**Minocycline and celecoxib as adjunctive treatments for bipolar depression: a multicentre, factorial design randomised controlled trial**

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**Research in Context**

**Evidence before this study:** Converging evidence indicates that an activated inflammatory response may be associated with bipolar disorder. Previous open-label and proof-of-concept trials suggest that anti-inflammatory agents such as minocycline and celecoxib may be useful adjunctive treatments for patients with bipolar depression. We searched trial registry databases Clinicaltrials.gov and ISRCTN as well as bibliographic databases Medline and Google Scholar with search terms “minocycline”, “celecoxib” and “bipolar” on December 20th, 2019. The search was not restricted by language and results did not yield any large, confirmatory randomised controlled trial assessing the putative antidepressant efficacy of minocycline and/or celecoxib in bipolar depression.

**Added value of this study:** To our knowledge, this is the largest trial of anti-inflammatory agents as adjunctive treatments for a mood disorder conducted to date. The study was conducted in Pakistan, a low resource setting.We found no evidence for difference in changes on the 17-item Hamilton Depression Rating Scale between participants receiving minocycline or celecoxib plus routine clinical care and those receiving placebo plus routine clinical care. Baseline inflammatory biomarker (white cell count, C-Reactive Protein levels) did not moderate a response to treatment nor did changes in biomarkers mediate a response to either anti-inflammatory medication.

**Implications of all the available evidence:** Minocycline and celecoxib provide no additional therapeutic benefit for symptoms of bipolar depression compared to routine clinical care. However, since the current study was conducted in a low to middle-income country, findings may not be generalizable to populations in high-income countries.

**Abstract**

**Background:** Several small studies suggest that the adjunctive use of anti-inflammatory agents may improve depressive symptoms in bipolar disorder. However, there are few well-designed, appropriately powered clinical trials assessing the efficacy of these novel treatment strategies.

**Methods:** This 12-week, randomised, double blind, placebo-controlled trial, was conducted in four centres in Pakistan. It aimed to assess the efficacy of adjunctive minocycline or celecoxib in the treatment of depression in adults with bipolar disorder. It used a 2x2 factorial design and involved adults (age: 18-65) with a DSM-5 bipolar I/II disorder and a major depressive episode. Participants were enrolled between May 1, 2016 and March 31, 2019; the modified intent-to-treat analysis was performed from July 1 to August 31, 2019 using mixed models. Participants were randomised to receive: (i) active-minocycline plus active-celecoxib; (ii) active-minocycline plus placebo-celecoxib; (iii) placebo-minocycline plus active-celecoxib; (iv) placebo-minocycline plus placebo-celecoxib. The primary outcome measure was mean change from baseline to week 12 on the 17-item Hamilton Depression Rating Scale (HAMD-17) scores. The trial was registered on Clinicatrials.gov (NCT02703363) on March 8, 2016.

**Findings:** A total of 266 participants were randomized to minocycline plus celecoxib (n = 68), minocycline plus placebo (n = 66), celecoxib plus placebo (n = 66) or placebo plus placebo (n =  66) across study sites. Overall baseline-to–end point reduction in HAMD-17 total score was observed across the four groups however, the reduction in depressive symptoms did not differ significantly between groups. There were no main effects for either minocycline or celecoxib (mean adjusted difference [95% confidence interval] for minocycline vs. non-minocycline: 1·48 (-0·41, 3·36), t(df) 1·5 (268·9), p=0·123, celecoxib vs. non-celecoxib: -0·74 (-2·62, 1·14), t(df) -0·8 (267·9), p=0·443). There was no evidence for differences in adverse effects between groups. A planned secondary analysis indicated that changes in C-Reactive Protein and white cell count from baseline to end-point did not mediate response to either treatment.

**Interpretation:** No evidence was found that minocycline or celecoxib were superior to placebo for the treatment of bipolar depression. This large RCT casts doubt on the potential therapeutic benefits of adjunctive anti-inflammatory agents for the acute management of bipolar depression.

