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Correlation between interleukin-6 levels and methadone maintenance therapy outcomes

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ABSTRACT

Background: The outcome of methadone maintenance therapy (MMT) varies in each patient with opioid use disorder (OUD). Opioid abuse activates proinflammatory processes by increasing cytokine production and impairing neurotrophic factor expression, and possibly leads to a vicious cycle that hinders recovery. Therefore, we investigated whether markers of inflammation and neurotrophic expression correlate with the MMT outcomes in OUD patients.

Method: We investigated OUD patients undergoing MMT and followed them up for 12 weeks. We measured plasma tumor necrosis factor (TNF)-α, C-reactive protein (CRP), interleukin (IL)-6, IL-1β, transforming growth factor (TGF)-β1, brain-derived neurotrophic factor (BDNF), urinary morphine tests, and plasma morphine levels at baseline and on weeks 1, 4, 8, and 12 during MMT. Multiple linear regressions and generalized estimating equations (GEEs) were used to examine the correlation between the cytokine and BDNF levels and MMT outcomes.

Results: We initially enrolled 104 patients, but only 78 patients completed end-of-study assessments. Plasma levels of CRP, TGF-β1, and BDNF fell during MMT. Plasma IL-6 levels were significantly associated with plasma morphine levels ($P = 0.005$) and urinary morphine-positive ($^{+}$) results ($P = 0.04$), and significantly associated with poor compliance ($P = 0.009$) and early dropout from MMT ($P = 0.001$). However, other cytokine and BDNF levels were not consistently associated with MMT outcomes.

Conclusion: Higher IL-6 levels were associated with poor MMT outcomes. Additional studies on regulating IL-6 expression to improve treatment outcomes in OUD patients might be warranted.

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1. Introduction

Opioid use disorder (OUD) is highly prevalent and causes global public health problems (Degenhardt et al., 2014). Methadone maintenance treatment (MMT) is a widely used and effective treatment for OUD (Soyka et al., 2011). Numerous studies have shown that MMT markedly reduces illicit opioid use, drug-related health consequences, crimes, and the transmission of HIV and hepatitis (Alavian et al., 2013; Marsch, 1998; Shi et al., 2007). The social productivity, personal relationships, and health of OUD patients treated with MMT also improve (Chou et al., 2013; Gearing and Schweitzer, 1974; Hsiao et al., 2015). However, the outcomes of MMT vary in different patients. Longitudinal studies (Hser et al., 2014) have reported that the mean duration of MMT is 141.3 ± 50.8 days and that only about 74% of patients complete the 24 weeks of therapy. Heroin use persists in some patients undergoing MMT: 31.7% of patients had opioid-positive ($^{+}$)urine specimens and an average of 4.4 days of heroin use during the past 30 days of MMT at the final follow-up (Hser et al., 2016). Some factors may affect the retention and outcomes of MMT, including age, drug use history, ethnicity, methadone dose, clinic accessibility, client-provider interaction, and treatment models, but the results were mixed (Che et al., 2010; Hser et al., 2014; Kelly et al., 2010; Lin et al., 2010; Simpson et al., 1997), partly due to the limitation of epidemiological study design. In this study, we tried to investigate other possible factors that may be correlated with MMT outcomes from different perspectives.

Emerging evidence shows that dysregulated neuroinflammation is involved in the development of addiction (Coller and Hutchinson, 2012). Larger substance doses and long-term heroin abuse cause systemic inflammation and upregulate proinflammatory cytokines like TNF-α, C-reactive protein (CRP), IL-6, and IL-1β (Dyuizen and Lamash, 2009; Kapasi et al., 2000; Pacifici et al., 2000; Reece, 2012), and downregulate the anti-inflammatory cytokine transforming growth factor (TGF)-β1 (Lu et al., 2017; Nabati et al., 2013). Systemic inflammation simultaneously causes widespread activation of microglia (Sandiego et al., 2015) and damages neurons in the brain (Wang et al., 2012). This drug-induced activation of central immune signaling contributes substantially to increasing the engagement of classical mesolimbic dopamine reward pathways and withdrawal centers (Coller and Hutchinson, 2012; Narita et al., 2006), which causes vulnerable individuals to develop an addiction. Conversely, chronic heroin users have lower BDNF expression levels (Angelucci et al., 2007). Postmortem data also shows that long-term heroin users progressively lose brain neurons (Li et al., 2005). Therefore, changes in the expression levels of proinflammatory cytokines and BDNF might be associated with OUD severity, and they might be feasible biological markers for predicting MMT outcomes.

