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EDITORIAL

Hoping for a better future

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Dear all,

We are culminating another year, and with it, the hopes and fears that we had at the beginning of it have been presented; some happened for the better, others became cruel realities that we have to face. One great hope was that the coronavirus-19 would finally be eradicated and that consequently the population would return to normality: students to their classrooms, workers to their jobs, professionals to their daily development, hoping that economic activity would increase leading to generating wealth and welfare in the population.

However, worldwide vaccination has not been enough. Even though most of the population has responsibly taken instructions at a central level, the problem prevails in our country. We are still unable to normalize our academic, labor, and professional activities, and the economy continues to suffer alterations that have led to devaluation and inflation.

It seems illogical that we want to solve serious issues, when we have allowed them to progress to the point of making regrettable decisions. We have just approved abortion when it would seem more critical and healthier to educate young people on sexuality, self-respect, and the importance of responsibility when having sex. We shorten the school plans in the university curriculum trying to promote efficiency, but by forming less prepared professionals with fewer resources to generate social welfare. Despite knowing the importance of primary prevention to optimize health, we continue to settle for secondary prevention, which in most cases is too late to help our patients.

It is the end of another year in the life of this publication. I want to thank those who read and follow this journal on behalf of all of us who make its bimonthly publication possible, even in adversity. I am incredibly grateful to the readers, who consider it a tool to continue updating their knowledge in neurosciences; to the peer reviewers who, with their efforts, make the authors improve their articles and enrich each paper with their criticisms and comments. And to the authors, who have trusted this journal to share their experiences within the medical and scientific community and who broaden the knowledge and help many patients indirectly.

I would also like to extend my gratitude for the exceptional work of Dr. Maria Ahn and the editorial team at Publicaciones Permanyer, who oversee the production of each publication from the reception of the papers, peer-review compliance, the design of galleys, and the responses to each query generated in all papers before they get published.

We end another year with the greatest hope that in 2022 we can finally hold face-to-face gatherings, meet with friends, spend more time with our families, and generate wealth in a country that needs education, health, security, and general welfare.

I wish you all a happy end of 2021, and may the year be one filled with health, success, joy, and love.

Happy 2022!

Sincerely yours,

Ildefonso Rodriguez Leyva

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ORIGINAL ARTICLE

High cognitive demand tasks may detect mild alterations in executive functions in American football players: A pilot study

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Abstract

Background: *Repeated head trauma associated with sports activities can cause subtle cognitive alterations in amateur players, but these are difficult to detect.* **Objective:** *The objective of this pilot study was to determine if there is an association between executive functions performance and different sports practice variables in a sample of amateur American football players.* **Methods:** *A pilot transversal study with amateur American football male players without previous neurological or psychiatric illnesses, drug abuse, or consumption of psychotropic medications were carried out and evaluated executive functions performance using automated test. In addition, the levels of stress, impulsivity, and symptoms of anxiety and depression were evaluated, as well as multiple variables related to sports practice such as previous concussions, time of sports practice, weekly training time, and position within the game.* **Results:** *Fourteen men players were assessed, with an average age of 20.57 (standard deviation [SD] ± 1.61) years, played 7 (50%) in an offensive position, 7 (50%) in defensive position, and 3 (21%) presented previous sport-related brain trauma. The average time of practice football was of 35.07 (SD ± 43.10) months, starting age of football playing 17.71 (SD ± 3.64), and hours of training during the week 5.75 (SD ± 2.83). There was no association between cognitive performance and any sports practice variable, however, the offensive position showed significant association with impairments in the highest span of visual working memory task ($\beta = 0.53$, SE = 0.16, $p = 0.001$).* **Conclusion:** *The results suggest that executive tasks with high cognitive demand may reveal alterations in the short term in amateur American football players.*

Key words: *Traumatic brain injury. Concussion. Executive functions. Sports. Cognition.*

Tareas de alta demanda cognitiva pueden detectar alteraciones leves en funciones ejecutivas en jugadores de futbol americano. Estudio piloto

Resumen

Antecedentes: *El trauma craneal repetido asociado a actividades deportivas puede provocar alteraciones cognitivas sutiles en jugadores amateurs, pero estas son difíciles de detectar.* **Objetivo:** *El objetivo de este estudio piloto fue determinar si existe asociación entre el rendimiento en las funciones ejecutivas con distintas variables de práctica deportiva en jugadores*

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de futbol americano amateur. **Material y métodos:** *Se realizó un estudio piloto transversal en jugadores de fútbol americano amateur sin antecedentes de enfermedades neurológicas, psiquiátricas, abuso de drogas o consumo de psicofármacos, y se evaluó el desempeño de las funciones ejecutivas mediante una prueba computarizada. Además, se evaluaron los niveles de estrés, impulsividad y síntomas de ansiedad y depresión, así como múltiples variables relacionadas con la práctica deportiva.* **Resultados:** *Se evaluaron 14 jugadores del sexo masculino, con una edad promedio de 20.57 años (DE ± 1.61), 7 (50%) jugaban en posición ofensiva y 7 (50%) en posición defensiva; 3 (21%) presentaron trauma craneal deportivo previo. El tiempo promedio de práctica deportiva fue de 35,07 meses (DE ± 43.10), la edad de inicio fue de 17.71 años (DE ± 3,64) y las horas de entrenamiento semanal de 5.75 (DE ± 2.83). No hubo asociación entre el rendimiento cognitivo con ninguna variable de la práctica deportiva, sin embargo, la posición ofensiva mostró una asociación significativa con las alteraciones en el rango más alto de la tarea de memoria de trabajo visual ($\beta = 0.53$, $SE = 0.16$, $p = 0.001$).* **Conclusiones:** *Los resultados sugieren que las tareas de funciones ejecutivas con alta demanda cognitiva pueden revelar alteraciones a corto plazo en jugadores de fútbol americano amateurs.*

Palabras clave: *Daño cerebral traumático. Concusión. Funciones ejecutivas. Deportes. Cognición.*

Introduction

The study of repeated head trauma (RHT) in contact sports has recently become more relevant because association between chronic RHT and long-term development of serious neurodegenerative diseases such as chronic traumatic encephalopathy (CTE), but also due to recent studies, suggests an increase in the incidence of concussion related to sports activities in the past decades, particularly in young women[1-3]. This global concern has led to the recent publication of several guidelines for the diagnosis and management of concussion associated with sports activities[4,5], as well as an effort to develop and validate the use of serum, cerebrospinal fluid, and neuroimaging biomarkers to support the diagnosis of CTE *in vivo*[6,7].

The development of cognitive and behavioral disturbances in RHT related to contact sports seems to require a significant exposure time, such as CTE, whose presentation latency ranges from a few years to several decades after stopping the sports activity[8]. In this sense, various studies have shown that the number of years of sports practice as well as the age of onset in sport activity have an influence on the cognitive alterations that are present in sport-related brain trauma[9,10]. Acute alterations (0-24 h) after trauma have been consistently demonstrated in concussive and subconcussive trauma, including cognitive and other post-concussional symptoms (headache, dizziness, sleep disturbances, etc.). Nevertheless, the follow-up reports show a reversal of those alterations in a period of a few days to 1 month[11]. Despite this, some studies reported that subjects with a history of prior trauma display a slower recovery, suggesting a cumulative effect of repeated injuries[12].

However, most populations of athletes previously studied often have multiple "neuroprotective" factors (youth, regular physical activity, diet, educational level, etc.). The effects of RHT are challenging to detect especially in the short term, because the subjects may compensate cognitive deficits until the damage reaches a "threshold" level[13]. On the other hand, amateur athletes may have different conditions than professional athletes: (a) fewer hours of physical preparation and training (which makes them more prone to suffer injuries) and (b) having a lesser quality of protective equipment or neuroprotective factors (especially in developing countries).

In the study of cognitive alterations in RHT, emphasis has been placed on the processes that have shown sensitivity to indicate alterations in athletes, such is the case of working memory, decision-making, planning, inhibition, and reaction times[14,15]. In this sense, the use of computerized tests represents an advantage in the study of repeated blows to the head, since it allows the randomized presentation of the stimuli to avoid the learning effect of a pencil and paper test and the measurement of times reaction rate as a measure of processing speed[16].

For all of the above, it is important to study the cognitive effects of RHT on this group of athletes, especially in developing countries, where this type of research is practically non-existent[17,18]. With all these in mind, the objective of the present research was to determine the association between the performance of executive functions with variables related to sports practice in amateur American football players.

Methods

The present study was adhered to the principles of the Helsinki Declaration revised in 2008 and was approved by the research and ethics committee of the

School of Medicine at the Universidad Nacional Autónoma de México (UNAM), and all participants signed and received a copy of the informed consent. An observational, cross-sectional pilot study was carried out in 14 amateur American football players belonging to the team from the School of Medicine at UNAM, Mexico City. The inclusion/exclusion criteria were as follows: male sex, being an active and regular player, without: previous neurological or psychiatric illnesses (included non-sport-related brain trauma), drug abuse, or consumption of psychotropic medications; high levels of stress, impulsivity, or important traits of anxiety and depression that may affect cognitive performance were discarded by the assessment of Hamilton, Beck's, SISCO inventory, and Barratt impulsivity scales. All evaluations were applied before the start of the game season and in an examination-free period. Executive functions (visual-spatial and verbal working memory, inhibition, and speed processing) were assessed by automated tests of the PEBL version 2.1 software (http://pebl.sourceforge.net/). The digit span, Flanker test. and Corsi cubes were the selected sub-tests. These tasks require the subject to identify, remember, and effectively manipulate information about numbers, words, and figures quickly and with increasing complexity. These tasks were chosen because the previous studies suggest that they are more sensitive in detecting changes in patients with sport-related concussion[16]; in addition, the use of the PEBL software allowed to quantify very precisely the errors made in the tasks and also the reaction times of participants, which would not be possible using traditional pencil and paper tests[16]; Likewise, different variables related to their sports activity (age of onset, years of sports practice, position in the game, amount of training days, previous sports concussions, etc.) were collected. Statistical analysis: The data analysis was performed with the statistical package SPSS version 23 (IBM Corp., 2014). To assess the association between variables related to sport activity on score and reaction time in every executive task (dependent variable), with a robust estimator of the maximum likelihood. a generalized linear model of main effects was made. For analyses, the decision rule was set to a value $p \leq 0.05$ with two-tailed hypothesis tests.

Results

In this study, a total of 22 male players were evaluated (the entire team), however, when applying the inclusion and exclusion criteria, eight participants were eliminated (due to personal history or use of psychotropic drugs) and a final sample of 14 men players was included, with an average age of 20.57 (standard deviation [SD] ± 1.61) years, played 7 (50%) in an offensive position, 7 (50%) in defensive position, and 3 (21%) presented previous brain trauma. The average time of practice football was of 35.07 (SD ± 43.10) months, starting age of football playing 17.71 (SD ± 3.64), and hours of training during the week 5.75 (SD ± 2.83). There were no differences between offensive and defensive players in time of practice, $p = 0.989$, starting age of football playing, $p = 0.773$, and hours of training during the week, $p = 0.353$. Furthermore, there were no differences between offensive and defensive players regarding global performance of digit span test, flanker test, and Corsi test (Table 1). Finally, a significant effect of offensive position controlled for previous cranial trauma was found in the Omnibus test $\chi 2$ (2) = 10.81, $p = 0.004$, but only in the highest span of visual working memory task (Corsi task level 5) (Table 2).

