Author’s response to reviews

Title: Hypocholesterolemia is an independent risk factor for depression disorder and suicide attempt in Northern Mexican population

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Answers to questions from Reviewers and modifications BMC Psychiatry

Reviewer 1: Helen Ruth Daniels, Ph.D., BSc:

Thank you for your comments.

Reviewer 2: Jan Christopher Cwik, Dr.

1. The manuscript is in not line with the manuscript guidelines of BMC Psychiatry. For example the authors list multiple references in the manuscript as follows: [2,14,15,16]. Instead, these references should be listed that way: [2,14-16]. Additionally, in the reference section some references are listed inconsequently and not in line with the
journals recommendations. I would recommend using citation software and the BMC Psychiatry citation style to solve this problem.

ANSWER: Thank you for your observation Dr. Cwik, we have all the necessary modifications within the manuscript with regard to the reference style for multiple references that you have pointed out. Also, we have now modified all references to suit the reference style of BMC Psychiatry. This includes DOI references for those published in more recent years.

2. On page 3 (line 54-58) the authors state: "Although roughly 60% of all suicides occur in the context of depressive disorders [6,7] it is still challenging for clinicians to predict suicide risk in patients with depression. For this reason, increased attention has been paid to potential biomarkers for suicide in patients with major depression disorder (MDD) and suicidal behavior [1,8]". It is unclear how clinicians (especially non medical clinicians) could utilize biomarkers to predict suicide risk in their daily practice. Additionally, do the authors have any information of the content of explained variance of suicidal behavior by such biomarkers? This should be considered in the manuscript.

ANSWER: In daily clinical practice, there is a need to find accessible and more economic biomarkers for clinicians trying to predict the risk for suicide in patients with depression. Many suicides occur in the context of major depressive disorder and it continues to be a challenge to predict suicide in such patients. For this reason, increased attention should be placed on the identification of biomarkers that can be easily obtained in the general population, but with a predictive value.

3. As a second hypothesis the authors assume: "2) Hypocholesterolemia is a risk factor associated with depression and suicide attempt." As the authors correctly state in the limitations, the study design is not suitable to make any predictive assumptions. This hypothesis should be deleted or rephrased.

ANSWER: We decided to rephrase our second hypothesis to the following: Hypocholesterolemia is associated with depression and suicide attempt. We agree in that the transverse nature of our study impedes us from ascertaining that hypocholesterolemia is a risk factor for depression and suicide attempt, and it would be necessary to conduct a longitudinal study in order to prove the direction of the relationship between our two variables. However, our statistical analysis shows that these variables are clearly associated. (MDD: OR 4.229 CI 95% 2.555-7 p < .001, hypocholesterolemia and suicide attempt: OR 5.540 CI 2.824-10.866 p < .001).

4. According to the methods section, MDD was diagnosed according to DSM-5 diagnostic criteria. It is completely unclear in what way the criteria were checked? Did the authors use structured diagnostic interviews? Or self-rating questionnaires? Or open clinical interviews? This should be stated and possibly considered as a limitation, if only self-rating scales or open clinical interviews were used.

ANSWER: Diagnosis was made by trained psychiatrists at three participating hospital settings according to DSM-5 criteria. Once diagnosis was confirmed, our group applied a physical evaluation with somatometric parameters, an in-hospital interview followed by a clinical history
questionnaire. We have rephrased this information in the Methods section (page 4, line 84). Since diagnosis was made by trained psychiatrists and we applied further questionnaires and interviews that were limited to collecting data which permitted the clinical profile of the subjects. We do not feel that this was a limitation in our study.

5. The authors report that they used a multiple linear regression analysis to determine the association between hypocholesterolemia, and the presence of depression and suicide attempt. I was wondering whether the data was checked for multicollinearity?

ANSWER: We considered your suggestion to check for multicollinearity and, in an effort to determine if there was a relationship between our variables, a non-parametric Spearman's rho was used. The analysis compared our three groups (MDD, MDD with suicide attempt, and healthy controls) with hypo, normo and hyper cholesterolemia. This analysis determined a correlation coefficient of -.185 (p < .001). We did not see it pertinent to utilize other correlation coefficients like Pearsons given the categorical classification of our dependent variable.

6. In their conclusions, the authors state that their findings "… could have important implications for public health policies…". At first, facing the fact that this study has a cross-sectional design, this assumption is a bit prematurely out of my point of view. Additionally, the clinical implications of the study's results are only marginally discussed in the manuscript.

ANSWER: We have rephrased the text in the following manner: "This finding, if consistent in more studies in our population, could influence public health policies focused in the prevention of mental health disorders". Conclusions section (page 14 line 239-241) In response to your question regarding the clinical implications of the study in the Conclusion: As previously mentioned, there is currently a need to find accessible and more economic biomarkers for clinicians trying to predict the risk for suicide in patients with depression. Measuring cholesterol levels would potentially be, an inexpensive and simple way to predict suicide risk in low-income groups in our population. In hospital settings, this would permit clinicians to efficiently obtain a laboratory result that, once combined with clinical evaluations and symptoms, could permit a more timely diagnosis. We have added the clinical implications to the manuscript and have rephrased our original text regarding implications. Conclusion section (page 13 line 228 to page 14 line 236).

7. The manuscript needs some language corrections before being published. For instance check the following sentences on page 5 (line 115-119): "... Post-hoc analysis demonstrated that patients with MDD were associated with suicide attempt (p 0.000) and subjects with only MDD (p 0.000) had significantly lower total cholesterol levels than the control group. Also, patients with MDD who were associated with suicide attempt had significantly lower total cholesterol levels than subjects with only MDD (p 0.016)." Or the sentence on page 6 (line 124): "However, only in females, did we observe significant differences between MDD-only group compared with the control group". Additionally the Oxford comma should be used consequently.
ANSWER: We have rephrased the sentences that you have pointed out. These now read as follows: "Post-hoc analysis demonstrated association between patients with MDD and suicide attempt (p < .001). While, subjects with MDD only (p < .001), had significantly lower total cholesterol levels compared to the control group. Also, patients with MDD who were associated with suicide attempt had significantly lower total cholesterol levels compared to subjects with MDD only (p 0.016)." Results segment (page 5 line 118 - page 6 line 121) "However, only in women, did we observe a significant difference between the MDD-only group versus the control group". Results segment (page 6 line 133-134.) Lastly, we added the Oxford comma where needed and these are highlighted in yellow.

Minor Points:

1. Please do not write DSM-V. Since the fifth edition the APA uses Arabic numerals: DSM-5. Additionally, the reference of DSM-5 should be listed.

ANSWER: DSM-V was changed to DSM-5 in all cases and highlighted in yellow.

2. The first sentence of the background sections sounds as if suicide is a CONSEQUENCE of psychiatric treatments. Please rephrase this sentence.

ANSWER: We have changed the first sentence and it now reads as follows: "Suicide is one of the most disastrous outcomes of psychiatric disorders." We believe that this no longer suggests it is a consequence of psychiatric treatments.

3. Please do not use p = .000. This is impossible from a theoretical point of view. Instead you should write p < .001.

ANSWER: We changed all p =0.000 to p < .001 in all cases as well as in Table 1 and Table 2. These are now highlighted in yellow.

4. Title: Considering the fact that this study is based on a Mexican population exclusively, this fact should be reflected by the title of the study.

ANSWER: We have considered your suggestion and have now changed the title to: Hypcholesterolemia is an independent risk factor for depression disorder and suicide attempt in Northern Mexican population.

5. Abbreviations like OR or CI should always introduced once.

ANSWER: OR has now been introduced as Odds ratio and CI has been introduced as Confidence interval in the manuscript. Statistical Analyses segment page 5 lines 110-111. The text reads as follows: "Odds ratio (OR) and 95% confidence interval (95%CI) were determined, while p value <0.05 defined the statistical significance."
6. Statistical parameters in Latin letters (SD, p, n, OR, CI) should always be written in italic letters. Please also check the tables.

ANSWER: In Table 1 and Table 2 we changed SD, p, n, OR and CI to italic form.

7. Two references are not cited correctly: page 11, line 191-197: "da Graça et al. in 2015 ... [57]" and „2016 by Pearson et al., ... [58]".

ANSWER: We have removed the references within the text to follow citation rules of the journal using numbers only (Discussion, page 12, lines 201-205)

8. There are some typos in the manuscript, e.g., on page 11 (line 186-187): „However, others studies..." → „However, other studies...".

ANSWER: The sentence now reads as follows: "However, other studies on the association between gender and serum cholesterol have been inconclusive". Discussion (page 12 lines 196-197)

Reviewer 3 Emily Goldmann, MPH, PhD

Introduction:

- The case for this study needs to be further described. Why is it important to study the relationship of interest in this population specifically? Why might findings be different? Are these issues of particular concern in this population? Or is the goal of the study to provide further evidence on a relationship that is inconsistent in the literature? How does this study help us figure out what's really going on, i.e., how does it improve on other studies?

ANSWER: In Mexico the rate of suicide is a current health problem that is accentuated by the fact that we are a developing nation. Therefore, we feel that a biomarker that is economically within reach is needed in our population. This is both at a national level as well as a local (state) level. Rather than different results, we would expect similar results to those obtained in other nations. However, we would like to give our result a more clinical application. In a previous meta-analysis, only one study that was done in Mexico appears (Almeida-Montes et al.) It was done in 33 patients with a major depressive episode and 18 patients who attempted suicide. We therefore saw a need for more studies in our population. Mexico has presented a high suicide rate. Between 2000 to 2014 we saw an increase in the suicide rate from 3.5 to 5.2 cases per 100,000 inhabitants.

- It would be helpful to briefly summarize the hypothesized explanations for both the positive and negative associations between cholesterol levels and mental health outcomes reported in the literature. How do previous studies try to explain their findings? What are the mechanisms (briefly)?
ANSWER: In our discussion, (page 10, line 160 to page 12 line 193) we specifically mention a variety of mechanisms such as regulatory mechanisms associated with synaptic lipid rafts in suicide, the 5HT receptors that participate in neurotransmitter signalling, as well as associations with suicide through reduced 5-HT activity. Unfortunately, these mechanisms were not mentioned in our introduction. We have now added a brief explanation of these mechanisms in our introduction (page 3 lines 64 to 67) together with a reference.

Methods:

- Does the study population comprise adults (those 18 years or older) only? Please clarify.

ANSWER: All participants were above 18 years of age given that the participating hospital only admits patients who are older than this age. Any one below 18 is still treated as a pediatric patient.

- Important: The factors used for matching, as well as the exclusion criteria, need to be justified. Specifically, why did the authors match on BMI? The biggest question is why were subjects with the chronic disease listed and those taking lipid lowering drugs excluded from the study? It is possible that previous studies found a positive association between cholesterol level and MDD because high cholesterol is associated with chronic disease, which is then in turn associated with poor mental health outcomes. Does anyone in the study population have high cholesterol levels? It is possible that there is a non-linear relationship between cholesterol level and MDD (threshold effects), which may explain the inconsistent findings in the literature. This is something that the authors should consider. The exclusion criteria need to be justified given that they may make the study less generalizable and difficult to compare to other studies in terms of key findings.

ANSWER: BMI was used as a matching criteria in order to exclude nutritional state as a confounding factor. Also there is a previous association between BMI and cholesterol levels (Shamai et al., Obes Surg. 2011). We considered that statin use and its antiinflammatory effect would be a confounding factor due to the variety of effects it may have (Bu et al., Curr Opin Lipidol. 2011). Especially the effect it may have over our dependent variable: depression. (Redlich et al., BMC Psychiatry, 2014). We also excluded those with chronic diseases due to their association with dyslipidemia. (Dalal et al., Indian J Endocrinol Metab. 2012, Schofield, Diabetes Ther. 2016) We agree with your assumption that the positive correlation you mention between cholesterol level and MDD was probably associated with high cholesterol found in chronic disease and eventually poor mental health. In our studied population, we found that some participants did, in fact, present high cholesterol levels. Specifically, 82 (17.4%) of our 472 total participants presented levels above 200 mg/dL. The frequencies of the three groups (hypo, normo and hyper cholesterolemia) were as follows: 136 (28.8%) presented hypocholesterolemia levels below 150 mg/dL, 254 (53.8%) presented normocholesterolemia levels between 150 mg/dL and 199 mg/dL, and 82 (17.4%) presented levels above 200 mg/dL, as already mentioned. For the MDD with suicide attempt group, we observed the following frequencies: 29 (49.2%) presented hypocholesterolemia, 21 (35.6 %) presented normocholesterolemia and and 9 (15.3%) hypercholesterolemia. For the MDD only group, we observed the following frequencies: 79 (38.2%) presented hypocholesterolemia, 81 (39.1%) presented normocholesterolemia and 47...
(22.7%) hypercholesterolemia. Lastly, for the control group, we observed the following frequencies: 28 (13.6%) presented hypocholesterolemia, 152 (73.8%) presented normocholesterolemia, and 26 (12.6%) presented hypercholesterolemia. Chi Square Value: 64.737 (p < .001), likelihood ratio value: 66.493 (p < .001), linear-by-linear association Value: 12.296 (p < .001).