Consistent with the hypothesis that OUD is associated with systemic inflammation, cross-sectional studies (Chan et al., 2015; Ghazavi et al., 2013) have shown that plasma levels of inflammatory cytokines were significantly higher in long-term OUD patients than in healthy controls. However, others (Sacerdote et al., 2008) have reported that the immune function as demonstrated in cytokine expression can be restored to almost normal range after MMT, which indicates there should be a dynamic change in cytokine expression during MMT. We therefore longitudinally followed up the cytokine and BDNF levels in OUD patients undergoing MMT for 12 weeks. We hypothesized that the changes in cytokine and BDNF levels during MMT would correlate with outcomes, including the frequency and volume of heroin use and patient adherence to treatment. Thus, we examined the correlations between plasma TNF-α, CRP, IL-6, IL-1β, TGF-β1, and BDNF levels in patients with OUD and MMT outcomes.

2. Methods

2.1. Patients

The research protocol was approved by the Institutional Review Board for the Protection of Human Subjects at National Cheng Kung University Hospital (NCKUH). The study was done in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. The procedures were fully explained to each participant before they were asked to sign the informed consent. OUD patients were recruited from the NCKUH MMT program.

This study is a subgroup analysis of clinical trials for OUD (Trial registration: NCT01189097 and NCT01189214 at https://register.clinicaltrials.gov/). The original study was a randomized, double-blind, controlled 12-week trial that investigated whether treating OUD with MMT plus add-on dextromethorphan or memantine is more effective than treating it with MMT alone. Because the aim of the current study was to examine the relationship between the changes in cytokine and BDNF levels in OUD patients and the treatment outcomes of MMT, we used only patients from the placebo group for this subgroup analysis to avoid the influence of add-on dextromethorphan and memantine, which are not routinely used for treating OUD. Each patient was initially interviewed and diagnosed by a board certified psychiatrist (author Tzu-Yun Wang) and then were also screened by a research team member well trained in using the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (American Psychiatric Association, 1994) criteria and the Chinese Version of the Mini International Neuropsychiatric Interview (MINI) (Sheehan et al., 1998). The MINI was used because completing 4–6 h of structured interviews, such as the Chinese Version of the Modified Schedule of Affective Disorder and Schizophrenia-Lifetime (SADS-L) (Endicott and Spitzer, 1978), is difficult for OUD patients. The MINI has good reliability and has been widely used in clinical trials and epidemiological studies (Ritchie et al., 2004), and its interrater reliability in Chinese Version was approximately 0.75 in previous studies (Kuo et al., 2003; Lung et al., 2008). Inclusion criteria were being an adult male or female between 18 and 65 years old who met the DSM-IV criteria for current opioid dependence. Exclusion criteria were having a cognitive disorder, being pregnant or nursing an infant, having taken any anti-inflammatory medications within 1 week before the study, and having a history of one or more uncontrolled major physical conditions such as diabetes mellitus or hypertension. Patients with other psychiatric comorbidities, such as anxiety, depression, alcohol use disorder, amphetamine-type stimulants use disorder (ATSUD), and antisocial personality disorder (ASPD), were not excluded from the study.

