Original article

IL-10 is associated with increased mu-opioid receptor levels in major depressive disorder

Suhaer Zeki Al-Fadhel[a], Hussein Kadhem Al-Hakeim[b], Arafat Hussein Al-Dujaili[c], Michael Maes[d,e,f,]*

[a] Department of Clinical Laboratory Sciences, Kufa University, Najaf, Iraq
[b] Department of Chemistry, College of Science, University of Kufa, Iraq
[c] Faculty of Medicine, Kufa University, Iraq
[d] Department of Psychiatry, Medical University of Plovdiv, Plovdiv, Bulgaria
[e] Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Thailand
[f] Impact Strategic Research Center, School of Medicine, Barwon Health, Geelong, Australia

A B S T R A C T

Objective: Activation of the immune-inflammatory response system (IRS) and the compensatory immune-regulatory system (CIRS) and aberrations in endogenous opioids play a role in the pathophysiology of major depressive disorder (MDD). There are no studies which examined the associations between both systems in MDD. The aim of the present study was to examine the relation between β-Endorphin (β-EP), Endomorphin-2, and their mu-opioid receptor (MOR) as well as interleukin (IL)-6 and IL-10, an anti-inflammatory cytokine, in MDD patients.
Method: The study included 60 depressed drug-free male patients and 30 matched controls. Serum β-EP, Endomorphin-2, MOR, IL-6 and IL-10 levels were measured using ELISA techniques.
Results: The results revealed a significant increase in serum β-EP, MOR, IL-6 and IL-10 in MDD patients versus healthy controls. MOR levels were strongly associated with IL-10 levels. There were no significant correlations between endogenous opioids and IL-6 and IL-10.
Conclusion: The results show that MOR levels may function as a possible component of the CIRS whilst there is no evidence that β-EP and EM-2 may modify the IRS. The significant correlation between MOR levels and IL-10 may be explained through central activation of the HPA-axis and increased B-cell numbers expressing MOR as a response to cytokine over-secretion in MDD.

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1. Introduction

Major depressive disorder (MDD) is a psychiatric disorder which is a state of extreme sadness characterized by a combination of symptoms including biochemical, environmental, genetic, and psychological factors that interfere with a person's ability to work, sleep, study, eat, and enjoy once-pleasurable activities [1]. There is now evidence that MDD is characterized by an activation of the immune-inflammatory response system (IRS), as indicated by increased levels of pro-inflammatory cytokines including interleukin (IL)-6 [2–5]. Moreover, recently it was shown that this primary IRS also induces the compensatory immune-regulatory system (CIRS), which attenuates an overzealous inflammatory response, as indicated by increased levels of IL-10, a negative immune-regulatory cytokine [6]. Altered functioning of the IRS and CIRS is implicated in multiple medical morbidities of MDD including neuroinflammatory and autoimmune disorders [7–9].

Many patients with MDD report somatic pain symptoms, which are considered to be a multidimensional experience that entails sensory as well as emotional, cognitive and behavioral aspects [10–12]. The most important biological pathways related to pain include pain matrix alterations, pro-inflammatory cytokines, including IL-6, and opioid levels [12,13]. Some studies showed that MDD is not only associated with IRS/CIRS activation [14], but also with changes in endogenous opioids [15] and opioid receptors [16,17]. Endogenous opioid peptides including β-endorphin (β-EP) and endomorphin-2 (EM-2), are small molecules that are naturally produced in the central nervous system (CNS) and in various glands throughout the body, such as the pituitary and adrenal glands. These peptides produce the same effects as the well-known

* Corresponding author at: Department of Psychiatry, Faculty of Medicine, Chulalongkorn University, Bangkok, Thailand.
E-mail addresses: suhaeralfadel@yahoo.com (S.Z. Al-Fadhel), headm2010@yahoo.com (H.K. Al-Hakeim), arafat.aldujaili@uokufa.edu.iq (A.H. Al-Dujaili), dr.michaelmaes@hotmail.com (M. Maes).

