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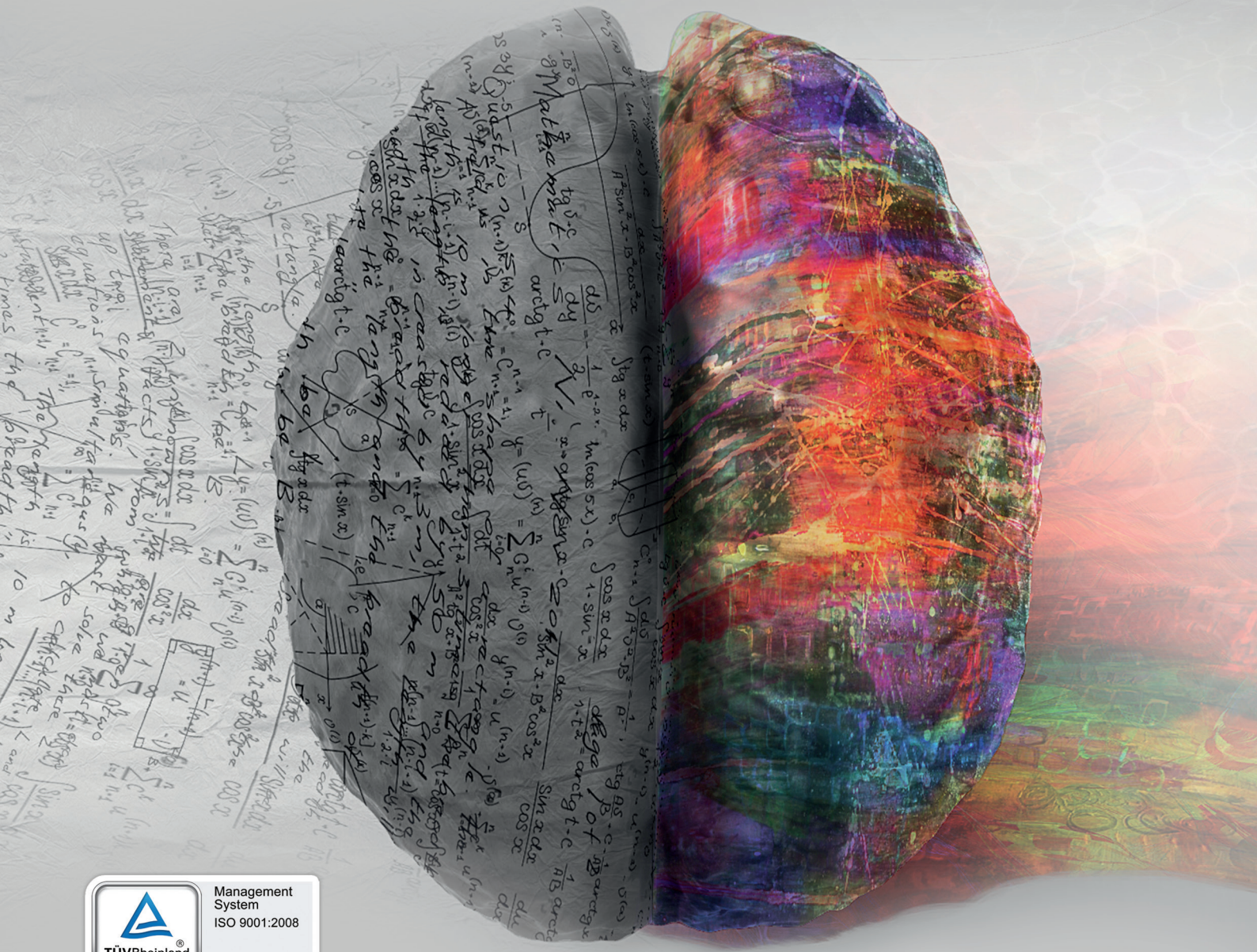

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**The Journal of Neurobehavioral Sciences (JNBS) is a peer-reviewed open-access neuroscience journal without any publication fees.
**JNBS published both electronically and hard copy printed forms 3 times a year by Uskudar University.
**JNBS accepts articles written in English language.

ABOUT THIS JOURNAL

Publication Policy

The Journal of Neurobehavioral Sciences (J Neuro Behav Sci) is a peer-reviewed open-access neuroscience journal without any publication fees. All editorial costs are sponsored by the Üsküdar University Publications and the Foundation of Human Values and Mental Health. Each issue of the Journal of Neurobehavioral Sciences is specially commissioned, and provides an overview of important areas of neuroscience from the molecular to the behavioral levels, delivering original articles, editorials, reviews and communications from leading researchers in that field. JNBS is published electronically and in the printed form 3 times a year by Uskudar University. The official language of JNBS is English. The average time from delivery to first decision is less than 30 days. Accepted articles are published online on average on 40 working days prior to printing, and articles are published in print at 3-6 months after acceptance. Please see our Guide for Authors for information on article submission. If you require any further information or help, please email us (jnbs@uskudar.edu.tr)

Aims & Scope

JNBS (J. Neuro. Behav. Sci) is a comprehensive scientific journal in the field of behavioral sciences. It covers many disciplines and systems (eg neurophysiological, neuroscience systems) with behavioral (eg cognitive neuroscience) and clinical aspects of molecules (eg molecular neuroscience, biochemistry), and computational methods in health.

The journal covers all areas of neuroscience with an emphasis on psychiatry and psychology as long as the target is to describe the neural mechanisms underlying normal or pathological behavior. Pre-clinical and clinical studies are equally acceptable for publication. In this context; the articles and treatment results of computational modeling methods of psychiatric and neurological disorders are also covered by the journal.

JNBS emphasis on psychiatric and neurological disorders. However, studies on normal human behavior are also considered. Animal studies and technical notes must have a clear relevance and applicability to human diseases. Case Reports including current neurological therapies or diagnostic methods are generally covered by JNBS.

Besides; The scope of JNBS is not limited to the abovementioned cases, and publications produced from the interdisciplinary studies established in the following fields and with the behavioral sciences are included in the studies that can be published in JNBS.

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• System neuroscience
• Molecular neuroscience
• Computational Neuroscience
• Neuromodulation, Neurolinguistic, Neuromarketing
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• Computational and simulation methods and interdisciplinary applications in medicine
• Artificial Intelligence (AI) and interdisciplinary applications in medicine
• Brain imaging
• In vivo monitoring of electrical and biochemical activities of the brain
• Molecular Biology
• Genetics
• Bioinformatics
• Psychiatric Nursing

INSTRUCTIONS FOR AUTHORS

Prior to submission, please carefully read and follow the submission guidelines entailed below. Manuscripts that do not conform to the submission guidelines may be returned without review.

Submission

Submit manuscripts electronically (.doc format with including all figures inside) via the online submission system of our website (https://review.jow.medknow.com/jnbs).

Assoc. Prof. Dr. Turker Tekin Erguzel, Ph.D Co-Editor, Journal of Neurobehavioral Sciences Department of Psychology

Uskudar University Altunizade Mh., Haluk Türksoy Sk No: 14, İstanbul-Turkey

General correspondence may be directed to the Editor's Office.

In addition to postal addresses and telephone numbers, please supply electronic mail addresses and fax numbers, if available, for potential use by the editorial and production offices.

Masked Reviews

Masked reviews are optional and must be specifically requested in the cover letter accompanying the submission. For masked reviews, the manuscript must include a separate title page with the authors' names and affiliations, and these ought not to appear anywhere else in the manuscript. Footnotes that identify the authors must be typed on a separate page. Make every effort to see that the manuscript itself contains no clues to authors' identities. If your manuscript was mask reviewed, please ensure that the final version for production includes a byline and full author note for typesetting.

Similarity Rate: The similarity of the submitted articles with the Ithenticate program is determined. The similarity rate should be below 20%.

Types of Articles: Brief Reports, commentaries, case reports and minireviews must not exceed 4000 words in overall length. This limit includes all aspects of the manuscript (title page, abstract, text, references, tables, author notes and footnotes, appendices, figure captions) except figures.

Brief Reports also may include a maximum of two figures.

For Brief Reports, the length limits are exact and must be strictly followed. Regular Articles typically should not exceed 6000 words in overall length (excluding figures).

Reviews are published within regular issues of the JNBS and typically should not exceed.

10000 words (excluding figures)

Cover Letters

All cover letters must contain the following: A statement that

the material is original —if findings from the dataset have been previously published or are in other submitted articles, please include the following information:

*Is the present study a new analysis of previously analyzed data? If yes, please describe differences in analytic approach.

*Are some of the data used in the present study being analyzed for the first time? If yes, please identify data (constructs) that were not included in previously published or submitted manuscripts.

*Are there published or submitted papers from this data set that address related questions? If yes, please provide the citations, and describe the degree of overlap and the unique contributions of your submitted manuscript.

*The full postal and email address of the corresponding author; *The complete telephone and fax numbers of the same;

*The proposed category under which the manuscript was submitted;

*A statement that the authors complied with APA ethical standards in the treatment of their participants and that the work was approved by the relevant Institutional

Review Board(s).

*Whether or not the manuscript has been or is posted on a web site;

*That APA style (Publication Manual, 6th edition) has been followed;

*The disclosure of any conflicts of interest with regard to the submitted work;

*A request for masked review, if desired, along with a statement ensuring that the manuscript was prepared in accordance with the guidelines above.

*Authors should also specify the overall word length of the manuscript (including all aspects of the manuscript, except figures) and indicate the number of tables, figures, and supplemental materials that are included.

Manuscript Preparation

Prepare manuscripts according to the Publication Manual of the American Psychological Association (6th edition).

Review APA's Checklist for Manuscript Submission before submitting your article. Double-space all copy. Other formatting instructions, as well as instructions on preparing tables, figures, references, metrics, and abstracts, appear in the Manual.

Below are additional instructions regarding the preparation of display equations and tables.

Display Equations

We strongly encourage you to use MathType (third-party software) or Equation

Editor 3.0 (built into pre-2007 versions of word) to construct your equations, rather than the equation support that is built into Word 2007 and Word 2010. Equations composed with the built-in Word 2007/Word 2010 equation support are converted to low-resolution graphics when they enter the production process and must be rekeyed by the typesetter, which may introduce errors.

To construct your equations with MathType or Equation Editor 3.0:

Go to the Text section of the Insert tab and select Object.

Select MathType or Equation Editor 3.0 in the drop-down menu.

If you have an equation that has already been produced using Microsoft Word 2007 or 2010 and you have access to the full version of MathType 6.5 or later, you can convert this equation to MathType by clicking on MathType Insert Equation. Copy the equation from Microsoft Word and paste it into the MathType box. Verify that your equation is correct, click File, and then click Update. Your equation has now been inserted into your Word file as a MathType Equation.

Use Equation Editor 3.0 or MathType only for equations or for formulas that cannot be produced as word text using the Times or Symbol font.

Tables

Use Word's Insert Table function when you create tables. Using spaces or tabs in your table will create problems when the table is typeset and may result in errors.

Abstract and Keywords

All manuscripts must include an English abstract containing a maximum of 250 words typed on a separate page. (It should contain headings such as Background, Aims and Objectives, Materials and Methods, Results, Conclusion etc.) After the abstract, please supply up to five keywords or brief phrases.

References:

Vancouver is a numbered referencing style used in JNBS.

Citations to someone else's work in the text, indicated by the use of a number. A sequentially numbered reference list at the end of the document providing full details of the corresponding in-text reference.

General rules of in-text citation:

- A number is allocated to a source in the order in which it is cited in the text. If the source is referred to again, the same number is used.
- Use Arabic numerals (1,2,3,4,5,6,7,8,9).
- Either square [ ] or curved brackets ( ) can be used as long as it is consistent.
- In the publication, source numbers are indicated in parentheses or as superscripts at the end of the sentence - name - in which the source is used.
- If the sources with consecutive numbers are to be displayed at the same time, the first and last numbers are separated with "-"

According to some estimates, the prevalence of ADHD has increased up to 30% in the last 20 years.[1]
S variant is associated with the lower transcriptional activity of the promoter when compared to the L variant.[4,7-9,11]

The Reference Section:

• Journal Article:

Russell FD, Coppell AL, Davenport AP. In vitro enzymatic processing of radiolabelled big ET-1 in human kidney as a food ingredient. Biochem Pharmacol 1998;55(5):697-701. doi: 10.1016/s0006-2952(97)00515-7.

Gonen, M. Planning for subgroup analysis: a case study of treatmentmarker interaction in metastatic colorectal cancer. Controlled Clinical Trials 2003;24 : 355-363. doi: 10.1016/s0197-2456(03)00006-0.

• Authored Book:

Lodish H, Baltimore D, Berk A, Zipursky SL, Matsudaira P, Darnell J. Molecular cell biology. 3rd ed. New York: Scientific American; 1995.

Millares M, editor. Applied drug information: strategies for information management. Vancouver: Applied Therapeutics, Inc.; 1998.

Figures

Graphics files are welcome if supplied as Tiff, EPS, or PowerPoint files. Multipanel figures (i.e., figures with parts labeled a, b, c, d, etc.) should be assembled into one file.

The minimum line weight for line art is 0.5 point for optimal printing

PUBLICATION ETHICS AND PUBLICATION MALPRACTICE STATEMENT (ETHICAL GUIDELINES FOR PUBLICATION)

The publication of an article in the peer-reviewed journal JNBS is an essential building block in the development of a coherent and respected network of knowledge. It is a direct reflection of the quality of the work of the authors and the institutions that support them. Peer-reviewed articles support and embody the scientific method. It is therefore important to agree upon standards of expected ethical behaviour for all parties involved in the act of publishing: the author, the journal editor, the peer reviewer, the publisher and the society of society-owned or sponsored journals.

Uskudar University, as publisher of the journal, takes its duties of guardianship over all stages of publishing extremely seriously and we recognise our ethical and other responsibilities.

We are committed to ensuring that advertising, reprint or other commercial revenue has no impact or influence on editorial decisions. In addition, Editorial Board will assist in communications with other journals and/or publishers where this is useful to editors. Finally, we are working closely with other publishers and industry associations to set standards for best practices on ethical matters, errors and retractions - and are prepared to provide specialized legal review and counsel if necessary.

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(These guidelines are based on existing COPE's Best Practice Guidelines for Journal Editors.)