The non-linear relationship you mention between cholesterol levels and MDD threshold effects may in fact explain the inconsistent results, as you have mentioned. We agree with your assumption and would like to add that a recent model study associated hypercholesterolemia with depression in hypercholesterolemic mice via monoaminergic metabolism. They found increased monoamine oxidase (MAO) A and B activity in the hippocampus of mice (Engel et al. Behav Brain Res. 2016), (Svensson et al. Acta Psychiatr Scand. 2017). Therefore, both elevated and decreased levels of cholesterol are able to produce depression, via independent mechanisms.

Our exclusion criteria included hepatic disorders, diabetes mellitus, hypertension, and cardiovascular disease. We included these criteria because in hepatic disorders, the synthesis of cholesterol would be affected. As for diabetes mellitus, hypertension and cardiovascular disease, all three have a previous association with dyslipidemia. It is for these reasons that we decided to exclude them from our study.

- In the DSM 5, I believe it's "major depressive disorder" not "major depression disorder"

ANSWER: We have made the change from major depression disorder to major depressive disorder. (Abstract, page 2, line 37), (Background, page 3, line 56).

- Please describe how suicide attempt was defined/measured in this study

ANSWER: -For our purposes, we utilized the CDS's definition of suicide attempt: "a self-directed, potentially injurious behavior with an intent to die". For our recruitment, we considered only participants that required hospitalization.

- If presence of MDD and suicide attempt are the dependent variables, I assume they are dichotomous, which would mean logistic regression, not linear regression, was used

ANSWER: We agree with your comment and we confirm that we have mistakenly written multiple linear regression on line 105 when in fact it was logistic binary regression. We have made the correction in the text and thank you for observing this error. Statistical Analyses (page 5, line 105)

- Important: How was matching accounted for in the analysis? Was McNemar's test used? Or conditional logistic regression models? Is that what was meant by "adjusted" and "multiple" when describing the regressions? The study design needs to be reflected in the analysis or, if it has been, this needs to be made more clear. Were regression models adjusted for any additional variables, besides the matching variables? In the models comparing those with and without suicide attempt, depressive symptom severity might be a confounder...
ANSWER: In answer to your question, we used logistic regression models and that is the reason why we used the terms "adjusted" and "multiple" when describing the regressions. Hypcholesterolemia was our independent variable and depression and suicide attempt was our dependent variable. Based on your suggestion have now modified the description of our statistical analysis based on the design of the study. (page 5, lines 105-107)

There were no additional variables considered in the adjusted regression models. Severity levels were determined using Hamilton depression scores and have now been analyzed and consider that our lack of adjustment for severity is a limitant of our study.

Results

- Table 1 findings need to be further described in the text. It looks there are some conflicting results -- for some cholesterol variables, levels are lower among those with MDD and suicide attempt; for others, levels are higher. The authors might also explain why all of these different cholesterol variables were assessed in this study. Did the authors hypothesize that there might be different relationships with the outcome? The paper also focuses primarily on hypocholesterolemia when other cholesterol variables were also examined...

ANSWER: The description of Table 1 in the manuscript has been modified. We have now added data related to LDL, VLDL and hypocholesterolemia between groups. (Results, page 6, lines 118-128). We have made modifications in the manuscript with regard to triglycerides and LDL levels in our results (Results, page 11, line 200-201), (Results, page 11, line 205). We also made modifications in the text regarding LDL levels and have added an additional reference (Results, page 11, lines 204-210). Our hypothesis refers to serum lipid levels in general and not exclusively to total cholesterol. We therefore, expected similar results to those previously reported. On pages 12 and 13, lines 204 to 214, we justify the reasons why we analyzed a variety of other parameters such as triglycerides, HDL and LDL. Several studies are cited within this justification. Although hypocholesterolemia is the main focus, the references cited made us consider those other variables in our study design and analysis. Others such as VLDL were included in the analysis simply to complement the lab lipid profile results that were reported.

- Analysis stratified by sex should be described in the Methods under Statistical Analysis

ANSWER: Analysis stratified by sex has now been mentioned in the Methods section under the statistical analysis section (page 5, line 105).

- p.6 line 127: did the authors mean "showed significant differences in both genders (p<0.05)" instead of p>0.05?

ANSWER: Thank you for your observation. We meant to say (p<0.05). This correction has been made in the text (page 6, line 136).
- Regression model results only presented for hypocholesterolemia as the independent variable. What about the other cholesterol variables?

ANSWER: Our regression model analyses have now included the other variables. Triglycerides were analyzed and we found the following Odds Ratios and 95% Confidence Intervals: for hypertriglyceridemia in MDD [3.528 (2.326-5.352); p < .001]; for hypertriglyceridemia in MDD with suicide attempt [2.626 (1.411-4.885); p = 0.002]. This finding coincides with a study that found a correlation between depressive symptoms and triglyceride levels (Huang et al., Psychiatry Clin Neurosci., 2004) and suggestions by others that postulate that high triglyceride levels are associated with Type A personality traits, such as hostility, anger and domineering attitudes (Tzavellas et al., JAMA, 2009). Upon further analysis, no significant association in all other variables analyzed was found. Our results have been anexed to our statistical analysis section (pages 6-7, line 143-147).

Discussion:

- p. 11 line 190, "Our results showed lower levels of triglycerides in subjects with MDD..." -- This is not what is presented in Table 1; it looks like it is the opposite.

ANSWER: Thank you for your observation. This correction has been made in the text.

- VLDL levels seem to be higher in MDD as well; this is not discussed

ANSWER: We have now added a brief discussion about VLDL results as well as a reference. Discussion (page 11, lines 207-210).

- p. 12 line 204: the study design is case-control not cross-sectional. Perhaps the authors meant that all measures in the study are prevalent not incident and timing was not assessed, so there is some temporal ambiguity.

ANSWER: We agree with your suggestion that it be changed to a cases vs. controls study. We initially considered it as a cross-sectional study because of the fact that we had three groups and not the traditional two group analysis. We have made the change accordingly. Methods (page 4, line 78) and Discussion(page 13, line 221).

- It is important to note whether previous studies also excluded individuals with chronic disease and those who were taking lipid lowering medications -- this may make comparison between previous studies' findings and the findings from this study difficult

ANSWER: We have taken your suggestion into account and have reviewed previous studies and exclusion criteria including chronic diseases and lipid lowering medication treatment. As previously mentioned, we found it necessary to apply the exclusion criteria the way we did because in hepatic disorders, the synthesis of cholesterol would be affected. In diabetes mellitus, hypertension and cardiovascular disease, there is a reported association with dyslipidemia. For example, a previous study by Maes et al. Acta Psychiatr Scand. 1997 excluded those taking lipid lowering drugs and metabolic disorders.
p. 12 line 208 -- is there evidence of this in the literature? Please provide a reference.

ANSWER: We have added a reference to support this information (Bretillon et al., J Lipid Res. 2000). Discussion (page 12, lines 225-226).

- p. 12 lines 211-214: Not clear what is being said. Please clarify.

ANSWER: We considered your suggestion and have rephrased the text with hope that it will be more clear. Discussion (pages 12 and 13, line 228-236).

- The authors state that subjects in the study were from the same sociocultural and economic background. Can the authors confirm that there is really no heterogeneity among subjects in terms of socioeconomic status? This could be an important confounder, though perhaps not available in the dataset.

ANSWER: All participating subjects received (had a right to) access to similar health care options depending on the type of job they held. This in itself is an approximation of their socioeconomic status and was used in the pairing process between cases and controls.

- How does exclusion of those taking lipid lowering drugs control for potential bias? What bias? This should be clarified in the Methods.

ANSWER: Statins' properties (anti inflammatory and antioxidant) could affect both the dependent (depression) and independent variables (cholesterol). As far as which bias, we consider that these properties of statins could generate confusion (confounding factors) when compared to a physiological reduction or increase in cholesterol levels that is not influenced by medication.

- Generalizability is also a limitation of the study given the exclusion criteria and specific characteristics of the study population

ANSWER: It is evident that these results may be extrapolated only to a population that is similar to our own with similar exclusion criteria. This may be considered as another limitation of our study. Discussion (page 13, line 232-233).

TABLES:

Tables:

- Where are the percents for female/male distribution?

ANSWER: Percentages have now been added for female/male distribution.

- Decimal places should be consistent
ANSWER: Decimal spaces have been checked for inconsistencies. With the exception of p values and the rest of the numerical values we did not find inconsistencies in the decimal places in both tables.

- What comparison is the p-value column reporting? There were three tests for significant differences, but only one p-value is shown.

ANSWER: P Values were determined by ANOVA. All additional symbols represent Bonferroni post-hoc test. This has been added in the Statistical Analysis description (page 5, line 103-104).

- Any p=0.000 should be presented as p<0.001

ANSWER: P Values were changed to p < .001 in tables and in the text.