depression rating scale (MADRS) by clinicians at the pretreatment baseline, 24 h after each infusion and again 14 days after the 6th infusion (day 26). A response was conventionally defined as a 50% or more reduction from baseline in MADRS score at 24 h after the 6th infusion (day 13). Remission was defined as a MADRS total score ≤ 10 at day 13.

Pain intensity was measured using the short-form McGill Pain Questionnaire (SF-MPQ). The main component of the SF-MPQ consists of the sensory index, affective index, present pain intensity (PPI) index and visual analog scale (VAS). The VAS was assessed at the same timepoint as the MADRS, but the sensory index, affective index and PPT were assessed at baseline, day 13 and day 26. Based on the presence or absence of pain using the SF-MPQ, 36 (46.2%) MDD patients had pain symptoms at the baseline.

Inflammatory cytokine measurements

The patients provided blood samples at baseline, day 13 and day 26, and HCs provided only a single blood sample. Blood samples were collected into EDTA tubes between 8:00 and 10:00 AM after an overnight fast. Tubes were immediately stored at + 4°C and then centrifuged (3000 rpm/min at + 4°C) for 10 minutes within 1 hour. Plasma was obtained and stored at − 80°C.

Plasma inflammatory cytokine levels were detected using the Human High Sensitivity T Cell Magnetic Bead Panel (Millipore, Billerica, MA, USA, HSTCMAG-28SK) performed with a Luminex 200 multiplex immunoassay system based on the manufacturer's instructions. Nineteen cytokines, including interferon-inducible T cell alpha chemoattractant (ITAC), granulocyte-macrophage colony stimulating factor (GM-CSF), fractalkine, interferon (IFN)-γ, IL-10, macrophage inflammatory protein (MIP)-3a, IL-12P70, IL-13, IL-17A, IL-1β, IL-2, IL-4, IL-23, IL-5, IL-6, IL-7, IL-8, MIP-1β, and TNF-α, were quantified twice. The detailed detection process and interassay coefficients of variability have been described in our previous studies [27].

Statistical analysis

Of the 78 MDD patients included, 7(9.0%) lacked blood sample at day 26, four of them were in non-pain group and three in pain group. Thus, these data were analyzed based on the intent-to-treat with expectation maximization algorithm interpolation method.

First, baseline demographic variables and clinical symptoms between groups (pain vs. non-pain) were statistically evaluated using Student's t-test for continuous variables with a normal distribution and chi-square test for categorical variables.

Next, the group comparisons of baseline cytokine levels among the pain group, non-pain group and HC group were examined using multivariate analysis of covariance (MANCOVA), with age, sex and body mass index (BMI) as covariates. Post hoc analysis was used to compare the differences within each group. Cohen's d was calculated to measure their difference. Prior to the analyses, data for inflammatory cytokines were natural log-transformed.

Then, changes in MADRS scores, VAS, sensory index, affective index and PPT over time and group differences were assessed using linear mixed models with group (pain vs. non-pain) and time (baseline, 24 h after each infusion or day 13, day 26) as factors. Baseline demographic and clinical variables that differed between groups were entered as covariates in linear mixed models. Bonferroni-corrected post hoc comparisons were used to calculate the group differences at each follow-up point.

Based on our previous study, the concentrations of several cytokines, including GM-CSF, fractalkine, IFN-γ, IL-10, IL-12p70, IL-17A, IL-1β, IL-2, IL-4, IL-23, IL-5, IL-6, IL-7 and TNF-α, were downregulated after six ketamine infusions in patients with MDD regardless of the presence of comorbid pain, so we further compared the changes in cytokine levels from baseline to follow-up points (day 13 and day 26) between the two groups using analysis of covariance, with their baseline levels as covariates.

Finally, linear regression analyses were used to further test whether changes in depressive symptoms individually correlated with changes in inflammatory cytokine levels in the pain group and the non-pain group and whether changes in pain symptoms correlated with changes in inflammatory cytokine levels in the pain group. Mediation analyses using the Sobel test (http://www.quantpsy.org/sobel/sobel.htm) were performed to assess whether the relationships between changes in inflammatory cytokine levels and changes in MADRS scores were mediated by changes in VAS scores in the pain group.

All statistical analyses were performed using IBM SPSS Statistics version 22, and p-values < 0.05 were considered statistically significant. *P*-values were adjusted for multiple comparisons using false discovery rate correction.

Results

Demographics

At baseline, BMI and years of education in the pain group were significantly greater than in the non-pain group; other baseline demographic or clinical characteristics showed no statistically significant differences between groups.