Reporting standards

Authors of reports of original research should present an accurate account of the work performed as well as an objective discussion of its significance. Underlying data should be represented accurately in the paper. A paper should contain sufficient detail and references to permit others to replicate the work. Fraudulent or knowingly inaccurate statements constitute unethical behavior and are unacceptable. Review and professional publication articles should also be accurate and objective, and editorial 'opinion' works should be clearly identified as such.

Authors are required to state in writing that they have complied with the Declaration of Helsinki Research Ethics in the treatment of their sample, human or animal, or to describe the details of treatment.

Data access and retention

Authors may be asked to provide the raw data in connection with a paper for editorial review, and should be prepared to provide public access to such data (consistent with the ALPSP-STM Statement on Data and Databases), if practicable, and should in any event be prepared to retain such data for a reasonable time after publication.

Originality and plagiarism

The authors should ensure that they have written entirely original works, and if the authors have used the work and/or words of others, that this has been appropriately cited or quoted.

Plagiarism takes many forms, from 'passing off' another's paper as the author's own paper, to copying or paraphrasing substantial parts of another's paper (without attribution), to claiming results from research conducted by others. Plagiarism in all its forms constitutes unethical publishing behavior and is unacceptable.

Multiple, redundant or concurrent publication

An author should not in general publish manuscripts describing essentially the same research in more than one journal or primary publication. Submitting the same manuscript to more than one journal concurrently constitutes unethical publishing behavior and is unacceptable.

In general, an author should not submit for consideration in another journal a previously published paper. Publication of some kinds of articles (e.g. clinical guidelines, translations) in more than one journal is sometimes justifiable, provided certain conditions are met. The authors and editors of the journals concerned must agree to the secondary publication, which must reflect the same data and interpretation of the primary document. The primary reference must be cited in the secondary publication. Further detail on acceptable forms of secondary publication can be found at www. icmje.org.

Acknowledgement of sources

Proper acknowledgment of the work of others must always be given. Authors should cite publications that have been influential in determining the nature of the reported work. Information obtained privately, as in conversation, correspondence, or discussion with third parties, must not be used or reported without explicit, written permission from the source. Information obtained in the course of confidential services, such as refereeing manuscripts or grant applications, must not be used without the explicit written permission of the author of the work involved in these services.

Authorship of the paper

Authorship should be limited to those who have made a significant contribution to the conception, design, execution, or interpretation of the reported study. All those who have made significant contributions should be listed as co-authors. Where there are others who have participated in certain substantive aspects of the research project, they should be acknowledged or listed as contributors.

The corresponding author should ensure that all appropriate coauthors and no inappropriate coauthors are included on the paper, and that all co-authors have seen and approved the final version of the paper and have agreed to its submission for publication.

Hazards and human or animal subjects

If the work involves chemicals, procedures or equipment that have any unusual hazards inherent in their use, the author must clearly identify these in the manuscript. If the work involves the use of animal or human subjects, the author should ensure that the manuscript contains a statement that all procedures were performed in compliance with relevant laws and institutional guidelines and that the appropriate institutional committee(s) has approved them. Authors should include a statement in the manuscript that informed consent was obtained for experimentation with human subjects. The privacy rights of human subjects must always be observed.

Disclosure and conflicts of interest

All authors should disclose in their manuscript any financial or other substantive conflict of interest that might be construed to influence the results or interpretation of their manuscript. All sources of financial support for the project should be disclosed.

Examples of potential conflicts of interest which should be disclosed include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding. Potential conflicts of interest should be disclosed at the earliest stage possible.

Fundamental errors in published works

When an author discovers a significant error or inaccuracy in his/her own published work, it is the author's obligation to promptly notify the journal editor or publisher and cooperate with the editor to retract or correct the paper. If the editor or the publisher learns from a third party that a published work contains a significant error, it is the obligation of the author to promptly retract or correct the paper or provide evidence to the editor of the correctness of the original paper.

Duties of editors

(These guidelines are based on existing COPE's Best Practice Guidelines for Journal Editors.)

Publication decisions

The editor of a peer-reviewed journal is responsible for deciding which of the articles submitted to the journal should be published, often working in conjunction with the relevant society (for societyowned or sponsored journals). The validation of the work in question and its importance to researchers and readers must always drive such decisions. The editor may be guided by the policies of the journal's editorial board and constrained by such legal requirements as shall then be in force regarding libel, copyright infringement and plagiarism. The editor may confer with other editors or reviewers (or society officers) in making this decision.

Fair play

An editor should evaluate manuscripts for their intellectual content without regard to race, gender, sexual orientation, religious belief, ethnic origin, citizenship, or political philosophy of the authors.

Confidentiality

The editor and any editorial staff must not disclose any information about a submitted manuscript to anyone other than the corresponding author, reviewers, potential reviewers, other editorial advisers, and the publisher, as appropriate.

Disclosure and conflicts of interest

Unpublished materials disclosed in a submitted manuscript must not be used in an editor's own research without the express written consent of the author.

Privileged information or ideas obtained through peer review must be kept confidential and not used for personal advantage.

Editors should recuse themselves (i.e. should ask a co-editor, associate editor or other member of the editorial board instead to review and consider) from considering manuscripts in which they have conflicts of interest resulting from competitive, collaborative, or other relationships or connections with any of the authors, companies, or (possibly) institutions connected to the papers.

Editors should require all contributors to disclose relevant competing interests and publish corrections if competing interests are revealed after publication. If needed, other appropriate action should be taken, such as the publication of a retraction or expression of concern.

It should be ensured that the peer-review process for sponsored supplements is the same as that used for the main journal. Items in sponsored supplements should be accepted solely on the basis of academic merit and interest to readers and not be influenced by commercial considerations.

Non-peer reviewed sections of their journal should be clearly identified.

Involvement and cooperation in investigations

An editor should take reasonably responsive measures when ethical complaints have been presented concerning a submitted manuscript or published paper, in conjunction with the publisher (or society). Such measures will generally include contacting the author of the manuscript or paper and giving due consideration of the respective complaint or claims made, but may also include further communications to the relevant institutions and research bodies, and if the complaint is upheld, the publication of a correction, retraction, expression of concern, or other note, as may be relevant. Every reported act of unethical publishing behavior must be looked into, even if it is discovered years after publication.

Duties of reviewers

(These guidelines are based on existing COPE's Best Practice Guidelines for Journal Editors.)

Contribution to editorial decisions

Peer review assists the editor in making editorial decisions and through the editorial communications with the author may also assist the author in improving the paper. Peer review is an essential component of formal scholarly communication, and lies at the heart of the scientific method. JNBS shares the view of many that all scholars who wish to contribute to publications have an obligation to do a fair share of reviewing.

Promptness

Any selected referee who feels unqualified to review the research reported in a manuscript or knows that its prompt review will be impossible should notify the editor and excuse himself from the review process.

Confidentiality

Any manuscripts received for review must be treated as confidential documents. They must not be shown to or discussed with others except as authorized by the editor.

Standards of objectivity

Reviews should be conducted objectively. Personal criticism of the author is inappropriate. Referees should express their views clearly with supporting arguments.

Acknowledgement of sources

Reviewers should identify relevant published work that has not been cited by the authors. Any statement that an observation, derivation, or argument had been previously reported should be accompanied by the relevant citation. A reviewer should also call to the editor's attention any substantial similarity or overlap between the manuscript under consideration and any other published paper of which they have personal knowledge.

Disclosure and conflict of interest

Unpublished materials disclosed in a submitted manuscript must not be used in a reviewer's own research without the express written consent of the author. Privileged information or ideas obtained through peer review must be kept confidential and not used for personal advantage. Reviewers should not consider manuscripts in which they have conflicts of interest resulting from competitive, collaborative, or other relationships or connections with any of the authors, companies, or institutions connected to the papers.

The Journal of Neurobehavioral Sciences

Volume 10 ¦ Issue 1 ¦ January-March 2023

Contents

The Burden of Rarity: Rare Diseases and Future Perspectives

Dear Editor,

A disease is considered rare disease (RD) when it affects less than one in every 2000 people.[1] The World Health Organization defines RD as a debilitating disease or lifelong condition with a prevalence of one or less per 1000 people. However, several developed and developing countries have their own definitions. It is a health condition that affects a smaller size of the individual patient population as compared to other prevalent diseases in the general population and has a startling social cost. The most common RDs include autoimmune diseases and lysosomal storage disorders, such as Pompe disease, Hirschsprung's disease, Gaucher's disease, cystic fibrosis, hemangiomas, and specific types of muscular dystrophies. The most common RDs that affect children are hemophilia, thalassemia, sickle cell anemia, and primary immunodeficiency.

RDs are often chronic, debilitating, and life-threatening in nature and can develop at any stage of life. Few of these diseases are preventable and can lead to unfavorable living conditions.[2,3] Less than five to seven individuals out of 10,000 are affected by RDs, although 6%–8% of the world's population is affected globally.[4] According to the Ministry of Health and Family Welfare, 72–96 million Indians are affected by RDs.[5] According to Orphanet data, among the 6172 unique RDs, 71.9% were genetic and 69.9% were disproportionately impacting children.[6] The scientific knowledge and medical understanding of RDs is inadequate due to their complexity, heterogeneity, and ever-evolving field. RD awareness, as well as a lack of treatment alternatives, may make the entire process from diagnosis to therapy difficult and uncertain. RDs have emerged as a primary public health priority owing to the challenges imposed by their low prevalence, especially their continuous, life-threatening nature and lack of information and expertise.[7]

There is inadequate epidemiological data to quantify the burden, and research and development options are limited. It is difficult to estimate the exact number of people affected by RDs, as the majority of RDs are not documented, except in certain developed countries. Government indecision on the provision of health-care access is caused by gaps in the maintenance of electronic health records or registers of cases noticed in the underprivileged and vulnerable sectors of society. Clinical trials are a crucial component of the drug discovery process. It is completed in stages and involves identifying numerous individuals suffering from particular medical conditions and developing a robust trial design to demonstrate the efficacy of the medicine. This issue significantly worsened in the case of RDs. Finding patients qualified for clinical trials typically involves significant search expenses. Given the rarity of patients and in their entire professional careers, a significant percentage of doctors in practice reported never having seen a patient with a RD,[8] finding the correct diagnosis takes a substantial amount of time. Clinical trials are expected to be expensive because pharmaceutical companies will most likely have to spend a substantial amount of money.

RDs affect not only the individuals who suffer from them but also their families and communities. Due to the prohibitively high cost of treatment, the impact on families is frequently disastrous in terms of both the emotional and financial tolls. Often, the patients' and their caregivers' medical and social needs are unmet, which leads to experiencing a severe psychosocial burden.[9] The individuals with RDs and their family caregivers' vocational aspects (education and employment) will be adversely affected due to the difficulty in accessing health-care medical services for the management of RDs, which leads to poor health outcomes.

A qualitative study from the USA reported that individuals with RDs had experienced three types of stigma: Structurally enacted, interpersonally enacted, and felt stigma.[10] There is a need to enhance the awareness, advocacy, and implementation of outreach programs about these RDs in low-income countries, among marginalized, poor literacy populations. However, research on the impact of RDs on psychosocial, economic, and vocational aspects of individuals with RDs and their family caregivers globally is lacking. Individuals with RD's cannot afford health-care services due to the high cost of treatment, diagnostic evaluations, medications, etc., especially in developing countries.

Usually, healthcare end-up spending is spent on people who are diagnosed late. This may often result in a terminal patient who needs to be managed with expensive therapies and rehabilitation. The government can implement the early detection programs, as it can save time and resources and reduce costs. A patient can be managed with less costly interventions while providing better care and quality of life. Early screening and diagnosis of RDs are critical for the prevention of perinatal and neonatal disorders such as Angelman syndrome, Carpenter syndrome, craniofacial disorder, Hutchinson-Gilford progeria syndrome, and Wiedemann-Rautenstrauch syndrome. In addition, drug development can be performed using an artificial intelligence algorithm that can potentially reduce costs and preclinical work compared to a fraction of traditional methods. The electronic health records of patients with RDs should be should be introduced and maintained so as to aid in future researches on RDs. From this data, crucial health information related to RD patterns can be identified regionally.

Ethics committee approval: There is no need for ethics committee approval.

One of the best methods for a proactive approach to therapy is networking and the exchange of experiences. Networking is one of the most valuable assets for people with RDs. Having a community will not only be a poignant reminder of the energy that people with RDs put into their lives every day but it will also be a valuable opportunity for them to feel less alienated in their ongoing struggles.

In summary, the fact that effective therapies are frequently unavailable adds to the amount of agony and suffering experienced by patients and their families. The government must establish a plan to raise awareness of the severity and impact of RDs on patients and their families, and simultaneously conduct research and plan for better diagnosis and treatment, develop pharmaceuticals drugs that are accessible, affordable, and provide insurance coverage for treatment. Furthermore, patients with RDs typically face challenges owing to their low prevalence and lack of knowledge among their healthcare providers. As there is an urgent need to bridge the gap in physicians' understanding of RDs, it is prudent to introduce additional courses on these diseases in the medical curriculum and postgraduate training for physicians. The internet is the primary source of information on RDs, and e-learning programs and courses should be implemented for all medical practitioners.

Patient informed consent

Patient informed consent was obtained.

Ethics committee approval

There is no need for ethics committee approval.

Financial support and sponsorship

No funding was received.

Conflicts of interest

There are no conflicts of interest to declare.

Author contribution area and rate

- Srikanth Pallerla (50%): Contributed to writing, and review of the manuscript, and literature search.
- Shweta Kapote (50%): Contributed to writing, and review of the manuscript, and literature search.

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Original Article

Low-frequency Prefrontal Cortex Magnetic Stimulation Improves Autism Spectrum Disorder Symptoms: A Pilot Study

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Abstract

Aim: Autism spectrum disorder (ASD) is a common neurodevelopmental disorder affecting multiple levels of social and cognitive skills and causing a significant health-care burden. Currently, there is no approved treatment for ASD. **Methods:** In this study, 10 children with ASD between the ages 6 and 19 years (M = 12.3, standard deviation = 3.94) were recruited. Repetitive transcranial magnetic stimulation (rTMS) was applied and symptom severity was measured before and after treatment using the Childhood Autism Rating Scale (CARS) and Autistic Behavior Checklist (ABC). All children received sessions of low-frequency rTMS to the bilateral prefrontal cortices. **Results:** The results showed that the children improved according to both symptom ratings. Specifically, both the relating ($z = -2.02$, $P < 0.05$), body and object use ($z = -2.03$, $P < 0.05$) and language ($z = -2.21$, $P < 0.05$) subscale scores and the total score of ABC ($z = -2.37$, $P < 0.05$) decreased. Regarding CARS, visual response ($z = -2.06$, $P < 0.05$), verbal communication ($z = -2.12$, $P < 0.05$) subscale scores, and the total score ($z = -2.52$, $P = 0.01$) decreased significantly after TMS therapy. **Conclusion:** Our study was open label and in terms of sample size should be considered a pilot study. Although the results should be evaluated cautiously, the findings suggest that rTMS might be a safe and useful tool for improving deficits related to ASD in children.