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**Key Words:**

Bipolar Disorder

Bipolar Depression

Inflammation

Anti-Inflammatory

Minocycline

Celecoxib

**Introduction**

Bipolar disorder affects approximately 2% of the population globally and is associated with significant morbidity and mortality.1 Bipolar depression remains a significant treatment challenge, with a paucity of evidence-based treatments. Only three pharmacological treatments for acute bipolar depression (olanzapine-fluoxetine combination, quetiapine, and lurasidone) are recommended by the British Association of Psychopharmacology2 and the Canadian Network of Mood and Anxiety Treatments (CANMAT) guidelines3, and are -approved by the US Food and Drug Administration (www.fda.gov). Other medications often used for the treatment of bipolar depression are those primarily used to treat mania or psychosis (i.e., lithium; antipsychotics) or major depressive disorder (i.e., antidepressants). Lamotrigine, which is recommended by international guidelines as a maintenance treatment for bipolar disorder to prevent depressive recurrence, has limited efficacy for acute bipolar depression.4 Current medication options are also limited by adverse effects, including renal and thyroid impairment with long-term lithium therapy and weight gain and metabolic abnormalities with atypical antipsychotics.4 Hence, there is a clear need for novel and efficacious treatments for bipolar depression.

Several lines of evidence indicate that neuroinflammation may be a potential treatment target for bipolar disorder. First, in some post-mortem studies, inflammatory processes such as microglial activation are associated with neuropsychiatric conditions including bipolar disorder.5,6 Second, neuroimaging studies using positron emission tomography (PET) have identified microglial activation in euthymic patients with bipolar disorder compared with healthy controls.7 Third, a large number of studies have demonstrated that bipolar disorder is associated with abnormal levels of circulating pro- and anti-inflammatory biomarkers.8 Finally, the frequent comorbidity of bipolar disorder with chronic inflammatory physical illnesses such as systemic lupus erythematosus,9 chronic infections such as Toxoplasma gondii,10 cardiovascular disease,11 and metabolic disorders such as diabetes, obesity, and hypercholesterolemia,11 strengthen the notion that bipolar disorder is a multisystem inflammatory disease.

Evidence from recent meta-analyses suggests that adjunctive anti-inflammatory medication may be effective in the treatment of mood disorders including bipolar depression.12,13 One of these medications, minocycline, is a tetracycline antibiotic that has good penetration through the blood-brain barrier and affects multiple systems including anti-inflammatory, anti-oxidant, anti-apoptotic, glutamatergic, and monoaminergic pathways. These pathways have all been implicated in the aetiology of mood disorders.14 Results from open-label trials suggest that minocycline may be beneficial for bipolar depression. 15,16

Similarly, in several small clinical trials the selective cyclooxygenase (COX)-2 inhibitor celecoxib has shown promise in the treatment of depressive symptoms.12 COX-2 is the key enzyme involved in synthesis of prostaglandin; it is expressed in inflammatory cells and induces pro-inflammatory cytokines. Pre-clinical models have shown that COX-2 disrupts production of serotonin in the CNS and its inhibition reduces depressive-like behaviours.17,18 Two small clinical trials have investigated the use of celecoxib in bipolar depression with promising results.19,20

In this context, we conducted a multicentre, 12-week, four-arm factorial design, double blind randomised controlled trial, to determine the efficacy and tolerability of minocycline or celecoxib as adjuncts to routine clinical care in adult patients with bipolar disorder experiencing a major depressive episode. We hypothesised that adjunctive minocycline or celecoxib would improve depressive symptoms, compared to placebo.

**Methods**

*Study Setting*

The study was conducted in outpatient psychiatric clinics in Hyderabad, Karachi, Lahore, and Rawalpindi, Pakistan.

*Participants*

Inclusion criteria were: Males or females aged 18–65 years; DSM-5 diagnosis of bipolar I or II disorder and current major depressive disorder; experiencing current depressive symptoms for at least 4 weeks (17-item Hamilton Depression Scale (HAMD-17)21 score ≥18); willing and able to provide informed consent; taking current medication for at least 4 weeks prior to baseline assessment; able to take medication in tablet form; if female and of child-bearing age, agreed to use contraception and consents to monthly pregnancy tests.

Exclusion criteria were: Serious physical health condition (eg, HIV, renal, hepatic, cardiac, and serious dermatological disorders such as exfoliative dermatitis and systemic lupus erythematosus); history of allergies/adverse effects to any of the tetracyclines or other anti-inflammatory medication; current treatment with penicillin; current use of anticoagulant medication; history of seizures; current use of other antibiotics, other NSAIDs, acetazolamide, or methotrexate; change of psychiatric medications within the preceding 4 weeks; history of substance misuse or dependence within the preceding 3 months according to DSM-5 criteria; pregnant or breast-feeding; diagnosis of primary psychotic disorder; high risk of suicide; three or more concurrent manic/hypomanic symptoms.