Differences in cytokine levels at baseline

MANCOVA showed significant differences in the levels of 12 of the 19 measured inflammatory cytokines, including GM-CSF, fractalkine, IL-10, MIP-3α, IL-13, IL-17α, IL-1β, IL-2, IL-4, IL-23, IL-5, IL-6, IL-7 and MIP-1β, among the pain group, no-pain group and HC group. GM-CSF, IL-1β and IL-6 levels were significantly higher in the pain group than in the no-pain group based on post hoc analyses (Fig. 1 and Supplementary Table 1).

Efficacy of ketamine treatment

Baseline MADRS scores were not significantly different between the two groups ($P > 0.05$). The linear mixed model with MADRS scores showed a significant group main effect (F = 8.066, $P = 0.006$), time main effect (F = 86.986, $P < 0.001$) and group-by-time interaction (F = 3.461, $P = 0.001$). The pain group had lower MADRS scores than the non-pain group from the 2nd infusion to the 6th infusion and on day 26 (all $P < 0.05$).

The patients with pain showed a significantly shorter time to an antidepressant response (F = 2.122, $P = 0.036$) and to depression remission (F = 2.172, $P = 0.032$) and had a higher response rate ($\chi^2 = 6.026$, $P = 0.014$) and remission rate ($\chi^2 = 4.373$, $P = 0.037$) than the patients without pain.

MADRS scores at baseline did not differ between the pain group and the non-pain group. The linear mixed model with MADRS scores showed a significant group main effect (F = 8.066, $P = 0.006$), time main effect (F = 86.986, $P < 0.001$) and group-by-time interaction (F = 3.461, $P = 0.001$). Significant reductions in MADRS scores were found at 24 h after the first infusion compared to baseline scores, and these reductions were maintained over the subsequent infusion period as well as on day 26 in both groups (all $P < 0.05$, Fig. 2 and Supplementary Table 2). The pain group had lower MADRS scores than the non-pain group from the 2nd infusion to the 6th infusion and on day 26 (all $P < 0.05$). The largest significant differences in MADRS scores between the pain group and the non-pain group were observed at the 6th infusion (Cohen's d = 0.565).

The VAS scores, sensory index, affective index and PPI in the pain group at baseline are summarized in Table 1. The linear mixed model showed a significant group main effect, time main effect and group-by-time interaction with VAS scores (group: F = 57.124, $P < 0.001$; time: F = 390.614, $P < 0.001$; interaction: F = 63.651, $P < 0.001$), sensory index (group: F = 45.354, $p < 0.001$; time: F = 33.782, $P < 0.001$; interaction: F = 48.499, $P < 0.001$), affective index (group: F = 71.426, $P < 0.001$; time: F = 46.391, $P < 0.001$; interaction: F = 64.352, $P < 0.001$) and PPI (group: F = 77.267, $P < 0.001$; time: F = 35.039, $P < 0.001$; interaction: F = 62.254, $P < 0.001$). In the pain group, large and significant reductions in VAS scores were found at 24 h after the first infusion compared to baseline scores and were maintained over the subsequent infusion period as well as on day 26 (all $P < 0.05$), and significant reductions in sensory index, affective index and PPI were found at the 6th infusion and on day 26 compared to baseline scores (all $P < 0.05$, Fig. 2, 3 and Supplementary Table 2).

Table 1
Demographics and clinical characteristics of ketamine in depressed patients with and without pain.