Keywords: *Autism, children, language, prefrontal cortex, repetitive transcranial magnetic stimulation*

Introduction

Deficits in social communication and interaction in addition to restrictive and repetitive patterns of behavior and interests or activities are characteristic symptoms of autism spectrum disorder (ASD).[1] Today, the prevalence of autism is estimated to be 1 in 100 children.[2] As ASD is a very frequent and pervasive developmental disorder affecting multiple areas of cognitive functioning, the burden of the disorder on health-care system and the families is often high.[3] Currently, there is no cure for ASD and apart from behavioral interventions and medications that symptomatically improve aggression and irritability, there is no evidence-based treatment.[4]

Besides medications and behavioral interventions, another possible approach for treatment could be neuromodulation techniques such as transcranial magnetic stimulation (TMS). Neuromodulation methods have the advantage of carrying less adverse effect potential as compared to medical treatment. As compared to behavioral interventions, they are also easier to apply and less time-consuming. However, the main disadvantage of TMS is relative sensitivity to movement as it may be difficult for children to sit still during the session. Second, there is a small but significant risk of epilepsy, especially in people with a previous history. Given a significant proportion of individuals with ASD will have epilepsy, this issue should be assessed carefully. In addition, the suitable lower age limit for the application of TMS is not clearly defined, there have been studies reporting the safety of repetitive TMS in children at 6 years of age.[5] In 2018, there had been 23 eligible reports

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of TMS (four case reports, seven noncontrolled clinical trials, and 12 controlled clinical trials) and meta-analyses revealed a moderately significant impact on stereotypic and social behaviors and the number of errors on executive function measurements and five of these studies reported persistence of the gains for up to 6 months.[6] Another point is that, in the majority of studies, TMS was applied to the dorsolateral prefrontal cortex. The medial prefrontal cortex and motor area were preferred as other application areas. The predominantly applied frequency value was between 0.5 Hz and 1 Hz.[7] Although a temporary and short-term mild headache was stated as the only significant side effect,[7] a recent meta-analysis revealed that the most common adverse effect due to TMS in the pediatric population is facial discomfort and irritability in addition to headache.[8] The prevalence of seizures related to TMS in ASD is limited by a single case and was to a programming error.[9]

Regarding the stimulation site and protocol, there is an inconsistency among previous studies. The dorsolateral prefrontal cortex (DLPFC) either unilateral or bilateral is the most commonly stimulated area, followed by the motor cortex and parietal areas. Both high and low-frequency stimulation protocols were used[10] and some even used theta-burst stimulation (TBS) which is associated with a higher risk of seizures.[11] It is not certain how DLPFC stimulation could be beneficial in ASD, however, speculatively one might say that inhibition of lateral areas related to the task-positive network may enable the activation of task-negative social areas such as the medial prefrontal cortex through reciprocal inhibition. Barahona-Corrêa *et al*.[6] have also noted an increment of the positive effects in the areas of social relations, decline in repetitive behaviors, and improvement in the selective attention process and visual processing areas.

In this pilot study, we aimed to assess the efficacy and applicability of repetitive TMS (rTMS) in a group of children aged 6–19 years. We specifically aimed to record both parent and physician ratings before and after TMS sessions to see if both evaluations would agree.

Materials and Methods

The ethics committee approval has been obtained from Uskudar University Clinical Studies Ethical Commitee. Ethical Permission is approved on October, 27 2017 with the document number of 61351342/2017/20.

Participants

Ten children with ASD have participated in the study. All parents gave verbal and written informed consent and the study protocol was approved by Uskudar University Clinical Studies Ethical Committee. The children with a history of seizures and/or with epileptic seizures detected based on the electroencephalography data obtained in the neurological examination were excluded as well as children with a very high level of hyperactivity who will not tolerate TMS. Inclusion criteria were being diagnosed with ASD by a child psychiatrist or neurologist and being between the ages of 6 and 19 years (M = 12.3, standard deviation (SD) = 3.94) as presented in Table 1. The children who were using medications, vitamins, supplements, or getting behavioral/occupational therapy were allowed to continue the same therapy during the TMS, and no change in the medical treatment was made during the course.

Instruments

Childhood Autism Rating Scale

The Childhood Autism Rating Scale (CARS) is a 15-item behavioral rating scale developed to distinguish individuals with intellectual disability (autism index) without autism from those with autistic symptoms. The CARS test enables autistic individuals to be clinically classified as mild, moderate, and moderate-severe. Each item consists of an evaluation with a half value between one and four points and the scores changes between 15 and 60. Individuals scoring between 15 and 29.5 are far from autism. A score of 30–36.5 indicates mild–moderate autism, and a score of 37–60 indicates severe autism. It is recommended to use 28 points for autistic symptoms and 35 points for

Table 1: Demographic characteristics of participants (*n*=10)

Variable	Value
Age	12.3 (6-19, ±3.94)
Male (%)	100

Age values are presented in years in mean (range and standard deviation)

Table 2: The descriptive statistics and statistical testing for pre–postchange in autistic behavior checklist (*n*=10)

Subtest	Mean±SD	*Z*	*P*
Sensory			
Pretest	8.9±7.5	−1.83	0.07
Posttest	6.2±6.4		
Relating			
Pretest	16.9±10.5	−2.02	0.04
Posttest	13.2±8.3		
Body and object			
Pretest	11.7±9.0	−2.03	0.04
Posttest	8.7±5.8		
Language			
Pretest	16.9±7.6	−2.21	0.03
Posttest	13.2±7.2		
Social and self-help			
Pretest	10.3±5.7	−1.83	0.07
Posttest	8.5±6.2		
Total			
Pretest	64.7±35.4	−2.37	0.02
Posttest	49.8±28.3		

SD: Standard deviation

severe autism. Evaluation can be conducted in the light of classroom evaluation and information received from parents. The Turkish validity and reliability studies of the scale were first performed by Sucuoğlu *et al*.,[12] and the analyzes were expanded by İncekaş Gassaloğlu *et al*.[13]

Autistic Behavior Checklist

The scale was first developed by Krug, Arick, and Almond.[14] It is a test consisting of 57 questions and five subscales: sensory, relating, body and object use, language, and social/self-help. While the lowest score on the scale is 0 and the highest score is 159. Yılmaz-Irmak *et al*.[15] evaluated the validity and reliability of the Autistic Behavior Checklist (ABC) test for our country and determined that it was a usable criterion and determined the cutoff point of the scale as 39. The scale is scored by teacher evaluation. The Cronbach's alpha and Spearman–Brown split-half test reliability coefficients of the scale were found to be. 92. For the reliability of each subtest, the Cronbach's alpha values ranged from 65 (social and self-help) to 82 (relating), and Spearman Brown's two-half test reliability values were similarly 61 (social and self-help) and between 84 (relating).

Transcranial magnetic stimulation application

Each child received 20 sessions of rTMS. The first 10 sessions were given to the left DLPFC and the rest were given to the right DLPFC. In each session, the children received 600 pulses with 90% of the resting motor threshold. We aimed to give TMS 6 days a week but due to interruptions of the schedule, the children received treatment between 23 and 30 days.

Results

All children were between 6 and 19 years of age (M = 12.3, SD = 3.94) and all were male. The means and SDs for the ABC autism checklist are given in Table 2 together with group comparison tests according to the Wilcoxon signed-rank test. According to these results, the relating ($z = -2.02$, $P < 0.05$), body and object use ($z = -2.03$, $P < 0.05$), and language ($z = -2.21$, $P < 0.05$) categories showed a significant decline after TMS treatment. The total score showed also a significant decline ($z = -2.37$, $P < 0.05$). Furthermore, CARS results changed significantly in total ($z = -2.52$, $P = 0.01$); in addition, visual response ($z = -2.06$, $P < 0.05$) and verbal communication ($z = -2.12$, $P < 0.05$) scores decreased significantly after TMS therapy [Table 3]. Even if the other subtest evaluations did not change significantly, analyses pointed out a downward trend in adaptation to change ($z = -1.807$, $P = 0.07$), listening response ($z = -1.890$, $P < 0.05$), activity level ($z = -1.84$, $P = 0.06$), and intellectual response ($z = -1.89$, $P = 0.06$).

Table 3: Childhood Autism Rating Scale test results (n=10)

Subtest	Mean±SD	*Z*	*P*
Relating to people			
Pretest	2.60±0.91	−1.604	0.11
Posttest	2.25±0.75		
Imitation			
Pretest	2.35±1.03	−1.604	0.11
Posttest	1.90±0.84		
Emptional			
Pretest	2.60±0.94	−1.604	0.11
Posttest	2.10±0.74		
Body use			
Pretest	2.40±0.88	−1.633	0.10
Posttest	2.10±0.74		
Object use			
Pretest	2.20±0.92	−0.921	0.36
Posttest	1.95±0.72		
Adaptation to change			
Pretest	2.15±0.82	−1.807	0.07
Posttest	1.75±0.63		
Visual response			
Pretest	2.20±0.82	−2.060	0.04
Posttest	1.75±0.43		
Listening response			
Pretest	2.20±0.70	−1.890	0.06
Posttest	1.75±0.50		
Taste, smell, and touch response			
Pretest	2.05±1.19	−0.816	0.41
Posttest	1.85±0.88		
Fear or nervousness			
Pretest	2.55±0.80	−1.63	0.10
Posttest	2.15±0.71		
Verbal communication			
Pretest	2.90±0.84	−2.12	0.03
Posttest	2.60±0.88		
Nonverbal communication			
Pretest	2.05±0.93	−1.00	0.32
Posttest	1.95±0.73		
Activity Level			
Pretest	2.4±0.94	−1.84	0.07
Posttest	2.0±0.82		
Level and consistency of intellectual response			
Pretest	2.0±0.62	−1.89	0.06
Posttest	1.75±0.59		
General impressions			
Pretest	2.55±0.80	−1.63	0.10
Posttest	2.30±0.67		
Total			
Pretest	35.2±10.74	−2.52	0.01
Posttest	30.15±7.86		

SD: Standard deviation

Discussion

Our results are consistent with previous studies.[10] In that low-frequency, TMS to bilateral prefrontal areas may be beneficial for children with ASD. According to the results, both parent interviews and clinical evaluations filled in by

the child psychiatrist showed consistent improvement. The subtest analysis reviled two separate evaluations by the parent and the psychiatrist agreed on language abilities. This improvement in our study results corroborates findings in studies examining the effect of TMS on language recovery, especially in aphasias (with and without stroke), revealing the potential of TMS to direct neuroplastic changes that facilitate language recovery.[16-18]

Another important aspect of the result of the study is that we applied rTMS to a relatively younger population as compared to previous TMS studies and none of the children left the study due to side effects. On the other hand, it is worth mentioning that we excluded children with a high level of hyperactivity or aggression. For those children who may not tolerate regular TMS, shorter treatments with theta-burst-type stimulation may be suitable. Looking at the recommendations in the literature, for instance,[19] conducted an open-label study suggesting that intermittent TBS (iTBS) would modulate synaptic plasticity more efficiently than TMS and could be a promising modality for neuropsychiatric disorders such as ASD. Researchers have revealed results that indicate improvement in some cognitive functions and proposed the necessity of further controlled studies of iTBS.

The most important shortcoming of our study is that our sample size was relatively small. However, it should also be kept in mind that our sample size was comparable to previous TMS treatment studies with ASD children. Another limitation is the lack of a control group. A sham-controlled double-blind treatment study might yield more reliable results in terms of the effectivity of TMS in ASD. Therefore, with continuity and optimal stimulation of what is reflected in the research, you can keep the numbers and duration.

Conclusion

This study, evaluating rTMS treatment for children with ASD in the mean age of 12 years, has revealed that both total score and and three subtests (the relating, body and object use and language categories) of ABC test showed significant decline after TMS treatment. Also, significant change in total scores of CARS, visual response and verbal communication subtest scores decreased significantly after rTMS. Other subtests results were not significant, whereas non-significant decrease in adaptation to change, listening response, activity level, and intellectual response was detected.

Patient informed consent

Patient informed consent was obtained.

Ethics committee approval

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Conflicts of interest

There are no conflicts of interest to declare.

Author contribution subject and rate

- Nevzat Tarhan (20%): Organized the research and contributed with comments on manuscript organization
- Muammer Aydoğdu: (20%): data collection and analyses
- Yelda İbadi (20%): Contributed on manuscript organization and write-up.
- Emel Sarı Gokten (20%): Design the research, data collection and analyses
- Barış Metin (20%): Design the research, contributed with comments on manuscript organization and write-up.

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Original Article

Effect of Coronavirus Vaccine on Depressive and Anxiety Symptoms of Health-care Professionals Developed through the Pandemic

Abstract

Aim: COVID-19 pandemic response measures adversely affected the psychological effects of health-care professionals due to disruption of daily life, sense of uncertainty, fear of getting sick, and the perception of working in a dangerous environment. In this study, we assessed the level of depression and anxiety symptoms in health-care professionals who had interaction with COVID-19 patients both before and after vaccination. **Materials and Methods:** The participants in this prospective cohort study, which took place between July 24, 2020 and April 30, 2021, were 233 health-care workers who were employed in the hospital's COVID area. Participants were divided into two groups as pre-COVID-19 vaccine group (Group 1; n = 98) and postvaccine group (Group 2; n = 135), both groups received the Hospital Anxiety and Depression Scale. **Results:** The mean score of the Group 1 anxiety subscale was 15.64 ± 2.112, and the mean score of the depression subscale was 15.19 ± 1.762. The same scores were 9.65 ± 5.535 and 9.13 ± 4.984, respectively, in Group 2. There was a statistically significant difference between the groups (P = 0.001). **Conclusion:** In our research, we have seen that the application of the vaccine has positive effects on the psychological state of health workers who are directly exposed to COVID-19 patients. We think that the therapies or preventive measures that are developed during the pandemic phase will lessen the possibility of sadness and anxiety in health-care personnel and boost the effectiveness of the effort to combat the disease.