A structured diagnostic interview (the Mini-International Neuropsychiatric Interview, MINI22) was used to confirm a diagnosis of DSM-5 bipolar disorder (type I and type II) and current major depressive episode. The criteria for study withdrawal were: (1) pregnancy; (2) at the participant’s request; (3) at the discretion of the responsible physician or trial investigator (e.g., an adverse event, poor compliance).

The study was conducted in accordance with the principles of the Declaration of Helsinki. The ethics committee of Karachi Medical and Dental College (KMDC) in Karachi, Pakistan provided Institutional Review Board (IRB) approval for the study (reference number 016/16). All participants provided written informed consent after reading the information in English or Urdu.

An independent Trial Steering Committee (TSC) that included a senior physician and a service user monitored the trial. The TSC had the responsibility for data and safety monitoring to assess for any potential harm to the participants from taking part in the trial.

*Study Design*

The study was a four-arm trial a 2x2 factorial design: Placebo-minocycline plus placebo-celecoxib, active-minocycline plus placebo-celecoxib, placebo-minocycline plus active-celecoxib, and active-minocycline plus active-celecoxib.

*Randomisation and masking*

A restricted randomisation (permuted block randomisation) method was used in which participants were randomly allocated to each block (target n=60) to ensure that equal numbers of participants received each drug/placebo combination. An off-site statistician, who was not involved in clinical assessments, determined the randomisation sequences. The statistician assigned the treatment code to participants after they provided consent and after the baseline assessment confirmed their eligibility.

Participants, their carers, referring psychiatrists and the researchers carrying out assessments were blind to the study drugs until study completion. A single pharmacy at each centre was responsible for dispensing minocycline, celecoxib, and placebo in identical tablet form, matched for size, shape and colour.

*Clinical Procedures*

In brief, minocycline was started at a dosage of 100 mg daily and was increased after two weeks to 200 mg daily (two tablets of 100 mg), taken as a single dose to encourage adherence. Celecoxib was started at a dosage of 200 mg daily and was increased after two weeks to 400 mg daily (two tablets of 200 mg), also taken as a single dose. Study medication was dispensed at each study visit, at which point research assistants (RAs) conducted pill counts to assess adherence.

The clinical teams at each centre continued to provide routine care for each participant and were permitted to make changes to concomitant psychotropic medication at the discretion of the treating psychiatrist. RAs could be contacted for the duration of the study to respond to any concerns. Routine clinical care of bipolar disorder in Pakistan consists of regular outpatient psychiatric follow-up and treatment with psychotropic medications (i.e., traditional mood stabilizers, antipsychotics, antidepressants, anxiolytics, and sedative/hypnotics). To the best of our knowledge, there is minimal access to supportive and specific psychological interventions for bipolar disorder in Pakistan.23

*Outcomes*

The primary outcome measure was mean change in HAMD-1721 scores from baseline to week 12. Secondary outcomes included rates of response and remission with response defined as a reduction of 50% or more of the HAMD-17 score, and remission as a score of ≤7 of the HAMD-17. The additional 7 items from the 24-item Hamilton scale were also assessed.24 Ratings were made on the basis of a semi-structured clinical interview at baseline and at every 2-weekly follow-up visit until week 12.

Other outcomes were the Clinical Global Impression (CGI) scale, an overall measure of illness severity,25 the 9-item Patient Health Questionnaire (PHQ-9),26 a measure of depression severity, the 7-item Generalized Anxiety Disorder scale (GAD-7),27 a measure of severity for GAD, and the EuroQoL EQ-5D,28 a measure of health-related quality of life. All instruments have been validated for use in the Urdu language and have been used in previous studies in Pakistan.29 Adverse effects were monitored using scales that have been specifically designed for minocycline and celecoxib and were used by the investigators in a previous study.30

### *Biomarkers*

Participants were requested to provide two blood samples (at baseline and at week 12) for analysis. This was optional and did not influence recruitment to the study. The biomarkers analysed included complete blood count (CBC) and C-Reactive Protein (CRP), which was quantified using Spinreact CRP Latex Agglutination Test.

*Inter-rater reliability*

Inter-rater reliability was measured throughout the study by local investigators. To establish inter-rater reliability, training videos were used while two raters coded them independently. Ratings were then assessed with the intraclass correlation coefficient.

*Sample Size*

The study was powered assuming *a priori* that an effect size of 0·45 or larger would be clinically meaningful, congruent with the published criteria for response in clinical trials of antidepressants.31 Thus, the sample size was calculated on the basis of a group standardised mean difference (SMD) of 0·45 on the Hamilton Depression scores (HAMD-17), for either minocycline or celecoxib. This is a medium effect size that would be of clinical interest. Also, we assumed that there would be no interaction between the two medications and thus powered the study to measure only for the main effects of either minocycline or celecoxib. The sample size was adjusted for a 20% attrition rate due to loss to follow-up or dropout. With these assumptions, a sample size of 262 would give 90% power to detect a standardized mean difference (SMD) of 0·45 with a level of significance set at 0·05. This naive power calculation did not include the gain in statistical efficiency from adjusting for baseline variables.32 For a given sample size, the gain is approximately equal to the square root of 1-ρ, where ρ is correlation between baseline and outcome. Assuming this is 0·5 (as shown for patient reported outcomes32) this would give an effective sample size of 280 allowing us to adjust for multiple comparisons as necessary.

*Statistical Analysis*

Analyses were carried out following the modified intention-to-treat principle (ITT). All participants were included in the analysis according to their allocated treatment group at randomisation. Statistical tests were two-sided bench-marked at p < 0·05 as the family wise error rate for significance. The p < 0·05 threshold, was adjusted for multi-comparisons as appropriate. All analyses were carried out in R 3·6 and STATA 15·0.

The primary outcome was change in the 17-item Hamilton depression rating scale score from baseline to week 12. Study outcomes were analysed using data from all post-randomisation timepoints; 2, 4, 8 and 12 weeks. As there was statistical dependency between repeated measures, (generalized) linear mixed models were used to assess the two experimental conditions: minocycline vs. non-minocycline and celecoxib vs. non-celecoxib at 12 weeks. As there were two treatment contrasts of interest for each outcome the significance threshold was Bonferroni corrected to p < 0·025 for the primary and other outcomes. Fixed effects consisted of treatment conditions, time, and their two-way interaction to allow the treatment effect at each timepoint to be calculated. Estimates of treatment effects (and 95% CI) were adjusted for baseline scores and randomisation centre and predictors of missing observations at 12 weeks. As observations by time were nested within individuals, a random intercept for participant was included in the model and the need for a random effect (slope) of time assessed by comparing models with and without the slope using a likelihood ratio test (the slope was included in all models). If these random effect terms were significant, they were included in the model. Standardised effect sizes were computed by dividing the estimated mean treatment group difference by the SD of the baseline outcome. Small effects were considered to range from 0 – 0·39, medium from 0·40 to 0·59 and large over 0·60. Binary outcomes were analysed with mixed effects logistic models.

The 2x2 factorial design assumes there is no interaction between the two treatment conditions, which is typically tested via an interaction between them. To test this assumption here, a model with a three-way interaction between the anti-inflammatory conditions and time was compared to the model with separate treatment contrasts for minocycline and celecoxib. Mixed model assumptions were assessed by visual inspection of residuals for normality and looking for influential observations using Cook’s distance as a measure.

To investigate the role of inflammatory biomarkers we took two approaches. Firstly we explored whether baseline levels of inflammatory markers affected treatment effects by adding treatment by time by CRP or WBC interaction (and main effect) terms to the primary analysis model. CRP and WBC were included as continuous variables. Secondly, we used mediation models to see if any treatment associated changes in depression symptoms were mediated by changes in biomarkers at 12 weeks. For this mediation analysis we used a structural equation modelling framework, which allows the joint estimation of multiple regression equations with a principled approach to missing data via full information maximum likelihood (FIML). The mediation pathway hypothesized that treatment follows an indirect path with lower levels of inflammatory markers for individuals (*a* pathway) being associated with lower levels of depression symptoms (*b* pathway). The indirect effect is then calculated as the product of the *a* and *b* coefficients. The *a* and *b* pathways were adjusted for the same covariates as in the primary analysis model (baseline severity and predictors of missing data) in addition to baseline values of the inflammatory marker. As there were 2 treatment conditions, there were 2 *a* pathways for minocycline and celecoxib (am and ac) to inflammatory marker, but only one *b* pathway. For the direct path there were two c coefficients cm and cc. As for the primary analysis, the two treatment pathways were assumed to be independent (cf. Appendix page 1). All variables were entered into the model as manifest variables. As the product of coefficients is not normally distributed, to estimate 95% confidence intervals percentile bootstrap estimates using 5000 repetitions were used.33 The lavaan package (v 0·65) within R was used to run the analysis.