We recruited 104 OUD patients and examined their plasma TNF-α, CRP, IL-6, IL-1β, TGF-β1, and BDNF levels at baseline and on weeks 1, 4, 8, and 12. Plasma morphine and urinary morphine tests were examined at the same times. However, some OUD patients were not tested for plasma morphine level because it was not included in the add-on memantine trial (NCT01189214). Their MMT and psychosocial interventions were maintained during the follow-up, which included health education, supportive psychotherapy and service linkages. According to the Taiwan Ministry of Health and Welfare (MOHW) MMT guideline, an appropriate initial methadone dose is between 10 and 40 mg per day, but preferably not over 30 mg/day. The guideline also suggests 5-mg increments and decrements of methadone. Therefore, methadone doses were adjusted based on the clinician's evaluation, the patient's subjective responses (withdrawal symptoms and methadone tolerance), and the MOHW guideline. In Taiwan, OUD patients are not allowed to take methadone home according to MOHW policy. They need to go to methadone clinics to take methadone everyday under the supervision of medical staffs. MMT outcome assessments included: (1) plasma morphine levels; (2) urinary morphine tests; (3) MMT compliance (compliance to scheduled follow-up appointment and to take methadone); (4) early MMT dropout (< 12 weeks).

2.2. Blood samples and cytokine analysis

After 20 mL of blood had been drawn from each participant, the plasma was isolated from the whole blood after it had been centrifuged at 3000 *g* for 15 min at 4°C, and then it was immediately stored at −80 °C. TNF-α, CRP, IL-6, IL-1β, TGF-β1 and BDNF levels were quantified using an antibody pair assay system (Flexia; BioSource Intl., Camarillo, CA). All laboratory procedures were double-blind, and all assays were done in duplicate.

2.3. Plasma and urinary morphine analysis

Once heroin is absorbed into the body, heroin will be metabolized by removing the acetyl groups and be reformed as morphine. Thus, we used a rapid screening test to screen for urinary morphine (negative/positive) at every visit. Plasma morphine concentrations were determined using high performance liquid chromatography (HPLC) analysis as previously described (Chen et al., 2005, 2012). The human plasma was filtered (Amicon Microcon YM-3 centrifugal filters; Millipore, Billerica, MA, USA) (molecular weight [MW] cutoff: 3000) at 17,800 × g for 40 min at 4 °C. The recovery rate of the filtration was 100%. The filtered sample was then injected into the HPLC system to measure free-form morphine concentration (Chen et al., 2012).

2.4. Statistical analysis

Because there were repeated assessments, the generalized estimating equation (GEE) method (Zeger et al., 1988) was used for multiple linear regression in repeated-measures analyses that accommodate randomly missing data (Shen and Chen, 2012). We used GEE analysis to investigate the correlations of changes in plasma cytokine and BDNF levels, and of MMT outcomes. Arithmetic transformations were used to produce approximately normal distributions for further analysis; log (x + 1) was used for cytokine levels. Potential prognostic factors included age, sex, methadone dose, disease duration, psychiatric comorbidities, and treatment duration (from baseline to week 12). When we examined the correlation between plasma and urinary morphine results and cytokine and BDNF levels, we controlled for age, sex, methadone dose, disease duration, psychiatric comorbidities, and treatment duration. When we examined the correlation between compliance and early dropout, we controlled for cytokine and BDNF levels, age, sex, methadone dose, disease duration, psychiatric comorbidities, and morphine$^+$ urine tests. Significance was set at $P < 0.05$. SPSS 18.0 was used for all statistical analyses.

3. Results

At baseline, there were 104 OUD patients (mean age: 36.6 ± 7.5 years old; male: 87.5%; and mean duration using heroin: 6.9 ± 5.2 years) (Table 1). About three quarters of the participants had morphine + urine tests and were undergoing MMT (mean dose: 36.39 ± 27.17 mg/day); and 26.9% of the patients had psychiatric comorbidities other than OUD (Table 1).

After 12 weeks of MMT, 78 patients (mean age: 37.0 ± 7.9 years old; male: 88.5%; and mean duration using heroin: 7.1 ± 5.7 years) had completed the assessment. The mean dose of methadone (39.96 ± 24.47 mg/day) was higher than at baseline, but the rate of morphine + urine tests was lower (50.0%) (Table 1).