Variables	Total (n = 78)	Non-pain (n = 42)	Pain (n = 36)	Statistics	*P*
Age, mean ± SD	34.5 ± 11.4	33.4 ± 11.5	35.7 ± 11.4	-0.891	0.376
Male gender, n(%)	33(42.3%)	16(38.1%)	17(47.2%)	0.662	0.416
Education (years), mean ± SD	12.3 ± 3.2	13.1 ± 3.0	11.4 ± 3.3	2.454	0.016
Employed, n(%)	37(47.4%)	22(52.4%)	15(41.7%)	0.892	0.345
BMI (kg/m^2), mean ± SD	22.5 ± 3.5	21.6 ± 3.5	23.6 ± 3.2	-2.605	0.011
Treatment resistance, n(%)	66(84.6%)	34(81.0%)	32(88.9%)	0.938	0.333
With suicidality, n(%)	44(56.4%)	24(57.15)	20(55.6%)	0.020	0.888
Duration of illness (months), mean ± SD	84.6 ± 74.9	76.8 ± 67.8	93.7 ± 82.5	-0.989	0.326
Psychiatric comorbidity (yes), n(%)	13(16.7%)	9(21.4%)	4(11.1%)	1.486	0.223
Family history of psychiatric disorders (positive), n(%)	24(30.8%)	12(28.6%)	12(33.3%)	0.206	0.650
Dose of antidepressant (mg/day), mean ± SD	39.7 ± 21.8	37.0 ± 22.0	43.0 ± 21.4	-1.191	0.237
On antipsychotic, n(%)	45(57.7%)	26(61.9%)	19(52.8%)	0.662	0.416
On mood stabilizers, n(%)	15(19.2%)	8(19.0%)	7(19.4%)	0.002	0.956
On benzodiazepines, n(%)	35(44.9%)	17(40.5%)	18(50.0%)	0.711	0.399
Baseline MADRS score, mean ± SD	32.1 ± 7.5	31.5 ± 8.2	32.7 ± 6.7	-0.713	0.478
Response rate after six infusions, n(%)	47(60.3%)	21(50.0%)	26(72.2%)	3.997	0.046
Remission rate after six infusions, n(%)	29(37.2%)	11(26.2%)	18(50.5%)	4.705	0.030
Days to response, mean ± SD	11.0 ± 8.3	12.9 ± 9.0	8.8 ± 6.8	2.256	0.027
Days to remission, mean ± SD	14.4 ± 7.7	16.0 ± 7.1	12.4 ± 8.0	2.090	0.040
Baseline VAS score, mean ± SD	2.4 ± 2.9	-	5.1 ± 2.0	NA	NA
Baseline sensory index, mean ± SD	2.1 ± 3.3	-	4.4 ± 3.6	NA	NA
Baseline affective index, mean ± SD	2.2 ± 3.1	-	4.7 ± 3.0	NA	NA

Abbreviations: BMI, body mass index; MADRS, Montgomery-Asberg Depression Rating Scale; VAS, visual analogue scale; PPI, present pain intensity.

Variables	Total (n = 78)	Non-pain (n = 42)	Pain (n = 36)	Statistics	*P*
Baseline PPI, mean ± SD	1.1 ± 1.4	-	2.5 ± 1.1	NA	NA

Abbreviations: BMI, body mass index; MADRS, Montgomery-Asberg Depression Rating Scale; VAS, visual analogue scale; PPI, present pain intensity.

Significant differences between the pain group and the non-pain group in VAS scores were found during the preceding five infusions and day 26. No significant differences between groups in sensory index, affective index or PPI were observed at day 13 and day 26 (all $P > 0.05$) .

Differences in cytokine changes after ketamine treatment

Based on the mean change in the levels of inflammatory cytokines, most of them decreased after ketamine treatment (Fig. 4 and Supplementary Table 3). The pain group showed a significantly greater decrease in IL-6 levels on day 13 and a greater decrease in IL-10, MIP-3α, IL-1β, IL-5 and IL-6 levels on day 26 than the non-pain group (all $P < 0.05$).

Clinical effects and inflammatory cytokine alterations after ketamine infusions

In the non-pain group, linear regression analyse showed that none of the cytokine alterations were associated with the reduction in MADRS scores following ketamine on day 13 or day 26 (all $P > 0.05$). In the pain group, the changes in IL-6 levels were associated with reductions in VAS ($\beta = 0.347$, $t = 2.159$, $P = 0.038$) and MADRS ($\beta = 0.590$, $t = 4.201$, $P < 0.001$) scores at day 13 and associated with a reduction in VAS scores at day 26 ($\beta = 0.440$, $t = 2.854$, $P = 0.006$). Thus, the mediation model in the pain group was further explored at day 13. A significant association was shown in the pathway from reductions in VAS scores to reductions in MADRS scores ($\beta = 0.440$, $t = 5.854$, $P = 0.007$), representing the indirect effect of changes in IL-6 levels on reductions in MADRS scores. A significant association was also shown in the pathway from changes in IL-6 levels to reductions in MADRS scores after reductions in VAS scores were taken into account ($\beta = 0.497$, $t = 3.490$, $P = 0.001$). The Sobel test showed significant indirect effects between decreases in IL-6 levels and reductions in MADRS scores (Sobel test: $Z = 2.026$, $P = 0.043$) at day 13.

Discussion

Three important findings of the present study are as follows: First, the depressed patients with comorbid pain had an elevated inflammatory response compared with the depressed patients without pain and healthy controls. Second, repeated subanesthetic doses of ketamine had significantly superior antidepressant effects in the depressed patients with comorbid pain compared with the patients without pain. Third, ketamine exerted greater downregulation effects in the depressed patients with pain than in the patients without pain. This study is the first to examine the role of plasma inflammatory cytokines in

clinical individual differences in depression comorbid with pain. In addition, this is the first study to explore clinical individual differences in ketamine's antidepressant effects in individuals with comorbid depression and pain, as well as the role of plasma inflammatory cytokines in ketamine's antidepressant response in those patients.

