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Editor-in-Chief

Psychoneuroendocrinology

Background: the authors compare brain derived neurotrophic factor (BDNF), tissue plasminogen activator (tPA), glucocorticoid receptor (GR), heat shock protein 70 (HSP70), tumour necrosis factor-alpha (TNF-alpha>) messenger ribonucleic acid (mRNA) of 20 male, drug-free manic patients, their remission period, and 20 male, healthy controls. Their results show that compared to controls BDNF and tPA mRNA expressions were down-regulated, and GR, HSP70, TNF alpha mRNA expressions were up-regulated in mania. In remission BNDF, tPA, HSP70 mRNA levels increased, GR and TNF-alpha mRNA levels decreased compared to mania. BDNF and tPA changes were statistically significant between mania and controls. Between mania and remission only BDNF reached statistical significance. The authors concluded that current results suggest that tPA and BDNF may be a biomarker of BD.

Overall feedback: I appreciate the opportunity to review this interesting paper focusing on mRNA levels of stress and inflammation related markers in BD patients before and after remission and HC as it targets an intriguing research area with currently no real empirical evidence. I commend the authors for a number of strengths of their work including the inclusion of both glucorticoid, BDNF, and inflammatory markers and the comparisons of patients before and after remission. This is a neat study design. Considering these strengths, though, as I read the manuscript I found some areas in which I would have appreciated greater clarity. I believe the paper could be further strengthened by addressing the following points:

1. Abstract
   1. Please provide participants’ demographics such as age, gender and IQ (if available). Under methods please provide a general list of the clinical measures used (e.g. YMRS and please provide full name of each questionnaire before mentioning the acronym). Please add reference to the repeated measure nature of this study (blood tests taken before and after, mean number of months in between blood draws). When the authors mention that “tPA may be a biomarker of BD like BDNF” it is unclear what they mean. I assume they mean that in the literature BDNF is a well-established marker of the disease but this should be explicitly stated beforehand.
2. Title: the authors could consider reformulating the title by either referring to remitted manic patients and stress/inflammation as these are relevant keywords and points of novelty of the study.
3. Introduction
   1. Please provide references for the statement “brain responds to acute and chronic stress by changing chemistry, even morphology”.
   2. Please explain why you want to study gene expression vs levels of BDNF and other markers. A brief references would provide a more robust rationale.
   3. How long does it take for mRNA levels of these markers to change? What does previous literature suggest?
   4. The fact that neuroprogression in BD leads to BD is still speculative and no solid evidence exists at this stage. I would recommend that the authors use more cautious language “may lead/is associated to BD”.
   5. Explain why you decided to pick the reported stress/inflammatory markers and whether some of them (e.g. BDNF) have been reported in previous literature in brain function and cognition in the literature (e.g. Peruzzolo et al. 2015, Bauer et al. 2014, Aas et al. 2013, Rosenblat et al. 2015). Hint: This would help the reader explain why the authors compare tPA to BDNF in their conclusions for instance.
4. Methods
   1. Overall provide full name of all markers/questionnaires prior to providing acronyms (e.g. YMRS)
   2. Please explain why only males were selected and whether age was an inclusion/exclusion criteria
   3. Why only bipolar I?
   4. Were comorbidities such as substance use allowed? Please explain why yes/no
   5. Did the authors consider other sources of stress during the “treatment period”? e.g. divorce, marriage, employment status change, in both groups? if they did not this could be mentioned in the discussion.
   6. Which medical illnesses were excluded? e.g. TBI, epilepsy, neurological disorders, cardiovascular diseases…
   7. Was the current health state of participants assessed at the time of the blood draws? I am wondering whether viruses/cold/flu/sepsis would have been exclusion criteria for instance.
   8. The authors mention the participants’ BMI in their results and their conclusions but not in their methods. Could the authors explain if cardiovascular disorders, obesity, diabetes were exclusion criteria? Was BMI a source of concern given the nature of the study and being obesity considered an inflammatory condition (Bond et al. 2015).
   9. Did the authors assess the severity of symptoms using scales other than YMRS? If not why?
   10. Why were blood draws conducted between 8am and 10am?
   11. The abstract mentions that the authors used peripheral blood monuclear cells but the methods section does not appear to mention this. I was wondering if the authors could clarify what kind of cells they used,e.g. lymphocytes? Also have other studies used the same types of cells (if so please provide reference) or rather serum?
   12. Were blood draws conducted at time 1 done when BDs were still inpatients or outpatients?
   13. Were there exclusion/inclusion criteria regarding the “waiting time” between pre and post-remission blood draws? When did the authors conduct the second blood draw in HC? Were BDs and HCs matched in terms of time between blood draw 1 and 2?
   14. Did HCs take medication/supplements at the time of the study? If so which ones?
   15. Did the authors plan to match HC and BDs in terms of demographics for instance? Please address this in methods or discussion.
5. Analyses
   1. Please mention what kind of statistical software you used.
   2. Were results Bonferroni corrected?
   3. Did the authors consider including covariates e.g. age, gender, BMI or even time between blood draw 1 and 2 if HC and BD differed in those terms? If not why? Please discuss in methods/discussion.
6. Results
   1. I would recommend that the authors structure the results section using 3 headers: e.g. demographics/clinical, BD vs HC, and pre- and post/remission vs HC. This would improve readability and understanding of the results.
   2. When mentioning that “BDNF levels were statistically significant” please indicate if they mean they were significantly higher/lower. The authors often reported results without indicating whether the levels were higher/lower, and whether the comparison group (remission/HC). Please make this clearer.
   3. Please indicate where you would place table 1 and figure 1 in the manuscript.
   4. In table 1 please add mean/SDs for age, education, IQ (if available), clinical measures (YMRS), GAF etc. in both BD and HC. Also please provide F/X2 or T and corresponding p values/post-hoc p values.
   5. In table 2 please add a column providing p values for mania vs remission. Please indicate if these p values are corrected for multiple comparisons.
   6. Figure 1: please provide results for HCs too. In the caption please add 1 sentence summarizing the primary finding.
7. Discussion
   1. Could the authors explain how they corrected for differences in medication in the BD during treatment? How do primary BD medications affect inflammation/stress and mRNA levels of these markers? Please provide an overall take-home message.
   2. Overall discuss further how you interpret the fact that only BDNF and tPA varied between pre and post-remission. Could this be due to the time to remission? And are BDNF mediators/catalyzers for changes in the other markers such as GR, HSP70 etc. In other words do the authors think that the other markers will eventually change too?
   3. Overall I elaborate a bit further in terms of limitations, strengths, future directions and clinical implications. The authors wrote a very short paragraph about strengths and limitations and did not provide an overall conclusion. I would suggest that they shorten and tighten their discussion up. They could then have sufficient space/words available to discuss further what an ideal time to remission would be and why. Further, would BDI differ from BD II? Were remission time and ageing considered in the study design? Why would cortisol have been beneficial to the study? What about gender related differences? These are just examples of how the authors could improve their discussion
8. Highlights

Authors could discard the first highlight and rather mention that 1. They compared BD patients before and after remission; they used mRNA levels of stress/inflammatory markers

1. Overall: I would highly recommend that the authors get editing help from someone with full professional proficiency in English (a few typos and ambiguous sentences).