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Correlation of cytokines, BDNF levels, and memory function in patients with opioid use disorder undergoing methadone maintenance treatment

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Abstract

Background: Patients with opioid use disorder (OUD) show memory deficiencies and impaired treatment outcomes. Emerging evidence suggests that opioid abuse activates proinflammatory processes by increasing cytokine production and impairing neuroprotection, which damages the memory function in OUD patients. Therefore, we investigated whether plasma inflammatory and neurotrophic markers correlate with memory function in OUD patients.

Method: OUD patients undergoing methadone maintenance therapy (MMT) were investigated and followed up for 12 weeks. Plasma tumor necrosis factor (TNF)- α , C-reactive protein (CRP), interleukin (IL)-6, transforming growth factor (TGF)- β 1, brain-derived neurotrophic factor (BDNF) levels, and Wechsler memory scale-revised (WMS-R) scores were assessed at baseline and after 12 weeks of MMT. Multiple linear regressions and generalized estimating equations (GEEs) were used to examine the correlation between cytokines and memory performance.

Results: We enrolled 89 patients at baseline; 47 patients completed the end-of-study assessments. Although Pearson correlations showed that CRP and TGF- β 1 levels were significantly negatively associated with some memory indices, the results were not significant after correction. The GEE results, controlled for several confounding factors and multiple testing, showed that changes in TNF- α levels were negatively correlated with changes in the visual memory index ($P = 0.01$), and that changes in IL-6 levels were negatively correlated with changes in the verbal memory index ($P = 0.009$).

Conclusion: Memory performance, TNF- α , and IL-6 levels in OUD patients were negative correlated. Additional studies on regulating TNF- α and IL-6 expression to improve memory function in OUD patients might be warranted.

Keywords

opioid use disorder; cytokines; brain-derived neurotrophic factor; memory; cognitive function

1. Introduction

The lifetime prevalence rate of opioid use disorder (OUD) is high—2.1% in the US (Grant et al., 2016) and 0.22% globally (Degenhardt et al., 2014)—and it causes substantial public health problems in the world (Degenhardt et al., 2014). The development of OUD is associated with multiple genetic, environmental, and developmental risk factors (Volkow et al., 2016). In addition to the classical etiology for OUD, emerging evidence also suggests that dysregulated neuroinflammation is involved in the development of addiction (Coller and Hutchinson, 2012). Larger doses and long-term heroin use are associated with dysregulation of the immune system, which damages neurons and triggers additional glial cells to upregulate the proinflammatory cytokines, such as tumor necrosis factor (TNF)- α and interleukin (IL)-6 (Chan et al., 2015; Dyuizen and Lamash, 2009; Pacifici et al., 2000; Wang et al., 2012). This drug-induced activation of central immune signaling contributes to increases in the engagement of classical mesolimbic dopamine reward pathways and

withdrawal centers (Coller and Hutchinson, 2012; Narita et al., 2006), and it is associated with developing OUD. The overactive microglial and inflammatory responses cause neurotoxicity in the hippocampus and amygdala via neuronal apoptotic pathways (Atici et al., 2004; Hu et al., 2002). Postmortem data also show that progressive neuron loss occurs in the brains of long-term heroin users (Li et al., 2005). Other findings suggest that chronic heroin users express lower levels of brain-derived neurotrophic factor (BDNF) (Angelucci et al., 2007). Collectively, these findings indicate that opioid abuse activates microglia and causes neurotoxicity by inducing the production of inflammatory cytokines and impairing neuroprotection, which consequently damages the brain in OUD patients.

The prolonged use of opioids, such as heroin, is associated with impaired attention, concentration, visual and verbal recall, and visual-spatial skills (Gruber et al., 2007). Methadone maintenance therapy (MMT) is the most-studied and it is an effective treatment for OUD (Soyka et al., 2011). However, changes in neuropsychological functioning persist in patients undergoing MMT (Prosser et al., 2006), especially in the domains of episodic (Curran et al., 2001), visual (Prosser et al., 2006), and working and long-term memory (Darke et al., 2000). Maladaptive memories associated with drug abuse might lead to relapses into drug-seeking behavior (Ross and Peselow, 2009). Therefore, increasing our understanding of the underlying mechanisms of the associated memory deficits in OUD patients undergoing MMT might help us design better treatment strategies.

