Author’s response to reviews

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

Authors:

Marcela Segoviano (marcela_segoviano@hotmail.com)
Manuel Cárdenas (long_xue_@hotmail.com)
José Salas (jsalas_pacheco@hotmail.com)
Osmel La Llave (ollave56@yahoo.es)
Marcelo Barraza (sbmaj30@hotmail.com)
Francisco Castellanos (xavier_castellanos@hotmail.com)
Fernando Vázquez (feralaniz1@hotmail.com)
Jazmin Méndez (jazminedmeh@gmail.com)
Ernesto Miranda (ernesto.miranda@mail.com)
Oscar Arias (arias@ciencias.unam.mx)
Edna Méndez (edna_madai@hotmail.com)

Version: 2 Date: 08 Nov 2017

Author’s response to reviews:

Reviewer 1, Dr. Ruth

Reviewer reports:

Helen Ruth Daniels, Ph.D., BSc (Reviewer 1): Please include all comments for the authors in this box rather than uploading your report as an attachment. Please only upload as attachments annotated versions of manuscripts, graphs, supporting materials or other aspects of your report which cannot be included in a text format.

Please overwrite this text when adding your comments to the authors.

ANSWER: Our group appreciates your previous comments.
Reviewer 2, Dr. Cwik

Jan Christopher Cwik, Dr. (Reviewer 2): I think the authors have dealt with the reviewers comments in a thoughtful and appropriate way which has enhanced the quality of the paper. I believe this paper should now be published.

ANSWER: Our group appreciates your previous and current questions and comments.

Reviewer 3, Dr. Goldman

Emily Goldmann, MPH, PhD (Reviewer 3): Dear Authors,

I would like to commend you on a thoughtful and thorough revision of this manuscript. There are still a few remaining issues that need to be addressed, in my opinion, as follows:

QUESTION 1.- You provide excellent responses to reviewer comments, particularly where the reviewer has asked for clarification or additional information. However, some of this information has not been added to the text, including:

- The additional rationale for conducting this study in this specific population. You make an excellent point about the high suicide rate (which is also increasing) in Mexico; this helps make the case for why this study is important and should be included in the Introduction's text

ANSWER: Dear Dr. Goldman, thank you for your most recent questions, comments, and suggestions. We have made an effort to address all of them as best as possible. Regarding your comment about suicide rate in Mexico, we have included a segment of our previous answer within the newly revised manuscript. We have also included a recent reference (a recently visited web site) to support our statement. The text we have added to the manuscript reads as follows:

"In Mexico the rate of suicide is a current health problem that is accentuated by the fact that it is a country with an emerging market economy. In recent years, Mexico has presented an increase in its suicide rate. For example, between 2000 and 2014 there was an increase in the suicide rate from 3.5 to 5.2 cases per 100,000 inhabitants."


- Please add to the Methods that this is an adult population. The reader will not know what the admitting practices are at the included hospitals.

ANSWER: In response to your comment, we have added the word "adult" according to your recommendation. You will now be able to find "adult patients" and "healthy adult volunteer controls" within the manuscript (Methods page 4, line 84 and line 85).
The authors provide good rationale for the matching variables and exclusion criteria in responses to the reviewer. It would be helpful to include some of this information in the manuscript as well in the Methods (rationale for matching and exclusion criteria) and discussion of non-linear relationship (Discussion)

ANSWER: We have taken your suggestion and comment into consideration and have now added the following text to the Methods section:

"The latter, in order to exclude nutritional state as a confounding factor and due to previous association between BMI and cholesterol levels." (Shamai et al., Obes Surg. 2011) [21].

"Use of lipid lowering drugs, such as Statins was considered a confounding factor due to the diverse effects it may have on cellular mediation of inflammation and immunity. (Bu et al., Curr Opin Lipidol. 2011) [22] in conjunction with its known effect on depression (Redlich et al., BMC Psychiatry, 2014) [23] and was, therefore, an exclusion criterion. Lastly, we excluded those with chronic diseases (hepatic disorders, diabetes mellitus, hypertension, cardiovascular disease) due to their association with dyslipidemia." (Dalal et al., Indian J Endocrinol Metab. 2012 [24], Schofield, Diabetes Ther. 2016) [25].

In response to your suggestion, we have added the following information to the manuscript (Discussion):

"A lack of consistency between different published reports coupled with the fact that, to date, it has not been possible to identify a cholesterol threshold level capable of precipitating a psychiatric disorder, suggests the presence of a non-linear relationship.

The existence of reports in which depression has been associated with increased cholesterol levels would support this hypothesis. A possible explanation for this, proposed the involvement of monoamine oxidase (MAO). The aforementioned model studies associated hypercholesterolemia with depression in hypercholesterolemic mice via monoaminergic metabolism. Specifically, they reported increased monoamine oxidase (MAO) A and B activity in the hippocampus of mice (Svensson et al. Acta Psychiatr Scand. 2017) [34], (Engel et al. Behav Brain Res. 2016) [57]. Thus providing one possible reason why elevated levels of cholesterol are able to produce depression much like decreased levels levels are able to, but via independent mechanisms. " (Discussion, pages 12-13, lines 214-223).

Please add how suicidal attempt was defined/measured to the Methods section

e). ANSWER: We defined suicide attempt as "a non-fatal, self-directed, potentially injurious behavior with an intent to die as a result of the behavior" as also defined by the Center for Disease Control and Prevention.
Reference: Violence Prevention.

This definition has now been included in the manuscript (Methods, page 4, lines 91-94).