alkaloid opiates, which include morphine and heroine. β-EP and EM-2 peptides function both as hormones and as neuromodulators [18]. There is strong evidence that opioid release is part of the organisms' defense mechanism against the harmful effects of stress [19], including increased HPA-axis activity, which is frequently observed in MDD [20]. Moreover, opioid receptors are involved in various physiological and pathophysiological activities, including pain modulation, emotional responses, immune functions, feeding, cardiovascular and respiratory control as well as neurodegenerative processes [21]. Many of these functions are disturbed in MDD and therefore the study of opioid receptor levels in this disorder is important in addition to their ligands β-EP and EM-2. Endogenous opioids, including β-EP and EM-2, as well as their μ-opioid receptor (MOR) are found throughout the central and peripheral nervous systems and in other tissues as well. Moreover, immunocytes express opioid receptors, including MOR, which additionally may modulate immune functions [22,23]. A molecular basis of bidirectional interactions between the opioid system and the immune system had been elucidated previously [24]. Furthermore, in animal models, pro-inflammatory cytokines affect opiate-dependent pathways by up-regulating the expression of MOR [25]. Exogenous opioids show immunosuppressive effects [26], while cytokines may elicit the release of endogenous opioids [27]. Thus, there are multiple reciprocal relationships between endogenous opioids and the immune system.

The present study aims to delineate the relation between IL-6, a pro-inflammatory cytokine, and IL-10, a CIRS cytokine, and two endogenous opioids (β-EP and EM-2) and MOR levels in MDD patients. The specific hypotheses are that MDD is accompanied by increased levels of IL-6, IL-10, β-EP and EM-2 and increased MOR levels.

2. Method

2.1. Participants

This case control study involved 60 depressed drug free male patients aged 14–70 year and 30 age matched healthy males as a control group. The samples were collected at "The Psychiatry Unit", Al-Hakeem General Hospital and a private psychiatric clinic run by an assistant professor in psychiatry, Najaf Governorate-Iraq during the period January to July 2017. C-reactive protein (CRP) was evaluated in all samples and we excluded subjects with CRP values >6 mg/L to eliminate any effects of overt inflammation on the results [3]. Patients were diagnosed by psychiatrists according to DSM-IV criteria (5th revision of the Diagnostic and Statistical Manual of Mental Disorders). Severity of clinical symptoms was assessed using the 24-item Hamilton Depression Rating Scale (HDRS) one or two days before blood was drawn. Only MDD patients with a total HDRS score >21 were included. Informed consent was obtained from all participants after approval from the ethics committee (IRB) of the College of Science, University of Kufa, Iraq.

Patients were evaluated by full medical history. We excluded subjects with systemic disease that may affect immune parameters, including diabetes, liver disease, and renal disease. We also excluded MDD patients who were medicated, and subjects with other-axis I diagnosis including substance abuse. Individuals with highly increased hsCRP values (CRP >6 mg/L) were also excluded to participate.

2.2. Measurements

Five milliliters of venous blood samples were drawn, utilizing disposable needle and plastic syringes, from patients and controls. The samples were transferred into a clean plain tube. Haemolyzed samples were discarded. The blood was left at room temperature for 15 min for clotting, centrifuged 3000 rpm for 10 min, and then serum was separated and transported into two Eppindroff tubes to be stored at −80 °C until analyzed. Serum CRP was measures using a kit supplied by Spinreact®, Spain. The test is based on the principle of the latex agglutination. Commercial ELISA sandwich kits were used to measure serum EM2 and MOR (MyBioSource®, Inc. CA, USA) and β-EP, IL-6, and IL-10 (CUSABIO® Co., China). The procedures were followed exactly without modifications according to manufacturer's instructions.

2.3. Statistical analysis

Normality of distribution of the variables was examined using the Kolmogorov-Smirnov test. The results were expressed as (mean ± standard deviation). Pooled *t*-test were used for the comparison between patients and controls. Pearson's correlation coefficients (r) were calculated to estimate the correlation between parameters. The differences between groups are considered to be statistically different when $p < 0.05$ (two tailed). The p-values were adjusted using the Benjamini–Hochberg method for multiple-hypothesis testing and false discovery rate (FDR). Statistical differences in categorical variables were evaluated by using χ2-test. All statistical analysis were performed using SPSS Statistics Version 25 (2017) by IBM-USA. Figures were made using the Excel program of Microsoft Office 2013.

3. Results

3.1. Demographic and clinical characteristics

The demographic and clinical characteristics of the patients with MDD and control groups are presented as observational data in Table 1. Patients with MDD and control groups showed no significant differences in age.

These data showed that most MDD subjects were married. About less than half of the patients were smokers. Most of them are urban and educated patients. All patients were newly diagnosed cases (drug free) in order to eliminate the effect of drugs on the measured parameter levels. The results in Table 1 showed a significantly lower BMI (p < 0.05) in MDD patients as compared with controls.

3.2. Comparison between patients with MDD and control groups

Serum β-EP, MOR, IL-6 and IL-10 concentrations in the MDD were significantly higher than in the control group (p < 0.05, Table 2), while EM-2 was not significantly different between both groups.

3.3. Comparison between smoking and nonsmoking MDD patients

Table 3 presents the comparison between smokers and nonsmokers MDD groups in addition to control group. There

Table 1
Demographic and clinical characteristics of patients and controls.

Parameter	Patients	Control	p-value
Gender: male(female)	60(0)	30(0)	
Age, year	32.48 ± 11.70	28.17 ± 12.42	NS
BMI, kg/m^2	24.69 ± 4.3	26.17 ± 5.2	NS
Demographic area: Urban (Rural)	45(15)	25(5)	NS
Education: Learned (Illiterate)	49(11)	28(2)	NS
Employment: Employed (Not Employed)	33(27)	26(4)	0.003
Marital status: Married (Single)	38 (22)	14(16)	NS
Smoking (Not smoking)	25(35)	1(29)	<0.001
Treatment: Treated (not-Treated)	0(60)	0(30)	

Table 2
Comparison between MDD patients and control group.

Parameter	Patients	Control	p-value
β-EP pg/ml	34.15 ± 3.65	24.83 ± 5.37	<0.001
MOR pg/ml	4.06 ± 1.45	3.02 ± 0.64	0.004
EM-2 pg/ml	3.24 ± 0.34	3.17 ± 0.41	0.417
IL-6 pg/ml	16.39 ± 3.04	11.44 ± 2.21	0.046
IL-10 pg/ml	9.52 ± 2.24	6.03 ± 1.40	<0.001

Table 3
Comparison between groups (control, nonsmoker MDD, and smoker MDD) in cytokine levels after adjusting for Age and BMI.

Parameter	Control	Smokers MDD	Nonsmokers MDD	Significance
IL-6 pg/ml	11.44 ± 2.21	12.95 ± 3.20	11.88 ± 2.86	NS
IL-10 pg/ml	6.03 ± 1.40	8.89 ± 2.15	8.90 ± 2.00	A,B
β-EP pg/ml	24.83 ± 5.37	34.90 ± 3.97	33.02 ± 2.75	A,B,C
MOR pg/ml	3.02 ± 0.64	4.44 ± 1.49	4.03 ± 1.27	A,B
EM-2 pg/ml	3.17 ± 0.41	3.28 ± 0.40	3.19 ± 0.21	NS

A: Significant difference (p<0.05) between Control and nonsmokers MDD groups.
B: Significant difference (p < 0.05) between Control and smokers MDD groups.
C: Significant difference (p < 0.05) between Smokers and nonsmokers MDD groups.

were no significant differences between smoking and nonsmoking MDD patients in IL-6 and EM-2. β-EP was significantly higher in both smoking and nonsmoking MDD patients than in controls (p < 0.05). IL-10 and MOR were significantly higher in both MDD subgroups than in controls, whilst there were no significant differences between both MDD subgroups (p < 0.05).

3.4. Correlation among parameters in MDD group

Correlation analyses among the parameters showed a significant correlation between IL-10 and MOR in the MDD group as seen in Fig. 1. This association remained significant after p-correction for FDR (p = 0.012). There were no significant correlations between IL-6 and any of the opioid system measurements and between IL-10 and either β-EP and EM-2.

4. Discussion

The first major finding of this study is that MDD is characterized by significant increases in serum β-EP and MOR levels. This increase may indicate stimulation of the synthesis of opioids and its receptor as a result of immune-inflammatory activation in MDD. The involvement of the opioid system in depression has been previously studied by several groups [17,28–30]. The results of β-EP assays in different papers is controversial. Hegadoren et al. [31]
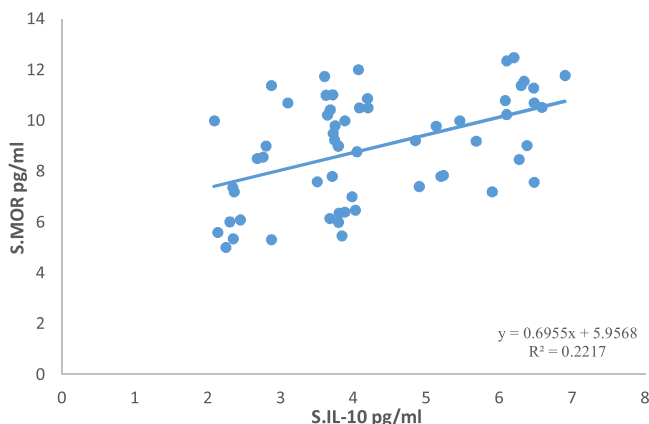

Fig. 1. Correlation between IL-10 and MOR in MDD group.

summarized findings on serum baseline levels of nineteen published studies. Of these studies, seven reported increases in β-EP levels in MDD compared to controls; five reported decreased β-EP levels; and seven reported no changes. Nevertheless, measurements of post-dexamethasone β-EP levels in MDD indicate that there are aberrations in the EP system in MDD. Thus, post-dexamethasone β-EP levels are significantly higher in MDD with melancholic and psychotic features as compared with controls, although there were no differences in baseline β-EP levels [28]. The variability in the β-EP results across studies may be explained by differences in subgrouping, comorbidity issues, sex difference, drug state of the patients, sample size, etc. (31). All in all, our results that β-EP levels are in increased in MDD are in agreement with a number of other studies revealing that plasma β-EP levels and MOR levels are elevated in MDD [32,33].

There is evidence that endogenous opioids may modulate human mood and that the endogenous μ-opioid tone is dysregulated in depression [34]. Accumulating evidence from animal research shows that MOR exerts a significant control over mood-related processes [35]. β-EP binds to opioid receptors thereby activating the endogenous analgesia system, which is located in the CNS. Moreover, this activation inhibits pain conduction and agitation of nociceptors to exert an analgesic effect [36]. These phenomena may be a reaction of the body against depression symptoms to overcome the psychological and somatic pain caused by MDD [37].

In the present study we found no significant differences in EM-2 levels between MDD patients and controls. To the best of our best knowledge, serum EM-2 levels in MDD were not previously studied. The possible role of endogenous opioid peptides in depression is supported by neurochemical and neurobehavioral findings [38]. It has been demonstrated that EMs and MORs are present in brain regions containing monoamine neurotransmitters (serotonin, dopamine, and noradrenaline), which play a role in the physiopathology of depressive disorders.

In fact, EMs have been shown to modulate dopaminergic [39], noradrenergic [40] and serotoninergic [41] neurotransmission. In animal studies, EMs and MOR may be involved in the pathophysiopathology of depressive disorders, whilst the endomorphinergic system could serve as a novel target for the development of antidepressant drugs [42]. The analgesia produced by EMs is short and its effect may not be significant because EMs are easily degraded by various proteases (poor metabolic stability) [43]. This effect may also limit possible increases in EMs in MDD patients. However, in a comprehensive survey [44], no consistent changes in endogenous opioids levels were observed in MDD patients.

Our results that IL-6 is increased in MDD is in agreement with several studies, reviews and meta-analyses [2,3,45–47]. The IL-6 results indicate that MDD is accompanied by increased proinflammatory IL-6 trans-signaling [45]. Moreover, our results that IL-10 is increased in depression is in line with other studies and meta-analyses [3,47,48]. These results further support the view that MDD is accompanied by a simultaneous activation of the IRS (increased IL-6 signaling) and CIRS (increased IL-10 production) amongst other immune pathways [6]. In fact, increased IL-6 signaling is a key characteristic of the activated IRS in MDD, while IL-10 is one of the hallmarks of the CIRS [6]. Other findings [49] suggest that IL-10 may have a regulatory effect on the HPA axis. Furthermore, it is required for regulating immune functions by promoting the widespread suppression of immune responses through its pleiotropic effects [6]. Stress can induce nerve cells in the hypothalamus to produce and release CRH, which stimulates the release of β-EP. CRH is transported to the anterior pituitary gland where it stimulates production of a proopiomelanocortin (POMC), which is the precursor for a number of stress-related hormones, including adrenocorticotropic hormone (ACTH) and β-

EP. ACTH stimulates cells of the adrenal glands to produce and release the stress hormone cortisol [50]. Cortisol has many effects when it binds to glucocorticoid receptors, including effects on cardiovascular function, immunologic status, arousal, and learning and memory. All of these systems are affected when the HPA-axis is activated in response to stress [51]. Thus, the anti-inflammatory effects of cortisol are brought about by reducing proinflammatory cytokines, histamine secretion, stabilizing the membranes of cell components and lysosomes [52], and stimulating immune complements [53].