Their plasma levels of cytokines and BDNF at baseline and after undergoing 12 weeks of MMT are listed in Table 2. The levels of CRP, TGF-β1, and BDNF were significantly (P = 0.001, 0.001, and 0.01, respectively) lower after 12 weeks of MMT, but the levels of TNF-α, IL-6, and IL-1β were not (Table 2).

After we had corrected for multiple possible confounding factors (age, sex, methadone dose, disease duration, treatment duration, and psychiatric comorbidities), a GEE analysis showed the association between plasma and urinary morphine test results and changes in plasma cytokine levels in patients with OUD undergoing 12 weeks of MMT. Log IL-6 levels were significantly positively correlated with plasma morphine levels (P = 0.005) and urinary morphine$^+$ results (P = 0.04) (Table 3).

Table 1
Demographic data in OUD patients during 12 weeks of MMT.

Variable	Baseline	After 12 weeks	Paired *t*	P
Cases (n)	104	78		
Age (years, mean ± SD)	36.6 ± 7.5	37.0 ± 7.9	–	–
Sex (Male/Female)	91/13	69/9	–	–
Disease duration (years, mean ± SD)	6.9 ± 5.2	7.1 ± 5.7	–	–
Methadone Dose (mg/day, mean ± SD)	36.39 ± 27.17	39.96 ± 24.47	−2.59	0.01*
Psychiatric comorbidities (n/%)			–	–
Yes	61 (58.7)	51 (65.4)	–	–
No	43 (41.3)	27 (34.6)	–	–
Mood disorders (n/%)			–	–
Yes	13 (12.5)	7 (9.0)	–	–
No	91 (87.5)	71 (91.0)	–	–
ATSUD (n/%)			–	–
Yes	50 (48.1)	45 (57.7)	–	–
No	54 (51.9)	33 (42.3)	–	–
AUD (n/%)			–	–
Yes	17 (16.3)	17 (21.8)	–	–
No	87 (83.7)	61 (78.2)	–	–
ASPD (n/%)			–	–
Yes	2 (1.9)	2 (2.6)	–	–
No	102 (98.1)	76 (97.4)	–	–
Urine morphine (n/%)			–	–
Positive	77 (74.8)[a]	35 (0.5)[c]	–	–
Negative	26 (25.2)[a]	35 (0.5)[c]	–	–
Plasma morphine (pg/mL, mean ± SD)	19.88 ± 65.43[b]	36.90 ± 69.23[d]	−0.70	0.49

ATSUD: Amphetamine-type stimulants use disorder; AUD: Alcohol use disorder; ASPD: anti-social personality disorder.
[a] total case number = 103.
[b] total case number = 54.
[c] total case number = 70.
[d] total case number = 33.
* $P < 0.05$.

Fourteen patients dropped out of their MMT early during the 12 weeks. Six patients lost follow-up, five decided to discontinue their MMT, and three discontinued MMT because they were in prison. Forty-six patients missed at least one scheduled follow-up appointment or did not take at least one daily dose of methadone during the follow-up. We assigned these patients to a poor compliance group (Table 4). After covarying for age, sex, methadone dose, disease duration, psychiatric comorbidities, and urinary morphine and amphetamine test results, higher log IL-6 levels in OUD patients were significantly associated with poor compliance (P = 0.009) and early dropout (P = 0.001) from MMT, lower log IL-1β and log BDNF levels were significantly (P < 0.001 and P = 0.02, respectively) associated with early dropout from MMT. Log CRP and log TNF-α levels were associated with MMT compliance and early dropout with borderline significance (P = 0.07 and 0.05, respectively).

4. Discussion

Our most important finding was a significantly positive correlation between plasma IL-6 levels and MMT outcomes, including the severity of heroin addiction and OUD patient adherence to MMT. Additionally, higher IL-6 levels during MMT were also associated with poor compliance and dropping out of MMT early. However, other cytokine and BDNF levels were not consistently associated with MMT outcomes.

Although MMT is a standard treatment for OUD (Soyka et al., 2011),

Table 2
Changes of plasma cytokine levels in OUD patients undergoing 12 weeks of MMT.

Cytokines	Cytokine levels: Baseline (mean ± SD)	Cytokine levels: After 12 weeks (mean ± SD)	Changes at 12 weeks of MMT: B	Wald χ^2 (95% Wald CI)	P
TNF-α(pg/mL)	4.01 ± 3.95	3.56 ± 3.90	−0.01	2.84 (−0.03 to 0.002)	0.09
CRP(μg/mL)	3.35 ± 3.09	2.90 ± 2.42	−0.02	10.30 (−0.02 to −0.006)	0.001*
IL-6(pg/mL)	2.61 ± 2.45	2.29 ± 2.28	−0.008	2.67 (−0.02 to 0.002)	0.10
IL-1β(pg/mL)	1.47 ± 1.78	1.43 ± 1.35	−0.002	0.11 (−0.01 to 0.009)	0.74
TGF-β1(ng/mL)	28.34 ± 14.85	21.55 ± 15.63	−0.03	10.83 (−0.04 to −0.01)	0.001*
BDNF(ng/mL)	11.89 ± 7.58	9.21 ± 5.49	−0.02	6.63 (−0.03 to −0.004)	0.01*

TNF-α: tumor necrosis factor α; CRP: C-reactive protein; TGF-β1: transforming growth factor β1; IL-6: interleukin 6; IL-1β: interleukin 1β.
* $P < 0.05$.

its outcomes are not satisfactory for every patient. The severity of heroin addiction, which is generally measured using urinary morphine tests, and adherence to MMT are the most frequently evaluated outcomes of OUD patients. In addition to demographic data, we found that higher plasma IL-6 levels were not only associated with more heroin use, but also associated with poor MMT compliance and early drop-out. IL-6 is a proinflammatory cytokine and promotes the proliferation and activation of T cells, the differentiation of B cells, and the regulation of the acute-phase response (Hunter and Jones, 2015). Compelling evidence shows that stress induces significant increases in circulating IL-6, both in animal (Zhou et al., 1993) and in human studies (Steptoe et al., 2007). IL-6 also interacts with the hypothalamic-pituitary-adrenal (HPA) axis and mediates the stress response system. IL-6 activates the HPA axis during immunological challenges, even without hypothalamic input from corticotropin-releasing hormone (Bethin et al., 2000). Thus, IL-6 is a pleiotropic cytokine that affects immune reactions and alters neuropsychological behavior under stress (Rohleder et al., 2012). Opioids, like lipopolysaccharide (LPS), cause neuroinflammation (Wang et al., 2012). Injecting opioids and opioid withdrawal is also significant stress that causes upregulation of IL-6 expression. Moreover, activated systemic inflammation will lead individuals more vulnerable to addiction. Methylphenidate-induced dopamine upregulation is significantly greater when subjects were pretreated with LPS, demonstrated with a higher IL-6 levels, rather than with placebo (Petrulli et al., 2017). Therefore, IL-6 might be associated with addiction in two ways: first, using heroin, like stress, upregulates IL-6 levels; and second, higher IL-6 levels increase individual susceptibility to addiction. The association between plasma IL-6 levels (inflammation) and heroin use (addiction) severity is a vicious cycle, and the more serious the inflammation, the more likely the patient will drop out of MMT. Because OUD patients with higher plasma IL-6 levels use more heroin, we assume that they will not regularly attend our MMT clinics. It is also possible that poor MMT attendance will cause them to use more heroin and thus raise their IL-6 expression. Therefore, the causal relationship between inflammation and poor MMT attendance might be bidirectional.

However, the systemic inflammation level in each OUD patient might not be an accurate explanation of why patients drop out of MMT. Of our 14 patients who dropped out early, three were in prison and six lost follow-up: they, too, might have been in prison. The small number of dropouts might have caused an analytical bias. Additionally, it is difficult to associate being in prison with inflammation. Therefore, our finding of an association between IL-1β levels and early dropout risk were not consistent with our finding that higher IL-6 levels predicted poor outcomes. Further studies to investigate the relationship between dropping out of MMT and inflammation are required, but it is also necessary to enroll more patients and to exclude who will probably drop out because they will most likely be going back to prison.