To assess possible effects of concomitant medication we operationalized this as the number of psychotropic medications being taken and included this as a covariate in the primary analysis model.

As there was some missing data, we took the following two-step approach in which data could be assumed to be missing at random (MAR). We created an indicator variable for whether the primary outcome at 12 weeks was missing or not for each participant and looked for baseline predictors of missingness (with a liberal criterion of p < 0·2) using demographic and clinical features. In the second step, including these drivers of missingness in models fitted using maximum likelihood (ML) can produce unbiased estimates of treatment effects if the MAR assumption holds.34 The published protocol stated that we would use multiple imputation (MI) to deal with missing data, hence we also repeated the analysis for the primary outcome with MI using chained equations (k=50) as a sensitivity analysis.

This study is registered with Clinicatrials.gov, number NCT02703363.

**Role of the funding source:**

This study was funded by the Stanley Medical Research Institute (SMRI), grant ID-15T 004. The funder had no role in study design, data collection, data analysis, data interpretation, or writing of the report.

The corresponding author had full access to all of the data and the final responsibility to submit for publication.

**Results:**

Participants were recruited between 1st May 2016 and 31st March 2019. Figure 1 shows the flow of participants in the study, up to and including the 12-week visit (the end of the treatment phase). Of the 266 participants included in the ITT analysis, at the end of the study, 35 (13%) were withdrawn and 7 (3%) dropped out; of the 35 withdrawals, 31 (89%) were due to a manic switch.

Participants were randomized to receive adjunctive minocycline plus placebo, celecoxib plus placebo, minocycline plus celecoxib or placebo plus placebo. Baseline demographic data for participants in each of the 4 treatment groups is presented in Table 1. The median duration of bipolar illness was 2 years (IQR 1, 4) years. The majority of participants were prescribed a mood stabilizer (most frequently valproic acid) and an adjunctive antidepressant (most frequently escitalopram) (Table 1). None of the participants were receiving any psychological therapy during the trial.

Mean baseline HAMD-17 scores were within a small standardized effect size (SES, d) of each other between groups (maximum 0·12) and were in the severe range (Table 2). Secondary outcome measures were also similar between groups with all effect sizes (ES) being small (Table 2). Twenty-six participants had increased CRP concentrations (>10 mg/L) at baseline otherwise baseline CRP concentrations were unremarkable (i.e. <3 mg/L). Mean (SD) baseline white blood cell count (WBC) was 8340·6 cells/mm3 (3785 cells/mm3), which is within normal limits.

There was no evidence for treatment effects for either minocycline or celecoxib at 12 weeks as HAMD-17 scores showed no difference over treatment conditions. Scores were higher in minocycline over non-minocycline but this difference was not significant (mean difference for maximum likelihood (ML) = 1·48 [95% CI: -0·4, 3·36, p = 0·123] and multiple imputation (MI) = 1·69 [95% CI: -0·2, 3·56, p = 0·076] (Table 2 and Appendix page 3). HAMD-17 scores were slightly lower in celecoxib vs non-celecoxib but again this difference was not significant (ML mean difference = -0·74 [95% CI: -2·61, 1·14, p = 0·443] and for MI = -0·93 [95% CI: -2·83,0·97, p = 0·339]) (Table 2 and Appendix page 3). Nor was there any interaction between treatment groups at 12 weeks (0·71 [95% CI: -3·04, 4·48], SES = 0·12 [95% CI: -0·52, 0·78], p > 0·7). Evaluation of HAMD-17 throughout the study at follow-up timepoints shows that all treatment conditions showed little difference (Figure 2, Appendix page 4). In an exploratory moderator analysis, treatment effects at 12 weeks did not differ according to CRP and WBC at baseline. Effects of minocycline on HAMD-17 at 12 weeks were not moderated by level of CRP (interaction coefficient (b) = -0·16 [95% CI: -2·46, 2·13], p = 0·89]) or WBC (b = 2·2 [95% CI: -4·21, 8·60], p = 0·502). Similarly, effects of celecoxib were not moderated by CRP level (b = 0·28 [95% CI: -2·01, 2·57], p = 0·28) or WBC (b = -3·56 [95% CI: -9·96, 2·84], p = 0·28). For the secondary outcomes, the pattern of data observed with HAMD-17 was repeated. Mean scores on the other outcome measures - PHQ-9, CGI, GAD-7 and EQ-5D - improved similarly in all four treatment groups across the trial (Table 2 and 3) with no significant differences in rates of remission or response at p < 0·025.