The immune system modulate learning, memory, and neural plasticity (Yirmiya and Goshen, 2011). Manipulating individual cytokines can also modulate learning, memory, and synaptic plasticity (Donzis and Tronson, 2014). Some proinflammatory cytokines have been implicated in memory and plasticity deficits. Overexpression of TNF- α in neurons and glial cells impairs passive avoidance memory (Fiore et al., 2000), inhibits long-term potentiation (LTP) and synaptic plasticity (Butler et al., 2004; Cunningham et al., 1996), and hinders cerebellar learning (Paredes et al., 2010). TNF- α also mediates memory deficits after chronic lipopolysaccharide (LPS) treatment (Belarbi et al., 2012), which is consistent with the memory impairing effect of TNF- α . Overexpression of IL-6 or application of IL-6 cause broad memory impairments (Heyser et al., 1997; Wei et al., 2012), diminished LTP (Li et al., 1997), and a reduction of neurogenesis in the hippocampal dentate gyrus (Vallieres et al., 2002). Increased C-reactive protein (CRP) levels have been associated with a wide range of brain dysfunctions, including age-related cognitive decline (Laurin et al., 2009), all-cause dementia (Kravitz et al., 2009), and memory impairment in schizophrenia (Bulzacka et al., 2016). In contrast, cytokines that modulate the inflammation process might be neuroprotective and improve the cognitive deficits caused by inflammation. Transforming growth factor (TGF)- β 1 is normally present at low levels under physiological conditions, but it is rapidly upregulated after a brain injury (Zhu et al., 2000). TGF- β 1 protects neurons against different kinds of insults, including excitotoxic injury and neurotoxins (Qian et al., 2008), and preserves both cognitive function and memory (Li et al., 2013; Matias et al., 2017). In addition, the production and signaling of BDNF has been implicated in almost every aspect of neural and behavioral plasticity, including hippocampal-dependent memory (Barnes and Thomas, 2008; Heldt et al., 2007), LTP (Lu et al., 2008), and neurogenesis (Li et al., 2008). Increased BDNF signaling underlies the influence of immune processes on learning, memory, LTP, and neurogenesis (Yirmiya and Goshen, 2011). Therefore,

investigating the potential impacts of cytokines and BDNF on the memory function in OUD patients might reveal the underlying mechanism for opioid-induced memory deficits.

Few studies have investigated the association between the plasma levels of cytokines, BDNF, and memory function in OUD patients undergoing MMT. Because studies have reported that memory function might change after two months of MMT (Gruber et al., 2006), a longitudinal study controlled for potential confounding factors is more suitable for assessing the association between changes in memory function and in plasma levels of cytokines and BDNF. We therefore used a longitudinal study to assess the correlation between cytokines and BDNF levels and changes in the memory performance of OUD patients after 12 weeks of MMT.

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. 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 MMT program. Each patient was initially interviewed by an attending psychiatrist and then 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 inter-rater 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 and who used opioids daily. 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, or having a history of one or more uncontrolled major physical conditions such as diabetes mellitus or hypertension.

We asked patients whether they had taken any anti-inflammatory agents, such as: steroids, nonsteroidal anti-inflammatory drugs (NSAIDs), or other immune modulating agents (disease-modifying anti-rheumatic drugs, DMARDs) before enrollment. If they had taken these agents, we would not enroll them in our study until they had stopped taking these medications for at least one week. We also informed our patients that we would follow up their cytokine expression levels during the follow-up and that taking any anti-inflammatory agents would interfere with the results. If they reported taking these agents during follow up, we excluded their cytokine data. Glucocorticoids inhibit the expression and action of most cytokines, including IL-1 β , TNF- α , IL-2, IL-3, IL-4, IL-5, IL-6, IL-8, IL-10, IL-12 and IFN- γ (Tsuboi et al., 1995). NSAIDs might also affect the responses of IL-2, IL-6, IL-8, and

monocyte chemoattractant protein-1 (Lisboa et al., 2017). DMARDs inhibit the induction of cytokines in monocytes and macrophages (Bondeson, 1997) and interfere with IL-6 and TNF- α expression (Mangoni et al., 2017). Therefore, the current study excluded patients taking anti-inflammatory agents.