Keywords: *Anxiety, COVID-19, depression, health-care professionals, vaccination*

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Introduction

The novel coronavirus (severe acute respiratory syndrome coronavirus 2 [SARS-CoV-2]), which first surfaced in Wuhan, China, in December 2019, is believed to be the cause of the coronavirus 2019 (COVID-19), which has the potential to proceed clinically with viral pneumonia.[1] On February 11, 2020, the World Health Organization (WHO) designated this new coronavirus as COVID-19. Its origin is unknown, despite the fact that it shares traits with prior coronavirus outbreaks (SARS, Middle East Respiratory Syndrome).[2] The WHO classified the illness a pandemic on March 11, 2020.[3]

Comments such as the disruption of routine life, the feeling of uncertainty, the fear of getting sick, the feeling of living, or working in an unsafe area after the measures taken with the declaration of the pandemic have shown that the pandemic has psychological effects as well as physiological effects.[4] As they work in the same environment as those who are infected or are likely to be carriers, health-care professionals are at a higher risk than the general population of contracting COVID-19 and experiencing stress. When the COVID-19 outbreak was at its worst, 1716 health-care professionals were reported to be infected with the virus in China.[5]

Elements like the rise in the quantity of cases and death rates in daily tests, inadequacy in medical equipment and devices, active and intense work tempo, and stress can cause a feeling of burnout in health-care professionals. Accompanying the feeling of burnout with depression and as a result of professional dissatisfaction can

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negatively affect the health workers. At a poll of more than 1200 health-care professionals in 34 hospitals in Wuhan, the city where the coronavirus pandemic first surfaced, almost 14% of doctors and 16% of nurses showed signs of mild-to-serious sadness.[6] Mak *et al*. in a study that aimed to evaluate long-term psychiatric morbidities in SARS survivors, health-care workers who survived the SARS epidemic showed depression, high rates of anxiety, and somatization symptoms during or after the epidemic.[7] In a study that compared the secondary traumatization, anxiety, and depression scores of health care and nonhealth workers who were directly exposed to and not exposed to patients with a diagnosis of COVID-19, the health workers who directly encountered COVID-19 obtained the highest score from the scales, whereas the nonhealth workers group received the lowest score.[8]

The coronavirus vaccine was administered for the first time in England, at Coventry University Hospital, on Tuesday, December 8, 2020, at 6.45 am. In Turkey, giving a priority to health-care professionals, it started to be implemented on January 13, 2021. Anxiety is a complex experience with psychological, behavioral, and cognitive symptoms. Anxiety, which is a universal part of the human condition, is considered abnormal if its severity and duration are inappropriate or occur without a specific threat. Anxiety disorders describe deviations from normal. Depression is an important public health problem because it is a common disease that can affect all the layers of society, has a high risk of chronicity and morbidity, and has a lethal potential, and is a treatable disease.[9,10] In this study, we assessed the intensity of depressive and phobic symptoms in health-care professionals who had close contact with COVID-19 patients during the pandemic before and after vaccination.

Materials and Methods

The Research Protocol Was Approved by The Gazi Yasargil Research and Training Hospital, Health Sciences University Clinical Research Ethics Committee (Date and Number: July 24, 2020 535).

Study participants

Between July 24, 2020 and April 30, 2021, this prospective cohort research was carried out, at hospital with the participation of health-care professionals working in the COVID area. Informed consent of the participants was obtained. Interviews were conducted face to face. In accordance with the Declaration of Helsinki, the research protocol received approval from the Hospital Clinical Research Ethics Committee (date and number: July 24, 2020-535).

Initial information was gathered between July 24, 2020 and November 30, 2020. Afterward, the study was suspended until a possible treatment or vaccine is developed. Two weeks after the first vaccination of the health-care professionals, the final data were collected by using the same questionnaire.

Data collection and psychometric measurements

A sociodemographic questionnaire and the Hospital Anxiety and Depression Scale (HADS) were used to gather the research data. The volunteers were questioned regarding their past history of mental illness, alcohol usage, and other drug use. After participants were made aware of the study, both verbal and written agreement was obtained.

The HADS was created by Zigmond and Snaith to screen for clinically significant anxiety and depression in outpatient medical settings. In addition, it can track the changes in those symptoms.[11] Aydemir *et al*. conducted a study on the validity and reliability of the tool, and it was found to be a suitable instrument.[12] The scale consists of a total of 14 questions, where depression and anxiety are measured by even and odd questions, respectively. It includes the HADS-A (7-question) and HADS-D (7-question) subscales for anxiety and depression, respectively. The maximum score for each sub-scale on the 4-point Likert scale for each item is 21. In the Turkish study, the cutoff values for the subscales measuring depression and anxiety were 7/8 and 10/11, respectively. As a result, people with higher scores are more likely to have mental illness.

Exclusion criteria

Those with a history of any psychiatric disorder or alcohol/substance abuse and those with COVID-19 disease were being left out of the study.

Statistics

The SPSS (Statistical Package for the Social Sciences, SPSS Inc., Chicago, IL, USA) programme was used to analyze the data. Descriptive statistics were used to assess the information on the anxiety and depression states (mean standard deviation). A significant outcome was considered to be one with a $P < 0.05$ after variables were assessed with a 95% confidence level.

Results

A total of 233 health-care professionals working in areas designated as COVID areas in the hospital between July 24, 2020 and April 30, 2021 were included in the study. The subjects joined to our survey before vaccination were grouped as Group 1 ($n = 98$) and after vaccination as Group 2 ($n = 135$). After recruitment, HADS has been implemented. The reliability coefficients for the Turkish population were evaluated as 0.85 for depression subscale and 0.78' for anxiety subscale. Cronbach's alpha values for our study were found to be 0.866 and 0.817, respectively.

Group 1 anxiety subscale mean score was calculated as 15.64 ± 2.112 and depression subscale mean score as 15.19 ± 1.762. On the other hand, Group 2 scores were calculated as 9.65 ± 5.535 and 9.13 ± 4.984, respectively.

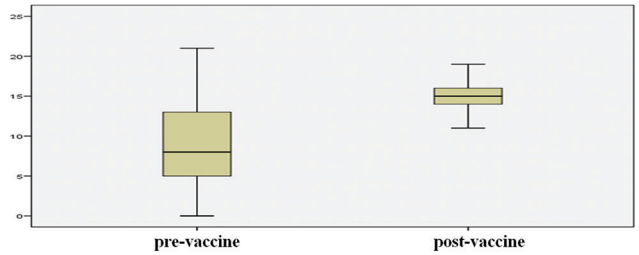

Figure 1: Depression symptom levels of study participants before and after the administration of COVID-19 vaccine. pre-vaccine post-vaccine

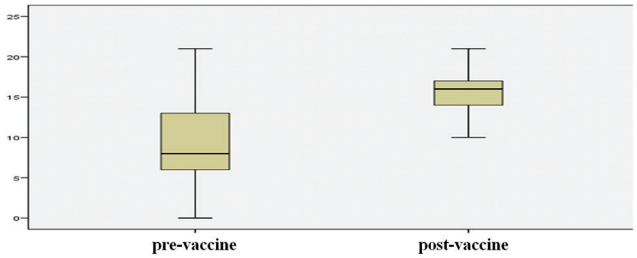

Figure 2: Anxiety symptom levels of study participants before and after the administration of COVID-19 vaccine. pre-vaccine post-vaccine

There was a difference between the groups, according to the statistics [P = 0.001; Table 1 and Figures 1 and 2].

Table 1: Anxiety and depression scores of both groups

	Group 1 (n=98)	Group 2 (n=135)	P (n=233)
Anxiety	15.64±2.112	9.65±5.535	0.000
Depression	15.19±1.762	9.13±4.984	0.000

Discussion

The COVID-19 epidemic has significantly altered everyone's lives, which has had an impact on their psychosocial well-being. The literature has shown that epidemics have profound psychosocial effects on both individuals and societies. When viewed at an individual level, it is observed that people experience fear of contamination, feelings of helplessness, or fear of death due to illness.[13] Rubin *et al*. conducted a study of swine flu (influenza A H1N1 v) between May 1, 2009 and January 10, 2010 in the UK, where they conducted an average of 36 weekly surveys at weekly intervals. The survey's findings indicated that 10% to 30% of people were extremely or extremely concerned about catching the virus.[14] Another study conducted between 2013 and 2016 found that when schools and workplaces were closed due to the Ebola virus epidemic in Guinea, Liberia, and Sierra Leone, where the illness was most commonly transmitted, people's negative feelings soared dramatically.[15] During an outbreak of an infectious disease, healthcare practitioners' psychological reactions might be challenging. Concerns about scarce resources, a sense of vulnerability or loss of control, worries about one's own health, the spread of a virus, worries about infecting family members, worries about the health of the patients they are caring for, adjustments at work, and a desire to withdraw are a few examples.[16]

Early COVID-19 studies showed that the public's psychological morbidity grew dramatically as a result of the epidemic. Twenty-nine percentage of respondents to an online survey of more than 1200 people in China between January and February 2020 reported having moderate-to-severe anxiety. In another study, moderate-to-severe depression was found to be present in 9%–17% of the population, while psychological distress (for example, depression, hopelessness, and irritability) was found in 8%–36% of adults.[17]

A March 2020 online survey of more than 1000 Americans revealed that 36% of respondents experienced a major negative impact on their mental health as a result of the new coronavirus epidemic.[18]

The impression of personal risk may be heightened by the knowledge that COVID-19 is contagious,[19,20] is linked to high morbidity, and has the potential to be lethal.[21] The pressure and concerns felt by health-care workers may also be increased by anticipated supply shortages and a rise in the suspected and confirmed COVID-19 cases.[22]

Previous research has demonstrated that health-care personnel are more susceptible to negative psychological effects when working in emergency departments, intensive care units, and infectious disease services.[23] Health-care professionals who treat patients with COVID-19 had a significant prevalence of mental health problems, according to a cross-sectional assessment of 1257 participants in China. Front-line work has been found to be a standalone risk factor for negative psychological consequences. Overall, 50.4% of all individuals displayed the signs of depression, compared to 71.5% who displayed signs of anxiety, 44.6% who displayed signs of distress, and 34% who displayed signs of insomnia.[24] Another Italian study discovered that during the COVID-19 pandemic, medical professionals had higher levels of anxiety and risk perception than the general public.[25] The data from the first stage of our investigation were comparable to all other studies in light of all these facts. In this study, we aimed to evaluate the depression and anxiety symptoms change of health-care workers after vaccination. Our data show that the vaccine significantly reduces the depression and anxiety levels of health-care workers. Since there is no similar study to our study, we believe that our study will be evaluated as a guide for future studies on health workers or normal population.

Conclusion

It should be highlighted that throughout the COVID-19 pandemic phase, health-care workers may also have mentally negative effects and may require psychiatric attention, similar to the general population. Health professionals felt more secure with the development of the

COVID-19 vaccination. Following immunization, health professionals' levels of depression and anxiety dropped. Healthcare personnel continued to work more selflessly and with improved energy efficiency when a safer working environment was made available. We believe that the preventive measures or treatments that can be found during the pandemic process will reduce the depression and anxiety that may occur in health-care professionals and will enable them to work more efficiently in the fight against the disease.

Limitations

Since our study is regional, the effect size may relatively be small. Thus, larger studies should be done. In addition, since this study was conducted on actively employed individuals, the data values may also be higher. Furthermore, our data may be insufficient about vaccine types and results, as the results of the vaccine were not waited. Finally, our study was conducted in an active pandemic hospital that has been resulted in a longer data collection and evaluation period than expected.

Patient informed consent

There is no need for patient informed consent

Ethics committee approval

The Research Protocol Was Approved by The Gazi Yasargil Research and Training Hospital, Health Sciences University Clinical Research Ethics Committee (Date and Number: July 24, 2020 535).

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No funding was received.

Conflicts of interest

There are no conflicts of interest to declare.

Author contribution subject and rate

- Öner Avınca (%25) and Mahmut Taş (%25): Create the research, gather the data, do the analyses, and write the entire manuscript.
- Abdullah Sen (%10), Mehmet Diyadin Güleken(%10), Remzi Çetinkaya(%10), Baran Arı(%10), Ahmet Yeşil(%10): Create the research, gather the data, do the analyses, and write the entire manuscript.

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Original Article

Evaluation of Ethanol Extract of *Curcuma longa* in Lead-induced Hippocampal Neurotoxicity

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Abstract

Background: Heavy metals such as lead are ubiquitous elements at exposure causing deleterious effects on the brain and leading to neurodegenerative diseases. **Aim:** In this investigation, the neurotherapeutic effects of ethanol extract of *Curcuma longa* (EECl) against lead-induced hippocampal neurotoxicity in rats were examined. Biochemical examination for antioxidant enzyme activity and lipid peroxide level (malondialdehyde [MDA], superoxide dismutase [SOD], and glutathione [GSH]) was evaluated, the Barnes maze for learning and memory, and histological analysis (H and E stain) for general histoarchitectural features to investigate the neurotherapeutic characteristics of EECl. **Materials and Methods:** Six groups totalling 36 rats were created (n = 6). In the first group, rats received distilled water (2 mg/kg), in the second, lead acetate (LA) (120 mg/kg), in the third, ascorbic acid (100 mg/kg), and the 4th, 5th, and 6th groups, rats received LA (120 mg/kg) and EECl (375 mg/kg, 750 mg/kg, and 1500 mg/kg, respectively) for 14 days. **Results:** A significant learning and memory deficit was seen in the LA-treated group's results, but a significant improvement was seen in the EECl-treated group. Increased oxidative stress was seen in the LA-treated group, as evidenced by an increase in MDA levels and a decrease in antioxidant enzymes (SOD and GSH). A decline in MDA levels and an increase in SOD and GSH activity was the evidence of the ameliorative effects of EECl treatment. Cytoarchitectural distortions relative to the control were observed with the LA-treated group. Mild distortion was however detected with EECl treatment. **Conclusion:** EECl has possible neurotherapeutic properties against LA-induced pathological changes in the hippocampus of Wistar rats. EECl may have neuroprotective effects against degenerative alterations brought on by LA.