The present study showed that 46.2% of patients with MDD had comorbid pain, consistent with previous reports that the comorbidity rate of chronic pain and depression is approximately 40–60% [3, 4]. Our findings that depressed patients with or without pain showed similar severity of depressive symptoms suggested that painful symptoms were independent of the degree of depression, which was inconsistent with previous results that patients with MDD comorbid with chronic pain suffered from more severe depression [30, 31].

A wealth of evidence supports the hypothesis that excessive activation of inflammation contributes to the pathophysiology of the comorbidity of pain and depression. Microglial activation in the hippocampus and thalamus was found in patients suffering from chronic fatigue syndrome who exhibited pain and depression using positron emission tomography scans [32], and microglial activation and increased inflammatory cytokine expression were found in pain- and mood-related brain regions in rodent models of depression-pain comorbidity [13, 33]. Activation of the inflammatory response was also found in comorbid depression and pain patients. For example, higher plasma IL-6 levels were found in patients with chronic back pain and comorbid depression and in patients with burning mouth syndrome and depressive symptoms than in healthy controls [9, 10]. In addition, higher serum IL-6 levels in patients suffering from burning mouth syndrome and depressive symptoms exhibited more pain [9]. In the present study, in addition to IL-6, other inflammatory cytokines, including GM-CSF, fractalkine, IL-10, MIP-3α, IL-13, IL-17α, IL-1β, IL-2, IL-6 and MIP-1β, were elevated in depressed patients with pain, while the levels of IL-4 decreased. Furthermore, plasma levels of GMCSF, IL-1β and IL-6 in depressed patients with pain were higher than those in patients without pain. An excessive inflammatory response may contribute to individual differences in risks for the comorbidity of pain and depression. A preclinical study reported increased levels of the inflammatory cytokines IL-6, IL-1β, TNF-α, IL-4 and IL-10 in spared nerve ligation rats with a depression-like phenotype but not rats without a depression-like phenotype [34]. Polymorphisms related to the inflammatory response may be moderators of depressive reactions to stress. IL-1β genetic variation and attendant increased IL-1β expression were found to be associated with high risks for stress-induced depression in a large cohort of youths [34]. Thus, it is likely that genetic variants that enhance immune reactivity might create vulnerability to pain and depression comorbidity. Further studies examining differences in immune gene expression between depressed patients with or without pain are needed to confirm this speculation.

Pain adversely affects the treatment response and prognosis of depression and vice versa. Patients with comorbid pain and depression were reported to experience a worse response to analgesic therapy than those without depressive symptoms [35]. Patients who had more severe pain symptoms prior to selective serotonin reuptake inhibitor treatment experienced poorer responses [30]. For depressed patients with pain in our study, the rate of response to six infusions of ketamine was 73.1%, and the remission rate was

48.1%, which were significantly higher than those without pain. Moreover, patients with comorbid pain also showed a significantly shorter time to achieve treatment response and remission. The better antidepressant outcomes in depressed patients comorbid with pain indicated that ketamine works on the brain through mechanisms different from the mechanisms of common antidepressants. Interestingly, the pain group also showed mild pain during ketamine treatment, even after ketamine treatment. A systematic review reported that headache is the most common acute side effect after ketamine treatment, especially in patients given intravenous ketamine [36]. In the present study, pain symptoms were reported during 84 (17.9%) infusions from among the 468 total infusions of ketamine. Although most of them reported that the pain resolved shortly after dose administration, their VAS score, sensory index, affective index and PPI still reflected pain symptoms because these assessments covered a 24-h postinfusion period.

Ketamine showed analgesic effects in patients suffering from acute and chronic pain, as well as rapidly robust antidepressant effects in patients with MDD. Several clinical studies have supported that subanesthetic doses of ketamine may be ideal for the treatment of pain and depression comorbidities. Subanesthetic ketamine can reduce depressive symptoms in chronic pain patients, even in patients with refractory neuropathic pain syndromes [23, 24]. Daily oral ketamine for 6 weeks also effectively improved depressive symptoms in patients with chronic pain with mild-to-moderate depression [26]. Furthermore, in animal studies, ketamine has been reported to relieve pain-induced depression, which is independent of its antinociceptive effect. The present study is the first to examine the efficacy of repeated ketamine in depressed patients with pain. The results again proved ketamine's antidepressant and analgesic effects and gave rise to an interesting finding that depressed patients with pain achieved greater antidepressant outcomes than those without pain and took a shorter time to reach those outcomes.