We recruited 89 OUD patients at the beginning of our study. At baseline, each patient was assessed for peripheral inflammatory cytokines and BDNF levels, and for memory function using the Wechsler Memory Scale-Revised (WMS-R). Their MMT and psychosocial interventions were maintained during the follow-up. After 12 weeks of follow-up, we assessed their plasma levels of inflammatory cytokines, and BDNF, and their WMS-R performance again. At the endpoint of 12 weeks, 47 OUD patients completed the assessment.

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. Cytokine and BDNF levels were quantified using an antibody pair assay system (Flexia; BioSource Intl., Camarillo, CA). Sample processing and data analysis were done according to the manufacturer's instructions. The immunological parameters—TNF- α , CRP, IL-6, TGF- β 1, and BDNF levels—were measured. All laboratory procedures were performed double-blinded and all assays were done in duplicate.

2.3. Memory function assessment

The WMS-R is designed to understand all factors of memory, and it is considered a major assessment of cognitive function (Wechsler, 1987). The WMS-R consists of 13 subtests and five indices (General Memory, Verbal Memory, Visual Memory, Attention/Concentration, and Delayed Recall). The Information and Orientation Questions (the first subtest) were used to screen for disorientation and intact sensory functioning. Attention and working memory were assessed using the Attention/Concentration index subtests (Kent, 2013). The index-score reliability coefficients range from 0.70 to 0.90 (Elwood, 1991). The test-retest reliability after 2 to 12 weeks is 0.62–0.82 (Wechsler, 1987). Inter-rater reliability is more than 0.9 (O'Carroll and Badenoch, 1994).

2.4. Statistical analysis

Pearson χ^2 analysis was used to examine the categorical variables. Fisher's exact test was substituted for the χ^2 test when values were smaller than expected (< 5). Pearson correlations were used initially to test the correlation between WMS-R index scores and the cytokine and BDNF levels. However, 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). In the current study, GEE analysis was used to investigate the changes in cytokine levels and WMS-R individual index scores from baseline to endpoint, and the correlations of changes in plasma cytokine and BDNF levels, and in WMS-R scores. Time effects (treatment period from baseline to week 12), age, sex, methadone dose, disease

duration, psychiatric comorbidities, and morphine-positive urine tests were covarying. Significance was set at $P < 0.05$. SPSS 18.0 was used for all statistical analyses.

3. Results

At baseline, there were 89 OUD patients (mean age: 35.99 ± 7.24 years old; male: 83.2%; and mean duration using heroin: 6.20 ± 4.87 years). All of them used intravenously injected heroin. The participants had a high rate (71.6%) of morphine-positive urine tests, and were undergoing MMT (mean dose: 34.61 ± 27.75 mg/day). Almost a quarter (24.7%) of the patients had psychiatric comorbidities other than OUD (Table 1). Their baseline plasma levels of cytokines and BDNF, and WMS-R scores are listed in Table 1.

After 12 weeks of MMT, 47 patients had completed the assessment. The endpoint plasma levels of TNF- α , CRP, IL-6, TGF- β 1, and BDNF levels were significantly lower than at baseline (Table 1). The endpoint verbal, visual, and general memory and delayed recall indices were significantly higher than at baseline (Table 1). Pearson correlations showed that CRP levels were significantly negatively associated with all five indices (Table 2). IL-6 levels were significantly negatively correlated with the verbal, general memory, and delayed recall indices (Table 2). TGF- β 1 levels were significantly negatively correlated with the general memory and attention/concentration indices (Table 2).