Table 1. Comparison of players global performance in executive functions tasks

	Offensive (n = 7)	Defensive (n = 7)	Stat.	p.
Span digit test*				
Total corrects	7.29 ± 2.4	7.71 ± 1.1	20.50	0.62
Total time (ms)	4.08 ± 0.4	3.79 ± 0.7	18.01	0.45
Flanker test*				
Total errors	48.2 ± 76.3	50.7 ± 71.4	23.50	0.90
Total time (ms)	514.8 ± 196	545.6 ± 174	17.50	0.38
Corsi test*				
Total corrects	9.8 ± 1.5	10.5 ± 1.2	18.00	0.45
Total time (ms)	4.85 ± 1.4	4.95 ± 0.4	19.50	0.53

*Data reported by mean (standard deviation)) and compared using the Mann–Whitney U-test. Stat.: statistic.

Discussion

The results of the present pilot study suggest a significant association between player's offensive position (controlled by previous cranial trauma), with a low cognitive performance but only in the highest span of visual memory task. Likewise, the tasks that evaluate visuospatial working memory with higher cognitive demand (greater number of elements) may be more sensitive to detect the early alterations in amateur athletes who receive repetitive cranial trauma and have multiple factors of neuroprotection.

Table 2. Multivariable association between sport practice variables and highly demand visual-spatial working memory task

Parameter	B	β	SE (β)	CI 95% (β)	χ^2 Wald	p
Intersection	4913	0.030	0.1590	(–0.28, 34)	0.035	0.85
Position (offensive)	–771	0.536	0.1650	(0.21, 0.85)	10.54	0.001
TCE (presence)	1032	–0.552	0.0876	(–0.72, –0.38)	39.79	0.0001
(Scale)	232,992	0.402	0.1521	(0.19, 0.84)		

B: beta no standardized; β: beta standardized; SE: standard error; CI: confidence interval.

Our findings are in agreement with that reported by Baugh et al., 2015, who point out that the player's position is a risk factor in athletes exposed to RHT, since according to their location on the playing field, athletes are exposed to a greater or lesser number of head trauma[19,20].

Another factor observed in this research is the history of previous head trauma, since a player who previously suffered a trauma is more prone and more vulnerable to the effects of a second trauma, and may also have a cumulative effect[21].

On the other hand, neuropsychological assessment is considered crucial in the assessment of RHT in athletes[22]. The evidence suggests cognitive impairments in specific processes such as working memory, inhibition, cognitive flexibility, planning, and processing speed[23]. This emphasis on the evaluation of executive (frontal) functions is mainly related to the biomechanics of trauma, in which it is proposed that there is greater damage in frontal orbital and dorsolateral regions, as well as in the anterior and basal portion of the temporal lobes[24,25].

Based on our preliminary results, we suggest that future research emphasizes on the evaluation of the right frontal functions, especially in tasks with high cognitive demand, and in a larger group of amateur football players. On the other hand, regarding amateur sports practice, we can suggest the rotation of offensive and defensive positions to try to reduce the risk of brain damage. The final purpose of this type of studies is to generate alternatives so that players can carry out a safer sports practice.

Conclusion

In this group of amateur football players, the offensive position controlled by a history of previous head trauma was related to poor performance in visuospatial working memory task with a higher cognitive demand.

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Conflicts of interest

All authors declare that they have no conflicts of interest with this research or with the publication of its results.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors have obtained the written informed consent of the patients or subjects mentioned in the article. The corresponding author is in possession of this document.

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ORIGINAL ARTICLE

Depression is associated with CD4 levels in people living with HIV in Ecuador

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Abstract

Background: *HIV can cause neurotoxicity and neuronal apoptosis, hence the importance of neuropathogenesis mechanisms in HIV. People in AIDS phase with CD4 count under 250, may be at greater risk of suffering from depression.* **Objective:** *Evaluate whether there is a relationship between T-CD4 lymphocyte count and depression in people living with HIV but have not started antiretroviral treatment.* **Method:** *This was a cross-sectional cohort study conducted between October 2020 and January 2021 in the HIV Unit of the Eugenio Espejo Hospital in Quito. A CD4 test was performed, and the Hamilton Depression Scale was used in patients after 1 month of being diagnosed with HIV but before receiving antiretroviral treatment.* **Results:** *A total of 108 subjects were recruited, mainly men (79.6%), of which 82.4% were between 18 and 45 years old. The subjects in the AIDS phase were 25% of which 92.6% presented depression (p = 0.000), it was noted that the older the age, the prevalence of depression increases (p = 0.007). As for the female sex, it seems to have a risk of practically double with respect to men of suffering from depression (p = 0.005).* **Conclusions:** *In our cohort study, at PLWH in Ecuador, patients in AIDS phase present a considerable risk of suffering from depression; at the same time, as the age increases, the probability of presenting depression is greater. As well as, female sex is a risk factor. Therefore, the follow-up of these patients is an essential part of care and treatment process.*

Key words: *CD4. HIV. Depression.*

Asociación entre conteo de CD4 y depresión en personas que viven con VIH en Ecuador

Resumen

Antecedentes: *El VIH puede causar neurotoxicidad y apoptosis neuronal, de ahí la importancia de los mecanismos de su neuropatogénesis, las personas en fase de SIDA con conteo de CD4 inferior a 250 pueden tener mayor riesgo de padecer depresión.* **Objetivo:** *Evaluar si existe relación entre el recuento de linfocitos T-CD4 y la depresión en personas viviendo con VIH en pacientes que no han iniciado tratamiento antirretroviral.* **Método:** *Se trata de un estudio de cohorte transversal realizado entre octubre de 2020 y enero de 2021 en la Unidad de VIH del Hospital Eugenio Espejo de Quito. Se realizó una prueba de CD4 y se utilizó la Escala de Depresión de Hamilton en pacientes un mes después de ser diagnosticados con VIH y antes de recibir tratamiento antirretroviral.* **Resultados:** *Se reclutaron 108 sujetos, principalmente hombres (79.6%), de los cuales el 82,4% tenían entre 18 y 45 años. Los sujetos en fase SIDA fueron 25% de los cuales 92.6% presentaron depresión (p = 0.000), a mayor edad aumenta la prevalencia de depresión (p = 0.007). En el sexo femenino el riesgo parece*

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duplicar con respecto a los hombres de padecer depresión (p = 0,005). **Conclusiones:** *En nuestro estudio de cohorte, en PLWH, los pacientes en fase SIDA presentan un riesgo considerable de padecer depresión, a medida que aumenta la edad, la probabilidad de presentar depresión es mayor y el sexo femenino es un factor de riesgo. Por tanto, el seguimiento de estos pacientes es una parte fundamental del proceso de atención y tratamiento.*

Palabras Clave: *CD4. VIH. Depresión.*

Introduction

As shown, people living with HIV tend to experience prolonged episodes of depression throughout their illness, which directly affects adherence to treatment and their quality of life[1]. HIV can cause neurotoxicity and neuronal apoptosis[2], hence the importance of mechanisms in HIV neuropathogenesis, since pro-inflammatory cytokines/chemokines released by infected macrophages/microglia, excitotoxicity, and oxidative stress are evidenced as the main causes of neuronal injury, in addition to the one directly caused by viral proteins[3]. Traditionally, the histopathological impact of HIV brain infection included widespread reactive astrocytosis, activation of microglia, microglial nodules, and multinucleated giant cells in central white and deep gray matter, monocytoid cell infiltrates, and myelin paleness[4]. The persistence of HIV predicts the dysregulated metabolism of catecholamine precursor amino acids[5] with a clear incidence in the pathophysiology of depression. Serotonergic neurons play a crucial role in brain function and dysfunction, as in major depressive disorder; however, the complexity of serotonergic projections seriously hinders the elucidation of their precise mechanisms[6]. Therefore, the central nervous system of a patient with HIV would be notably aged in relation to his age group[7]; this neurodegenerative process would be related to the increase in the chances of the appearance of depressive symptoms. This study focuses on the relationship between CD4 T-lymphocyte count and depression, since there is still not enough scientific evidence to clarify whether patients with a CD4 lower than 250 cells/ml (AIDS) present more depressive symptoms in relation to those who are not in the AIDS phase[8].

Subjects and methods

This was a cross-sectional cohort study conducted between October 2020 and January 2021 in the HIV Unit of Hospital Eugenio Espejo in Quito, Ecuador. This hospital, one of the largest in Ecuador, is responsible for providing care for at least 10% of PLWHA nationwide. The study was authorized by the Ethics Committee for Research in Human Beings validated by the Ministry of Public Health of Ecuador. The informed consent of each subject was obtained.

All NAIVE patients who attended the HIV clinic in this period with an HIV diagnosis were invited to participate in the study. A total of 108 subjects were recruited, with a diagnosis of HIV through the third and fourth generation ELISA test with a reactive result, as indicated by the guidelines of the Ministry of Public Health of Ecuador. A CD4 test was performed at the national reference laboratory of the Ministry of Health. After signing the consent form, each one was evaluated using the Hamilton depression scale[9], which consists of five categories: no symptoms of depression (<7 points), and then mild (8-13 points), moderate (14-18 points), serious (19-22 points), and very serious (> 23 points). The Spanish version of the Hamilton Depression Scale has been previously validated[10]. The evaluation with this scale was carried out one month after diagnosis and before the start of antiretroviral therapy. Subjects with a previous history of depression and psychiatric illness were excluded. The captured data were entered into an electronic database and descriptive statistics, the statistical analysis contemplated a 95% confidence interval, using Chi-square, for which the SPSS version 23 program was used. In all cases, $p < 0.05$ was statistically significant.

Results

A total of 108 subjects were recruited, mainly men (79.6%) (Table 1), of which 82.4% were between 18 and 45 years old. Of the people aged between 46 and 60 years, 63.1% had mild-to-moderate depression (n = 12), the individuals aged between 31 and 45 years 45.8% showed some type of depressive symptoms (n = 12) (n = 22). In the 18 to 30-year-old category, 26.8% (n = 11) had some sign of depression (Table 2). The evaluation with the scale of depression of Hamilton, 58.3 % (n = 63) of patients had no depression, and 41.7 % (n = 45) presented some degree of depression. The

Table 1. Sex

	Frequency	Percentage	Valid percentage	Accumulated percentage
Valid				
Man	86	79.6	79.6	79.6
Woman	22	20.4	20.4	100.0
Total	108	100.0	100.0	

Table 2. Age

	Frequency	Percentage	Valid percentage	Accumulated percentage
Valid				
18-30	41	38.0	38.0	38.0
31-45	48	44.4	44.4	82.4
46-60	19	17.6	17.6	100.0
Total	108	100.0	100.0	

Table 3. CD4

	Frequency	Percentage	Valid percentage	Accumulated percentage
Valid				
AIDS	27	25.0	25.0	25.0
No AIDS	81	75.0	75.0	100.0
Total	108	100.0	100.0	

Table 4. Cross table

	CD4		Total
	AIDS	No AIDS	
Depression			
Absent			
Count	2	61	63
% within CD4	7.4%	75.3%	58.3%
Mild			
Count	11	19	30
% within CD4	40.7%	23.5%	27.8%
Moderate			
Count	14	1	15
% within CD4	51.9%	1.2%	13.9%
Total			
Count	27	81	108
% within CD4	100.0%	100.0%	100.0%

subject considered phase AIDS was 2.5%. Of the subjects in the AIDS phase, 92.6% manifest depressive symptoms and only 7.4% (n = 2) did not present depression. By contrast of the subjects considered non-AIDS, 75.3 (n = 61) were not positive for depression, a considerably lower number 24.7% (n = 20) scored positive in some measure for depression. As per the women who participated in the study, 68.2% presented depression (n = 15) (Tables 3 and 4).

It was found that there is a relationship between depression and age (p = 0.007). Similarly, there is a positive association between sex and depression (p = 0.005). Finally, there is a highly significant association between patients in the AIDS phase and depression (p = 0.000) (Table 5).

Discussion

In the present study, it was found that around 40% of PLWHA treated at the HIV Clinic at the Eugenio Espejo Hospital in Quito, Ecuador had some degree of depression, much more than previously reported in a similar population in Ecuador[11]. It is important to mention that mental health is a generally neglected area among health services in Ecuador, which leads to failure in the diagnosis of psychiatric problems among the general population, not to mention among PLWHA. Most of the subjects, who are in the AIDS phase in this study, present symptoms of depression in line with the few studies carried out in reference to this topic[12], which shows a possible role of the neurotrophic factor derived from the brain in the symptoms of depression and other aspects of the state of mind that until now have been superficially studied. It has also been noted that there are more positive cases of depression as age increases, these results are in accordance with other studies carried out[13]. Age appears to be a predictor of comorbidity. The female sex also seems to be associated with a higher incidence of

Table 5. Chi-square tests

	Value	gl	Asymptotic significance (bilateral)
Pearson's Chi-square	55,539[to]	2	0.000
Likelihood ratio	56,951	2	0.000
Linear by linear association	53,710	1	0.000
N of valid cases	108		

depression in PLWHA, as verified in other studies[14], studies in Spanish-speaking America in this regard are scarce, so this mood disorder could be related to other variables. Lower levels of brain-derived neurotrophic factor BDNF were associated with AIDS diagnosis and CD4 count, but not with viremia or duration of infection, which, in turn, has a direct correlation with depression[12] confirming our premise that CD4 levels are directly related to depression. Studies conducted in the United States show that age, sex, race, baseline CD4 count, and viral load are significant factors associated with increased risk of mental illness[15], which supports our findings. While we recognize that the screening tools used for depression may have increased the number of diagnosed patients, we believe that the criteria used allowed us to select the most clinically significant cases. Furthermore, another limitation was that the subjects were recruited from only one center, although one of the largest, and there was no control group due to national guidelines for the care of PLWHA. In any case, our results support that patients in the AIDS phase have a higher risk of suffering from depression, as well as gender and age are factors that contribute to both the frequency and the severity of depression cases among PLWHA in Ecuador.

Conclusions

In our cohort study, in PLWHA in Ecuador, patients in the AIDS phase have a considerable risk of suffering from depression, in the same way, at an older age; there is a greater probability of presenting depression as well as being a female sex a risk factor. Therefore, the follow-up of these patients is essential as part of the care and treatment process.

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Conflicts of interest

The authors declare that they have no conflict of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that they have followed the protocols of their work center on the publication of patient data.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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REVIEW ARTICLE

Quality of life in primary caregivers of patients with cerebrovascular disease

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Abstract

Stroke is the leading cause of motor and neuropsychological disability in adults worldwide, requiring a primary caregiver (PC) during rehabilitation. The relationship between PC and a patient with cerebrovascular sequelae (PC-PCVD) is complex and bidirectional. Indeed, literature shows a serious deterioration in the PC's quality of life during the follow-up. Through a narrative review of articles published in the last 20 years, this study aims to know the quality of life of PC-CVD, analyze the determinants of the vulnerability of PC-PCVD, and identify the most used test. PC-PCVD was found to have a lower-than-expected quality of life in physical and emotional domains. Being a woman, single, of mayor age, 3 h of daily care, limited income, and symptoms of anxiety or depression were the main risk factors associated with lower quality of life among PCs. The main assessment tools of quality of life were the World Health Organization Quality of Life BREF and the SF-36 Health Survey.

Key words: *Caregiver. Main caregiver. Quality of life. Stroke.*

Calidad de vida en cuidadores primarios de pacientes con enfermedad vascular cerebral

Resumen

La enfermedad vascular cerebral (EVC) es la principal causa de discapacidad motora y neuropsicológica en adultos a nivel mundial, demandando en el proceso de su rehabilitación la presencia de un cuidador primario (CP). La interacción del CP con el paciente con secuelas de EVC (CP-PEVC) es compleja y de acuerdo a evidencias recientes es además bidireccional. De hecho, la mayoría de la información coincide en un deterioro grave en la calidad de vida de los CP durante el seguimiento. Mediante una revisión narrativa de artículos publicados en la literatura en los últimos 20, el presente trabajo tuvo como objetivos conocer la calidad de vida del CP-PEVC, analizar los factores que determinan su vulnerabilidad e identificar las principales pruebas en la evaluación de su calidad de vida. Se encontró que los CP-PEVC presentan niveles de calidad de vida por debajo de lo esperado, específicamente en dominios físico y emocional. Ser mujer, soltera, de mayor edad, con al menos tres horas de cuidado diarios, con ingresos económicos limitados y síntomas de ansiedad o depresión fueron los principales factores de riesgo asociados a menor calidad de vida en los CP. Las principales pruebas utilizadas fueron el World Health Organization quality of Life BREF (WHOQOL-BREF) y el Short Form 36 Health Survey Questionnaire (SF-36 Health Survey).

Palabras clave: *Calidad de vida. Cuidador. Cuidador primario. Enfermedad vascular cerebral.*

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Introduction

Cerebrovascular disease (CVD), either ischemic or hemorrhagic, represents the second cause of death in developed countries and the first cause of disability in adults worldwide[1]. Over 90% of CVD survivors have several consequences, while 30-50% present disability for activities of daily living, both basic and instrumentals[2,3], these patients require a sophisticated infrastructure and organization by the health system, as well as the participation of competent community personnel and family members or primary caregivers (PC) at home.

The PC plays a key role in the rehabilitation of patients with CVD. However, due to the absence – in the worst case – or failures and inconsistencies in the hospital or out-of-hospital support network, it is possible that the burden of PC is excessively high and produces stress, anxiety, depression, sleep disorders, physical pain, and poor quality of life.

The complex concept of quality of life quality was introduced since the 1980s. It was defined by the United Nations Organization[4] as the perception of an individual about his position in life, in the context of his culture and his value system and about his goals, expectations, standards, and concerns; however, there is heterogeneity of definitions and therefore, each author describes various components or domains for this concept.

The analysis of the quality of life of both the patient with CVD and the caregiver favors the understanding of the impact it has on other aspects of cerebral vascular disease that are not usually addressed. In this narrative review, it is intended to answer the following questions: (a) which are the affected quality of life domains in the PC of CVD patients (PC-PCVD)?; (b) what factors determine the level of quality of life of PC-PCVD?; and (c) what are the most used instruments in the evaluation of quality of life for PC-PCVD?

Methods

This article is based on unsystematic research in *Google Scholar and PubMed* for original manuscripts about: “Cerebral Vascular Disease,” “Cerebrovascular Disease,” “Cerebral vascular accident,” “Stroke,” “Cerebral infarction,” “Intracerebral hemorrhage,” “Caregiver,” “ PC,” and “Caregiver quality of life” followed for a discretionary selection of publications. Some of the references used as “clinical evidence sources” are commented on the reference list.

The inclusion criteria were: (a) studies published between 2000 and 2020; (b) Spanish or English language; (c) general or specific objective was to analyze the quality of life of PC-PCVD and/or to find the determinants of quality of life, (d) publications with CVD of ischemic disease (cerebral infarction) or hemorrhagic disease (intracerebral hemorrhage).

The exclusion criteria were: (a) studies of paid caregivers and (b) reviews of the literature, reflections, or editorial notes on the subject.

The information analysis was about content. It was carried out with the help of tables, where each article was categorized by objective, year, sample size, methodology, and conclusion.

Results

During the period of the last 20 years, 244 eligible abstracts were identified. However, only 28 articles were selected according to the inclusion and exclusion criteria described above. Indeed, figure 1 explains the complete selection process for this non-systematic review.

Out of the 28 articles, only 3 articles were published in Spanish and were developed in Colombia and Spain. On the other hand, the 25 English articles were directed in countries as Sweden, the United States, Canada, Mongolia, Japan, China, Korea, Portugal, Brazil, Ireland, and Italy.

To accomplish the objectives, the results of this analysis are presented in three different sections: (I) quality of life of PCs of patients with cardiovascular disease, (II) determinants of quality of life in PC of patients with cerebrovascular disease, and (III) quality of life tests more frequently used in evaluating PC of patients with CVD.

Besides, it is essential to establish that due to the heterogeneity in the methods (study designs and tests) of the reviewed articles, there are not specified numerical values of the performances of neither the caregivers nor patients.

Quality of life of PC of patients with cerebrovascular disease

In a Swedish study, the quality of life of PC of patients with ischemic (89.1%), hemorrhagic (10.6%), and undefined (0.3%) CVD was compared at 4 and 16 months[5]. The Short Form 36 Health Survey Questionnaire (SF-36) was used to assess eight domains through subjective personal perception: physical functioning (degree of limitation on activities of daily living due to physical health); physical role (limitations on instrumental and work

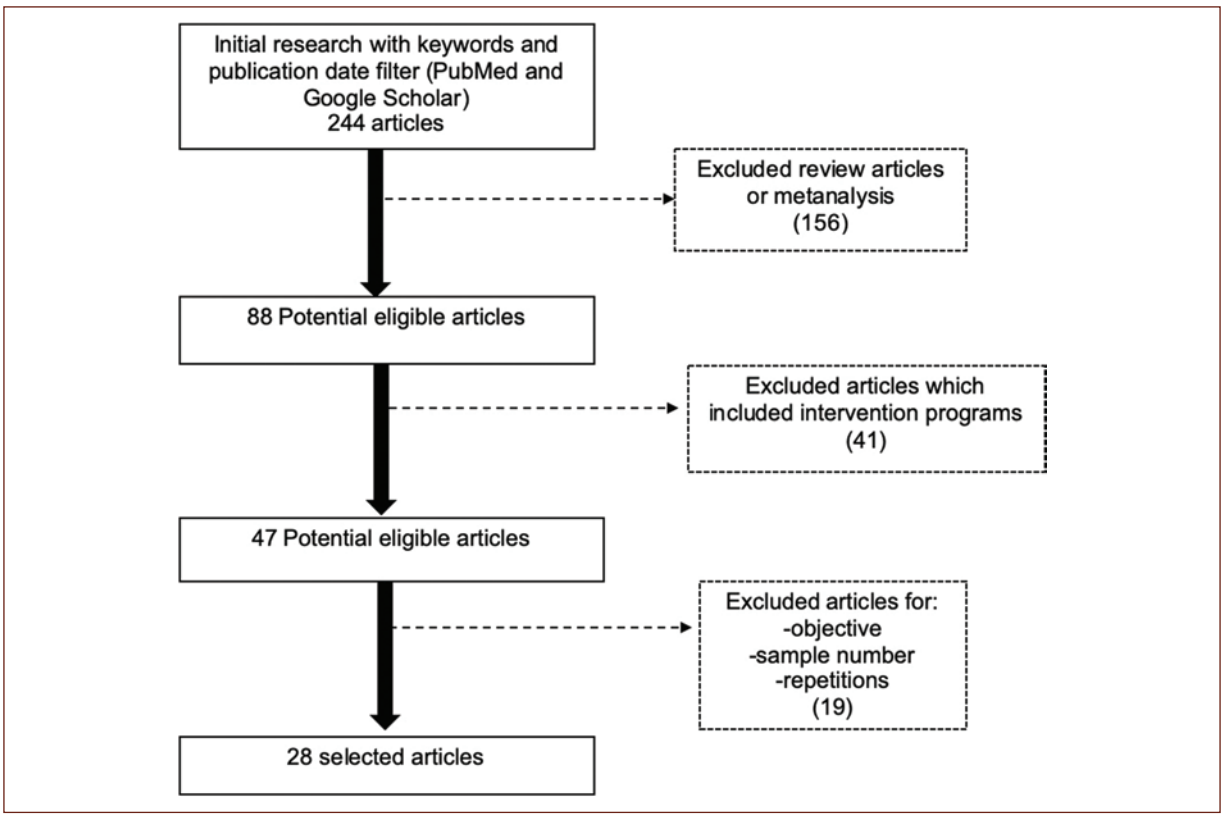

Figure 1. Diagram of the selection process of the articles.

activities due to alterations in physical health); bodily pain (limitation due to physical pain); general health (includes the perspective of health and resistance to illness); vitality (amount of energy); social functioning (degree of social life impact); emotional role (level at which emotional difficulties affect daily and work activities); and mental health (depressive symptoms, anxiety, behavioral and emotional disturbances). Each domain is scored from 0 to 100, with the highest score meaning better quality of life[6].

Overall, caregivers scored better than patients in the short and long term, with the exception of the emotional role domain, which was the only domain with a significantly lower performance for PC[5].

With the support of the same assessment instrument, and therefore, following the same theoretical aspects of quality of life, McPherson et al., in a Canadian study, found out that when comparing the quality of life of ischemic (71%), hemorrhagic (25%), and mixed (4%) PC-PCVD with the non-caregiver subjects from the normative sample of the SF-36 Health Survey, there was a lower score in all eight domains among caregivers. Although, the most significant were physical role, physiological functioning, and emotional role[7].

Another study conducted in the United States compared the well-being of PC-PCVD (92% ischemic, 2% hemorrhagic) versus non-caregivers after 3 years of follow-up. Well-being was considered as the absence of depressive symptoms, quality of life (physical and mental aspects), and satisfaction with life and with leisure activity. Evaluations were obtained at nine, 18, 27, and 36 months. At 9 months, PC-PCVD performed lower than controls in every expected area in the quality of life physical domain. Likewise, at long-term evaluation, it is shown an improvement in the physical domain but without reaching the same level as non-caregivers. However, satisfaction with leisure activity was persistently low during the 3 years for PC-PCVD[8].

In Mongolia, Chuluunbaatar et al. carried out a quality of life study in PC-PCVD of ischemic (58%) and hemorrhagic (42%) types, in the acute phase at 7 and 10 days and at 12 months considering four domains: physical health, psychological health, social relationship, and environmental domain. This study showed that during the first year, caregivers improved significantly in the psychological health and environmental domain, but not in physical health. In other words, at the acute phase, caregivers tend to suffer a physical damage; however,

they seem to adapt by having more positive feelings, better concentration, higher self-esteem, higher spirituality, better self-perception, more social assistance, effective use of financial resources, ease of transportation, and broader knowledge of the disease[9].

An additional study from Pucciarelli et al. (2017) considered the same four quality of life domains. This investigation evaluated PC and patients with ischemic (80%) and hemorrhagic CVD (20%) in the acute phase and at 12 months follow-up. The findings were that in the acute phase both caregivers and patients showed lower than expected quality of life level, although caregivers had a better quality of life than patients. At 12 months, patients improved in both physical and psychological health aspects, while caregivers continued to have a better quality of life[10].

In Latin America, there is a work published in Colombia in 2010, which evaluated PC-PCVD through the Ferrel scale, which divides quality of life into four aspects: physical, psychological, social, and spirituality[11]. This study found out greater deterioration in physical and social aspects in most PC, specifically sleep disturbance and fatigue, as well as insufficient support from others and disturbances in personal relationships. On the contrary, the least affected area was spiritual domain[12].

Moreover, an Asian study in Malaysia compared the quality of life of PC and PCVD (93.3% ischemic and 6.7% hemorrhagic). It reported that caregivers had a better quality of life than the patients[13]. However, a Japanese project reported greater impairment of quality of life in PC than in patients with unspecified CVD[14].

Determinants of quality of life in PC of patients with cerebrovascular disease

Gender

Women caregivers have been reported to have higher levels of burden and depression than men. In fact, it has been reported that women caregivers have greater difficulty in asking for help and support and that they often spend more hours caring for patients with CVD[9,15]. Furthermore, it has also been found that the longer the caring times, the greater quality of life for CVD survivors (mainly in the ischemic type). In other words, being a woman seems to be a factor of vulnerability as a caregiver, but a protective factor for the patient[16].

However, other findings suggest that male caregivers are related to worst PC quality of life in specific areas such as energy/vitality and mental health[5]. It is important to note that being a woman seems to be a risk factor for the low quality of life as PC, but it is essential to highlight that this finding may be due to the majority are women caregivers' cases in most of the studies[17,18].

Age

In addition to the sex of the PC, age has been also identified as a determining factor in their own quality of life. Some studies indicate that younger age of the patient and caregiver determines a better quality of life in favor of PC[5,7]. It has been described more specifically that those caregivers over 60 years old are more likely to have a low quality of life scores. However, it has also been stated that those caregivers under 40 years of age present a greater complaint of physical pain compared to older people[17]. On the other hand, there are some studies where no correlation between the age of PC and quality of life has been found[19].

Time of care

Some evidence highlights the negative effect of a higher number of caring hours on the PC-PCVD quality of life. These findings are of great relevance since they not only refer to the investment of time and fatigue due to the long day of care but also highlight the reduction of time dedicated to other activities as resting and recreation by the PC, showing they have higher levels of frustration and depressive symptoms[20].

In this way, other studies have provided information on the impact of hours of care on the burden and low quality of life of PC, considering it a risk factor for mental health[18]. In fact, it has been specifically described that caregivers with more than 3 h of care per day have worse performance in most of the determinants of quality of life[21], while those who work as caregivers 12 or more hours per day have worse performance and a significant deterioration in all quality of life aspects[22].

However, not only the hours of care per day matter but also the intensity of it. In other words, spending several days or months taking care of a patient with CVD without a rest period has also been assessed. It is shown that at 1-year post-CVD-without specifying the type-no significant differences in quality of life have been found, inferring that the negative impact on quality of life from the PC occurs in the first 12 months[22,23].

Employment/financial situation

There are direct or indirect costs caused by CVD in the medium or long term. By example, the costs of care,

rehabilitation, secondary prevention, partial or total disability, loss of productive activities, among others. These costs are relevant for the patient, for the health care system, and also for the family, including caregivers[24]. However, this aspect has been scarcely studied in the literature.

Unemployed PC has a lower quality of life compared to their peers, greater burden, and a negative impact on mental health[25]. This finding is relevant, considering that the majority of the caregiving wives of patients with CVD are unemployed or retired[17]. Additional information highlights that 36% of PC with current employment were reassigned or looked for another job because to care for their relative; this occurred between the 3rd and 9th month after CVD[26].

In a 2013 Brazilian study, it was found that PC-PCVD non-specified type, regardless of gender, obtained one to three minimum monthly wages (678-2,034 Brazilian real's or 4,109-12,329 Mexican pesos). This income was explained by the need for part-time jobs and/or with minimal school demands and flexible schedules[17].

The anxiety generated by the reduction or lack of economic income has been associated with a lower score in the quality of life; this affects the vitality and mental health of PC[9,17]. This generates a vicious circle because low levels of quality of life are associated with the hospital readmission of the patient who does not have medical insurance, and therefore expenses have a negative impact on the family economic situation[27].

In 2012, it was reported that those PC that lives in rural areas have a lower quality of life. This is mainly explained by the difficulty of accessing social and medical support[28]. Furthermore, a relationship between the economic situation and the educational level exists. In the case of PC patients with chronic diseases and dependence, it has been identified that a high educational level determines a better quality of life in caregivers[29].

Relationship and marital status

Delcourt et al. analyzed the interaction of ischemic and hemorrhagic PC-PCVD and marital status, observing that married caregivers had a better quality of life. This was due to a better support network and/or perception of support[30]. Likewise, Costa et al. found that being the patient's spouse was reflected in a better quality of life, specifically in mental health[17].

In contrast, other studies have described that single caregivers have a better quality of life; this indicates that married PC with children have worse scores in the measurement of quality of life[21].

Being the spouse of a patient with an ischemic stroke guarantees functional improvements at 1 year of follow-up at the cost of a decrease in the quality of life of the spouse[31]. However, in another comparative study between spousal and non-spousal caregivers of patients with unspecified CVD during the first 2 months, it was found that the four aspects of quality of life evaluated with better scores were obtained by the spouses of patients with CVD. The aspects evaluated were: physical health, psychological health, social and environment relationships, particularly in the section on social relationships, there were higher scores[32].

Regardless of the relationship that exists between the PC and the patient, cohabitation gives higher scores in the quality of life[33].

Anxiety/depression

Mood, anxiety, and depression disorders have been studied in PC due to their strong relationship with quality of life. In fact, there are significant correlations between the mental component of quality of life and symptoms of anxiety and depression[21]. Furthermore, it has been reported that around a fifth of PC suffers from anxiety, while a quarter suffers from depresión[34]. Indeed, equivalent depression and anxiety scores have been found between PC and patients, indicating that the psychological sequelae of CVD affect both PC and the patient because of an effect called "emotional contagion"[13].

More precisely, Wan-Fei et al. found that high levels of depression in PC-PCVD, both ischemic and hemorrhagic, have an impact on their own quality of life, specifically in physical appearance, lower vitality, and limitations in daily life due to his physical health. In this same study, high levels of anxiety among caregivers had a strong impact on their quality of life, being more significant in mental aspects, greater limitation in life activities, and less social coexistence, among others[13]. The presence of pain, depressed mood, and burden has been associated with a poorer quality of life in PC-PCVD of unspecified type[18].

Patients disability

Any type of CVD could be the cause of diverse sequelae among patients, highlighting motor and speech disorders as the most frequent. Nowadays, it is known that the greater the physical dependence of the patient, the lower the quality of life of the PC[5,33]. Likewise, it has been found that the greater the severity of the patients sequelae and their inability to communicate, the

PC will present lower scores than expected in the mental health components in the quality of life construct[21,20]. It is also recognized that a poor social interaction on the part of the patient negatively affects the quality of life of his PC[5].

Regarding cognitive and psycho-affective sequelae in patients with CVD, it has been described that the greater the cognitive deterioration and the greater the perception of alterations in mental functions by the PC, the greater the symptoms of anxiety and depression in the latter[34,35].

The comorbidity of patients with CVD, such as diabetes, kidney failure, osteoporosis, cataracts, heart failure, and many more, is very important deleterious factors to consider in the quality of life of PC[36].

Finally, a relevant aspect is an opposite effect, that is, the influence that the PC's quality of life exerts on the patient's quality of life. It has been found that all aspects of the SF-36 Health Survey aimed at evaluating the quality of life in the caregiver have a significant correlation with the measures of well-being and quality of life of patients with CVD[7].

Quality of life tests more frequently used in evaluating PC of patients with cerebrovascular disease

Quality of life is a complex construct that is composed of several domains that have not been yet sufficiently standardized. Therefore, various methods and tests have been developed for its evaluation. Table 1 shows some of the most frequently used assessment instruments, which included the WHOQOL-BREF[4], SF-36 Health Survey[6], 12-Item Short Form Health Survey[37], Ferrell Iinstrument[11], and the EuroQoL-5D (EQ-5D)[38], emphasizing their theoretical foundation. Most of the tests are presented in self-administered formats and are intended to assess the subjective perception of various aspects of the physical and mental functioning of patients.

The physical aspect refers to the loss of energy, tiredness, fatigue, or difficulty in performing any physical activity and impact on work performance. All of them, activities that were previously carried out without problems, without pain, discomfort, or difficulty even resting, or sleeping. The mental aspect refers in most cases to symptoms of anxiety or depression, hope for recovery, contextualization of their current situation, self-esteem, self-perception, and limitations in activities of daily life due to emotional disorders.

Table 2 shows some of the main studies on quality of life in caregivers of patients with CVD and the tests used, as well as the most relevant findings.

Discussion

Risk factors, causes, diagnosis and acute treatment, mortality, secondary prevention, and physical and cognitive rehabilitation are usually studied in CVD, leading to few studies about the quality of life of their PC. It is known that the 1st year is the most critical and challenging period for both the patient with CVD and his or her PC[5,9,10].

After reviewing the literature about quality of life in PC-PCVD, this work concludes the following: quality of life of PC-PCVD shows a significant impairment compared with non-caregivers[7,8]. Besides that, by being compared with the CVD patients under their care, patients exhibit lower scores in most quality of life domains in the acute phase; but a greater improvement over time. In other words, PC shows a worse prognosis and adaptability[5,10,14].

Likewise, there is heterogeneity of quality of life domains as well as assessment tools. The outcome is an inherent inconsistency of results. Despite the heterogeneity of the quality of life concept, it was possible to find out the most common tests for its evaluation. In the first place, the WHOQOL-BREF, which mainly reveals a moderate to severe disturbance in quality of life of PC-PCVD. In addition, it shows a greater effect in areas as fatigue, mobility, and pain, sleep disturbances, meaning physical domain. In the second place, the SF-36 Health Survey, which points out as the most affected domain the limitations in usual role activities because of emotional problems, meaning the role emotional of PC-PCVD[5,7,10,18].

Being female, single, and older, with at least 3 h of care per day, with limited income, and anxiety or depression symptoms are the main characteristics associated with poorer quality of life in PC-PCVD. Although not all of the determinants of poor quality of life have theoretical explanations, some researches have a possible hypothesis. For example, it is proposed that caring hours does not imply a problem by itself, but rather the peripheral situations such as lack of leisure time or lack of social support network[20]. Likewise, it is referenced how limited income has a negative effect on the quality of life of PC through stress and worries rather than lack of supplies[9,17].

Finally, it is mentioned that a married caregiver tends to have better support networks resulting in an inherent protective factor[32]. In several longitudinal investigations, the 1st year has been determined to be the most complicated for PC-PCVD because of the number of abrupt changes[5,10]. While the quality of life

Table 1. Quality of life assessment instruments more frequently used in primary caregivers of patients with cerebrovascular disease

Quality of life instruments	Domains	Description of domains
World Health Organization Quality of Life BREF (WHOQOL-BREF)[4]	Physical	Administration of medications, energy and fatigue, mobility, pain and discomfort, sleep and rest, and capacity to work
	Psychological	Body image, appearance, negative feelings, self-esteem, spirituality, religion, personal beliefs, thinking, learning, memory, and concentration
	Social	Social relationships, social support, and sexual activity
	Environmental	Finances, freedom, security, quality of health and social assistance, domestic environment, recreation, traffic pollution
Short Form 36 Health Survey Questionnaire (SF-36 Health Survey)[6]	Physical functioning	Limitations in physical activities because of health problems
	Physical role	Limitations in usual role activities because of physical health
	Emotional role	Limitations in usual role activities because of emotional problems
	Vitality	Energy or fatigue
	Mental health	General mental health, i.e., psychological distress and well-being
	Social functioning	Limitations in social activities because of physical or emotional problems
	Bodily pain	Intensity of pain and its effect on daily life
	General health	General health perception
12-Item Short Form Health Survey[37]	Reduced version of SF-36 Health Survey. Includes the exact same eight domains	Usually used for large samples because its duration is 2 min approximately
Ferrell Instrument[11]	Physical	Functionality and general health
	Psychological	Personal characteristics as depression, fear, happiness and sense of control
	Spirituality	Meaning and purpose in life, hope, uncertainty, and significance
	Social	Interrelated components of quality of life including family distress, social isolation, finances and sexual activity
EuroQoL-5D (EQ-5D)[38]	Mobility	Walking, moving, being in bed
	Self-care	Daily activities as bathing and getting dressed
	Usual-activities	Working, cleaning, family time, and free time
	Pain and discomfort	Physical or emotional pain
	Anxiety and depression	Symptoms of anxiety and depression
	It evaluates the five domains in five different levels: no problem, slight problem, moderate problem, severe problem, and unable to. In addition, it gives a ranking from 0 to 100 of general health perception	

remains a vulnerable issue throughout disease evolution, surprisingly, improvements in the environmental domain (financial status, safety, quality of health and social services, home environment, recreation, pollution, and traffic) have been demonstrated after the 1st year of the event[9].

The majority of the reviewed articles were conducted in non-Spanish speaker population. As a matter of fact, the only investigation done in Latin America by Torres et al.[12], included a spirituality domain, being the most preserve aspect in PC-PCVD. This reflects the urgent need to analyze the quality of life in Latin countries. In

Table 2. Factors for lower quality of life in primary caregivers of patients with cerebrovascular disease

Reference	Sample (n)	Instrument	Most affected domains	Factors for PC lower quality of life
Jönsson et al.[5] (2005)	304 CVD patients and 234 PC	World Health Organization Quality of Life BREF (WHOQOL-BREF)	Emotional role	Being female, older age, lower functionality, and lower social participation of patient
Torres et al.[12] (2010)	97 PC	Ferrell Instrument	Social and physical well-being	Being female, older age and lower incomes
McPherson et al.[7] (2011)	56 PC	SF-36 Health Survey	Physical functioning, Physical and emotional role	Older age either in caregiver and patient. Perception of unbalanced relationship for give-and-take between caregiver and patient
Costa et al.[17] (2015)	136 PC	SF-36 Health Survey	Bodily pain	Younger age
			Mental health and vitality	Lower income
			Emotional role	Being single
			Physical functioning	Being female
Haley et al.[8] (2015)	235 PC and CVD patients	12-Item Short Form Health Survey	All of them except physical functioning	Younger age, lower education level, and health problems
López-Espuela et al.[18] (2015)	48 PC	EuroQol-5D Questionnaire	Pain and discomfort; anxiety and depression	Caring time and Sleeping disorders
Chuluunbaatar et al.[9] (2016)	155 CVD patients y 88 PC	World Health Organization Quality of Life BREF (WHOQOL-BREF)	Physical domain	Being female, poor health, and financial difficulties
Efi et al.[21] (2017)	150 PC	12-Item Short Form Health Survey	Physical health	Poor health, type of CVD; anxiety and depression
			Mental health	Anxiety and depression; daily care and patient's aphasia
Caro, Costa and Da Cruz[19] (2018)	30 PC	World Health Organization Quality of Life BREF (WHOQOL-BREF)	Social domain	Burden
Pucciarelli et al.[33] (2018)	244 PC	World Health Organization Quality of Life BREF (WHOQOL-BREF)	Environmental domain	Older age, lower education, cohabitation with patient, lower functional independence of the patient

PC: primary caregivers; CVD: cerebrovascular disease.

spite of the inclusion of every type of CVD case for this review, it is clear a majority of ischemic CVD, coinciding with the higher prevalence showed in literature. All the investigations cited in this review analyzed several aspects of both PC and patients with CVD to understand the determinants of quality of life.

With regard to the patient, the main focus was on motor, independence, and speech disturbances; however, the variety of possible neuropsychological disturbances was ignored. This opens a path for future investigations. Finally, it is important to mention that every study used the quality of life concept in their description; however, the "health-related quality of life" concept turned out to be present in several test explanation. This finding might support future investigations about the difference between both concepts.

Conclusion

The quality of life of PC-PCVD is mainly affected in physical and emotional domains. Most of the analyzed studies highlight the importance of PC in the well-being of patients, such as their vulnerability. This leads to the proposal of broader and more controlled investigations.

Studies about PC-PCVD are limited in our context despite its relevance. Prevention and intervention plans are urgently needed to improve quality of life.

The determinants of quality of life for PC-PCVD were summarized in seven sociodemographic characteristics of the caregiver: sex, age, caring hours, financial situation, relationship with the patient, anxiety and depression symptoms, and patient sequelae.

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Conflicts of interest

None.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

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REVIEW ARTICLE

Effects of binaural beats and isochronic tones on brain wave modulation: Literature review

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Abstract

This systematic review is dedicated to deepening the study of two phenomena: binaural beats and isochronic tones. Data from the scientific literature suggest the existence of a promising therapeutic potential in neurology and psychophysiology due to their influence on specific frequencies of brain waves and their implications for mental health and homeostasis of brain neurotransmitters. Prolonged audio stimuli in repetitive and synchronized manner may induce changes in brain waves patterns and, consequently, modulating neurophysiological, and behavioral responses. The literature review was conducted using PUBMED, MEDLINE, LILLACS, and SCIENCE DIRECT online platforms using the search words: "audio brain entrainment," "auditory beat stimulation, " "binaural beats," "brainwave entrainment (BWE)," and "isochronic tones." The search yielded 674 studies, of which 49 were in duplicate, and 592 were out of the scope of this review, and, therefore, were excluded from the study. The remaining studies were analyzed according to the Cochrane Handbook for Systematic Reviews, resulting in 33 randomized, controlled clinical trials that were then evaluated by the Jadad scale. From that, 17 studies obtained a score of three points or more on the Jadad scale. These studies were fully read and critically analyzed. Binaural beats were used in 15 studies (88.25%), whereas isochronic tones were used only in two studies (11.76%). Although most of the studies reviewed here indicated audio BWE effectiveness, some positive outcomes may require further investigation, with more refined and appropriate evaluation tools, better suited for each specific type of intervention and/or therapeutic target. Considering these limitations, the performance of additional studies with more adequate experimental design and data analysis is recommended, particularly focusing on the neurophysiological and behavioral effects of brain wave entrainment on mental states.

Key words: *Audio brain entrainment. Binaural beats. Brainwave entrainment. Isochronic tones.*

Efectos de los tonos binaurales y isocrónicos en la modulación cerebral: Revisión de la literatura

Resumen

Esta revisión sistemática está dedicada a profundizar en el estudio de dos fenómenos: latidos binaurales y tonos isocrónicos. Los datos de la literatura científica sugieren la existencia de un potencial terapéutico prometedor en neurología y psicofisiología debido a su influencia en frecuencias específicas de ondas cerebrales y sus implicaciones para la salud mental y la homeostasis de los neurotransmisores cerebrales; los estímulos de audio repetitivos y sincronizados pueden inducir cambios en los patrones de ondas cerebrales y modular las respuestas neurofisiológicas. La revisión de la literatura

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se realizó con plataformas en línea PUBMED, MEDLINE, LILLACS y SCIENCE DIRECT, con las palabras de búsqueda: "arrastre cerebral de audio", "estimulación del ritmo auditivo", "ritmos binaurales", "arrastre de ondas cerebrales" y "tonos isocrónicos". La búsqueda produjo 674 estudios, de los cuales 49 estaban duplicados y 592 estaban fuera del alcance de esta revisión y fueron excluidos. Los estudios restantes se analizaron de acuerdo con el Manual Cochrane de Revisiones Sistemáticas, que tuvo como resultado 33 ensayos clínicos controlados aleatorios que fueron evaluados por la escala de Jadad. A partir de esto, 17 estudios obtuvieron una puntuación igual o superior a tres puntos y fueron leídos íntegramente y analizados críticamente. Los tonos binaurales se utilizaron en 15 estudios (88. 25%) y los tonos isocrónicos sólo en 2 estudios (11. 76%). Aunque la mayoría de los estudios revisados demuestran la eficacia del arrastre de ondas cerebrales, algunos resultados pueden requerir más investigación con herramientas más refinadas y apropiadas, para cada intervención y/u objetivo terapéutico. Teniendo en cuenta estas limitaciones, se recomienda que se lleve a cabo un diseño experimental y análisis de datos más adecuados, centrándose en los efectos neurofisiológicos del arrastre de ondas cerebrales y los estados mentales.

Palabras clave: *Estimulación cerebral con audio. Tonos binaurales. Arrastre de ondas cerebrales. Tonos isocrónicos.*

Introduction

Brain waves consist of rhythmic patterns of neuronal activity or synchronized electrochemical pulses from groups of neurons in the central nervous system (CNS)[1]. There are several well-established brainwave range patterns: gamma (30-70 Hz), beta (13-30 Hz), alpha (8-13 Hz), theta (4-8 Hz), and delta (1-4 Hz)[2]. Each one of these frequency bands has been correlated with different states of consciousness, such as awake, relaxed, rapid eye movement (REM) sleep, as well as non-REM sleep stages[3].

According to Gruzelier[4], prolonged audio stimuli in repetitive and synchronized manner may induce changes in brain waves patterns and, consequently, may modulate neurophysiological and behavioral responses. More specifically, repetitive external or environmental stimuli may temporarily affect the predominance of specific brain wave frequencies, a phenomenon namely brainwave entrainment (BWE)[5-7]. Therefore, BWE can be defined as rhythmic synchronization of brainwave oscillation with an external repetitive stimulus.

BWE is a recurrent phenomenon in nature and biologically present in living beings[8]. The principle of entrainment or harmonization was discovered around 1665, by the Dutch scientist Christian Huygens[9]. The synchronization obtained through the entrainment principle is the result of the harmonization principle, a physical phenomenon that occurs systematically in nature, and that is dependent on environmental stimuli, for example, visual, auditory, or tactile. These stimuli may be used to elicit synchronized brainwave patterns to match that of different mental states and/or levels of consciousness, as seen with data acquisition techniques, such as the electroencephalography (EEG). In this context, Oster[10] stated the possibility to improve, amplify or modulate brain wave patterns to conditioned events in the cerebral cortex[3,11]. The proposed therapeutic benefits have a wide scope, including the improvement of cerebral blood flow, neuroplasticity stimulation, and neurophysiological compensations between the cerebral hemispheres[3].

In this review, we focused on the most common forms of auditory BWE, that is, binaural beats and isochronic tones. Acoustic waves are characterized in Hertz (number of cycles per second). The audible sound spectrum for humans comprises frequencies between 20 Hz and 20,000 Hz, regardless of its complexity, and as long as it has an amplitude greater than 0 dB (decibel)[12,13].

Isochronic tones consist of distinct and repetitive regular beats of a single tone. The number of peaks per second in the signal amplitude is the isochronic frequency heard at regular and standardized time intervals. On the other hand, binaural beats represent the auditory experience that occurs when two sounds of close frequencies are presented separately to each ear with headphones or stereo speakers. The brain integrates the two signals, producing a third "phantom sound" representing the difference between the two auditory stimuli. For example, if a frequency of 114 Hz is presented to the right ear, and another of 124 Hz to left ear, a binaural beat of 10 Hz is created by the brain as a result of these stimuli. In this case, brain waves tend to match the binaural beat frequency, in this example 10 Hz, which corresponds to alpha brainwave pattern. Binaural beats are created in the superior olivary nucleus of the brain stem, the local of contralateral integration of auditory input[14] (Fig. 1).

The beat is neurologically transported to the reticular formation, which uses neurotransmitters to trigger changes in brain wave activity[10] that synchronizes with that of the stimulus generated. The mental features

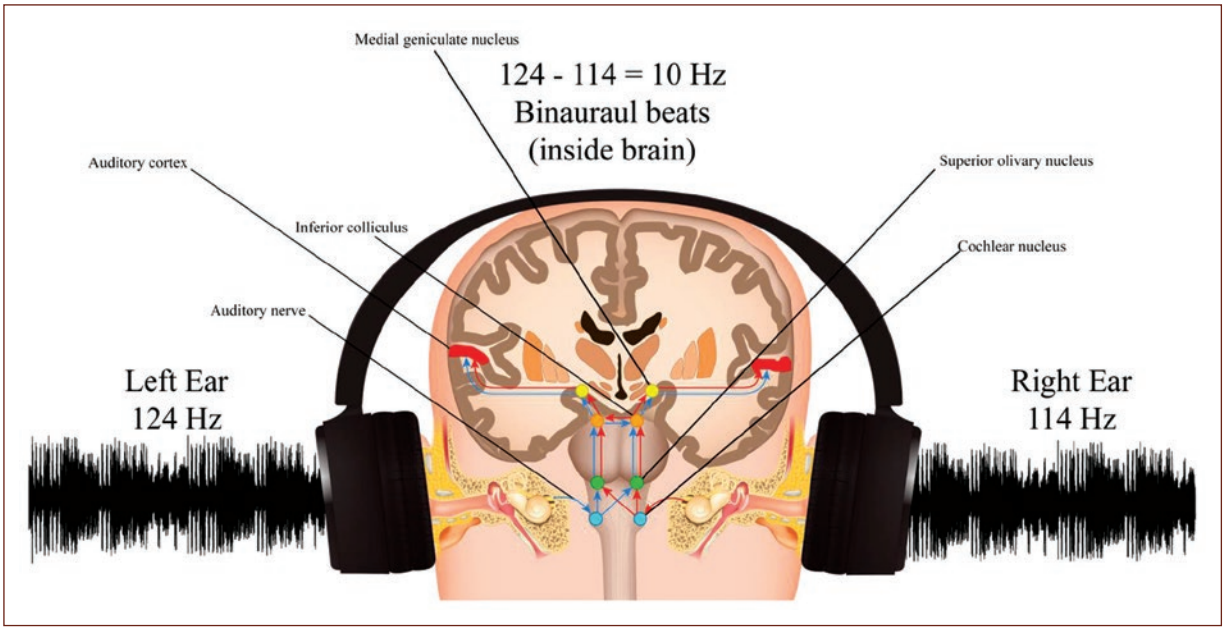

Figure 1. Binaural beats generated by the brain.

associated with each brain wave pattern can be elicited based on the scientific principle of harmonization, also known as "brain wave entrainment."

Most of studies with binaural beats and isochronic tones provides positive outcomes, indicating that audio brain entrainment may yield different benefits, both physically and mentally[15,16]. More specifically, brain entrainment can be applied to induce mental states and as adjunctive treatment to several brain disorders in a safe and non-invasive manner, such as for the treatment of depression and anxiety disorders[17]. For instance, it was demonstrated that a group of individuals exposed to 6 Hz sounds for 10 min presented a significant increase on theta wave (4-8 Hz) cortical activity in comparison to control group that did not receive the stimulus. These findings indicate a facilitatory effect on induction of a meditative state and altered states of consciousness[18]. In this context, the aim of this study was to review the scientific evidence on the therapeutic use of binaural beats and isochronic tones for the modulation of brain wave patterns and mood states.

Development

Methodology

The central question of the current study was: what are the therapeutic and/or neuroplastic and behavioral effects of binaural beats and isochronic tones on brain wave patterns and mood state modulation?

The following electronic databases were used for the studies search: PUBMED, MEDLINE, LILLACS, and SCIENCE DIRECT, and the following search words (key words) were used: "audio brain entrainment," "auditory beat stimulation," "binaural beats," "BWE," and "isochronic tones."

The bibliographic search was conducted independently by the authors from October to December 2019, and blindly to the results obtained by the other authors. The studies that met inclusion criteria (see below) were later compared and compiled. The Cochrane Handbook for Systematic Reviews[19] recommendations were followed to assess the risk of bias; and the Jadad scale[20] to evaluate methodological quality. The Jadad scale consists of five criteria items, ranging from 0 to 5 points. A score below 3 indicates low methodological quality and a score of 3 points or more indicates superior methodological quality. The scale consists of the following questions:

1. Was the study randomized?
2. Was the randomization method appropriate?
3. Was the study blinded? Double blinded?
4. Was the blinding method appropriate?
5. Were drop-offs properly reported?

For questions 1, 3, and 5, a single point for yes, or zero for no, is assigned. For questions 2 and 4, a single point for the use of the appropriate method, zero points for no

description of the method, or a single negative point for the inappropriate use of the method is assigned.

Inclusion criteria

The following criteria were included in the study:

1. Publication date: studies published from 2009 to 2019
2. Study design: clinical studies, such as case report, case series, case-control study, non-randomized controlled clinical trial, and randomized clinical trial
3. Population: without limitation
4. Intervention: stimulation with binaural beats or isochronic tones
5. Comparison: without limitation
6. Result: qualitative and quantitative.

Results

The search yielded a total of 674 studies (PUBMED: 74; MEDLINE: 396; LILLACS: 138; and SCIENCE DIRECT: 66). As illustrated in figure 2, from the total of studies, 49 studies were in duplicate, and 592 studies addressed other themes beyond the scope of this review, and, therefore, were excluded from the study.

The selected studies were analyzed according to the Cochrane Handbook for Systematic Reviews[19], resulting in 33 randomized, controlled clinical trials that were then evaluated by the Jadad scale[20] (Fig. 3). Only 17 studies obtained a score of three points or more on the Jadad scale[20], and them were fully read and critically analyzed; studies are summarized in Table 1. Binaural beats were used in 15 studies (88.25%) whereas isochronic tones were used in only two studies (11.76%) (Fig. 3). In 82.35% of these publications monaural and/or binaural audio stimulation were more effective in comparison to control group.

After the evaluation made by the Jadad scale, the 17 selected studies were classified according to the methodological criteria adopted in each experiment (Randomized Study (R); Double Blind Study (BD), Controlled Study with Placebo (PC), type of sample and number of individuals in the study and results obtained by each researcher. The summary of therapeutic targets and forms of intervention and approaches of these selected studies are described in Table 2.

Main findings of the studies addressing the effects of brain waves on mental states

In this section is presented a description of the different brainwaves, their effects, and the discussion of the main findings obtained in the studies reviewed here. The study performed by Washington and collaborators[21] indicated that each brain wave frequency produces particular neurophysiological and cognitive effects, been associated with a specific state of consciousness.

Delta waves (< 4 Hertz): the slowest waves are associated with the deepest state of sleep and unconscious. These waves represent the ideal for sleep, physical and mental recovery, and deep meditation. Delta waves lead to a state of mental happiness and empathy where the person feels more connected with her/himself. This state improves intuition and memory. Delta waves are associated with the release of growth hormone[22], which is beneficial for cell regeneration, as well as the production of endogenous opioids[23]. None of the selected studies review here used delta waves.

Theta waves (4-8 Hz): this frequency pattern is related to the processes of creativity, enhanced intuition, more intense emotional connections that elevate sensitivity, and a sense of tranquility and reduced anxiety. Theta waves also contribute to the improvement of problem-solving skills and retention of much larger amounts of information in shorter period of time. This pattern is associated with decreased levels of serum cortisol and with the modulation of serotonin and melatonin. Theta waves generate a relaxed state of consciousness[24,25].

Eight of the studies reviewed here were conducted with Theta waves, with binaural beats as well as isochronic tones interventions. Major targets were cognitive and/or pathological states. Increased long-term memory performance was observed in patients with epilepsy who underwent 6-min 5 Hz brain entrainment sessions, once a week for 6 weeks with binaural and isochronic tones[26]. On the other hand, in non-epileptic adult subjects, 15-min 5 Hz brain entrainment sessions did not induce significant effects[27]. Visuospatial working memory and cortical connectivity were not altered following a single intervention of 5 min with 5 Hz therapy[28]. Another study using binaural beats also describes absence of significant effects in the working memory of healthy young adults[29]. Interestingly, 20-min 6 Hz binaural beat entrainment twice a week for 14 days effectively reduced the perception of pain severity[16].

In addition, in patients with temporal lobe epilepsy who underwent brain implants with EEG signal control, acute therapy of temporolateral (5 Hz for 5 min) increased memory acuity with EEG synchronization[17]. Moreover, in young adult's post-physical training, binaural beat entrainment (4-7 Hz for 20 min) increased parasympathetic

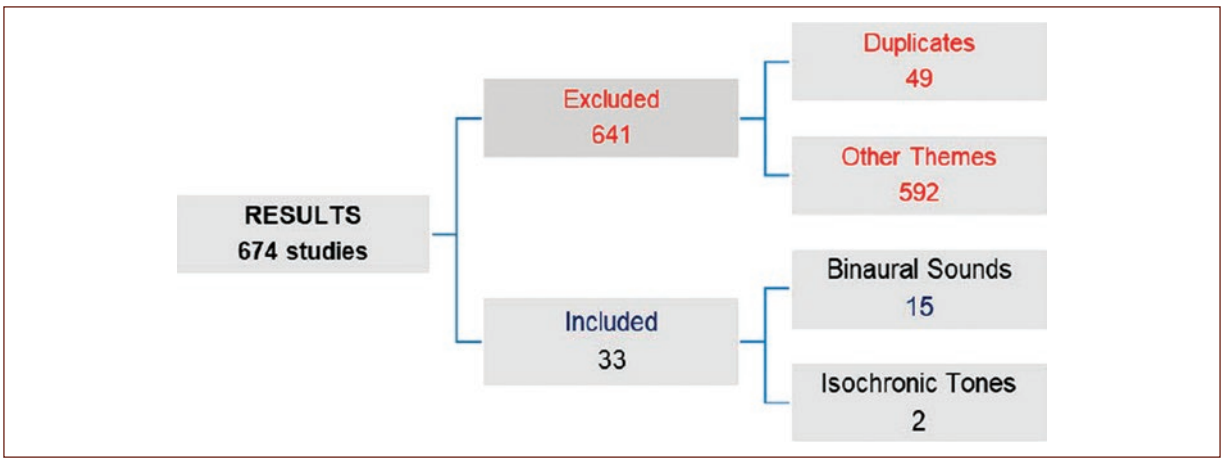

Figure 2. Randomization algorithm and study selection.

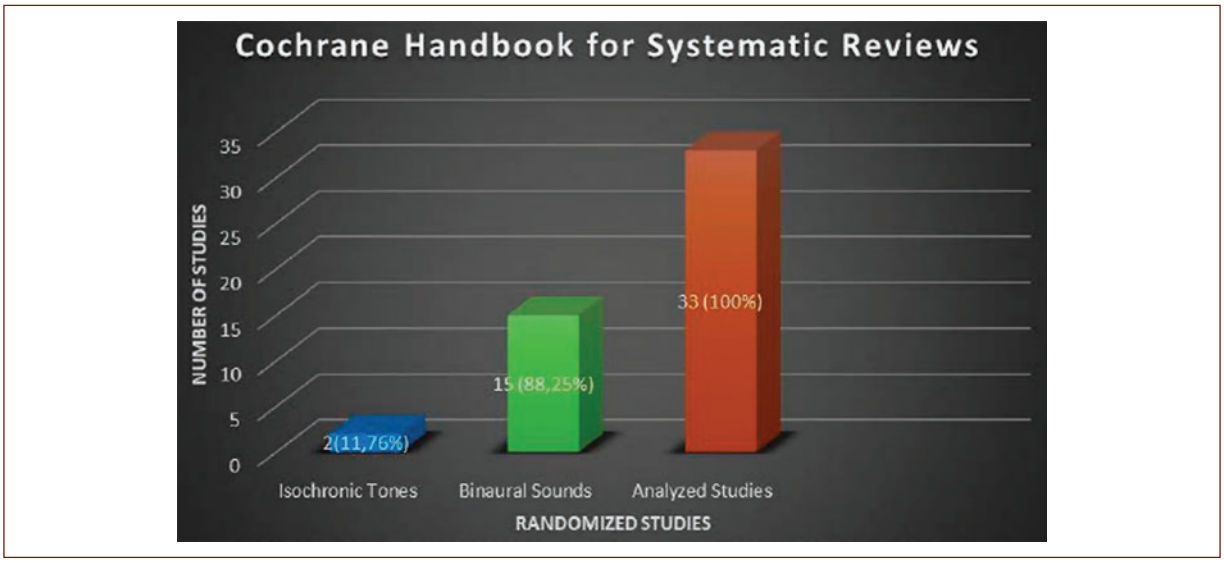

Figure 3. Summary of the selected studies - handbook for systematic reviews.

activation and self-reported relaxation[30]. Finally, a single intervention with isochronic tones at 6, 10 and 40 Hz during 5 min reduced anxiety and improved well-being reports of healthy individuals[31].

Alpha waves (8-13 Hz): this frequency pattern is related to mental relaxation[32], visualization, and creative processes; therapeutically uses include memory optimization, and modulation of pain perception threshold. The use of this wave pattern in the elderly population has been shown an excellent therapeutic potential to treat memory disorders[33].

Alpha waves were used with a therapeutic and/or neurocognitive focus in six studies reviewed here. Vernon et al.[34] reported that 10 Hz alpha pattern for 1 or 5 min did not elicit significant EEG alteration. However, isochronic stimulation with 7 Hz for 9 min with 3 min intervals induced temporal and parietal lobe activation with the potential to alter brain networks in adult's healthy young individuals[35]. Alpha binaural beats for 3 min in healthy young adults did not affect attentional blink (AB) control with the EBR method (predictor of mood states associated with dopamine levels)[36]. Moreover, patients with temporal lobe epilepsy who underwent brain implants, the acute exposure to 10 Hz isochronic tones for 5 min increased significantly medio temporal synchronization[17,30].

Table 1. Summary of the main findings of the 17 selected studies according to the therapeutic use of binaural beats and isochronic tones auditory brainwave entrainment

Study	Method and Sample size (N)	Population/Type/Special?	Number of interventions and frequency	Outcomes
Kennel et al., 2010[44]	R+DB+PC; 20.	Male and female children and adolescents with Attention-deficit/ hyperactivity disorder	3 weeks with three interventions per week with binaural beta-16 Hz- beats 10 min	Improved focus and attention
Vernon et al., 2014[34]	R, 22	Healthy male and female young adults	Acute intervention (single): 10 Hz (Alpha) 20 Hz (Beta)	Induced brainwave entrainment
McConnell et al., 2014[30]	R+DB+PC, 21	Young adults post-exercise	Single post-workout intervention of 4-7 Hz theta waves	Increased parasympathetic activation, increased sympathetic withdrawal, and increased self-reported relaxation after exercise
Becher et al., 2015[17]	R+PC, 10	Patients with temporal lobe epilepsy	Acute pre-surgical intervention, the duration of the main experiment varied between 15 and 40 min, and the total number of auditory stimuli varied between 87 and 214 Hz	Increased intracranial flow modulation in patients with temporal lobe epilepsy
Reedjik et al., 2015[36]	R+PC, 24.	Healthy male and female young adults	Applications of binaural frequencies of gamma frequency (40 Hz) and alpha (10 Hz), for 3 min before and during a global-local task	Measurement and control of attentional blink (AB), EBR method (predictor of dopamine levels and mood states)
Tirdad et al., 2015[35]	R+PC, 15	Healthy male and female young adults	Intermittent intervention of 3 min with 1 min of intertrial interval, 7 Hz applications of binaural tones during 9 min	Promoted brainwave entrainment
Colzato et al., 2016[54]	R+PC+DB, 40	Healthy male and female adults	Single 40 Hz gamma binaural beat intervention	Improved selective attention
Zampi, 2016[16]	R+PC, 36	Chronic pain patients	Sequential intervention for 14 days with Theta 6 Hz	Attenuated severity of pain
Beaucheneet al., 2016[28]	R+PC, 28	Healthy male and female young adults	Acute intervention (single) with six acoustic stimulation conditions: None, Pure Tone, Classical Music, 5 Hz, 10 Hz and 15 Hz binaural beats	Improved visuospatial working memory performance and cortical connectivity
Beauchene et al., 2017[29]	R+PC, 34	Healthy male and female young adults	Acute intervention (single) with six acoustic stimulation conditions: None, Pure Tone, Classical Music, 5 Hz, 10 Hz and 15 Hz binaural beats	Improved working memory performance
Colzato et al., 2017[53]	R+PC+DB, 36	Healthy male and female young adults	Acute intervention (single) applications of binaural frequencies of gamma frequency (40 Hz), for 3 min before and during a global-local task	High frequency binaural beats improved the attention
Lopez-Caballero and Escera, 2017[58]	R+PC, 40.	Healthy male and female young adults	Beats of 5 different frequencies (4.53 Hz - theta -, 8.97 Hz -alpha-, 17.93 Hz -beta-, 34.49 Hz -gamma -or 57.3 Hz-super-gamma) binaurally and acoustically for 3 min, preceded and followed. For periods of white noise of 90 s (baseline and post values, respectively)	Promoted brainwave entrainment

(Continues)

Table 1. Summary of the main findings of the 17 selected studies according to the therapeutic use of binaural beats and isochronic tones auditory brainwave entrainment (*Continued*)

Study	Method and Sample size (N)	Population/Type/Special?	Number of interventions and frequency	Outcomes
Nantawachara Jirakittayakorn, 2017[52]	R+PC, 40	Healthy male and female young adults	Applications binaural beats of gamma frequency (40 Hz), for 20 min before and after global-local task	Improved working memory function assessed by the word list retrieval task
Chaieb et al., 2015[55]	R + PC; 15	Epilepsy in pre-surgical situation Male and female adults.	6 interventions 1 time per week, 5 Hz theta binaural tones, 6 min with 3.5 s and 5 s intervals.	Improved long-term memory in epileptic patients
Chaieb et al., 2017[31]	R+PC; 25	Male and female young adults	Single progressive intervention with isochronic tones 6 HZ theta, 10 HZ alpha, and 40 HZ gamma	Reduced anxiety levels (modulation of mental states)
Gálvez et al., 2017[43]	R+DB+PC,14	Parkinson's disease patients	2 randomized and counterbalanced sessions of sound stimulation (14 Hz binaural beats) for 10 min separated by a minimum of 7 days of interval	Improved motor and non-motor symptoms including anxiety symptoms and cognitive deficits
Garcia-Argibay et al., 2017[27]	R+PC, 32	Healthy male and female adults	Single intervention of 5 Hz theta waves and another group with 20 Hz Beta	Improved long-term memory and attention

R: randomized study; DB: double-blinded study; PC: placebo-controlled study.

Beta waves (13-39 Hz): this frequency pattern is related to attention, focus, concentration, and cognition. Beta BWE was shown to be effective in improving fatigue and some symptoms of attention deficit hyperactive disorder (ADHD), including learning and attention deficits. Additional studies showed improvement in visual acuity, coordination, potential for the dyslexia treatment and low of concentration, as well as, to promote IQ gain in the range of 8-10 points[37]. Beta wave entrainment influenced self-confidence and socialization and makes people more optimistic and energetic[38-41]. Finally, it helps learning as well as sports-related abilities[3,42].

Five independent studies reviewed here used beta waves. Beauchene et al.[28] demonstrated that 15 Hz beta pattern for 5 min increased short-term visuospatial working memory and cortical connectivity in healthy young subjects. A single 15-min session of 20 Hz significantly increased long-term memory, improving the codification of new information without previous memories[24]. Interestingly, Vernon et al.[34] demonstrated that two daily sessions (14 Hz, during 10 min) increased motor and non-motor symptoms in Parkinson's disease (PD) patients. In addition, Gálvez et al.[43] showed a decrease of functional connectivity and optimization of working memory, with no changes in gait or anxiety levels in PD patients in comparison to control group. Kennel et al.[44] submitted a group of children and adolescents with diagnosis of ADHD to brain entrainment sessions (duration of 10 min, 3 days per week for 3 weeks). Results were not totally conclusive, although parents reported improvement in the performance of homework tasks after the interventions.

Gamma Waves (> 40 Hz): this frequency pattern is involved in blinking and processing of information from all parts of the brain. High gamma wave activity in the brain is associated with intelligence, compassion, self-control, and feelings of happiness[45-48]. In addition, gamma brainwaves have been associated with improved memory and a greater ability of reality perception[49,50]. Gamma brainwave activity has been shown to be increased in monks during meditation[51].

Six studies were conducted with gamma BWE. It was demonstrated that 40 Hz gamma stimulation for 20 min improved working memory performance and mnemonic function in healthy subjects[52]. AB control, using the EBR method (predictor of mood states associated with dopamine levels), was significantly affected by 3-min binaural gamma stimulation in healthy young adults, before and during a global-local task[36]. These benefits

Table 2. Summary of therapeutic applications of different brain waves patterns

Type of intervention	Method	Time	Wave frequency range
Isochronic tones 5,10,40 and 80 Hz	Acute Single sequential	15 to 40 min	Theta, alpha, beta, gamma, and super gamma
Isochronic tones 6,10 and 40 Hz	Acute Single sequential	5 min	Alpha, beta, and gamma
Binaural 5,10 and 15 Hz	Acute Single sequential	5 min	Theta, alpha and beta
Binaural 40 Hz	Acute Single sequential	20 min	Gamma
Binaural 5 and 20 Hz	Acute Single sequential	15 min	Theta and beta
Binaural 10 and 20 Hz	Acute Single alternate	1 min	Alpha and beta
Binaural 7 Hz	Acute Single	9 min	Theta
Binaural 10 and 40 Hz	Acute Single alternate	3 min	Alpha and gamma
Binaural 40 Hz	Acute Single	10 min	Gamma
Binaural 5,10,40 and 80 Hz	Acute Single sequential	15-40 min	Theta, alpha, beta, gamma, and super gamma
Binaural 4 a 7 Hz	Acute Single sequential	20 min	Theta
Binaural 5 Hz	Chronic 3 repetitions per week for 6 weeks	6 min	Theta
Binaural 6 Hz	Chronic 2 daily repetitions for 14 days	20 min	Theta
Binaural 14 Hz	Chronic 2 repetitions a day for 7 days	10 min	Beta
Binaural beta - performance range	Chronic 3 repetitions per week for 3 weeks	10 min	Beta

were observed only in individuals with low rates of spontaneous blinking, which indicates low levels of dopamine in the striatum. Colzato et al.[53] reported that 3-min 40 Hz binaural beats stimulation improved focus and attention in healthy young adults. Moreover, a study using 10-min 40 Hz stimulation in healthy adults[54] demonstrated that BWE acts by modeling specific brain oscillations, in cognitive processes sustained in the gamma wave frequency range, such as mental processes related to intelligence, self-control, and well-being. In epileptic patients subjected to brain implants, acute isochronic tones and binaural beats exposure, in the range of 40-80 Hz for 5 min significantly decreased synchronization in medio-temporal sites, demonstrating their potential as a non-invasive therapy for modulating intracranial flow in synchronization of the EEG signals[17]. A single intervention with isochronic tones 6, 10, and 40 Hz over 5 min reduced anxiety and, consequently, increased the subjects' well-being, however, without marked effects on cognition[55].

As illustrated in figure 3, in 82.35% of the reviewed studies, monaural, and/or binaural audio stimulation were more effective in comparison to control group. As previously mentioned, binaural beats were used as therapeutic modality in 15 studies (88.25%), and isochronic tones were only used in two studies (11.76%).

Discussion

The data reviewed in the present study indicates that binaural beats are more commonly used than isochronic tones, at least in research (as seen in Fig. 3). Unfortunately, in our view, there is no standard in the choice of therapy parameters, such as brainwave range (in Hz), treatment duration, frequency, and regimen, neither for the treatment of specific conditions or disorders, nor for simple increase of executive brain functions and stimulation of inherent mental processes, and neurocognitive performance. A relevant observation is the absence of properly controlled double-blinded studies, which compromises the validity of the results available in the literature. Nevertheless, the results reviewed here suggest that auditory BWE, although still scarcely explored in behavioral and neurophysiological therapy, may represent an effective and inexpensive therapeutic approach, with minimal side effects. It is well known that all brain activity occurs through the bioelectric activity of neural networks and that the brain wave phenomenon is produced as a result of the sum of bioelectric interactions of the billions of neurons and their trillions of synaptic connections[56]. Changes in states of consciousness (concentration, excitement, relaxation, sleep, dreams, etc.) are closed related to changes in the frequency of the vibratory pattern of brain waves, which varies according to the intensity of these bioelectric activities.

According to this system and considering that our state of consciousness is influenced by brain wave patterns, it is plausible to conclude that BWE can modulate many aspects of behavior, from states of consciousness to perception, learning, and cognition[57].

The adaptation to daily activities requires the brain ability to modulate brain wave activity, in consonance to external stimuli and signals, as well as when faced with challenges and/or problems to be solved. Each type of brain wave can modulate different neurotransmitter systems, inducing particular synaptic and neurochemical readjustments[12,56].

Conclusions

Based on the data reviewed in this study, binaural beats and isochronic tones BWE may effectively modulate mood states, improving attention, and memory processes. Promising results were also obtained in subjects suffering from different CNS disorders, including ADHD, PD, epilepsy, chronic pain, and anxiety disorders.

Despite the audio BWE effectiveness described in many studies reviewed here, it is important to emphasize that some positive outcomes may require further investigation, with more refined and appropriate evaluation tools, better suited for each specific type of intervention, and/or therapeutic target. Considering these limitations, the performance of additional studies with more adequate experimental design and data analysis is recommended, particularly focusing on the neurophysiological and behavioral effects of brain wave entrainment on mental states.

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Conflicts of interest

The authors declare that they have no conflicts of interest.

Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

Right to privacy and informed consent. The authors declare that no patient data appear in this article.

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REVIEW ARTICLE

The pathogenesis of autism spectrum disorder

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Abstract

This manuscript reviews the mechanisms that contribute to the production of the autism spectrum disorder (ASD), especially the genetic and immunological components. Knowing the participating elements and mechanisms are essential to establish preventive measures and look for early markers. The ASD can have subtle or devastating manifestations, and exerting immunomodulatory actions could be useful in the management of these patients. There seems to be different environmental insults that may act as triggers in genetically predisposed subjects; these insults can promote an inflammatory response in which interleukin-6 could participate actively at the level of neural stem cells and progenitors. The degree of involvement in neurogenesis and astrogenesis, and therefore, the observed clinical spectrum will depend on two facts that alter the neural circuits, including the brain region that loses proper input or output connectivity due to abnormal migration of a group of neurons, and the astrocytic survival.

Key words: *Autism. Pathogenesis. Risk factors. Immunology. