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Macrophage Migration Inhibitory Factor and microRNA-451a in Response to Mindfulness-based Therapy or Treatment as Usual in Patients with Depression, Anxiety, or Stress and Adjustment Disorders

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Abstract

Background: Macrophage migration inhibitory factor is a proinflammatory cytokine that has been associated with various psychiatric disorders. MicroRNA-451a can directly target macrophage migration inhibitory factor and downregulate its expression in cells. However, the role of macrophage migration inhibitory factor and microRNA-451a in psychiatric patients treated with psychotherapeutic interventions is unknown. In this study, our aim was to investigate levels of macrophage migration inhibitory factor and its regulating microRNA-451a in patients with depression, anxiety, or stress and adjustment disorders who underwent mindfulness-based therapy or treatment as usual.

Methods: A total of 168 patients with psychiatric disorders were included from a randomized controlled trial that compared mindfulness-based therapy with treatment as usual. Plasma levels of macrophage migration inhibitory factor and microRNA-451a were measured at baseline and after the 8-week follow-up using Luminex assay and qPCR.

Results: Macrophage migration inhibitory factor levels decreased significantly in patients posttreatment, whereas microRNA-451a levels showed a nonsignificant change. Macrophage migration inhibitory factor levels were inversely associated with microRNA-451a expression levels at baseline ($\beta = -0.04$, $P = .008$). The change in macrophage migration inhibitory factor levels (follow-up levels minus baseline levels) was associated with the change in microRNA-451a (follow-up levels minus baseline levels) ($\beta = -0.06$, $P < .0001$). The change in either macrophage migration inhibitory factor or microRNA-451a was not associated with improvement in psychiatric symptoms.

Conclusion: We demonstrate that the levels of macrophage migration inhibitory factor decreased after psychotherapeutic interventions in patients with psychiatric disorders. However, this reduction was not associated with an improvement in psychiatric symptoms in response to the treatment. We also found an association between macrophage migration inhibitory factor and its regulating microRNA. However, this association needs to be further examined in future studies.

Keywords: macrophage migration inhibitory factor; MiR-451a; depression; anxiety; stress and adjustment disorders

Significance Statement

Psychotherapeutic interventions such as mindfulness-based therapy or TAU may influence inflammatory responses that contribute to psychological health. In the present study, we investigated the role of MIF and its regulating miR-451a and found that MIF was significantly decreased after psychotherapeutic intervention. Another important finding is that MIF was inversely related to plasma miR-451a levels in patients with depression, anxiety, or stress and adjustment disorders. For the first time, it is shown that MIF and miR-451a may have a role in patients with psychiatric disorders.

Introduction

Macrophage migration inhibitory factor (MIF) is one of the first cytokine-like proteins that was discovered more than 50 years ago (B. R. Bloom and Bennett, 1966; David, 1966). MIF was named for its ability to recruit macrophages to sites of inflammation and prevent their random migration. It is synthesized by T- and B-lymphocytes, monocytes, macrophages, dendritic cells, neutrophils, eosinophils, mast cells, and basophils. MIF is widely distributed in tissues. High levels of MIF expression are noted in the endocrine system, especially in the organs that are involved in stress responses, for example, hypothalamus, pituitary, and adrenal glands (Waeber et al., 1997; Calandra and Roger, 2003; Fingerle-Rowson et al., 2003). MIF has pleiotropic effects on inflammation, chemotaxis, cell survival, and proliferation that act as a regulator of innate immune and inflammatory responses (Mitchell et al., 1999; Calandra and Roger, 2003; Bernhagen et al., 2007; Savaskan et al., 2012; J. Bloom and Al-Abed, 2014). Inducers for MIF releasing include microbial products such as lipopolysaccharides and proinflammatory cytokines. Once released, MIF acts in an autocrine or paracrine fashion to induce production of proinflammatory cytokines (Calandra et al., 1994; Calandra, 2003; Calandra and Roger, 2003; J. Bloom and Al-Abed, 2014). MIF also interferes with the antiinflammatory activity of glucocorticoids at a transcriptional and posttranscriptional level (Fingerle-Rowson et al., 2003). Circulating MIF is increased during episodes of inflammation, infection, and stress (Bernhagen et al., 1993; Beishuizen et al., 2001; Calandra and Roger, 2003).

MicroRNAs (miRNAs) are a class of small (21-23-nucleotide), noncoding, single-stranded RNAs that inhibit gene expression by promoting messenger-RNA (mRNA) degradation or inhibiting translation (Ambros, 2004). They influence a variety of physiological cell processes during development and tissue homeostasis by regulating the expression of around 90% of all human genes (Miranda et al., 2006). Numerous miRNAs have been recently detected in several body fluids, including serum, plasma, and cerebrospinal fluid (Weber et al., 2010). Blood cells extensively contact plasma; thus they can be the major contributors to extracellular miRNA content in plasma (Pritchard et al., 2012; Turchinovich et al., 2012; Makarova et al., 2016), which can be derived under different conditions (Turchinovich and Burwinkel, 2012). The biological significance of secreted miRNAs remains to be determined (Turchinovich et al., 2012). One explanation is that cells secrete miRNAs after reducing the target mRNA expression, while the unused miRNA is redundant (Squadrito et al., 2014). On the other hand, the presence of extracellular miRNAs involves cell-to-cell communication via selective miRNA export system (Villarroya-Beltri et al., 2013; Turchinovich et al., 2016; Thomou et al., 2017). Several miRNAs targeting MIF were identified in silico using bioinformatic programs such as TargetScan and miRSystem (version 20120229; and miRDB, version 4.0) (Lu et al., 2012). MIF was identified as a target of miR-451a and this was confirmed by multiple studies. For example, miR-451a can directly target MIF and downregulate its expression and can lead to decreased cell proliferation, colony formation, cell migration and invasion in vitro, suppress xenograft tumor growth in vivo, and modulate epithelial cell survival (Bandres et al., 2009; N. Liu et al., 2013; Graham et al., 2015; Tang et al., 2015; G. Liu et al., 2016). Furthermore, miR-451 is widely expressed in different cells, such as red blood cells, white blood cells, platelet samples, and serum/plasma (K. Wang et al., 2012). However, the relation between circulating miR-451a and MIF is largely unknown.

Dysregulation of the immune system and inflammatory response has been linked to the pathophysiology of certain psychiatric disorders, such as depression (Irwin and Miller, 2007). For example, the role of MIF in the pathobiology of depression has been investigated in different ways (J. Bloom and Al-Abed, 2014). MIF is expressed in the brain especially in the areas concerning behavioral symptoms of depression and anxiety (Conboy et al., 2011). Behaviorally, genetic deletion of MIF has resulted in increased anxiety- and depression-like behaviors, and the role of MIF in mediating the antidepressant action of exercise has been found (Conboy et al., 2011; Moon et al., 2012). In blood, the association between MIF and mood disorders may be the opposite. Previous studies have shown that blood MIF levels are increased in subjects with mild to moderate depression, major depression, and other mood disorders (Baugh and Donnelly, 2003; Hawkley et al., 2007; Musil et al., 2011; J. Bloom and Al-Abed, 2014). Treatment with antidepressants can reduce the blood levels of MIF (Cattaneo et al., 2013). MIF does not, however, cross the blood brain barrier (Bacher et al., 2002), so the blood level of MIF may be a consequence of different albeit related processes of depression (J. Bloom and Al-Abed, 2014).

Recently, the role of miRNAs in the development of depression and in antidepressant treatment has gained significant attention. Studies conducted on postmortem brains from subjects who suffered from depression and subsequently committed suicide showed alterations in 29 miRNAs compared with nonpsychiatric control subjects (Smalheiser et al., 2012). Several studies have reported on the role of miR-124-3p in depression. MiR-124-3p may be used as a biomarker for diagnosis and antidepressant treatment response, because miR-124-3p is a target of antidepressants and shows similar changes in the blood and brain of patients suffering with depression (Dwivedi, 2017; Roy et al., 2017). MiR-135a has also been found to be lower in the blood of patients with depression and was increased after treatment with antidepressants (Issler et al., 2014). To our knowledge, the role of miR-451a in psychiatric disorders has not been fully investigated (Camkurt et al., 2015; Wan et al., 2015). One research group found that treatment with antidepressant could reverse the stress-induced change of miR-451 expression in rat hippocampus (O'Connor et al., 2013). However, it is not known whether psychotherapeutic interventions can affect plasma levels of MIF or miR-451a in patients with psychiatric disorders. We therefore aimed to examine whether psychotherapeutic interventions (mindfulness therapy or treatment as usual [TAU], mainly cognitive-behavioral therapy [CBT]) are associated with

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MIF or miR-451a levels in patients with psychiatric disorders (depression, anxiety, and stress and adjustment disorders) who have been treated in primary health care. Mindfulness-based therapies and CBT are effective ways of treating depressive disorders and are associated with reductions in proinflammatory processes according to previous research (Steptoe et al., 2007; Irwin et al., 2015; Sundquist et al., 2015; Black and Slavich, 2016; Walsh et al., 2016; Memon et al., 2017).

Our study population was based on a previously published randomized controlled trial (RCT) conducted by our research group. The study population included patients with depression, anxiety, or stress and adjustment disorders from 16 primary health care centers in Sweden who were treated with mindfulness-based therapy or TAU for 8 weeks (Sundquist et al., 2015). In the present analysis, we analyzed MIF and miR-451a collected in the RCT at baseline and after the 8-week follow-up. Our overall aim was to investigate levels of MIF and its regulating miR-451a in patients with depression, anxiety, or stress and adjustment disorders who underwent mindfulness-based therapy or TAU. We first investigated the potential changes in plasma levels of MIF and miR-451a after 8 weeks of mindfulness-based therapies or TAU in the patients with depression, anxiety, or stress and adjustment disorders. Later we explored the potential association between plasma levels of MIF and miR-451a at baseline and between changes in levels of MIF and miRNA-451a posttreatment. Finally, we investigated whether the possible changes in MIF and miR-451a were associated with the improvement in psychiatric symptoms (assessed using MADRS-S) posttreatment.

Methods

Study Subjects and Sample Collection

The study population included 168 patients (age 21–65 years) with depression, anxiety, or stress and adjustment disorders. All the patients were recruited from the 16 primary health care centers that had participated in a randomized controlled trial (RCT) of mindfulness therapy compared with TAU, mainly CBT. A detailed description of the study design is provided in a previous article (Sundquist et al., 2015). Patients were recruited between January 4, 2012 and March 22, 2012 at the 16 primary health care centers in the county of Scania (Skåne in Swedish) in southern Sweden.

Patients who fulfilled the inclusion criteria were included in the study. All clinical diagnoses were made by doctors at the 16 primary health care centers, including one or more of the following ICD-10 psychiatric diagnoses: F32.0, mild depressive episode; F32.1, moderate depressive episode; F32.9, depressive episode, unspecified; F33.0, recurrent depressive disorder, current episode mild; F33.1, recurrent depressive disorder, current episode moderate; F41.0, panic disorder; F41.1, generalized anxiety disorder; F41.2, mixed anxiety and depressive disorder; F41.3, other mixed anxiety disorders; F41.8, other specified anxiety disorders; F41.9, anxiety disorder, unspecified; F43.2, adjustment disorders; F43.8, other reactions to severe stress; and F43.9, reaction to severe stress, unspecified; age 20 to 64 years; ability to speak and read Swedish; and a score of ≥10 on the Patient Health Questionnaire (PHQ)-9 or ≥7 on the Hospital Anxiety and Depression Scale (HADS) or a total score on the Montgomery-Åsberg Depression Rating Scale (MADRS-S) between 13 and 34 (mild to moderate depression). The exclusion criteria were as follows: severe personality disorder, risk of suicide, pregnancy, thyroid disease, current psychotherapy of any kind, and participation in any other psychiatric intervention study. Each