After covarying for multiple possible confounding factors (age, sex, methadone dose, disease duration, time effects, psychiatric comorbidities, and morphine-positive urine tests), a GEE analysis showed that changes in TNF- α levels were negatively correlated with changes in the visual memory index ($P = 0.01$) (Table 3), and that changes in IL-6 levels were negatively correlated with changes in the verbal memory ($P = 0.009$) and delayed recall indices ($P = 0.03$) (Table 3). After a Bonferroni correction for multiple testing, the negative correlations between TNF- α levels and the visual memory index, and IL-6 levels, and the verbal memory index, were still significant (Table 3).

4. Discussion

This is the first study that shows the relationship between memory test scores and peripheral inflammatory cytokines in OUD patients. We found a significantly negative correlation between TNF- α levels and the visual memory index, and IL-6 levels and the verbal memory index after controlling for multiple factors. We also found that memory performance and inflammatory status in OUD patients might improve during MMT treatment. Although CRP and TGF- β 1 were correlated with memory test scores in the initial analysis, the results were not significant after controlling for multiple confounding factors. These findings indicated that TNF- α - and IL-6-related pathways are involved in memory processes in opioid-addicted patients.

It has been hypothesized that immune processes in the brain, including microglial activation and inflammatory cytokine production, have a complex dual role in learning, memory, and neural plasticity and excitability (Yirmiya and Goshen, 2011). Locally controlled and properly timed activation of immune processes (Schneider et al., 1998; Stellwagen and Malenka, 2006) might increase the neural excitability that underlies neural plasticity and

memory consolidation. However, excessive immune activation and “cytokine storms” can hyperexcite neural circuits and disturb both memory and neural plasticity (Yirmiya and Goshen, 2011). If the immune processes are severely or chronically overactivated, excitotoxicity, apoptosis, and neurodegeneration might occur, which will reduce neural excitability and increase impairments in learning, memory, and neural plasticity (Yirmiya and Goshen, 2011). Our study results partly support this hypothesis and show that greater levels of inflammation might have negative effects on memory test scores.

The detrimental effect of TNF- α on memory was first reported in learning-impaired transgenic mice (Fiore et al., 1996) that overexpressed TNF- α in the brain in a passive-avoidance paradigm. Chronic ventricular administration of TNF- α for a week before water-maze training consistently impaired spatial learning and memory in mice (Bjugstad et al., 1998). After TNF- α had been intrahippocampally administered for 10 days in rats, their hippocampal-dependent working memory was also impaired (Matsumoto et al., 2002). Human studies have also shown that elevated TNF- α levels are associated with reduced verbal memory test scores in patients with psychotic disorders (Hoseth et al., 2016) and with breast cancer (Kesler et al., 2013), and are associated with short- and medium-term cognitive dysfunction after coronary artery surgery (Hudetz et al., 2011). Our study showed that TNF- α levels were negatively correlated with visual memory test scores in OUD patients, which partially supported the notion that the negative influence of TNF- α might be dose-dependent, and was restricted to the performance of tasks that depend on normal hippocampal functioning (Yirmiya and Goshen, 2011).

The role of IL-6 in memory is quite complex and in opposite ways under various conditions. Balschun et al. (Balschun et al., 2004) reported that LTP upregulated IL-6 gene expression in rats, but that treatment with anti-IL-6 antibodies 90 min prolonged the LTP. Thus, they suggested that endogenous IL-6 regulated or fine-tune the consolidation of long-term synaptic plasticity and hippocampal-dependent learning and memory during physiological conditions. However, chronic and pathological IL-6 overproduction might mediate age-related memory impairments (Weaver et al., 2002). Elevated IL-6 levels have also been associated with memory deficiencies in dementia (Engelhart et al., 2004), and in various pathologies accompanied by an increase production of the cytokine, e.g., cardiovascular and liver diseases and major depression (Grassi-Oliveira et al., 2011; Hudetz et al., 2011; Luo et al., 2012; Trapero and Cauli, 2014). After combining the findings of all these studies with our findings, we hypothesize that the negative effects of IL-6 and TNF- α on memory performance are the same in addicted patients as in those with other pathological conditions, and that they illustrate the pivotal role of IL-6 and TNF- α in memory function.