Then, we further analyzed whether alterations in inflammatory cytokines were related to individual differences in ketamine's effects on comorbid depression and pain. We observed that most of the 19 inflammatory cytokine levels decreased from the mean change values after six infusions of ketamine treatment, consistent with our previous findings in an overlapping sample [27]. By comparing changes in inflammatory cytokine levels after ketamine treatment, a greater decrease in IL-6 levels at 24 h after six infusions and greater decreases in IL-10, MIP-3α, IL-1β, IL-5 and IL-6 levels at two weeks after six infusions were observed in patients with pain than in patients without pain. We speculate that there is a relationship between downregulation of the inflammatory response and ketamine's superior antidepressant effects in patients with pain. Moreover, given that the depressed patients with pain exhibited higher plasma IL-1β, IL-6 and GM-CSF levels than the patients without pain before the ketamine intervention, it is likely that patients who have elevated inflammatory responses may more easily benefit from ketamine. In previous clinical studies, higher levels of IL-6 and IL-1β were reported as potential predictors of ketamine's antidepressant efficacy [37]. In an animal study, spared nerved ligation rats with a depression-like phenotype showed lower serum levels of IL-1β and IL-6 than nonresponders at baseline [34]. In addition, the results from rats subjected to inescapable electric shock suggested that peripheral IL-6 may contribute to resilience versus susceptibility to inescapable stress [38]. Thus, IL-6 was the only

inflammatory cytokine that displayed a greater decrease immediately after ketamine treatment in the patients with pain than in the patients without pain.

Interestingly, correlations between changes in IL-6 levels and both antidepressant and analgesic effects were found in the depressed patients with pain at day 13; however, further analysis showed that ketamine's analgesic effect mediated the association between decreases in IL-6 levels and its antidepressant effect. Previous studies have also suggested that ketamine can decrease the expression of inflammatory cytokines in MDD patients, but the results regarding the relationship between changes in cytokine levels and antidepressant efficacy have been inconsistent. Chen et al. found that the decrease in levels of TNF-α after a single dose of ketamine in patients with MDD was correlated with antidepressant efficacy [39], while no association was found in Park's clinical study [40]. In combination with the present results, the downregulated inflammation, especially the decrease in IL-6 levels, may play a more direct role in ketamine's analgesic effect than its antidepressant effect. However, the precise mechanisms underlying the relationship between elevated inflammatory responses and susceptibility to the comorbidity of pain and depression are currently unknown. Further preclinical studies are warranted to determine the precise anti-inflammatory mechanism of ketamine in combined models of depression and pain.

This study was associated with several limitations. First, the patient sample was relatively small. The small sample size made it impossible to perform subgroup analyses by the area of pain. Second, seven participants lacked inflammatory cytokine data at 2 weeks after ketamine treatment. The third limitation was that inflammatory cytokines were measured only in peripheral blood, which does not directly reflect the inflammatory response in the brain.

Our study suggested that an elevated inflammatory response plays a critical role in the individual differences among depressed patients with or without pain. Ketamine showed great antidepressant and analgesic effects in depressed patients with pain, which may be related to its anti-inflammatory effect. Further preclinical studies to address the precise anti-inflammatory mechanism of ketamine and future therapies based on such a mechanistic understanding can be developed to better serve those with a depression and pain comorbidity.

Abbreviations

BDNF: Brain-derived Neurotrophic Factor; DSM-V: Diagnostic and Statistical Manual of Mental Disorders; GM-CSF: Granulocyte-macrophage Colony Stimulating Factor; HAMD-17: 17-item Hamilton Depression Rating Scale; HCs: Healthy Controls; IFN: Interferon; IL: Interleukin; ITAC: Interferon-inducible T Cell Alpha Chemoattractant; MADRS: Montgomery-Asberg Depression Rating Scale; MDD: Major Depressive Disorder; MIP: Macrophage Inflammatory Protein; PPI: Present Pain Intensity; SF-MPQ: Short-form McGill Pain Questionnaire; TNF: Tumor Necrosis Factor; VAS: Visual Analog Scale.

Declarations

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Declaration of conflicting interests

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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Availability of data and materials

The datasets generated during and/or analyzed during the current study are not publicly available due to the intelligence rights owned by the hospital and the authors but are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

This study was approved by the Clinical Research Ethics Committee of the Affiliated Brain Hospital of Guangzhou Medical University. The study participants agreed on the participation of the study with written informed consent.