In the mediation analysis, plasma CRP levels and WBC were unaffected by treatment and thus showed no evidence for effects of changes in CRP or WBC on HAMD-17 scores (Appendix pages 5 to 8).

At baseline, participants were taking either 2 or 3 psychotropic medications. Including this as a covariate in the primary analysis model made little difference to the estimates of the treatment effect (minocycline = 1·54 (-0·33, 3·4), celecoxib = -0·64 (-2·51, 1·22)) compared to those in Table 2. We also identified patients who changed medication and analyzed whether treatment effects were different across the groups. Twenty-six patients changed medication during the course of the trial (8 in the placebo group, 4 in minocycline, 5 in celecoxib and 9 in the combined group). The mean group differences were a little bigger for changed medication participants versus no changed, but still within the 95% CI for the treatment effects. For minocycline, in the no medication change group, patients were 1·45 (-0·49, 3·5) higher on HAMD-17 scores. For the changed medication group, the treatment cost was 2·98, which is however still in the 95% CI of the no medication change group. Similarly for celecoxib, the treatment difference was in the same direction in both groups; in the medication change group HAMD-17 was -1·86, relative to -0·78 (-2·73, 1·16) in the non-change group. This difference was still within the 95% CI for the no change group.

A total of 31 participants were withdrawn from the study after experiencing a manic switch, which was considered a serious adverse event. A total of 2 participants were withdrawn for self-harm. One participant in the minocycline plus placebo group died following a motor vehicle accident during the study period. There were no statistically significant differences in rates of manic switch or any other adverse effects, among the four treatment groups (Table 4).

With regards to inter-rater reliability, agreement between the four main RAs when scoring videos of HAMD-17 interviews showed an excellent intra-class correlation of 0·898. Finally, with regards to medication adherence, RAs did not detect poor adherence in participants in any of the four groups from the pill counts conducted at each study visit.

**Discussion:**

In this study, neither the anti-inflammatory tetracycline minocycline nor the selective COX-2 inhibitor celecoxib were associated with an antidepressant effect compared with placebo in the treatment of bipolar depression. To our knowledge, this is the largest clinical trial of anti-inflammatory agents in mood disorders to date. Two RCTs have previously investigated the use of celecoxib and one other RCT has investigated the use of minocycline in the treatment of bipolar depression.19,20,35 A double-blind RCT of adjunctive celecoxib vs. placebo in 28 patients with bipolar depression found no significant differences between groups after 6-weeks of treatment.19 More recently, in an RCT of celecoxib vs. placebo added to escitalopram in 47 patients with bipolar depression, the celecoxib group had significantly lower HAMD-17 scores than the placebo group at weeks 4 and at endpoint (week 8).20 The small samples in both studies may in part account for their conflicting results.

Our factorial-design RCT is the first study to concurrently assess the efficacy of both minocycline and celecoxib as adjunctive treatments for bipolar depression. The negative finding on the efficacy of minocycline in our study is similar to the findings from another RCT with a 2x2 factorial design that assessed minocycline and aspirin in 99 patients with bipolar depression35. In that trial there was no significant main effect of either aspirin or minocycline on the scores of the Montgomery-Äsberg Depression Rating Scale (MADRS) after 6 weeks of treatment.35 However, a secondary analysis showed that the response to minocycline was significantly higher in participants in the minocycline + placebo group with higher interleukin (IL)-6 concentrations. Another open-label study has suggested a differential effect of minocycline in patients with bipolar depression and various inflammatory cytokines16. As our biomarker panel did not include an assessment of cytokines, we are unable to replicate these findings, but they suggest that minocycline may confer benefit to a subgroup of individuals with elevated IL-6. Further studies of minocycline in bipolar depression could test this hypothesis by enriching their samples with participants with elevated IL-6 at baseline.