Keywords: *Barnes maze, cytoarchitecture, hematoxylin and eosin, learning and memory, oxidative stress*

Introduction

Humans' daily interactions with the environment make them susceptible to pollution. Environmental pollution is the prevalence of pollutants in the soil, air, water, and subsequently in food that can harm environmental organisms.[1,2] In their daily interactions with their surroundings, both humans and animals are exposed to a variety of heavy metals such as lead, mercury, aluminum, and cadmium. These relationships with the environment happen through air, water, and food.[3] Heavy metals become harmful when they accumulate in soft tissues instead of being metabolized by the body.[4]

In underdeveloped and developing nations, lead toxicity is a common concern to public health because of human activities such as mining and farming.[5] Lead is a multi-organ toxin that damages several organs and is linked to several cancers, damage to the kidneys and nervous system, and problems with reproduction in both humans and animals. It can finally result in a child's death.[5,6] Several public health and occupational safety actions have been implemented to minimize lead exposure instances, yet several lead poisoning cases are still reported.

Lead crosses the blood − brain barrier and causes neurotoxicity through oxidative stress molecular mechanism.[7,8] Essential macromolecules such as proteins, lipids, and DNA are oxidized as a result of the increased formation of nitrogen-based radicals and oxygen and associated nonradical products.[9] Reactive oxygen

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species (ROS) are by-products of metabolic activities in aerobic organisms. Under normal circumstances, antioxidant enzyme activity, such as that of catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), and lipid peroxidase, regulate ROS concentration.[10] The imbalance between the scavenging and generation of ROS, which can harm the detoxification system and promote ROS production, causes oxidative stress.[11] However, during oxidative stress, the excessive free radical formation has detrimental consequences on cells, tissues, inflammatory responses, and apoptosis.[10,11] Neurodegenerative ailments such as Parkinson's and Alzheimer's are the main results of increased production of ROS in the body system.[12]

The hippocampus, a complex brain structure which is deeply ensconced in the temporal lobe of the cerebral cortex, is crucial for spatial navigation, learning and memory, emotional behaviour, and the regulation of hypothalamic functions.[13] Cornu ammoni (CA: CA1, CA2, and CA3) and dentate gyrus are the two major divisions of the hippocampus. These parts curve into one another and are separated by the hippocampal sulcus.[14] The dentate gyrus serves as the starting point for information flow through the hippocampus, which continues through CA3 to CA1 to the subiculum with extra input information at each stage and outputs at the two last stages. Even while CA2 makes up a very small percentage of the hippocampus and is sometimes overlooked in descriptions of hippocampal function, it is noteworthy that this tiny area appears remarkably resistant to situations that typically result in significant cellular damage, such as epilepsies.[14,15]

Curcuma longa (Turmeric) belongs to the *Zingiberaceae* family of ginger. It is extensively cultivated in nations with tropical climates such as India and China,[16] as well as Nigeria, where Hausa speaking Nigerians refer to it as *"Gangamau"* or "*Zabibi"* or "*Magina."* It has been employed in conventional medicine as a home treatment for several illnesses.[17] *C. longa* has numerous pharmacotherapeutic effects including antibacterial, anti-tumor, antioxidant, anti-inflammatory, hepatoprotective, and anti-viral activities.[18,19] *C. longa* offers a wide spectrum of medicinal potentials both *in vivo* and *in vitro*, including antioxidant and anti-inflammatory capabilities,[20] which makes it suitable for reversing the reactions and alterations brought on by lead toxicity in several organs as a result of the formation of ROS and promotion of an inflammatory response during oxidative stress.[21,22]

Materials and Methods

Ethical clearance for this study was provided by the Ethics Committee on Animal Use and Care, Ahmadu Bello University (ABU), Zaria: ABUCAUC/16.08.2021/100.

Materials

Plant

Fresh rhizomes were procured locally in a market in Samaru, Zaria, Nigeria. The rhizomes were taken for the identification and authentication in the Department of Botany's Herbarium, Ahmadu Bello University (ABU), Zaria. Specimen Voucher Number: ABU0551 was provided.

Plant extraction method

The preparation of the ethanol extract of *C. longa* (EECl) took place in the Department of Pharmacognosy and Drug Development, ABU, Zaria. The method for maceration as stated by Brain and Turner,[23] Harborne,[24] and Evans[25] was adopted for the extraction using ethanol.

Experimental animals

Thirty-six healthy male Wistar rats (weighing between 100 and 180 g) were procured from the Faculty of Pharmaceutical Sciences' Animal House. The animals were brought to the Animal Welfare, Department of Human Anatomy, ABU, Zaria, the animals were housed under standard laboratory conditions of 12-hrs dark and light cycles and fed with rat meal and water at will. The animals were allowed to acclimatized for 2 weeks before the commencement of the study.

Drugs

Lead acetate

Lead acetate (LA) was procured and utilized in this work as a neurotoxin. The manufacturer of the item (Product No. 5032) is Hopkin and Williams Chemical Ltd, England.

Ascorbic acid (Vitamin C)

To assess the therapeutic effectiveness of EECl, ascorbic acid (Vitamin C) pills were acquired and utilized as a comparison medication. The item is produced in Lagos, Nigeria, by Emzor Pharmaceutical Industries Ltd.

Ketamine

For anesthesia, ketamine (50 mg/ml Ketamine Hydrochloride injectable USP,) bought from Swiss Parenterals PVT Ltd. in Gujarat, India.

Experimental design

Six groups of six rats each were formed from 36 healthy male Wistar rats. Group I acted as the control, and distilled water (2 mg/kg) was given to the rats. Only LA (120 mg/kg) was given to the rats in Group II. In addition, LA (120 mg/kg) and ascorbic acid (100 mg/kg) were given to rats in Group III. Rats in Groups IV, V, and VI received doses of 120 mg/kg of LA and 375, 750, and 1500 mg/kg, respectively, of EECl. Fourteen days were allotted for administrations.

At the end of the experiment, the rats were anesthetized using 75 mg/kg ketamine intraperitoneally.[26] The brains of all the rats were harvested and cut into two halves, one was preserved in freshly prepared Bouin's fluid immediately for histopathological studies while the second half was homogenized in 0.1 M phosphate buffer solution (pH 7.4) for biochemical analysis.

Barnes maze

The neurobehavioral assessment was conducted using the Barnes maze apparatus (BMA). The Barnes maze test is an apparatus used in assessing the spatial memory and learning. The test was developed by Dr Caro Barnes in 1979. The primary purpose of the Barnes maze is to assess the capacity of a rat to learn and remember the location of a target zone and escape into the safety spot within a set amount of time.[27] BMA consists of a round surface with up to 20 circular holes around it. The diameter of the circular surface is 92 cm with a central circle measuring 10.5 cm in diameter. There are 20 holes around the circular surface and each hole is 5 cm in diameter. Each hole is at a distance of 7.5 cm from one other and also 2 cm from the circular surface. A wooden box is constructed beneath the circular board which is movable serving as the safest spot for the escape.

After the rat is being placed in the central area of the BMA, it was allowed to freely roam around it for 90 s. The equipment was cleaned with methylated spirit following each animal trial and allowed to dry before the next animal was tested. Behaviors scores were:

- Wrong Trial (WT): This is the frequency at which the rat tried to escape through the wrong holes
- Time Spent: This is the total time spent by a rat within the given time duration, till it was able to escape into the safety spot.

All of the animals underwent daily training in the BMA (habituation) for 5 days. On the 3rd, 6th, 9th, and 12th days following administration, repetition of this learnt activity was conducted.

Biochemical analysis

A digital weighing scale (Acculab Vicon VIC-511 Precision Balance/Scale, USA, 0.001 g) was used to calculate the weight of the brains. The brain tissues (1 g tissue/4 ml) were homogenized in 0.1 M phosphate buffer at pH 7.4.[28] The homogenate was centrifuged and the supernatant was collected in plain sample bottles for estimation of oxidative stress markers (Malondialdehyde [MDA], GSH, SOD). This was carried out in Human Anatomy Department, ABU, Zaria. According to Ohkawa *et al*.[29] and somewhat modified by Atawodi *et al*.,[30] MDA was identified as a thiobarbituric acid (TBA) reactive compound utilizing 15 percent trichloroacetic acid and 0.67% TBA (TBA). Analysis of SOD activity using the Fridovich method[31] and GSH activity using the Ellman method[32] were used to measure the enzyme-based antioxidant activity.

Histological studies

Histological methods were used to process fixed brain samples by cutting sections that were specifically aimed at the hippocampus (CA1 and CA3) using Rats Brain Atlas as a guide.[33] The Department of Human Anatomy's Histology Unit, ABU, Zaria produced, processed, and stained histological sections using hematoxylin and eosin (H and E) stains to display the histoarchitecture of the CA1 and CA3. The Laboratory for Microscopy and Stereology Researchof the same institution were used to conduct microscopy and micrography utilizing an optical microscope (HM-LUX, Leitz Wetzlar, Germany) and a digital microscopic camera (MA 500 AmScope®, USA).

Data analysis

The results obtained were analyzed using the IBM Statistical Package and Service Solution (version 26.0 Armonk, NY, USA) and the results were expressed as mean ± SEM The presence of significant differences within the means of the groups was analyzed using one-way analysis of variance (ANOVA) while two-way Repeated measures ANOVA was used to compare the mean differences for neurobehavioral studies. *P* values less than ($P < 0.05$) were considered to be statistically significant.

Results

Neurobehavioural study

Barnes maze was utilized to test spatial memory and learning.

The results obtained from the acquisition escape time (ET) test following 90 s of learning and memory test using Barnes Maze revealed a significant ($P < 0.05$) increase in group II treated with only LA (120 mg/kg) when compared to the control, Group IV administered LA (120 mg/kg) + 373 mg EECl revealed a significant decrease when compared to control, also, Group VI treated with 120 mg/kg Pb + 1500 mg EECl showed a significant decrease in ET in relation to Group II treated with only lead [Figure 1a].

The result obtained from the probe test ET following 90 s of learning and memory test using Barnes Maze showed a significant increase in the ET in Group II in relation to the control group. However, there was a substantial decrease in the ET in groups III, IV, and V when compared to groups I and II, there was also a significant decrease in the ET in group VI when compared to group II [Figure 1b].

The result obtained from the frequency of acquisition WT following 90 s of learning and memory test using Barnes Maze showed an increase in WT in group II which is not significant ($P > 0.05$). However, there was a substantial decrease in group VI when compared to the control and group II [Figure 1c].

The result obtained from the frequency of probe WT following 90 s of learning and memory test using Barnes Maze showed a substantial decrease ($P < 0.05$) in the control group when compared to LA-treated group. Also, there was a significant decrease in the frequency of WT in groups V and VI when compared to group II [Figure 1d].

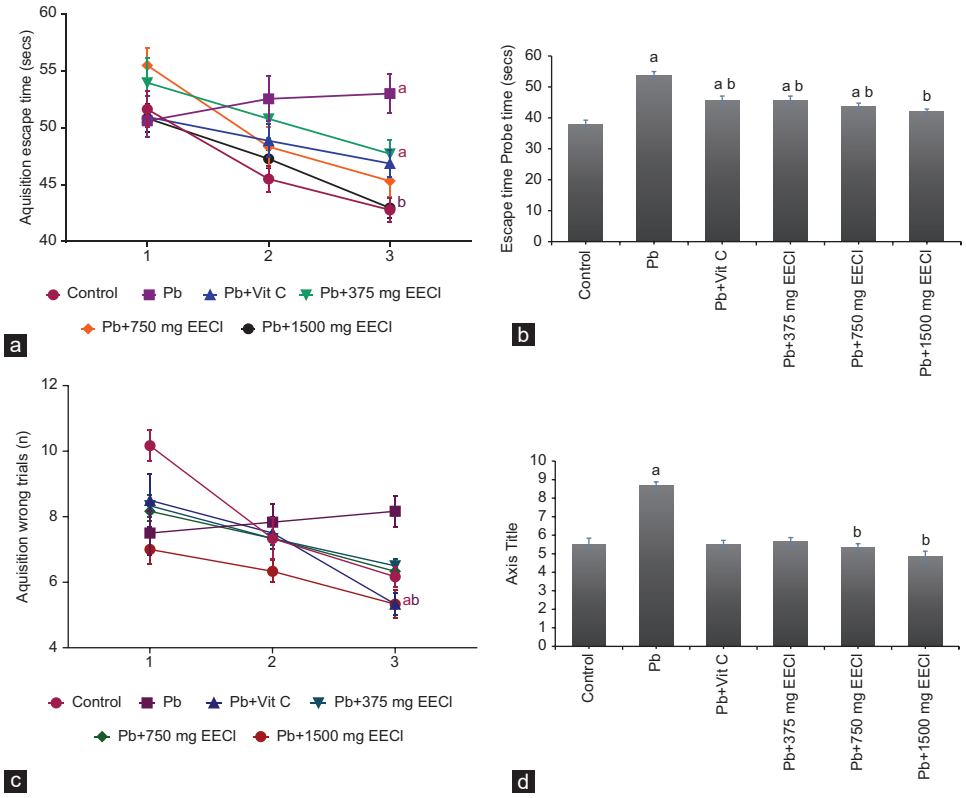

Figure 1: (a) Barnes maze acquisition escape time (s) of experimental animals following oral administration of $Pb2^+$ and *Curcuma longa*. *n* = 6; mean ± SEM, two-ways repeated measures ANOVA, Tukey *post hoc* test. $^aP < 0.05$ when compared to control, $^bP < 0.05$ when compared to $Pb2^+$. (b) Barnes maze probe test escape time (s) of experimental animals following oral administration of $Pb2^+$ and *Curcuma longa*. *n* = 6; mean ± SEM, one-way ANOVA, Tukey *post hoc* test. $^aP < 0.05$ when compared to control, $^bP < 0.05$ when compared to $Pb2^+$. (c) Barnes maze acquisition wrong trials of experimental animals following oral administration of $Pb2^+$ and *Curcuma longa*. *n* = 6; mean ± SEM, two-ways Repeated measures ANOVA, Tukey *post hoc* test. $^aP < 0.05$ when compared to control, $^bP < 0.05$ when compared to $Pb2^+$. (d) Barnes maze probe test wrong trials (n) of experimental animals following oral administration of $Pb2^+$ and *Curcuma longa*. *n* = 6; mean ± SEM, Kruskal − Wallis test, Dunn's *post hoc* test. $^aP < 0.05$ when compared to control, $^bP < 0.05$ when compared to $Pb2^+$. Pb: Lead, Vit C: Vitamin C, EECl: Ethanol extract of turmeric, ANOVA: Analysis of variance

Biochemical studies

MDA, SOD, CAT, and reduced GSH were tested for antioxidant enzyme activity and lipid peroxide levels in the brain tissue homogenate of Wistar rats.

Results on MDA showed a surge in the MDA level of rats administered with only LA (120 mg/kg) when compared to the control group which was not significant. MDA level significantly decreased ($P < 0.05$) in Groups V and VI when compared to the group treated with only LA [Figure 2a].

The level of SOD decreased in Group II treated with only LA 120 mg/kg as observed which was not significant. SOD level increased in Groups III and VI as observed when compared with Group II. However, the changes in the SOD level were not statistically significant [Figure 2b].

A decrease was also observed in the reduced GSH level of rats administered with LA (120 mg/kg) when compared to the control group and an increase in roups III, IV, V, and VI when compared to group II treated with only LA. However, the changes in GSH levels were not statistically significant as observed [Figure 2c].

Histological studies

The hippocampus of Wistar rat is subdivided into four regions, namely: CA1, CA2, CA3, and CA4 (CA stands for *cornu ammonis*), based on the size, density, and branching of the pyramidal cells' axons and dendrites. Each of these pyramidal cell sections is composed of three layers: The stratum molecular (molecular layer), the stratum pyramidale (pyramidal layer), which houses the bodies of the pyramidal cells, and the stratum multiforme (multiform layer). The continuation of CA3 in the concavity of the dentate gyrus (*fascia dentata*) is the CA4. Medium-sized cells are packed closely together in the CA1 region, giant cells are clustered closely together in the CA3 region, and the CA2 region is located between the CA1 and CA3 regions.

Histological examination of the Wistar rats' hippocampi, particularly the CA1 regions in the control group demonstrated their typical histoarchitecture of these regions which is essentially a sheet of organized large neurons (pyramidal cells). Relative to the control, histological examination of Wistar rats' hippocampal CA1 region exposed to lead demonstrated pathologic

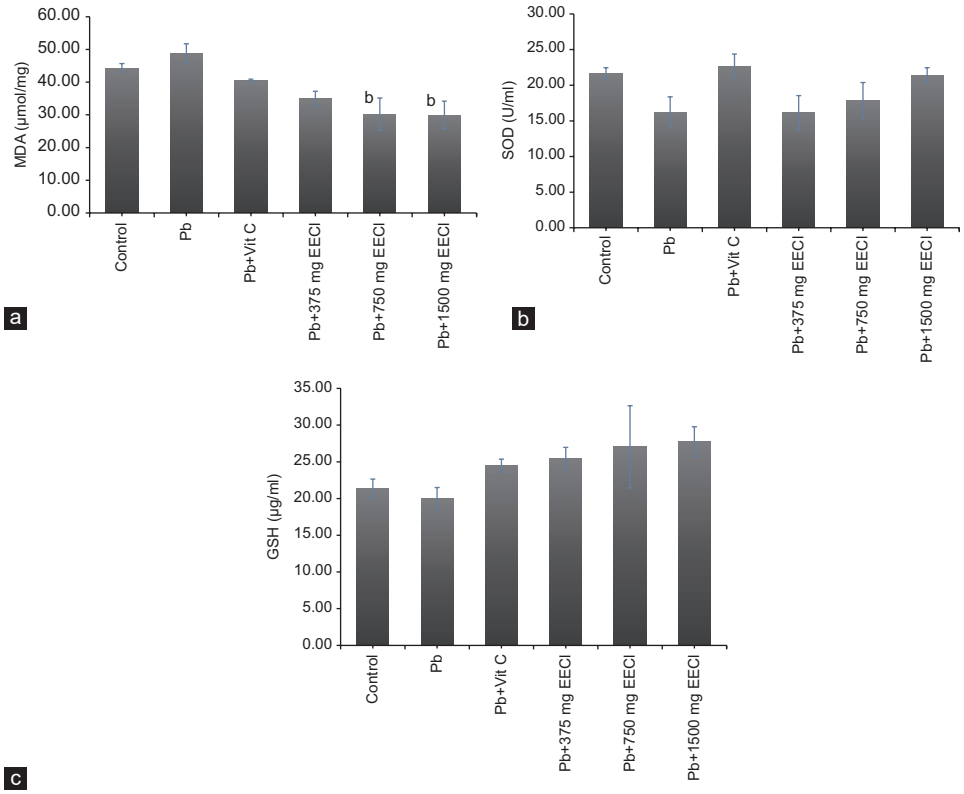

Figure 2: (a) MDA levels in experimental Wistar rats. *n* = 6; mean ± SEM, One-way ANOVA, Tukey *post-hoc* test. $^{b}P < 0.05$ when compared to lead $Pb2^{+}$ group. (b) SOD levels in experimental Wistar rats. *n* = 6; mean ± SEM, One-way ANOVA, $P > 0.05$ when compared to lead $Pb2^{+}$ group. (c) GSH levels in experimental Wistar rats. *n* = 6; mean ± SEM, One-way ANOVA, $P > 0.05$ when compared to lead $Pb2^{+}$ group. Pb: Lead, Vit C: Vitamin C, EECI: Ethanol extract of turmeric, MDA: Malondialdehyde, ANOVA: Analysis of variance, SOD: Superoxide dismutase, GSH: Glutathione

features such as irregular sizes of pyramidal cells of CA1 hippocampal neurons. Some pyramidal cells had a proliferation of glial cells (gliosis), fragmented nuclei (karyorrhexis), and dissolved nuclei (karyolysis). Vitamin C (100 mg/kg) and EECl (375, 750, and 1500 mg/kg)-treated groups revealed some restoration with mild distortions in the histoarchitecture of the pyramidal cells (P) with some pyknosis (Py), karyorrhexis (K), karyolysis (Ky), cytoplasmic vacuolation (cV), and gliosis (g) [Figure 3].

Histological examination of the Wistar rats' hippocampi, CA3 regions in the control group demonstrated typical histoarchitecture of these regions; the basic pattern of large neurons (pyramidal) whose cell bodies are all packed together. The big neurons are the enormous pyramids of CA3 with each neuron having a central vesicular nucleus with a prominent nucleolus. Relative to the control, histological examination of Wistar rats' hippocampal CA3 region exposed to lead demonstrated gross histoarchitectural distortions with karyorrhexis (K) and gliosis (g). Moreover, Ascorbic acid (100 mg/kg), EECl (375, 750, 1500 mg/kg) improved with mild distortion in the pyramidal neurons of the CA3 region of the hippocampus such as cytoplasmic vacuolation (cV), gliosis (g), karyorrhexis (K) [Figure 4].

Discussion

This work used neurobehavioral, pharmacological, and histological evaluation to examine the neurotherapeutic potential of an ethanol extract of *C. longa* on lead-induced hippocampal neurotoxicity in Wistar rats. The importance of the hippocampus in learning and memory cannot be overemphasized. Memory is known as the mental ability of an organism to store, retain and recall information.[34,35] Barnes Maze is a neurobehavioral task that is used in behavioural science and psychology, it serves as a behavioral model to evaluate learning and memory in Wistar rats.[27,36] Learning and memory were reflected by the rat's ability to find the safety spot with a reduced number of WT of treatment session compared to pretreatment session. Increased WT and ET as detected in the lead-treated group are indicative of learning and memory impairment. Results are in line with Naqi,[37] who stated that lead intake could induce cognitive hippocampal damage in adult Wistar rats. LA intake according to report causes mental retardation and reduce learning and memory.[38] Encephalopathy, a response to extremely high concentrations of lead, causes the development of irritability, mental dullness, headache, tremors, attention trouble, memory loss, and hallucinations within weeks of exposure, and it is one of the main neurological effects of lead exposure.[39]

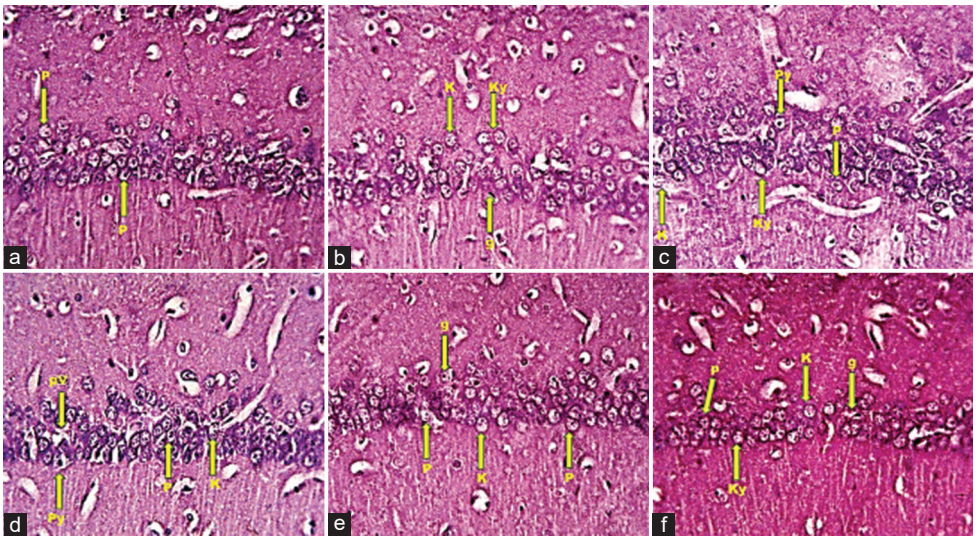

Figure 3: Micrograph of Hippocampus (CA1) of Wistar rats (H and E, × 250). (a) Control (distilled water 2 ml/kg) with normal histoarchitecture of the pyramidal cells (P). (b) Lead acetate (120 mg/kg) treated group with distortions in the histoarchitecture of the cells such as karyorrhexis (K), Ky and gliosis (g). (c) Lead acetate (120 mg/kg) and Ascorbic acid (100 mg/kg) treated group with mild distortions in the histoarchitecture of the hippocampus. Py, karyorrhexis (K), Ky, cV, and gliosis (g). (d) Lead acetate (120 mg/kg) and EECL (375 mg/kg) treated group with mild distortions in the histoarchitecture of the hippocampus. pV, Py, karyorrhexis (k), and some restoration of the histoarchitecture of the pyramidal cells (p). (e) Lead acetate (120 mg/kg) and EECL (750 mg/kg) treated group with some improvement in the histoarchitecture of the hippocampus. Karyorrhexis (k) and gliosis (g). (f) Lead acetate (120 mg/kg) and EECL (1500 mg/kg) treated group with marked improvement in the histoarchitecture of the hippocampus. Gliosis (g), karyorrhexis (K), Ky. pV: Perineuronal vacuolation, Py: Pyknosis, Ky: Karyolysis

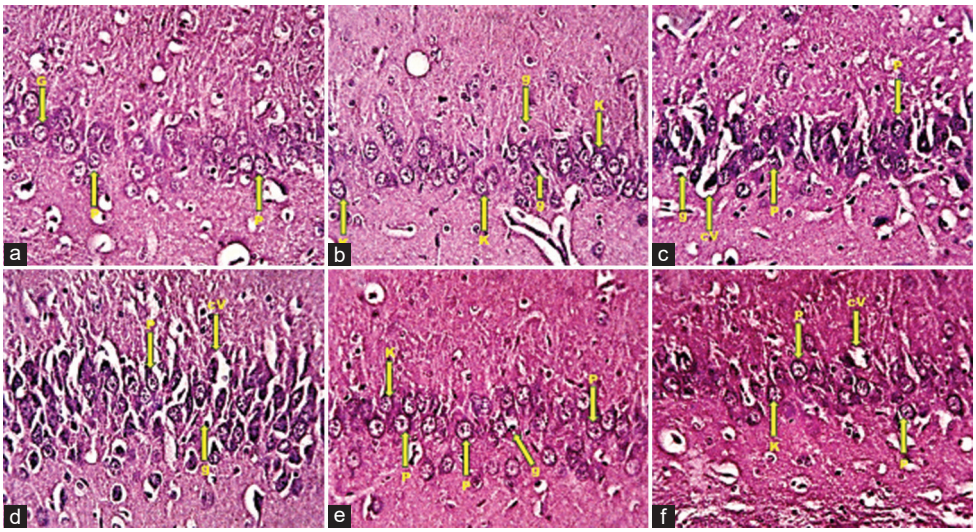

Figure 4: Micrograph of Hippocampus (CA2) of Wistar rats (H and E, × 250). (a) Control (distilled water 2 ml/kg) group with normal histoarchitecture of the pyramidal cells (P). (b) Lead acetate (120 mg/kg) treated group with distortions in the histoarchitecture of the cells such as karyorrhexis (K) and gliosis (g). (c) Lead acetate (120 mg/kg) and Ascorbic acid (100 mg/kg) treated group with mild distortions in the histoarchitecture of the hippocampus. pyramidal cells (P), cV and gliosis (g). (d) Lead acetate (120 mg/kg) and EECL (375 mg/kg) treated group with mild distortions in the histoarchitecture of the hippocampus. Gliosis (g), cV and some restoration of the histoarchitecture of the pyramidal cells (P). (e) Lead acetate (120 mg/kg) and EECL (750 mg/kg) treated group with some improvement in the histoarchitecture of the hippocampus. Karyorrhexis (k) and gliosis (g). (f) Lead acetate (120 mg/kg) and EECL (1500 mg/kg) treated group with marked improvement in the histoarchitecture of the hippocampus cV. cV: Cytoplasmic vacuolation

The Vitamin C-treated group showed decreased WT to the control. Furthermore, administration of EECl after day 14 of treatment had decreased WT in a dose-dependent manner when compared to the control and LA-treated group. This could be due to the ameliorative conclusion of Vitamin C and plant extract on the hippocampus which is in line with the previous studies.[40] Vitamin C is also very rich in biological properties which enhance memory through its bioactive constituents.[41,42] The administration of the plant extracts of turmeric has been reported to chelate the deleterious effects of lead-induced memory deficits and improve memory.[43] These findings imply that turmeric ethanol extracts may help to reduce lead-induced cognitive impairment.