Consent for publication

Not applicable.

Authors' contributions

YN conceived and designed the study. YZ drafted the manuscript. YZ, CW, XL and HL carried out data collection, data analysis, and interpretation. ZC and XL contributed inflammatory cytokine detection. All authors read and approved the final manuscript.

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Figures

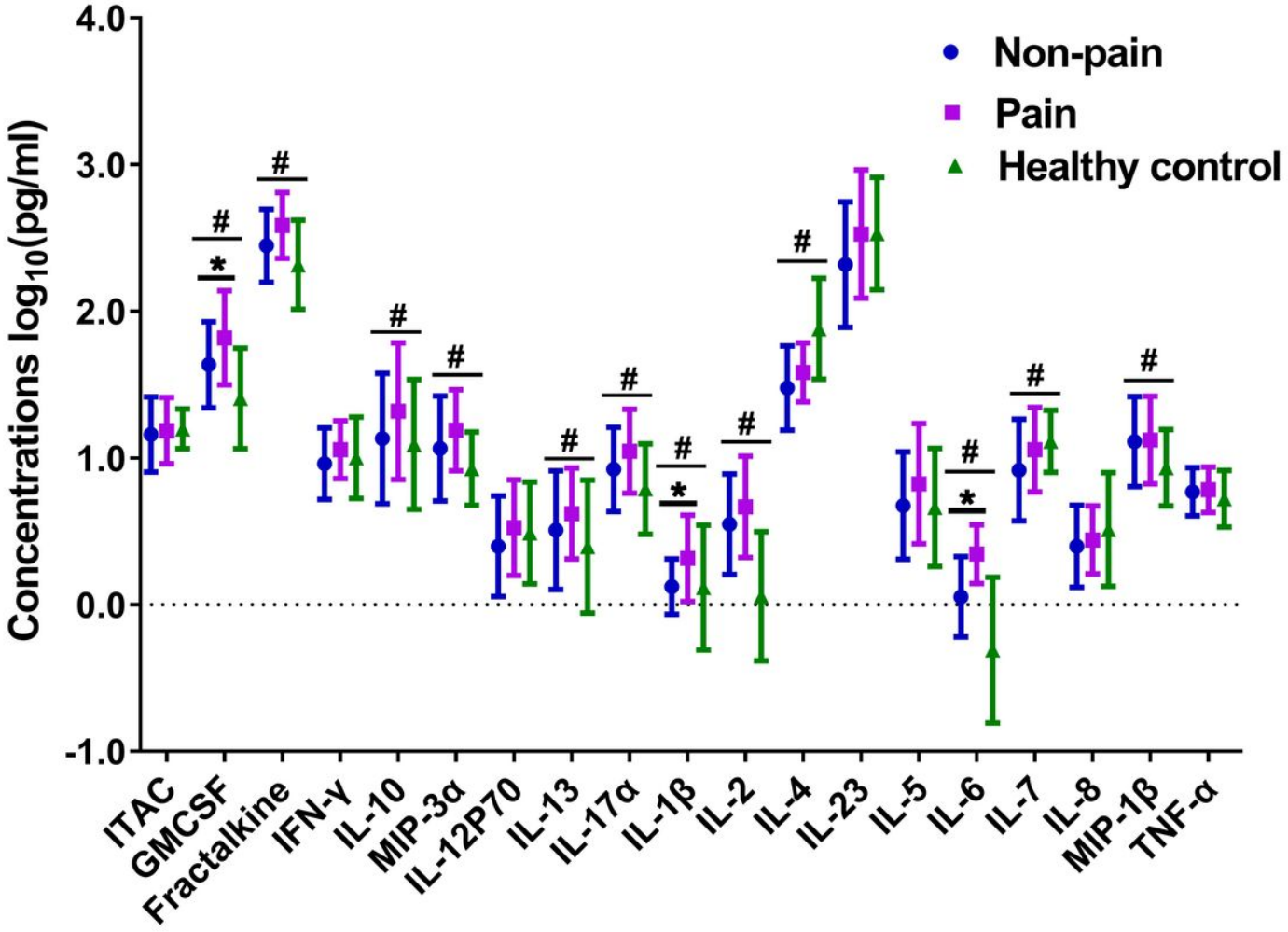

Figure 1

Baseline plasma levels of inflammatory cytokines in depressed patients with and without pain and healthy controls. Legend: # represents significant difference among pain group, non-pain group and healthy controls ($p<0.05$); * represents significant difference between pain group and non-pain group according to post hoc analysis ($p<0.05$). Abbreviations: ITAC, interferon-inducible T cell alpha chemoattractant; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN-γ, interferon gamma; IL, interleukin; MIP, macrophage inflammatory protein; TNF-α, tumor necrosis factor alpha.