The efficacy of MMT has been widely investigated, and showed that OUD patients undergoing MMT will markedly reduce illicit opioid use, drug-related health consequences, crimes, and transmission of HIV and hepatitis (Marsch, 1998; Shi et al., 2007) and have improvement in social productivities, personal relationships and health status (Chou et al., 2013; Hsiao et al., 2015). Our study results suggested an additional benefit in OUD patients undergoing MMT. They may have improvement in memory function and inflammatory status. Although past studies also showed significant improvements in verbal learning and memory, visuospatial memory, and psychomotor speed in opiate-dependent patients after 2

months of MMT, the study sample size was small (Gruber et al., 2006). Our study enrolled more OUD patients, adjusted more confounding factors and followed longer to reduce the learning effect of WMS-R, which will provide stronger evidence. However, further longer follow up studies and compared with controls may be required to examine how long the improvement will last and control for the learning effects.

Consistent with our finding, studies have shown that a dysregulated immune function in heroin abusers might be normalized by switching to long-term MMT (McLachlan et al., 1993; Sacerdote et al., 2008). Several possible factors have been proposed to explain the changes in the immune function during MMT. First, lifestyle changes like reducing how much heroin is injected, and reducing the risks of infectious diseases, malnutrition, stress, reversed sleep-wake patterns, and sleep deprivation may all attribute to restore immune function (McLachlan et al., 1993). Second, long lasting treatment with methadone normalizes the hypothalamic-pituitary-adrenal (HPA) axis, which is altered by withdrawal-induced stress (Gerra et al., 2007), and restores the disturbed immune function in heroin abusers (Kapadia et al., 2005; Kreek, 2001). Additionally, both preclinical and clinical studies suggest that not all opioid receptor agonists have the same immune-modulating properties (Sacerdote et al., 2008). Short-acting opioid drugs like heroin and morphine can markedly change the immune system (Pacifci et al., 2000), and long-acting opioid drugs like methadone can progressively restore the immune function and cytokine expression (Neri et al., 2002; Sacerdote et al., 2008). It is possible that improved immune responses require the constant activation of μ opioid receptors (MOR) provided by methadone and buprenorphine treatment (Sacerdote et al., 2008).

However, we found that BDNF levels and TGF- β 1 levels decreased during MMT. Some studies (Heberlein et al., 2011; Nabati et al., 2013; Zhang et al., 2014) reported that BDNF and TGF- β 1 levels were higher in OUD patients than in controls, partly because greater BDNF and TGF- β 1 expression occurs as a compensatory response to neuronal insults (Zhang et al., 2014; Zhu et al., 2000). Though previous study did not find the difference in serum BDNF levels among heroin addicts at baseline and one month after heroin cessation (Zhang et al., 2014), we supposed that the compensatory elevation of BDNF and TGF- β 1 may be normalized when reducing heroin injection a longer period of time.

Some researchers may argue that peripheral cytokines levels are irrelevant for the brain. However, inflammatory cytokines pass through the blood-brain barrier (BBB) and influence brain function through three pathways (Miller and Raison, 2016). The first pathway is the humoral pathway, which involves cytokine passage through leaky regions in the BBB, such as the circumventricular organs, and the binding of cytokines to active transport molecules on the BBB (Erickson et al., 2012; Miller and Raison, 2016). The second pathway is the neural pathway, which involves the binding of cytokines to peripheral afferent vagus nerve fibers that in turn stimulate ascending catecholaminergic fibers in the brain and then are translated back into central cytokine signals (Erickson et al., 2012; Miller and Raison, 2016). The last one is the cellular pathway, which involves the trafficking of activated immune cells, typically monocytes, to the brain vasculature and parenchyma (D'Mello et al., 2009). Therefore, it is possible that peripheral inflammatory responses drive inflammation in the brain and influence its cognitive function.