patient filled in 3 self-rated questionnaires (above-mentioned PHQ-9, HADS-A/HADS-D, and MADRS-S) at baseline and after 8 weeks of follow-up. The patients received antidepressants and tranquilizers (pharmacotherapy) if deemed necessary. Blood samples were collected at the same time as the assessment of self-rated symptoms before and after treatment. Patients with missing clinical information or poor-quality plasma samples, for example, hemolysis samples, were excluded from our study.

Plasma Collection

Whole blood (6 mL) was collected from each participant in EDTA tubes. Blood samples were centrifuged at 2000 g for 10 minutes at 4°C, and the plasma was then aliquoted and stored at -80°C before further processing. Blood samples were processed and the plasma was frozen within 8 hours of collection (Friebe and Volk, 2008).

Detection of MIF and miRNA in Plasma Samples

Plasma MIF levels were determined using the bead-based multiplex assay for the Luminex platform (R&D Systems Inc) based on the manufacturer's instructions. To summarize, 80 μL of plasma was diluted 1:2 in the dilution buffer and then incubated with antibody-coated magnetic bead. Protein levels were measured using the Bio-Plex suspension array system and data were analyzed with Bio-Plex Manager software (Version 4). Absolute concentrations were calculated from a standard curve generated from 8 serially diluted standards provided in the kit. The intra- and inter assay CV were 3.8% and 4.4%, respectively. Duplicate samples were assayed and all results were reported as means.

Plasma miRNA measurement was performed as previously described (X. Wang et al., 2014, 2015). In brief, 50 μL of total RNA was isolated from 200 μL of plasma using the miRNeasy Mini Kit (Qiagen GmbH) according to the manufacturer's protocol, with minor modifications. miRNAs were reverse transcribed using a Universal cDNA Synthesis kit (Exiqon). Quantitative real-time PCR (qPCR) was carried out in 384-well plates using the CFX38 real-time PCR detection system (Bio-Rad Laboratories). At present, there are different methods to detect hemolysis, including low levels, in plasma /serum (Blondal et al., 2013). (Kirschner et al., 2011; Shah et al., 2016). Blondal et al. suggested that the ratio (miR-451a/miR-23a-3p) of red blood cell-enriched miR-451a to miR-23a-3p, the latter not affected by hemolysis, could be used as an indicator of hemolysis. The ratio of miR-451a to miR-23a-3p was found to be the most sensitive method that could detect as little as 0.001% hemolysis in serum. Briefly, miR-451a/miR-23a-3p ratios of <5, 5–7, and >7 indicate low, mild, or high risk of hemolysis. Both miR-451a and miR-23a-3p were measured in all the plasma samples. Samples with a ratio of ≥7 were excluded in the analysis. Our measurements showed that the Ct value for miR-451a was 23.1 ± 0.8 (mean $\pm$ SD) and the Ct value for miR-23a–3p was 26.3 ± 1.4 in all the patient samples. This indicates that miRNA-451a levels displayed little variation between individuals. There were 6% of samples with a ratio of 5 to 7. For these 6% of samples, the Ct value for miR-451a was 22.7 ± 0.7 (mean $\pm$ SD) and the Ct value for miR-23a–3p was 28.2 ± 0.9. There were no significant differences in miRNA-451a levels between these 6% of samples and the whole study population. Therefore, we included these 6% in our analysis. At present, no generally accepted standards for normalization of miRNA PCR data have been established. Different methods have been suggested. Some studies have used spike-in oligonucleotides U6 as normalization controls in their study (Zampetaki et al., 2012; Graham et al.,