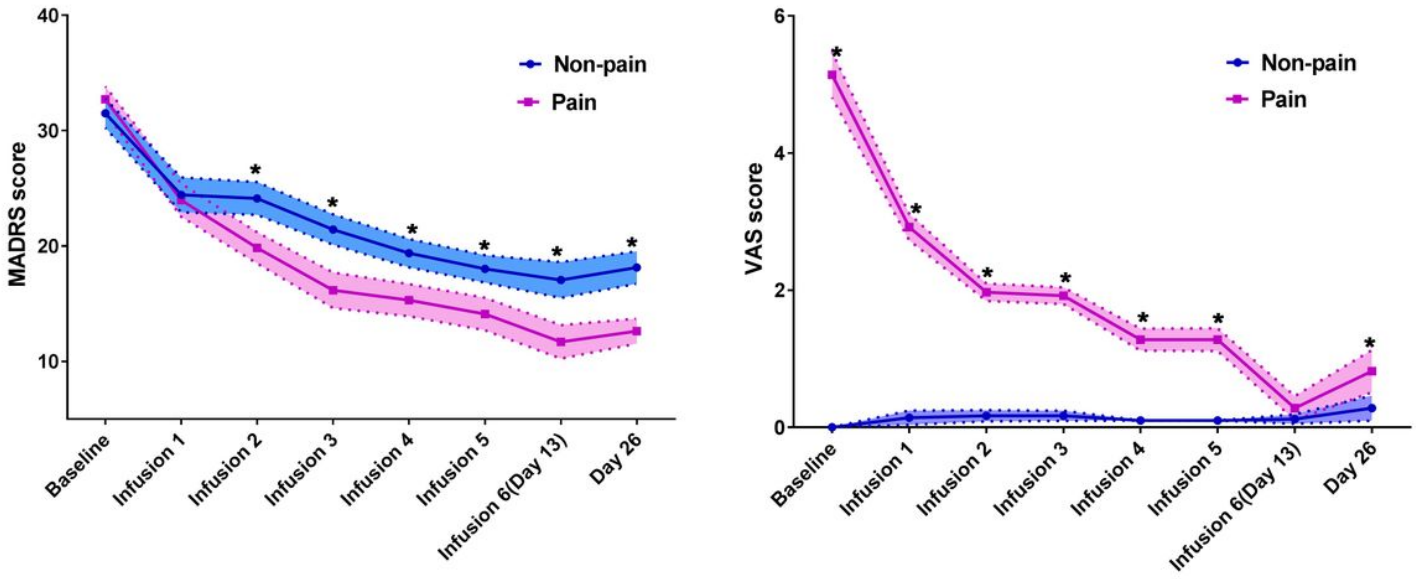

Figure 2

Change in depressive symptoms and pain intensity in pain group and non-pain group. Legend: * represents significant difference at given time point between pain group and non-pain group according to post hoc analysis ($p<0.05$). Abbreviations: MADRS, Montgomery-Asberg Depression Rating Scale; VAS, visual analogue scale.

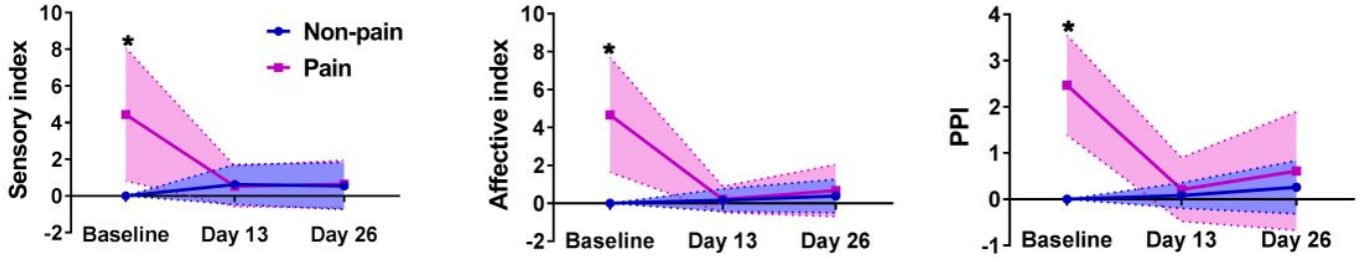

Figure 3

Change insensory index, affective index and present pain intensity in pain group and non-pain group. Legend: * represents significant difference at given time point between pain group and non-pain group according to post hoc analysis ($p<0.05$). Abbreviations: PPI, present pain intensity.