We found no significant associations between CRP, TGF- β 1, BDNF and memory test scores in WMS-R after we had controlled for several possible confounders and corrected for multiple testing. Although the initial correlation analysis showed a significant negative association between memory test scores and CRP, IL-6, and TGF- β 1 levels, the findings for CRP and TGF- β 1 did not remain significant after correction. Because we controlled for several factors, including age, sex, methadone dose, disease duration, time effects, psychiatric comorbidities and urine morphine-positive urine tests, our results differ from those of studies that found significant associations between CRP, TGF- β 1, and BDNF levels and memory function (Bulzacka et al., 2016; Laurin et al., 2009; Li et al., 2013; Zhang et al., 2014). In addition, we analyzed OUD patients undergoing MMT, which were also different from the types of patients in some of those other studies (Bulzacka et al., 2016; Laurin et al., 2009; Zhang et al., 2014). This suggests that these molecules may not have common pathways for impairing memory function in different psychopathologies.

Our study has some limitations. This is the first study that tests the hypothesis that peripheral inflammation markers are associated with memory performance in OUD patients. A larger sample size would provide more convincing results. Although we have tested five potential factors in this study, there are still many other proinflammatory and anti-inflammatory cytokines and neurotrophic factors that might also affect memory function. However, our study results underline the initial and important linkage between memory function and inflammation, which will provide a new perspective in addiction research. Although we tried to control for factors that might affect changes in plasma cytokine and BDNF levels, 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.

Additionally, we did not check urine screen tests for non-morphine opioids or other drug classes, like marijuana, cocaine, etc. It is less common to abuse other non-morphine opioids in Taiwan. The most commonly abused opioid in Taiwan are heroin (14,685 positive specimens in 2017) and codeine (10,699 positive specimens in 2017) (Taiwan Food and Drug Administration [TFDA] annual reports for drug abuse examination and statistics <<https://www.fda.gov.tw/TC/site.aspx?sid=1578>>). Because heroin and codeine will both metabolize into morphine and be excreted in urine, we did urinary morphine tests for each OUD patient. We hypothesized that the results would reveal their opioid abuse levels. In Taiwan, marijuana and cocaine use are not as common as in America or Europe (TFDA); thus, few Taiwanese OUD patients use them concurrently. However, in this study, we did not control for their concurrent methamphetamine and alcohol use, which is more common in Taiwan OUD patients. Our patients were not severely intoxicated when their memory function was assessed: they passed the Information and Orientation Questions in the first subset of the WMS-R, which indicated that their orientation and sensory functions were intact. But subtle effects from using alcohol or methamphetamine on WMS-R performance still cannot be excluded. These factors should be controlled for in future research. Also, all our patients were undergoing MMT, which might affect their memory function. We tried to control for the methadone dose in our analysis to reduce the effect, but to generalize our results to abstinent former opioid abusers who did not undergo MMT might require additional research. Therefore, our findings should be interpreted with caution.

5. Conclusion

We found a significantly negative correlation between memory function and TNF- α and IL-6 levels in OUD patients. Our findings provide preliminary evidence that changes in inflammation levels might be involved in memory function in OUD patients. Given that effective treatments for cognitive deficiency in OUD patients are scarce and that MMT might cause cognitive impairment (Verdejo et al., 2005), further studies are required to test the efficacy of agents that regulate TNF- α and IL-6 expression to improve memory function in OUD patients.

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Figure 1.
Scatter plot of plasma TNF- α levels and visual memory index in patients with OUD undergoing MMT.

Figure 2.
Scatter plot of plasma IL-6 levels and verbal memory index in patients with OUD undergoing MMT.

Table 1

Demographic data, cytokine levels, and mean WMS-R index scores in OUD patients before and after 12 weeks of MMT