In this study, light microscope examinations of routinely (Hematoxylin and Eosin, H and E) stained

histological sections of hippocampi (CA1 and CA3 regions) were conducted. Neurodegeneration is a process involved in both morphological and brain conditions.[35,44] Observed histoarchitectural distortions of the hippocampal CA1 and CA3 regions, such as an unequal neuronal arrangement of the CA1 and CA3 hippocampal neurons, and changes such as karyorrhexis, karyolysis, clumping of neuronal arrangement of both CA1 and CA3 neurons, also known as gliosis was observed which could be indicative of lead-induced neurodegenerative changes. The deranged CA1 nerve cells in the study imply treatment-related degeneration. This is similar to the work of Barkur and Bairy[45] who reported lead exposure significantly damaged neurons in the hippocampus, cerebellum, and amygdala regions in all lead-exposed groups. Mild degenerative changes in the CA1 and CA3 regions such as cytoplasmic vacuolation, karyorrhexis, karyolysis, pyknosis, and gliosis in the group treated with lead acetated followed by Vitamin C compared to control suggests lead toxicity. Once in the brain, lead may result in morphological changes in the brain that may persevere even after lead levels have decreased because it has an impact on numerous biological processes at the molecular, cellular, and intracellular levels.[46]

Oxidative stress is the difference between the amount of free radicals produced and the biological system's ability to quickly detoxify reactive intermediates or repair the damage they cause.[5] Another sign of oxidative stress is lipid peroxidation, which is also one of the effects of ROS on lipid membranes that have received the most attention. The created free radical harms the cell by stealing electrons from the lipids in the membrane.[47] MDA, a secondary outcome of lipid peroxidation is a decent biomarker of free radical-mediated damage and oxidative stress. In this study, it was observed that rats exposed to only 120 mg/kg LA had an increased concentration of MDA when compared to the control group and rats treated with vitamin C and varying doses of EECl also showed a not significant increase. This finding is not consistent with similar studies by Sudjarwo and Sudjarwo which reported an increase in MDA levels of lead-exposed rats when compared to groups treated with curcumin.[48] However, there was a decrease in the concentration of MDA in groups treated with 750 mg/kg EECl and 1500 mg/kg EECl, respectively, after exposure to LA.

In this study, it was also observed that the levels of SOD in rats administered with only 120 mg/kg LA were lower than that of the control group. Lead-induced oxidative stress is caused by both the generation of ROS and the depletion of antioxidant reserves. A decrease in antioxidant enzyme production may result in alterations in membrane integrity, thereby increasing the vulnerability of the membrane to lead exposure.[46] SOD requires calcium and zinc for their activities. These enzymes can be inactivated by lead by substituting the important co-factors for these enzymes, which are the calcium and zinc ions.[49] Administration of 750 mg/kg and 1500 mg/kg EECl after exposure to 120 mg/kg LA herein study was seen to be ameliorative. The SOD level of rats treated EECl was higher than that of rats administered with only LA although the increase was not significant. This study's findings are congruent with those of Abubakar *et al.* who reported an increase in SOD levels in rats treated with 100 mg/kg and 200 mg/kg curcumin after exposure to lead.[50] Ethanol extracts of EECl have been previously stated to possess very high antioxidant action[51,52] and therefore administration of EECl could have led to the recovery from oxidative stress initially caused by LA.

GSH is a key antioxidant that is present in cells. Reduced GSH is essential for metal scavenging and a precursor to phytochelatins because metals have a significant affinity for their thiol group.[53] In this study, there was a decrease in the concentration of GSH in rats administered with only 120 mg/kg LA when compared to the control group. Administration of 375 mg/kg EECl, 750 mg/kg EECL, 1500 mg/kg EECl, and 100 mg/kg Vitamin C after lead exposure resulted in a surge in the level of GSH when associated with the level observed in rats administered only LA. The finding of this present study could be due to the high antioxidant activity of the ethanolic extract of *C. longa* (turmeric) as earlier reported by Yuliani *et al*.[52] The finding of this study also agrees with other reports by Elsayed on the result of curcumin on the homogenate of mice brain where it was observed to enhance both nonenzymatic and enzymatic cellular antioxidants like CAT, SOD, GSH, and GSH peroxidase (GPx).[54] This result also agrees with prior research by Sidhu and Nehru, who found that GSH levels in the brains of Sprague − Dawley rats exposed to 50 mg/kg LA for 8 weeks were significantly decreased.[46] The finding of this study is also consistent with those of Olayinka *et al.* and Aigbiremolen *et al.* who also reported decreased GSH levels in Wistar rats exposed to LA.[55,56] Lead binds to the sulfhydryl groups of GSH, deactivating it. This causes the-glutamyl cycle, which is often ineffective at replenishing the supply of GSH, to synthesize GSH from cysteine. Lead binds to the sulfhydryl groups of GSH, deactivating it. This causes the-glutamyl cycle, which is often ineffective at replenishing the supply of GSH, to synthesize GSH from cysteine. Similarly, lead inactivates enzymes such as GSH reductase, δ-amino levulinic acid dehydratase, GSH peroxidase, and GSH S-transferase, which lowers the GSH levels even more.[5] The results of this study are also in line with those of a study by Shukla *et al*., who discovered that curcumin greatly decreased lead-induced damage while increasing GSH levels and antioxidant enzymes SOD and CAT in the brains of lead-poisoned rats.[57]

Conclusion

In conclusion, ethanol extract of turmeric possesses possible neuroprotective properties against Wistar rats' hippocampal

lead-induced neurotoxicity. The neuroprotective potentials could be attributed to many phytochemical contents of turmeric such as alkaloids, tannins, steroids, saponins, and flavonoids, among others. To enable the development of therapeutic formulations, more research can be done to determine the efficacy of turmeric as a treatment for physiological changes, oxidative stress-related biochemical disorders, and neuropathologies.

Patient informed consent

There is no need for patient informed consent

Ethics committee approval

Ethical clearance for this study was provided by the Ethics Committee on Animal Use and Care, Ahmadu Bello University (ABU), Zaria: ABUCAUC/16.08.2021/100.

Financial support and sponsorship

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Conflicts of interest

There are no conflicts of interest to declare.

Author contribution subject and rate

- Rimamnde Usman Elisha (40%): Design the research, data collection and analyses.
- Murdakai Tanko (30%): Research organization and supervision.
- Abubakar Adamu Sadeeq (30%): Research organization and supervision.

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Original Article

In silico Evaluation of Single-Nucleotide Polymorphisms in *CHRNA7* and *GRIN1* Genes Related to Alzheimer's Disease

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Abstract

Aim: The purpose of this study is to predict the possible impact of missense single-nucleotide polymorphisms (SNPs) in *CHRNA7* and *GRIN1* genes associated with AD on protein structure, function, and stabilization and to analyze gene–gene interactions via *in silico* methods. **Materials and Methods:** SIFT, PolyPhen-2, SNPsandGO, PROVEAN, SNAP2, PhD-SNP, and Meta-SNP were used to estimate high-risk SNPs. The impact of SNPs on protein stabilization was evaluated with I-Mutant 3.0 and MUpro software. Three-dimensional models of amino acid changes were determined with the Project HOPE software. Furthermore, the gene–gene interactions were analyzed via GeneMANIA. **Results:** According to the results of 603 missense SNPs in the *CHRNA7* gene, rs142728508 (Y233C), rs12899798 (W77G), rs138222088 (R227H), rs140316734 (R227C), rs199633275 (P322R), rs199819119 (L29F), rs200147286 (Q49P), rs200908085 (Y115C), rs201094833 (Q61R), rs201473594 (N69D), rs201210785 (E195K), and rs368352998 (S48W) polymorphisms were predicted as deleterious. Similarly, rs193920837 (P117 L), rs3181457 (I540M), and rs201764643 (R217P) polymorphisms in the *GRIN1* were estimated as deleterious. **Conclusion:** It is thought that the results of this study will provide useful information to guide future diagnostic and experimental strategies for AD.

Keywords: *Alzheimer's disease, CHRNA7, GRIN1, in silico, single-nucleotide polymorphism*

Introduction

Alzheimer's disease (AD) is known as a neurodegenerative disease (ND) that causes neurochemical deficiency in some parts of the brain, accumulation of amyloid-β, decreased cholinergic neurons, and formation of neurofibrillary tangles. Furthermore, pathologically, it causes amyloid-β accumulation outside the cell, while accumulation of tau proteins is observed inside the cell.[1-3] Lately, a range of research has reported that the amyloid-β peptide binds to the alpha 7 nicotinic acetylcholine receptor (α7nAChR) on neuronal cell surfaces, which results in the precipitate of amyloid plaque formation in AD.[4,5] *CHRNA7* gene encodes α7nAChR which are ligand-gated ion channels and substantially expressed in neuronal tissues.[6,7] Furthermore, the N-methyl-D-aspartate (NMDA) receptor is a subtype of glutamate receptors and has been reported to be closely related to neuronal activities. *GRIN1* (glutamate ionotropic receptor NMDA type subunit 1) gene encodes the GluN1 of NMDA receptors.[8]

Single-nucleotide polymorphisms (SNPs) are significant in investigating the risk of susceptibility to diseases and in detecting drug responses. Therefore, SNPs have a key role in the detection of ND.[9,10] Among the SNP types, missense SNPs cause amino acid substitution. Depending on its location, this change can have significant impacts on protein structure, function, and stabilization. The possible deleterious effects of missense SNPs in *CHRNA7* and *GRIN1* genes that lead to amino acid changes on protein function and structure can be estimated with the help of *in silico* analysis software with different algorithms, and the results can further guide diagnostic and experimental strategies.

The aim of this study is to predict the possible impact of missense SNPs in *CHRNA7* ve *GRIN1* genes associated with

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AD on protein structure, function, and stabilization and to analyze gene–gene interactions using *in silico* methods.

Materials and Methods

There is no need for ethics committee approval.

First, the SNPs in the CHRNA7 and GRIN1 genes were obtained from the NCBI dbSNP (https://www.ncbi.nlm.nih.gov/snp/). The sequences of the protein encoded by the CHRNA7 and GRIN1 genes were obtained from the UniProt (https://www.uniprot.org/). Second, publicly available software such as SIFT, PolyPhen-2 (HumDiv-HumVar), SNPs and GO, PROVEAN, SNAP2, PHD-SNP, and Meta-SNP were used to predict potentially harmful SNPs in CHRNA7 and GRIN1 genes. After, I-Mutant 3.0 and MuPro were used to estimate its effect on protein stabilization. Furthermore, three-dimensional (3D) modeling of proteins was created by the Project HOPE. Finally, the gene–gene interactions were determined with the GeneMANIA (https://genemania.org/) [Figure 1].

SIFT (Sorting Intolerant From Tolerant) predicts the impact of an amino acid substitution on the function of a protein according to the sequence similarity and physical features of amino acids.[11] PolyPhen-2 (HumDiv, HumVar) is a software that estimates the effects of an amino acid replacement on the structure and function of a given protein based on physical and comparative features.[12] SNPsandGO predicts whether a SNP is associated with diseases based on protein functional annotation.[13] PROVEAN is a software that makes a prediction on the impact of an amino acid change on the protein function.[14] SNAP2 predicts the functional effects of amino acid substitution based on a "neural network."[15] PhD-SNP (Predictor of human Deleterious SNP) is defined as a predictor of harmful SNPs in humans. The PhD-SNP algorithm was used for estimating the effect of human SNPs in both coding and noncoding sites.[16] The Meta-SNP was used to estimate whether a particular protein variation can be identified as disease-associated.[17]

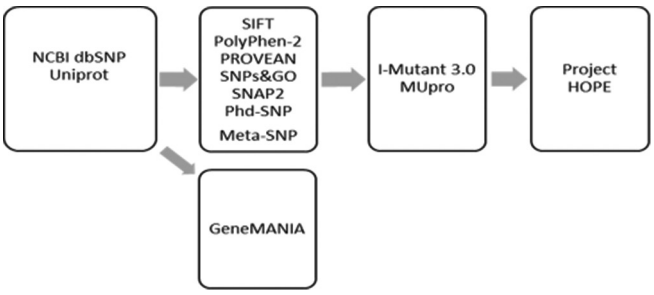


Figure 1: *In silico* analyses tools

MUpro[18] and I-Mutant 3.0[19] are support vector machine-based tools that estimate protein stability alterations due to SNPs. 3D modeling of proteins is created by Project HOPE. It also reports data on features of residues at polymorphism sites.[20] The interactions of *CHRNA7* and *GRIN1* genes with other genes were determined with the GeneMANIA software tool.[21]

Results

Results of gene–gene interaction

It was determined that there were 161 links between them when the interaction of the *CHRNA7* gene with 20 genes was examined. The maximum associated five genes with *CHRNA7* were *MAPK15, ADCY6, MAPKAPK5, MAPK4,* and *MAPK6*. Similarly, 624 links were determined between *GRIN1* and 20 genes examined. *GRIN2A, GRIN2B, FBXO2, GRIN3A,* and *DRD1* genes were determined as the top five genes which have the maximum association with *GRIN1* [Figure 2] (GeneMANIA).