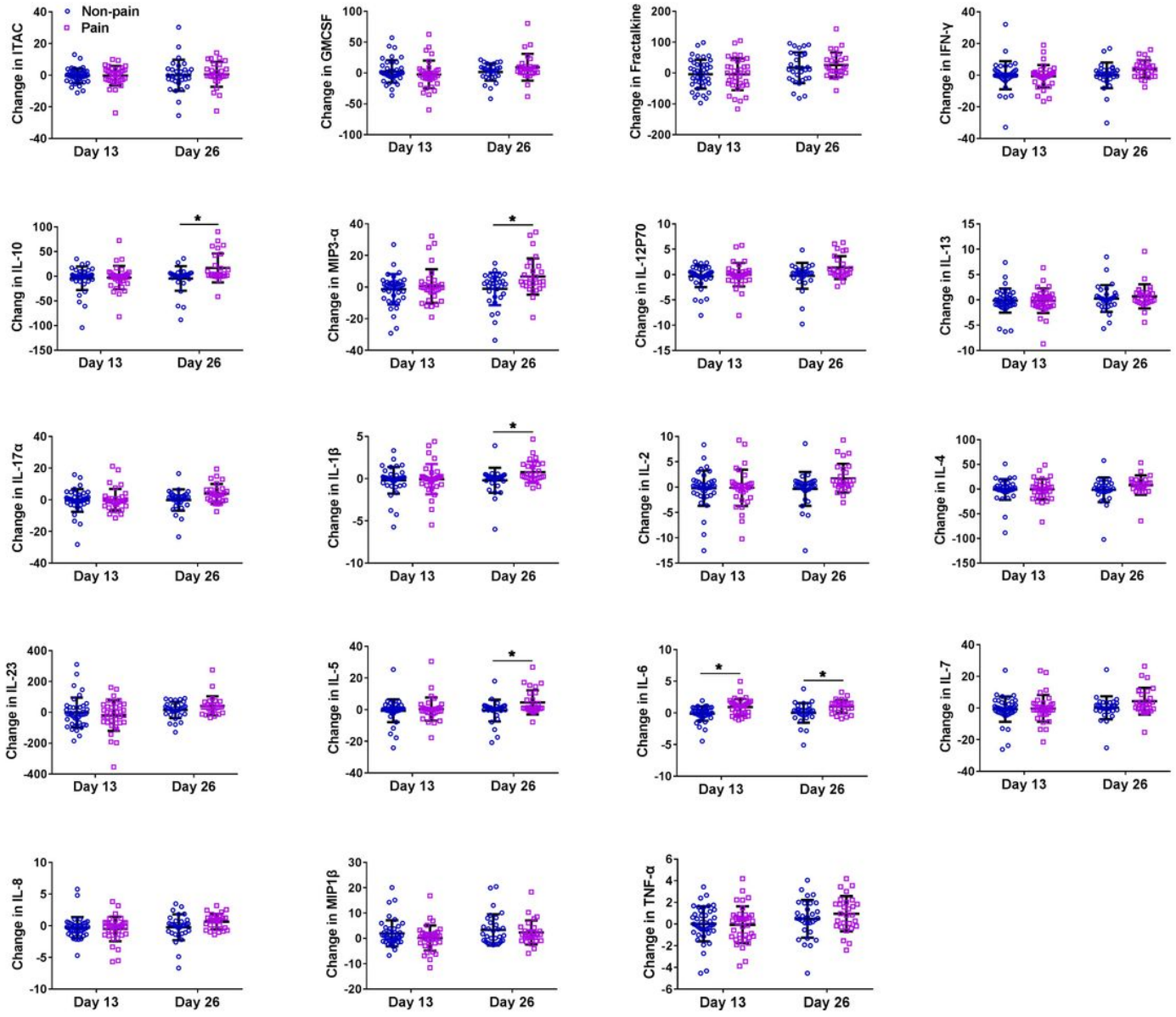

Figure 4

Comparison of changes in inflammatory cytokine levels from baseline to follow-up points (day 13 and day 26) between pain group and non-pain group. Legend: * represents significant difference between pain group and non-pain group ($p<0.05$). Abbreviations: ITAC, interferon-inducible T cell alpha chemoattractant; GM-CSF, granulocyte macrophage colony-stimulating factor; IFN-γ, interferon gamma; IL, interleukin; MIP, macrophage inflammatory protein; TNF-α, tumor necrosis factor alpha.

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