Our negative results are consistent with the negative results from a recent placebo-controlled RCT of another anti-inflammatory agent, the Tumour Necrosis Factor (TNF) antagonist, infliximab. The study recruited and stratified patients with bipolar I/II depression (n=60) who presented with either biochemical (i.e., CRP > 5 mg/L) or phenotypical (e.g., obesity, comorbid diabetes, inflammatory bowel disease, rheumatologic disorder, and migraine headaches) evidence of inflammation.36 Despite this stratification, infliximab did not significantly reduce depressive symptoms compared with placebo.

To our knowledge, our study is the largest RCT evaluating anti-inflammatory agents in adults with bipolar disorder. The large sample size and high retention rates (84% overall) in the intervention and placebo groups are clear strengths of this study and allowed us to detect a medium effect size for the main effects of minocycline and celecoxib. Also, we collected CRP levels in the majority of randomised participants; evidence supports elevated peripheral CRP as a marker of low-grade inflammation in unipolar depression.37 However, the use of CRP as the sole peripheral inflammatory biomarker was a limitation and a more direct measure of inflammation was not obtained, due to limited funding and the lack of infrastructure in Pakistan to measure more novel biomarkers (e.g., inflammatory cytokines). Future studies should collect more comprehensive inflammatory biomarkers.

Another putative limitation is that the study was powered on the reasonable assumption that there were no interactions between minocycline and celecoxib. Factorial design studies can often have unanticipated interactions between interventions. Although there was no evidence of any interaction here for the primary outcome or any of the secondary outcomes, it is possible that an adequately powered study might find an interaction of clinical significance. Whilst the result for minocycline appeared to be inconsistent with all but the smallest positive effect, the data for celecoxib was more inconsistent with 95% CI for the primary outcome not ruling out SES from moderate negative to positive (cf. Table 3). Further, there was weak evidence for an advantage for celecoxib with the HAMD-17 remission outcome (OR=3·02, p=0·036, not significant at the Bonferroni corrected p < 0·025). However, against this, no other outcomes showed positive evidence. Further trials of celecoxib in bipolar depression may be needed to address these inconsistent findings.

Including participants receiving non-standardized pharmacotherapy regimens is another limitation of our study. However, given the nature and scale of this trial, and the unpredictable course of bipolar disorder, it would not have been feasible to standardize treatment for outpatients in the community. The majority of patients recruited to the trial were on combination treatments involving mood stabilizers, atypical antipsychotics and antidepressants, and levels of concomitant psychiatric care were similar across groups at baseline. A further limitation is that all participants recruited to the study were permitted to change their regular medications under the supervision of their treating team. Although it can be argued that changes to concomitant treatment would add “noise” to the treatment signal, we were able to record all medication changes for each participant and found that these changes were similar across the four groups. Furthermore, a post-hoc secondary analysis found that baseline concomitant medication and changes to concomitant medication made little difference to the estimates of the treatment effect.

Although the 12-week duration of our study was consistent with most RCTs in mood disorders, it may be considered a limitation as a previous trial of the anti-oxidant, anti-inflammatory N-acetyl Cysteine in bipolar depression showed that an antidepressant response for this agent was only evident from week 20 onwards.38 However, Figure 3 shows similar symptom trajectories in all four groups in the current study. Another important factor to consider when interpreting these results is that the study was conducted in Pakistan, a low- to middle-income country where diet, nutritional status, and other lifestyle factors differ from those in high-income countries. While these factors should be balanced among the groups in a randomised study with a reasonably large sample size, our findings may not be generalizable to high-income countries.

Patients with bipolar depression often present with atypical features such as hypersomnia and hyperphagia, symptoms that are not adequately captured by our primary outcome measure, the HAMD-17.39 A further limitation of the current study is that we did not more adequate measures of bipolar depression. However, the probability of a false negative finding is reduced when considering the fact that we found no differences across groups on either on the CGI or the PHQ-9, which rates both insomnia or hypersomnia and loss of appetite or overeating.