Question 2. Thank you for revising the p-values as both reviewers suggested (p<0.001 instead of p=0.000). There is one remaining instance of p=0.000 on p. 6 line 132

ANSWER: Thank you for your observation Dr. Goldman, we have now changed the previously found (p 0.000) to (p < .001). (Results, page 7, lines 143-44).

Question 3. I'm not sure that the statistical analysis is fully appropriate given the matched design. I previously inquired if CONDITIONAL logistic regression and McNemar's test were used, as (to my knowledge) these are approaches to analyzing matched data, but I do not see a response about this from the authors. This is different from "adjusted" or multiple regression, where regression models include covariates, in addition to the independent variable. This is an important issue that I feel must be addressed prior to publication, as this could influence the study's results. It may be helpful to speak with a biostatistician. Also, no need to say "logistic binary regression"; "logistic regression" is sufficient. Additionally, p. 6 line 141: these are not bivariate regressions adjusted for age, sex and BMI; they are bivariate logistic regressions using data matched on age, sex, and BMI (and, as mentioned above, should be conditional logistic regressions).

ANSWER: With respect to your contribution suggesting the improvement of our statistical analysis, we would like to say that we have discussed this topic with a biostatistician. In conjunction with him, it was determined that the use of McNemar’s test is pertinent when comparing proportions between related samples. For example, when comparing the proportion of the same measured characteristic (on more than one occasion) for each one of the individuals or when comparing the frequency of a categorical condition before and after an intervention within the same group. Given the fact that our study groups do not correspond to related samples, but rather, to independent samples, we decided to utilize the Chi- squared test.

With respect to your suggestion to utilize conditional logistic regression, we here document that in case-control designs, the estimates for coefficients and variance matrix (parting from the functions of conditional and non-conditional verosimilarity) produce the same results. This is the case if and when the probabilities of selecting individuals in both groups are independent of the independent variables of the models. This condition becomes difficult to accomplish when a sample size is small. However, in case-control studies having a large sample size (as is the case with our study) a non-conditional estimation may be utilized.

In response to your suggested modification, we have changed the phrase "logistic binary regression" to "logistic regression" according to your recommendation. Also you will now be able to find " bivariate logistic regressions using data matched on " in place of "bivariate regressions…” within the manuscript (Methods, page 6, lines 121 and Methods, page 7, line 153).
Question 4. In the authors’ response to the reviewer comment on socioeconomic background (given that SES is likely a confounder of the relationship of interest), the authors state: "All participating subjects received (had the right to) access to similar health care options depending on the type of job they had" -- This suggests that subjects had different occupations, and therefore different SES... Then, the authors state: "This in itself is an approximation of their socioeconomic status and was used in the pairing process between cases and controls". I do not see any SES variable (e.g., occupation) included as a matching variable -- only age, sex, and BMI -- in the Methods. Was this another variable on which cases and controls were matched? This needs to be clarified in the text. If cases and controls were not matched on an SES variable, the authors should consider adding this variable as a covariate in regression analysis, as SES is a potential confounder here.

ANSWER: Our apologies for not having answered this question clearly in the previous revision. We would therefore, like to clarify our previous answer regarding socioeconomic background. Seguro Popular is a fairly recent model for health insurance in Mexico that belongs to our Social Protection in Health System and was founded in 2004. It’s main objective is to finance health services to all individuals who are not affiliated to other social security services. In our previous response to your question, we made reference to the fact that "all participating subjects received (had a right to) access to similar health care options depending on the type of job they had". Since all participants belong to Seguro Popular, it is understood (in our country) that they also belong to a low income group. All individuals who depend on Seguro Popular, are attended at designated clinics and/or General Hospitals, but do not have access to commonly used social security services such as IMSS (Instituto Mexicano de Seguro Social) or ISSSTE (Instituto de Seguridad y Servicios Sociales de los Trabajadores del Estado), normally reserved for government employees or for those who receive a salary. In addition, those with Seguro Popular are clearly unable to afford the cost of Mexico’s private health care system. Seguro Popular was designed to cover medical costs that occur under the most adverse conditions to families that receive low incomes and are uninsured. As previously mentioned, belonging to Seguro Popular is in itself an approximation of their (low) socioeconomic status.

With the objective of complementing the analysis of this variable, we applied a comparative analysis of occupational variables, education level, and marital status between the study groups and were able to observe the following frequencies:

Marital status: Healthy controls (single 8%, married 68%, widowed 0%, divorced 8%, and free union 16%), subjects with MDD (Single 11.4%, married 48.6%, widowed 5.7%, divorced 8.6%, and free union 25.7%) and in the MDD group associated with suicide attempt (single 13.5%, married 62.2%, widowed 0%, divorced 10.8%, and free union 13.5%) p 0.568.

Educational level: Healthy controls (uneducated 0%, primary school 11.1%, middle school 74.1%, high school 14.8%, professional 2.8%, and postgraduate 0%), group with MDD (uneducated 2.9%, primary school 14.3%, middle school 60.0%, high school 8.6%, professional career 11.4%, and postgraduate 2.9%), and in the MDD group with suicide attempt (uneducated 0%, primary school 13.9%, middle school 58.3%, high school 25%, professional career 2.8%, and postgraduate 0%), p 0.291.
Occupation: Healthy controls (unemployed 0%, housewife 48.1%, employed 11.1% and self-employed 40.7%), group with MDD (unemployed 2.9%, housewife 40%, employed 28.6% and self-employed 28.6%) and in the MDD group with suicide attempt (unemployed 2.7%, housewife 29.7%, employed 29.7%, and self-employed 37.8%), p 0.474.