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2015). Nonetheless, these synthetic miRNAs are not incorporated in microvesicles or protein lipid complexes. Therefore, extraction efficiency is not accounted for. Alternatively, endogenous miR-NAs that are detectable in all samples show lower variation of expression levels and have been used as internal controls previously (Bye et al., 2013; X. Wang et al., 2016). In the present study, the Ct values were normalized according to the ΔCt method with the internal controls miR-425-5p and miR-186-5p. These 2 reference miRNAs were selected based on the screening of miRNA expression using a Serum/Plasma Focus microRNA PCR Panel (Exiqon) comprising 179 LNA microRNA primer sets in the selected 11 samples (X. Wang et al., 2015). The geometric mean of 2 or more selected reference genes is more accurate than a single gene for normalization (Vandesompele et al., 2002). The normalization stability of these two miRNAs was confirmed in our study with geNorm software (Song et al., 2012) and the result is shown in supplementary Figure 1. These 2 miRNAs have also been used as reference miRNAs in previous studies (Bye et al., 2013; X. Wang et al., 2014; Chen et al., 2017). Our measurements showed that the Ct value for miR-186-5p was 29.4±0.9 (mean±SD) and the Ct value for miR-425–5p was 28.3±1.0 in all the patient samples. This indicates that those two reference miRNAs are robust. The relative expression of miR-451a was calculated with miR-425-5p and miR-186-5p using the following equation: $\Delta Ct = Ct_{\text{miR-425-5p\&miR-186-5p (geometric mean of two miRNAs)}} - Ct_{\text{miR-451a}}$. The changes of miRNA level after follow-up, the expression of miRNA at 8 weeks follow-up is compared to expression of the miRNA at baseline. It was calculated with the formula $\Delta\Delta CT = \Delta Ct_{\text{follow-up}} - \Delta Ct_{\text{baseline}}$.

Ethical Considerations

The study was performed according to the principles of the Declaration of Helsinki. It was reviewed and approved by the Ethics Committee of Lund University, prior to its commencement, on 5 October 2011 (application no. 2011/491). Written informed consent was obtained from all participants.

Statistical Analysis

Data are presented as mean and SD for age, body mass index (BMI), and baseline miR-451a levels. Median and interquartile range (IQR) were used for baseline MADRS-S and MIF, whereas sex, smoking, and alcohol status and antidepressant and tranquilizer use are presented as numbers and percentages (Table 1).

To estimate the change between baseline and the 8-week follow-up, we used the median and IQR for MIF and the mean and SD for miR-451a and tested it with a nonparametric test (Wilcoxon sign-rank test) for MIF and a paired *t* test for miR-451a (Table 2).

Linear regression models were used to test the association between MIF (transformed with the common logarithm because of a highly skewed distribution) and miR-451a at baseline, both unadjusted and adjusted for potential confounders (Table 3). The following potential confounders were considered, age, sex, BMI, smoking, and alcohol status and pharmacotherapy (antidepressants and/or tranquilizers). In addition, the associations between changes in MIF (follow-up levels minus baseline levels) and changes in miR-451a (follow-up levels minus baseline levels) were tested using linear regression and adjusted for the potential confounders (Table 4). These results are also shown in a figure format together with a Pearson correlation coefficient (ρ) and a coefficient of determination (R2) (supplementary Figure 2).