Much like other trials in mood disorders,36,40 the study is also limited by the significant placebo response rate in the heterogeneous sample that was recruited. Studies indicate that approximately 80% of symptom improvement experienced in participants receiving an intervention in antidepressant trials is also observed in the placebo comparison group.40 Factors implicated in the inflation of the placebo response include non-specific therapeutic effects of repeated study visits with research staff. This is particularly relevant in low-resource settings such as Pakistan where our study was conducted. In this setting, access to routine clinical care is limited and is most often privately funded. The pressure on local health services and in particular, the scarcity of mental health services in countries like Pakistan, may contribute to larger placebo effects in clinical trials. Other factors contributing to the placebo response specific to our study include the symptom severity threshold requirement for inclusion into the trial. High entry criteria requirements have been associated with greater placebo response rates, and can cause raters to overestimate severity of symptoms at entry but not thereafter, leading to bias.41

At this time, the results of this robust RCT, taken together with existing evidence, argue against the use of adjunctive anti-inflammatory agents for bipolar depression. Although previous small RCTs and open-label studies have suggested that anti-inflammatory agents may confer a moderate antidepressant effect in bipolar depression,12, 13 the lack of consistent post-mortem evidence of inflammation in bipolar disorder, paucity of Mendelian randomisation studies, and lack of prospective studies showing that immune biomarkers are associated with an increased risk for bipolar disorder, suggest that further investigation is needed to confirm whether abnormal inflammatory processes contribute to the pathophysiology of the disease. Further treatment trials of adjunctive anti-inflammatory agents in bipolar depression are only warranted if future studies demonstrate a plausible causal link between inflammation and disease activity.

**Abbreviations:**

CGI: Clinical Global Impression scale; DSM-5: Diagnostic and Statistical Manual-5; GAD-7: Generalised Anxiety Disorder-7 questionnaire; HAMD-17: 17-item Hamilton Depression Rating Scale; PHQ-9: Patient Health Questionnaire-9; RCT: Randomised controlled trial.

**Declaration of Interests:**

MIH is a PI for a trial sponsored by COMPASS Pathways Limited for which he receives salary support. IBC, JFWD and NH have given lectures and advice to Eli Lilly, Bristol Myers Squibb, Lundbeck, Astra Zeneca and Janssen pharmaceuticals for which they or their employing institution have been reimbursed. MIH, IBC and NH were previously trustees of the Pakistan Institute of Learning and Living. BHM currently receives research support from Brain Canada, the Canadian Institutes of Health Research, the CAMH Foundation, the Patient-Centered Outcomes Research Institute (PCORI), the US National Institute of Health (NIH), Capital Solution Design LLC (software used in a study funded by CAMH Foundation), and HAPPYneuron (software used in a study founded by Brain Canada). Within the past five years he has also received research support (medications for NIH-funded clinical trials) from Bristol-Myers, Eli Lilly, and Pfizer. He directly own stocks of General Electric (less than $5,000). AHY has been commissioned to provide lectures and advice to all major pharmaceutical companies with drugs used in affective and related disorders. AHY has undertaken investigator-initiated studies funded by Astra Zeneca, Eli Lilly, Lundbeck and Wyeth.

None of the companies listed above have a financial interest in this research.

**Data Sharing:**

Requests for sharing the anonymised trial database should be addressed to the lead author.

**Author Contributions:**

MIH conceived the idea for the study, contributed to design and coordination of the study, was involved in training and supervision of RAs, and drafted the manuscript. IBC contributed to design and coordination of the study and was involved with training and supervision of RAs and helped with drafting the manuscript. MAA, MOH, FAM and HAN contributed to design of the study, recruitment of patients, and were involved with training and supervision of RAs. JH contributed to design of the study, led and performed the statistical analysis, and helped with drafting the manuscript. AK contributed to recruitment of participants and undertook assessments. JFWD, AFC, JHM, and BHM contributed to the data analysis and helped with drafting the manuscript. NH conceived the idea for the study, contributed to the design of the study, was involved in assessments and in training and supervision of RAs and helped with drafting the manuscript. AHY conceived the idea for the study, contributed to design of the study and helped with drafting the manuscript. All authors reviewed and approved the final manuscript.

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