Other studies (Angelucci et al., 2007; Chan et al., 2015; Dyuizen and Lamash, 2009; Lu et al., 2017; Nabati et al., 2013; Reece, 2012) have reported different levels of TNF-α, CRP, IL-1β, TGF-β1, and BDNF in OUD patients than in controls. Our results, however, did not support the notion that they predict outcomes in OUD patients. Moreover, although we found that CRP levels decreased in OUD patients undergoing MMT, they were not consistently associated with MMT outcomes. CRP is an acute phase protein produced in the liver and secreted into the bloodstream during inflammation, mostly in response to IL-6 signaling (Del Giudice and Gangestad, 2018). Although CRP levels were significantly correlated with IL-6 levels in our study (Pearson correlations: $r = 0.24$, $P < 0.001$), there was no significant associations between CRP levels and heroin use severity. We hypothesized that because IL-6 is the upstream cytokine in the inflammatory cascade, it may be more sensitive to link with heroin use induced subtle inflammatory changes. Furthermore, our study was able to longitudinally follow up the cytokine changes in OUD patients undergoing MMT, and we controlled for several factors that might have affected outcomes. Compared with other studies (Angelucci et al., 2007; Chan et al., 2015; Dyuizen and Lamash, 2009; Lu et al., 2017; Nabati et al., 2013; Reece, 2012), our data from those biomarkers might be more reliable for predicting MMT outcomes. We therefore suggested that MMT generally attenuates systemic

Table 3
Association between plasma and urinary morphine test results and changes in plasma cytokine and BDNF levels in OUD patients.

Cytokines	Plasma morphine levels: B	Wald χ^2 (95% Wald CI)	P	Urinary morphine + group[a]: B	Wald χ^2 (95% Wald CI)	P
Log TNF-α	−1.06	0.004 (−32.18 to 30.05)	0.95	0.13	0.08 (−0.76 to 1.01)	0.78
Log CRP	−14.92	0.93 (−45.19 to 15.35)	0.33	−0.38	0.53 (−1.41 to 0.65)	0.47
Log IL-6	77.61	7.77 (23.04 to 132.17)	0.005*	1.10	4.43 (0.08 to 2.12)	0.04*
Log IL-1β	27.11	1.65 (−14.27 to 68.49)	0.20	0.35	0.43 (−0.70 to 1.41)	0.51
Log TGF-β1	−44.89	2.89 (−96.65 to 6.86)	0.09	−0.13	0.05 (−1.23 to 0.98)	0.83
Log BDNF	8.44	0.28 (−22.70 to 39.58)	0.60	0.66	1.15 (−0.55 to 1.87)	0.28

Covarying for age, sex, methadone dose, disease duration, psychiatric comorbidities, and treatment duration.
* $P < 0.05$.
[a] Reference group: [a]urinary morphine^{-} group.

Table 4
Association between poor compliance and early drop out from MMT and changes in plasma cytokine and BDNF levels in OUD patients.

	Poor compliance group[b]			Early dropout from MMT[c]		
Cytokines	B	Wald χ^2 (95% Wald CI)	P	B	Wald χ^2 (95% Wald CI)	P
Log TNF-α	0.08	3.21 (−0.007 to 0.17)	0.07	0.88	3.86 (0.003 to 1.77)	0.05*
Log CRP	−0.15	4.00 (−0.29 to −0.003)	0.05*	−0.62	0.82 (−1.96 to 0.72)	0.37
Log IL-6	0.17	6.78 (0.04 to 0.30)	0.009*	1.76	11.54 (0.75 to 2.78)	0.001*
Log IL-1β	−0.07	3.08 (−0.15 to 0.08)	0.08	−1.82	12.34 (−2.84 to −0.81)	< 0.001*
Log TGF-β1	−0.004	0.04 (−0.05 to 0.04)	0.85	−0.14	0.27 (−0.66 to 0.39)	0.61
Log BDNF	0.03	0.54 (−0.04 to 0.10)	0.46	1.06	5.39 (0.17 to 1.96)	0.02*

Covarying for age, sex, methadone dose, disease duration, psychiatric comorbidities, and urinary morphine and amphetamine test results.
[b,c]Reference groups: [b]good compliance group; [c]completed 12 weeks in MMT group.
* $P < 0.05$.

inflammation in OUD patients, shown in CRP levels, but that IL-6 is more specifically associated with MMT outcomes.