To assess the potential association between MIF/miR-451a and treatment response, we used linear regression analysis

Table 1. Characteristics of the Study Population at Baseline (n = 168)

Variables	Patients (n = 168)
Age, y	
Mean (SD)	42.2 (11.0)
Sex, n (%)	
Male	22 (13)
Female	146 (87)
BMI[a]	
Mean (SD)	26.9 (5.6)
Smoking status, n (%)[b]	
Yes	22 (13.1)
No	143 (85.1)
Alcohol status, n (%)[c]	
Standard size drinks per week	
≤1	144 (85.7)
>1	19 (11.3)
Antidepressants, n (%)[d]	
Yes	55 (33)
No	97 (58)
Tranquilizers, n (%)[e]	
Yes	24 (14)
No	121 (72)
Baseline MADRS-S	
Median score (IQR)	20 (10)
Baseline MIF (pg/mL)	
Median score (IQR)	5398 (4339)
Baseline miR-451a (ΔCt)	
Mean (SD)	5.75 (1.30)

Abbreviations: BMI, body mass index; IQR, interquartile range.
[a]9 patients had missing on BMI.
[b]3 (1.8%) had missing on smoking status.
[c]5 (3%) had missing on alcohol status.
[d]16 (9%) had missing on antidepressants.
[e]23 (14%) had missing on tranquilizers.

Table 2. MIF and miR-451a (ΔCt) for Patients at Baseline and 8-Week Follow-up (Mindfulness and TAU)

Variables	Baseline	Follow-up	Difference	P-value
MIF				
Median (IQR)	5398 (4339)	4561 (3131)	-718 (2797)	<.0001[a]
miR-451a				
Mean (SD)	5.75 (1.30)	5.80 (1.32)	0.05 (1.05)	.54[b]

[a]Difference tested by Wilcoxon sign-rank test.
[b]Difference tested by paired *t* test.

to analyze the changes in MIF/miR-451a and the changes in MADRS-S adjusted for MADRS-S at baseline, both unadjusted and adjusted for the potential confounders (Table 5). This was also examined by testing the potential difference in changes in MIF (using a T-test) between responders (defined as ≥50 % decrease in MADRS-S after follow-up) (Trivedi et al., 2009) and nonresponders (supplementary Table 1). The results from linear regression models for the associations between MADRS-S and MIF and miR-451a levels at both baseline and follow-up are shown in a scatterplot together with a Pearson correlation coefficient (ρ) and a coefficient of determination (R2) (supplementary Figure 3).

A sensitivity analysis was performed where the period between blood collection and isolation of the samples was also considered in the analysis, but it did not change our results (data not shown). Hence, it was not included in the final models.

and TAU on MIF and miR-451a levels in the patients. This analysis showed no significant difference in patients treated with mindfulness or TAU (data not shown).

The Association Between Plasma MIF and miR-451a in Patients

We further investigated the potential role of miR-451a in regulating MIF levels. Table 3 shows that MIF levels were significantly associated with age and smoking status, with higher age and smoking (yes vs no) associated with higher levels of MIF (β=0.006, P=.001 and β=0.12, P=.03). Higher BMI and an alcohol consumption of more than one standard size drink were also positively associated to MIF, although not significant. We performed linear regression analysis to assess the potential association between MIF and miR-451a levels at baseline. Unadjusted linear regression revealed that MIF levels at baseline were inversely related to miR-451a levels (β=−0.03, P=.02). Results remained significant after adjusting for age, sex, BMI, smoking, and alcohol status and pharmacotherapy (β=−0.04, P=.008).

In addition, we investigated the association between changes (follow-up level minus baseline level) in MIF and miR-451a posttreatment. With the linear regression analysis (as shown in Table 4), we found that the change in levels of MIF was significantly associated with the change in levels of miR-451a (β=−0.08, P<.0001), although miR-451a showed a nonsignificant change before and after treatment (Table 2). These results are also presented in supplementary Figure 2 (ρ=−0.40 and r^2=0.16). Adjustment for age, sex, BMI, smoking and alcohol status, and pharmacotherapy did not change the results (β=−0.06, P<.0001).

The Potential Role of MIF/miRNA-451a in the Improvement in Psychiatric Symptoms in Response to the Treatment

Moreover, we performed a linear regression analysis to evaluate the potential association between the changes in MIF/miR-451a and the improvement in psychiatric symptoms in response to the treatment (MADRS-S score). However, there were no significant associations between the change in either MIF or miR-451a and the change in MADRS-S (Table 5). Similar results were found for both HADS-A/HADS-D and PHQ-9 (data not shown in tables). This was also investigated by examining the difference in MIF changes between responders (defined as a percentage reduction of ≥50 % in the MADRS score after follow-up) and nonresponders, with the same conclusion of no significant association (supplementary Table 1). Scatterplots (supplementary Figure 3) were then used to examine potential associations between MIF/miR-451a and MADRS-S (symptoms) at baseline and follow-up separately. A statistically significant but weak association was found only for MIF and MADRS-S at follow-up (ρ=−0.17; R^2=0.03).

Discussion

This is, to our knowledge, the first study to explore the potential role of plasma MIF and miR-451a in patients with depression, anxiety, or stress and adjustment disorders treated with mindfulness-based therapy or TAU. Our main findings were the following: (1) the levels of MIF were significantly decreased after 8 weeks of treatment in patients, whereas miR-451a levels showed a nonsignificant change; (2) a significant association was found between levels of MIF and miR-451a in patients at baseline as well as between changes in MIF and changes in

miR-451a post-treatment; (3) no significant associations were found between the changes in either MIF or miR-451a and improvement of psychiatric symptoms.

It is of note that mindfulness-based therapy or TAU in our study reduced MIF levels. MIF is important in the homeostasis of the host immune response. MIF has been reported to be involved in the pathobiology of depression/anxiety (Musil et al., 2011; J. Bloom and Al-Abed, 2014). Edwards et al. reported that elevated MIF related to depressive symptoms, a smaller cortisol reactivity to acute stress, and lowered morning cortisol values, indicating that MIF may act as a neuro-immune mediator linking depressive symptoms with inflammation and hypothalamic–pituitary–adrenal axis dysregulation (Edwards et al., 2010). A previous study also showed that MIF levels were reduced after treatment with antidepressants in patients with depression (Cattaneo et al., 2013). However, it is not known whether mindfulness-based therapy or CBT have effect on MIF levels. In the present study, we found that MIF was significantly decreased after psychotherapeutic interventions. In line with our finding, Walsh et al. reported that a 4-week mindfulness-based intervention can reduce salivary IL-6 and TNF-α in women with depressive symptomatology (Walsh et al., 2016). Furthermore, Moreira et al. found that CBT significantly decreases the serum levels of IL-6 and TNF-α in patients with depressive symptoms (Moreira et al., 2015). Based on the evidence above, mindfulness treatment/CBT may also seem to have an effect on inflammatory immune response in depression, but the underlying mechanism is not well established.

There is not sufficient evidence to show that pharmacological antidepression treatment affects the miR-451a expression. We found only one study showing that treatment with antidepressants could reverse the stress-induced change of miR-451a expression in rat hippocampus. However, it is not known whether psychotherapeutic interventions can affect plasma levels of MIF or miR-451a in patients with psychiatric disorders. In the present study, we found a nonsignificant change in miR-451a after the treatment. We were unable to identify the exact mechanism behind this however, as most miRNAs regulate their targets at cellular levels. We have quantified the circulating miRNA that may not represent the whole effect of regulation by this miRNA at the cellular level; small changes in the circulating miR-451a may represent large changes in cellular miR-451a levels.

It is well known that miR-451a can directly target MIF and downregulate its expression in the cells (Bandres et al., 2009; N. Liu et al., 2013). The circulating miR-451a is usually secreted from their cells of origin (Arroyo et al., 2011; Valadi et al., 2007). However, the relation between circulating MIF and miR-451a has never been reported. In the present study, we found that there is a significant inverse association between plasma levels of MIF and miR-451 levels in patients with psychiatric disorders. Our study is the first to evaluate a possible association of MIF with circulating miRNA in patients with psychiatric disorders. Similarly, a recently published study reported another circulating miRNA (miR-939) regulates multiple proinflammatory genes in other disease also (McDonald et al., 2016). We also in this study show a significant association between the change in MIF levels and the change in miR-451a levels in patients with psychiatric disorders after psychotherapeutic interventions. However, the biological mechanism behind this relationship still needs to be examined. Therefore, in any future studies, it will be of interest to examine the relationship between MIF and miR-451a at cellular level in patients with depression, anxiety, and stress and adjustment disorders (Cattaneo et al., 2016) (McDonald et al., 2016).

We have previously shown that mindfulness therapy and TAU reduced psychiatric symptoms in patients with depression, anxiety, and stress and adjustment disorders (Sundquist et al., 2015). Therefore, we investigated whether the changes in MIF and miR-451a were associated with the improvement in psychiatric symptoms in response to psychotherapeutic interventions. However, we were unable to detect any significant associations between response and MIF or miR-451a. In agreement with our findings, a recent study reported that a mindfulness-based intervention can reduce salivary cytokines although the reduction was not related to the improvement in depressive symptoms (Walsh et al., 2016). Cattaneo et al also reported that treatment with antidepressants reduced the levels of MIF, but the reduction was not associated with treatment response (Cattaneo et al., 2013). Recent evidence shows that the expression levels of proinflammatory and inflammatory cytokines in postmortem brain have a positive relationship with the levels in plasma in patients with depression (Pandey et al., 2012; Miller and Raison, 2016). However, it is important to note that MIF does not cross the blood brain barrier (Bacher et al., 2002; J. Bloom and Al-Abed, 2014). Therefore, the changes in MIF in the plasma may differ from the changes in the brain. Furthermore, multiple preinflammatory cytokines are involved in the development of depression. It is possible that elevated levels of some of these inflammatory cytokines may not be the consequence of the depression but could be a result of brain dysfunction associated with depression and therefore may not be associated with response to treatment. Therefore, a simple reduction in MIF levels may not be enough to improve the depressive symptoms in response to mindfulness therapy or TAU. Taken together, MIF may not act as an independent predictive biomarker for mindfulness therapy or TAU in the circulation.

Our study has several strengths. We provide evidence that psychotherapeutic interventions may reduce MIF levels in patients with psychiatric disorders. MIF levels and miR-451a levels at baseline as well as changes in levels posttreatment were significantly associated with each other. These findings suggest that miRNA may interact with the inflammatory process linked to the psychotherapeutic interventions in patients with psychiatric disorders. As such, this novel contribution has never been shown in previous studies. Although our findings are promising, several general limitations should be considered. First, we do not have postmortem samples to further confirm our findings. Second, the blood samples used for the assays were frozen between 4 to 8 hours after collection, which may affect our results. However, according to Friebe et al., the cytokine levels in EDTA tubes are not affected if the samples are prepared within 8 hours at room temperature (Friebe and Volk, 2008). The time points for both blood collection and plasma isolation were, however, available in our patient samples; the results were adjusted for this time point and the results were not affected. Third, we did not perform separate analyses according to the subtype of psychiatric disorders. However, overlapping symptoms are relatively common among these conditions and the patients were therefore analyzed together. Fourth, our findings cannot be used to guide clinical practice methods at present; it needs to be confirmed in independent cohorts. Moreover, our study did not include patients that did not receive any therapy as a control. It might be possible that the changes in MIF and miR-451a were simply a reflection of variability over time.

In conclusion, our results suggest that 8 weeks of psychotherapeutic interventions can reduce the MIF levels in patients with psychiatric disorders. However, this reduction was not associated with an improvement in psychiatric symptoms in response to the treatment. The association between plasma levels of MIF and miR-451 may relate to the regulatory process occurring at the cellular level, even though the biological mechanisms need to be examined in future studies.

Supplementary Material

Supplementary data are available at *International Journal of Neuropsychopharmacology* online.

Acknowledgments

Statement of Interest

None.

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