Results of *in silico* analysis of *CHRNA7* and *GRIN1* genes

SNPs information for the CHRNA7 and GRIN1 genes was accessed from the NCBI dbSNP in September 2021. The total number of SNPs belonging to the CHRNA7 gene was 51693 and the number of missense SNPs was 603. A total of 913 different amino acid changes of these missense SNPs were determined. Among them, twelve missense SNPs were determined to be possibly harmful and the results of the analysis are showed in Table 1. For the GRIN1 gene, 591 missense SNPs were determined

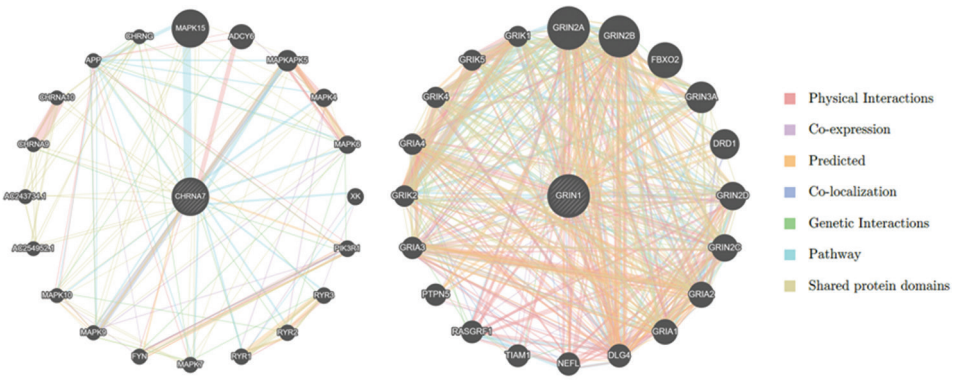


Figure 2: Gene–gene interaction of *CHRNA7* and *GRIN1* genes

Table 1: Prediction results of SNPs in the *CHRNA7* and *GRIN1* genes

Gene name	SNP number	Amino acid change	SIFT	Score	PolyPhen-2 HumDiv	Score	PolyPhen-2 HumVar	Score	PROVEAN score
CHRNA7	rs142728508	Y233C	Dlt	0	PD	1.000	PD	1.000	Dlt
	rs12899798	W77G	Dlt	0	PD	1.000	PD	1.000	Dlt
	rs138222088	R227H	Dlt	0.001	PD	1.000	PD	0.998	Dlt
	rs140316734	R227C	Dlt	0	PD	1.000	PD	1.000	Dlt
	rs199633275	P322R	Dlt	0.001	PD	1.000	PD	0.992	Dlt
	rs199819119	L29F	Dlt	0.002	PD	1.000	PD	1.000	Dlt
	rs200147286	Q49P	Dlt	0.024	PD	0.986	PD	0.983	Dlt
	rs200908085	Y115C	Dlt	0.022	PD	1.000	PD	0.997	Dlt
	rs201094833	Q61R	Dlt	0	PD	0.996	PD	0.977	Dlt
	rs201210785	E195K	Dlt	0	PD	0.999	PD	0.986	Dlt
	rs368352998	S48W	Dlt	0	PD	1.000	PD	0.999	Dlt
	rs201473594	N69D	Dlt	0.044	PD	0.982	PD	0.950	Dlt
GRIN1	rs193920837	P117L	Dlt	0	PD	1.000	PD	1.000	Dlt
	rs3181457	I540M	Dlt	0.002	PD	0.999	PD	0.996	Dlt
	rs201764643	R217P	Dlt	0	PD	1.000	PD	0.998	Dlt

Gene name	Score	SNPs and GO	Score	Meta-SNP results	Meta-SNP score	SNAP2	Score	Expected accuracy	PhD-SNP	RI
CHRNA7	-8.472	Disease	10	Disease	0.853	Effect	65	80%	Disease	8
	-10.612	Disease	10	Disease	0.885	Effect	90	95%	Disease	7
	-4.574	Disease	10	Disease	0.625	Effect	66	80%	Disease	7
	-7.398	Disease	10	Disease	0.761	Effect	61	80%	Disease	6
	-5.409	Disease	10	Disease	0.635	Effect	78	85%	Disease	3
	-3.293	Disease	10	Disease	0.720	Effect	2	53%	Disease	4
	-2.540	Disease	10	Disease	0.519	Effect	24	63%	Disease	1
	-7.538	Disease	10	Disease	0.883	Effect	30	66%	Disease	8
	-3.362	Disease	10	Disease	0.759	Effect	76	85%	Disease	7
	-3.675	Disease	10	Disease	0.792	Effect	57	75%	Disease	5
	-5.724	Disease	10	Disease	0.778	Effect	7	53%	Disease	5
	-4.235	Disease	10	Disease	0.763	Effect	18	59%	Disease	5
GRIN1	-7.801	Disease	10	Disease	0.773	Effect	64	80%	Disease	9
	-2.582	Disease	7	Disease	0.506	Effect	17	59%	Disease	8
	-5.362	Disease	9	Disease	0.634	Effect	80	91%	Disease	6

RI: Reliability index, PD: Probably damaging, Dlt: Deleterious, SIFT: Sorting Intolerant From Tolerant, SNPs: Single-nucleotide polymorphisms

among 13914 SNPs and 751 different amino acid changes were detected. Among them, three missense SNPs were determined to be harmful, and the analysis results are given in Table 1.

Table 2: Stability results of *CHRNA7* and *GRIN1*

Gene name	SNP ID	Amino Acid change	I-Mutant 3.0	DDG (Kcal/mol)	RI	MUpro	DDG
CHRNA7	rs142728508	Y233C	Decrease	0.00	7	Decrease	−0.95518426
	rs12899798	W77G	Decrease	−2.74	9	Decrease	−1.8496217
	rs138222088	R227H	Decrease	−1.22	8	Decrease	−1.198904
	rs140316734	R227C	Decrease	−1.49	3	Decrease	−0.60266667
	rs199633275	P322R	Decrease	−0.24	3	Decrease	−1.1710044
	rs199819119	L29F	Decrease	0.72	4	Decrease	−1.3135187
	rs200147286	Q49P	Decrease	−0.60	1	Decrease	−1.5366824
	rs200908085	Y115C	Decrease	0.85	1	Decrease	−0.77705861
	rs201094833	Q61R	Decrease	−1.02	3	Decrease	−0.91951906
	rs201210785	E195K	Decrease	−1.06	4	Decrease	−1.2298417
	rs368352998	S48W	Increase	−0.32	0	Decrease	−0.38466246
	rs201473594	N69D	Decrease	−1.20	8	Decrease	−0.43114591
GRIN1	rs193920837	P117L	Decrease	−1.43	6	Decrease	−0.33774896
	rs3181457	I540M	Decrease	−0.79	8	Decrease	−1.0170584
	rs201764643	R217P	Decrease	−1.28	4	Decrease	−1.6345586

DDG: Delta Delta G, RI: Reliability index, SNP: Single-nucleotide polymorphism

Table 3: Features of amino acids at polymorphism sites

Gene	SNP ID	Amino acid substitution	Size	Charge	Hydrophobicity
CHRNA7	rs142728508	Y233C	Wild type >Mutant type	-	Wild type <Mutant type
	rs12899798	W77G	Wild type >Mutant type	-	Wild type >Mutant type
	rs138222088	R227H	Wild type >Mutant type	Wild type: Positive Mutant type: Neutral	-
	rs140316734	R227C	Wild type >Mutant type	Wild type: Positive Mutant type: Neutral	Wild type <Mutant type
	rs199633275	P322R	Wild type <Mutant type	Wild type: Neutral Mutant type: Positive	Wild type >Mutant type
	rs199819119	L29F	Wild type <Mutant type	-	-
	rs200147286	Q49P	Wild type >Mutant type	-	Wild type <Mutant type
	rs200908085	Y115C	Wild type >Mutant type	-	Wild type <Mutant type
	rs201094833	Q61R	Wild type <Mutant type	Wild type: Neutral Mutant type: Positive	-
	rs201210785	E195K	Wild type <Mutant type	Wild type: Negative Mutant type: Positive	-
	rs368352998	S48W	Wild type <Mutant type	-	Wild type <Mutant type
GRIN1	rs193920837	P117L	Wild type <Mutant type	-	-
	rs3181457	I540M	Wild type <Mutant type	-	-
	rs201764643	R217P	Wild type >Mutant type	Wild type: Positive Mutant type: Neutral	Wild type <Mutant type

SNP: Single-nucleotide polymorphism

Results of protein stability

Stability analysis of proteins was performed with the I-Mutant 3.0 and MUpro software tools for variants that all online software tools predicted to be functionally harmful. The prediction results of are shown in Table 2.

Results of amino acids at polymorphism sites and three-dimensional models

The features of amino acid changes caused by variants in CHRNA7 and GRIN1 genes on protein structure and function were obtained with Project HOPE. The size, hydrophobicity, and charge differences between wild and variant amino acids as well as 3D structures of the protein were estimated. The results are given in Tables 3 and 4, respectively.

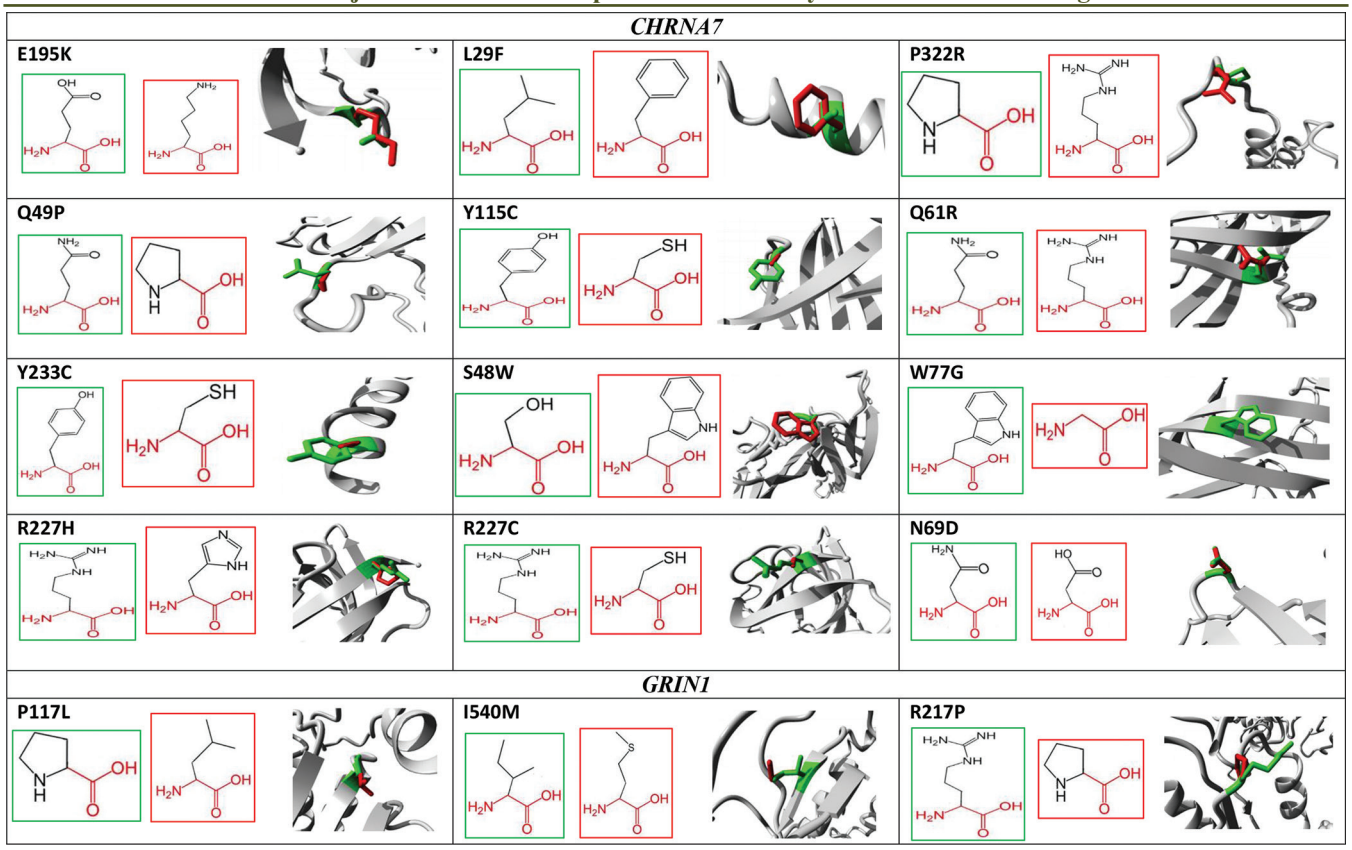
Discussion

In recent years, polymorphisms in the *CHRNA7* and *GRIN1* genes, which are associated with AD, have been the focus of attention. For example, the roles of polymorphisms in the *CHRNA7* gene in response to inhibitors in AD[22,23] and polymorphisms in the *CHRNA7* gene in AD[24] have been reported. Furthermore, the association studies between variations in the *GRIN1* gene and in various diseases such as type 2 diabetes mellitus,[25] paranoid schizophrenia,[26] and Parkinson's disease[27] have been reported. In this study, the possible effects of polymorphisms in these genes were determined by bioinformatics approach based on their roles on various diseases. The high-risk SNPs predicted using bioinformatics tools are rs142728508 (Y233C), rs12899798 (W77G), rs138222088 (R227H), rs140316734 (R227C), rs199633275 (P322R), rs199819119 (L29F) rs200147286 (Q49P), rs200908085 (Y115C), rs201094833 (Q61R), rs201210785 (E195K), and rs368352998 (S48W) in the *CHRNA7* gene and rs193920837 (P117 L), rs3181457 (I540M), and rs201764643 (R217P) in the *GRIN1* gene in this study.

The differences of features between wild and variant type amino acids of amino acid substitutions were investigated via Project HOPE [Table 3]. The protein stability changes caused by amino acid substitutions were estimated via I-Mutant and MUpro [Table 2]. Amino acid changes can affect the folding rate of a protein and depend mainly on the location and type of mutations.[28] Amino acid substitutions can alter the function of a protein with disruption of hydrogen bonds or salt bridges, changing of the physicochemical effects, and geometric constraint changes. These changes may cause destabilization of protein or some abnormal biological functions.[29]

The investigation of gene–gene interactions is significant in the etiology of some diseases such as cancer, cardiovascular, and immune system.[30] For this reason,

Table 4: Project HOPE results of proteins encoded by *CHRNA7* and *GRIN1* genes



gene–gene interaction map was determined in terms of genetic interaction, physical interaction, coexpression, colocalization, shared protein domains, pathways, and predicted interaction in *CHRNA7* and *GRIN1* genes [Figure 2].[31]

Conclusion

Consequently, it is recommended that SNPs, which are predicted to be high risk in *CHRNA7* and *GRIN1* genes as a result of bioinformatic analyses carried out, should be primarily evaluated and investigated in experimental and clinical studies related to AD. For this reason, it is thought that the findings obtained from the study will provide important data for future experimental studies.

Patient informed consent

There is no need for patient informed consent

Ethics committee approval

There is no need for ethics committee approval.

Financial support and sponsorship

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Conflicts of interest

There are no conflicts of interest to declare.

Author contribution subject and rate

- Arash Rezaeirad (40%): Data collection, in silico analysis, writing—original draft preparation
- Ömer Faruk Karasakal (30%): Organizing the research, designing the research and methodology, writing (review and editing).
- Ebru Özkan Oktay (15%): Writing (review and editing), contributed with comments on methodology.
- Mesut Karahan (15%): Writing (review and editing), contributed with comments on methodology.

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