Our study also showed that BDNF and TGF-β1 levels fell during the 12 weeks of MMT. Other studies report inconsistent findings on peripheral BDNF and TGF-β1 levels in OUD patients. Some studies (Heberlein et al., 2011; Nabati et al., 2013; Zhang et al., 2014) reported that BDNF and TGF-β1 levels were higher in OUD patients than in controls, possibly because of a compensatory response to neuronal insults; while another (Lu et al., 2017) reported that plasma BDNF and TGF-β1 levels were significantly downregulated in long-term heroin addicts and were highly correlated with changes in platelet counts and with impaired executive function. Our findings did not confirm or contradict those claims. Using animal studies might be more feasible for determining the association between downregulated BDNF and TGF-β1 expression, neuronal insults, and platelet counts and heroin and methadone treatments.

Our study has some limitations. Because this is the only study that tests the hypothesis that peripheral inflammation markers are associated with MMT outcomes in OUD patients, studies with more patients are needed to provide more conclusive findings. Although we tested six potential factors in this study, there are many other pro-inflammatory and anti-inflammatory cytokines and neurotrophic factors that might be correlated with OUD patients' outcomes. Nevertheless, our findings indicate the important association between inflammation markers and treatment outcomes in OUD patients; this might stimulate fruitful addiction research. Also the definition of poor compliance and drop out of MMT may be too strict in current study and could be revised in future study. The arbitrary classification of poor adherence group may cause the inconsistent findings in borderline significantly negative association between CRP levels and poor MMT adherence. The definition of poor compliance may be revised as less than 90% of attendance rate of methadone clinics, and OUD patients who were in jail may be excluded from the drop out group in future study. Additionally, we have tried to control for factors that might affect changes in plasma cytokine and BDNF levels, but other factors, e.g., metabolic profiles and smoking, might also have affected our findings. Most of our participants smoked, which might interfere with cytokine expression levels. These other factors should be controlled for in future research. Moreover, the methadone dose in our study was lower than the usual recommend dose (Fareed et al., 2010; Farre et al., 2002), which may affect the MMT treatment outcome. We tried to control the effects of methadone dose in our analysis, but the limitation of lower methadone dose may still affect our interpretation. Additionally, although our patients were diagnosed by a board certified psychiatrist and also screened by MINI, our study lacked structure diagnostic instruments to make psychiatric diagnosis. Finally, we followed OUD patients undergoing MMT for only 12 weeks, which might be insufficient for determining long-term outcomes. Other studies (Hser et al., 2014), however, have reported that about 25% of OUD patients drop out of MMT within the first 30 days, which indicates that the initial period of treatment might be critical for retaining patients. Thus, OUD patients who can remain in MMT programs more than 12 weeks might have better retention and link with better MMT outcomes. But longer follow-ups are still necessary. Because of these limitations, our findings should be interpreted with caution.

5. Conclusion

We found a significant correlation between IL-6 levels and MMT outcomes. Higher IL-6 levels were associated with higher heroin use and poor treatment adherence in OUD patients undergoing MMT. Additional studies on regulating IL-6 expression to improve treatment outcomes in OUD patients might be warranted.

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Contributors

RBL and TYW designed the study and wrote the protocol. SLC, SHC, and CHC supervised the laboratory work and the data analyses. TYW, SYL, NST, PSC, IHL, KCC, YKY, and RBL recruited participants. TYW wrote the first draft of the manuscript. SYL, YHC, SHL, PC and JSH reviewed the literature and contributed to the discussion. All authors contributed to and reviewed the final version of the manuscript.

Declaration of Competing Interest

All authors declare that they have no conflicts of interest.

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