Variable	Baseline	After 12 weeks	B	P
Number of cases	89	47		
Age	35.99 ± 7.24	37.19 ± 7.96		
Sex (Male/Female)	74/15	41/6		
Disease duration (years)	6.20 ± 4.87	6.72 ± 5.84		
Methadone dose (mg)	34.61 ± 27.75	42.91 ± 25.60		
Psychiatric comorbidities (+/-)	22/67	11/36		
Morphine-positive urine tests (+/-)	63/25 ^a	22/24 ^b		
Plasma cytokines				
TNF-α	3.81 ± 3.67	3.60 ± 4.29	-0.76	<0.001 *
CRP	3.51 ± 3.18	2.66 ± 2.63	-0.79	<0.001 *
IL-6	2.48 ± 2.10	1.96 ± 1.44	-0.43	<0.001 *
TGF-β1	26.41 ± 14.73	22.80 ± 16.94	-2.75	0.02 *
BDNF	11.09 ± 7.41	9.07 ± 5.83	-1.73	<0.001 *
Wechsler memory scale-revised				
Verbal Memory Index	92.30 ± 12.86	99.55 ± 19.40	6.83	<0.001 *
Visual Memory Index	98.07 ± 13.27	105.40 ± 15.91	7.59	<0.001 *
General Memory Index	93.78 ± 17.49	102.04 ± 18.91	7.96	<0.001 *
Attention/Concentration Index	104.36 ± 13.30	109.02 ± 12.44	1.97	0.06
Delayed Recall Index	96.97 ± 17.18	104.51 ± 19.81	7.24	<0.001 *

WMS-R: Wechsler memory scale-revised; OUD: opioid use disorder; MMT: methadone maintenance therapy; TNF-α: tumor necrosis factor α; CRP: C-reactive protein; IL-6: interleukin-6; TGF-β1: transforming growth factor-β1; BDNF: brain-derived neurotrophic factor.

Covarying for age, sex, methadone dose, disease duration, psychiatric comorbidities, and morphine-positive urine tests in GEE analysis.

^a n = 88;

^b n = 46;

* P < 0.05.

Table 2Pearson correlations (*r*) of plasma cytokine and BDNF levels and WMS-R index scores

Variable	Verbal Memory Index	Visual Memory Index	General Memory Index	Attention/Concentration Index	Delayed Recall Index
TNF- α	-0.04	-0.15	-0.08	0.07	-0.01
CRP	-0.19 *	-0.16 *	-0.22 **	-0.25 **	-0.21 *
IL-6	-0.28 **	-0.10	-0.24 *	0.01	-0.22 *
TGF- β 1	-0.12	-0.10	-0.16 *	-0.05 *	-0.16
BDNF	-0.07	-0.09	-0.10	-0.02	-0.12

WMS-R: Wechsler memory scale-revised; BDNF: brain-derived neurotrophic factor; TNF- α : tumor necrosis factor α ; CRP: C-reactive protein; TGF- β 1: transforming growth factor- β 1; IL-6: interleukin-6.

* $P < 0.05$;

** $P < 0.01$.

Table 3

Correlation of changes in WMS-R scores and in plasma cytokine and BDNF levels before and after 12 weeks of MMT

Indices: Variable	Verbal Memory			Visual Memory			General Memory			Attention/Concentration			Delayed Recall		
	B	Wald χ^2	P	B	Wald χ^2	P	B	Wald χ^2	P	B	Wald χ^2	P	B	Wald χ^2	P
TNF- α	0.02	0.01	0.92	-0.52	6.69	0.01**	-0.25	0.83	0.36	-0.14	0.46	0.50	-0.06	0.07	0.79
CRP	-0.40	0.72	0.40	-0.47	0.99	0.32	-0.78	2.21	0.14	-0.66	3.62	0.06	-0.73	1.54	0.22
IL-6	-1.92	6.79	0.009**	0.50	0.65	0.42	-1.19	2.16	0.14	1.02	3.21	0.07	-1.83	4.60	0.03*
TGF- β 1	-0.05	0.45	0.50	0.02	0.04	0.84	-0.06	0.41	0.52	-0.02	0.07	0.79	-0.02	0.03	0.87
BDNF	0.13	0.35	0.56	0.18	0.57	0.45	0.18	0.69	0.41	0.02	0.02	0.90	0.21	1.09	0.30

WMS-R: Wechsler memory scale-revised; BDNF: brain-derived neurotrophic factor; MMT: methadone maintenance treatment; TNF- α : tumor necrosis factor α ; CRP: C-reactive protein; TGF- β 1: transforming growth factor- β 1; IL-6: interleukin-6.

Covarying for age, sex, methadone dose, disease duration, time effects, psychiatric comorbidities, and morphine-positive urine tests.

* $P < 0.05$;

** $P < 0.01$.