The finding of the present study show that the differences in IL-10, MOR and β-EP between MDD patients and controls were not affected by smoking. Table 3 shows a significant difference in serum β-EP between smoking and nonsmoking MDD patients. In one study, β-EP level was found to be higher in light smokers (less than fifteen cigarettes daily) than in controls [54]. Increased nicotine induces transient increases in circulating β-EP [55], suggesting that there may be individual differences in the response of β-EP to nicotine, its derivatives, or other components of cigarette tobacco [54]. There are few studies reporting on the impact of smoking on inflammatory markers in MDD. Nunes et al. [56] showed that smoking and MDD act synergistically to increase inflammatory markers. Another paper found an association between IL-6 and smoking status where male current smokers had significantly higher levels of serum IL-6 compared to male former smokers [57]. Our negative MOR results with regard to smoking status are in accordance with Kuwabara et al. [58] who found no significant difference in MOR availability between controls and smokers.

The second major finding of this study is that IL-10 levels are strongly correlated with MOR levels (Fig. 1), while there was no significant correlation between endogenous opioids and IL-6 and IL-10. To the best of our knowledge, this is a first study that MOR levels are associated with IL-10, a key component of the CIRS. Cytokines regulate growth and proliferation of glial cells, modulate the activity of endogenous opioid peptides, release endogenous opioids, activate the HPA-axis [59] via increased production of corticotrophin releasing hormone (CRH) and elicit changes in neurotransmitter activity [27]. Endogenous opioids, in turn, exert a negative tonic inhibition on CRH secretion from the hypothalamus thereby inhibiting cortisol secretion [60]. This process may result in increased density of opioid receptors in peripheral nerve terminals, contributing to antinociceptive effects of opiates [59]. Furthermore, opioid administration may suppress the immune system thereby increasing vulnerability to infections, although endogenous opioids may not have such effects [61]. Recent studies show that the role of opioid receptors in immune function is very complex and entails various different mechanisms. Experimental and clinical studies have clearly shown that activation of peripheral opioid receptors with exogenous opioid agonists and endogenous opioid peptides are able to produce significant analgesic and anti-inflammatory effects, without central opioid mediated side effects (e.g., respiratory depression, sedation, tolerance, dependence [62]. β-EP, an anti-nociceptive neuropeptide [63], may be released in the brain in response to nociceptive stimuli indicating a possible mechanism for the organisms to cope with pain as observed in MDD patients [64].

Different opioids may have immunosuppressive effects and/or immunostimulatory effects [22]. Finley et al. [65] suggested a broad role of opioids in the modulation of the immune system function and concluded that the kappa opioid receptor induces an antiinflammatory response through down-regulation of cytokines, chemokines and chemokine receptor expression, while activation of MOR may favor a more proinflammatory response. Al-Hashimi et al. [66] concluded that opioids produce immune modulation in both humans and experimental animals. Furthermore, they found

that MOR receptors are probably the main target for classical opioid immune modulation [66]. Activated dendritic cells can be regulated by endogenous opioid through MOR expression. Moreover, MOR activation by EP activates IL-10 productions and suppresses IL-12 and IL-23 secretion [67], suggesting that MOR plays a role in the CIRS and attenuates some IRS components. In animal models, exogenous opioids (heroin and morphine) have a strong stimulatory effect on the release of anti-inflammatory cytokines including IL-10 [68]. Another possible pathway explaining the association between IL-10 and MOR revolves around B cell functions. Thus, B-lymphocytes express μ, δ, and κ opioid receptors [69], while μ, but not δ or κ, opioid receptors may regulate immune functions [23]. During the later phase of the adaptive immune response, increased production of T cell cytokines helps to activate B cells [70]. In MDD, Maes et al. [71] noticed a significant increase in B-cells number and percentage as compared with controls. Therefore, the correlation between the MOR and IL-10 could in part be explained by the increased number of B-cells that express MOR and are activated during an adaptive immune response.

5. Limitations of the study

One limitation of this study is that we included only males and excluded females in order to eliminate any effects of changes in female hormones, an irregular menstrual cycle or menopausal status on the results.

6. Conclusions

Serum β-EP, MOR, IL-6 and IL-10 concentrations are significantly higher in MDD than in controls, while EM-2 did not show a significant difference. Endogenous opioids (β-EP and EM-2) play a crucial role in relaxation and regulation of mood of depressed patients through the reduction of stress while attenuating immune responsivity. We found a significant correlation between IL-10 and MOR indicating that there is a possible link between immune system and endogenous opioid receptors in MDD that needs more mechanistic research for absolute proof.

Funding/support

Conflict of interest disclosures

None reported.

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