

Your review report

Manuscript

Estimation of Apolipoprotein E Genotypes and its Serum Concentration among Rheumatoid Arthritis Patients

Feedback for the author(s)

Comments to the author(s)

After conducting a thorough review of the manuscript titled "Estimation of Apolipoprotein E Genotypes and its Serum Concentration among Rheumatoid Arthritis Patients" with Submission ID 6fe986ca-419a-4af2-b343-8f1d5f5a899a, I have identified several areas that require attention and improvement:

1. The sample size is too small. Could you please provide the equation used to calculate the sample size?
2. It is essential to specify the diagnostic criteria for rheumatoid arthritis (RA) and include a reference to the relevant diagnostic guidelines.
3. In the "2.3.1. Detection of the APOE Gene Polymorphism" section, you have chosen 49 participants from a total of 84. Could you explain the rationale behind this selection? Since the initial sample size is already small, it seems that further reducing the sample may compromise the study's reliability.
4. In the "Primers Selection and Design" section, it appears that you did not design the primers as stated. Please remove any references to primer design throughout the manuscript. Additionally, in this section, you wrote "(rs SNP)"—this phrasing is unclear and should be rephrased for clarity.
5. Sections titled "2.3.2.1. Principle of the Assay" and "2.3.2.2. Procedure:" should be removed. Instead, you can simply state: "The determination of serum ApoE was performed using the ApoE ELISA kit (Manufacturer's name, Country) following the manufacturer's protocol."
6. Please provide the number and date of approval from the ethics committee.
7. In the "2.5. Statistical Analysis" section:
 - It appears that there are multiple mistakes and errors in the statistical analysis. Could you clarify how you determined if the data met the assumptions of parametric tests? Was a normality test conducted? Additionally, in the ANOVA analysis, were post-hoc tests performed? If so, please specify which post-hoc test was deemed suitable for your study.
 - Please provide detailed information on how you calculated the area under the curve (AUC) and created the receiver operating characteristic (ROC) curve. It would be helpful to include sensitivity, specificity, cut-off values, positive predictive value, and negative predictive value related to this analysis.

8. The section starting with "A total of 60 RA patients and 24 control were examined for ApoE, RF, ACCP, and CRP concentrations, while for assessing the APOE alleles and genotypes, 49 participants were selected from 84 participants (33 patients and 16 controls)" should be moved to the appropriate section dedicated to materials and methods.
 9. Table 4 should establish a correlation between serum ApoE levels and its corresponding genotype, similar to the correlation provided for RF, ACCP, and CRP in the table.
 10. In all tables, footnotes should thoroughly explain the information to ensure a comprehensive understanding without the need to refer back to the main text.
 11. Table 5 lacks important information, such as the p-value and odds ratio. It is crucial to include these values in the table and indicate whether this polymorphism is a protective factor or a risk factor. Please incorporate these details into the table.
 12. In all figures, please provide detailed figure captions that include relevant information, such as the T-test and any necessary abbreviations. The captions should be easily understandable without needing to refer to the accompanying text.
 13. In figures 1 and 2, please replace the number of patients with the p-value.
 14. In the "3.7. ROC Curve of ApoE" section, please add sensitivity, specificity, cut-off values, positive predictive value, and negative predictive value related to this analysis.
 15. In figure 4, the caption is unclear. Please provide a detailed explanation of the band size.
 16. On page 14, in paragraph 3, you mentioned that "This study's findings indicated that BMIs are risk factors for RA," but since it is statistically insignificant in your results, it cannot be considered a risk factor.
 17. On page 15, in paragraph 2, you mentioned the word "protective" twice. However, without an odds ratio, it becomes challenging to determine whether it is a risk or protective factor.
 18. On page 15, in paragraph 3, you wrote, "In the current study, the concentration of ApoE3 was slightly higher than the other genotypes." However, you have determined the serum ApoE concentration overall, not specifically ApoE3.
 19. On page 16, in paragraph 1, in the section "because of its high sensitivity and specificity," please clarify how you determined the sensitivity and specificity, as you only have the AUC.
- Overall, the sample size is insufficient, the statistical analysis has been conducted incorrectly, the discussion lacks strength, and the quality of English writing needs improvement.

Confidential feedback for the Editor

Your recommendation

- **Revise**

Is the study design appropriate to answer the research question (including the use of appropriate controls), and are the conclusions supported by the evidence presented?

- ***No, and there are fundamental issues that cannot be addressed***

Comments

The study design, interpretation of ApoE as a diagnostic biomarker, and regarding BMI as a risk factor require careful reassessment. Addressing these concerns will strengthen the

validity and reliability of the study and ensure that the conclusions are supported by the evidence presented.

Are the methods sufficiently described to allow the study to be repeated?

- ***No, and there are fundamental issues that cannot be addressed***

Comments

sample size utilized in the study is inadequate, which may impact the reliability and generalizability of the findings.

Is the use of statistics and treatment of uncertainties appropriate?

- ***No***

Comments

The statistical analysis has been conducted incorrectly, compromising the validity of the results.

Is the presentation of the work clear?

- ***No, it's not suitable for publication unless extensively edited***

Comments

The authors' presentation of the work lacks clarity as they base their decisions and conclusions on a comparison with other studies.

Are the images in this manuscript (including electrophoretic gels and blots) free from apparent manipulation?

- ***No***

Comments

The gel exhibits several unwanted extra bands, indicating a lack of primer specificity. The figures were presented inadequately, lacking informative captions and p-values.

Confidential comments to the Editor

Thank you for choosing me to revise manuscript, entitled "Estimation of Apolipoprotein E Genotypes and its Serum Concentration among Rheumatoid Arthritis Patients," with Submission ID 6fe986ca-419a-4af2-b343-8f1d5f5a899a.

After carefully reviewing the manuscript, I have identified several areas that require attention and improvement. Firstly, the sample size utilized in the study is inadequate, which may impact the reliability and generalizability of the findings. Additionally, the statistical analysis has been conducted incorrectly, compromising the validity of the results.

Moreover, the discussion section lacks sufficient depth and fails to provide a comprehensive interpretation of the findings. Furthermore, the quality of English writing throughout the manuscript is subpar and requires substantial improvement for clarity and coherence.

I recommend addressing these issues before resubmitting this manuscript. Specifically, consider increasing the sample size to enhance statistical power and ensure more robust conclusions. Additionally, revising the statistical analysis methods and seeking appropriate assistance in this regard is crucial.

Regarding the discussion section, the authors should expand upon the implications of your findings, explore possible mechanisms, and discuss any limitations of the study. This will strengthen the overall impact and relevance of your research.

Lastly, I strongly suggest seeking professional editing or proofreading services to improve the overall quality of the English writing in this manuscript. The authors should clear and concise language will significantly enhance the readability and understanding of your work.

Thank you again for considering these recommendations, and I look forward to reviewing the revised version of this manuscript.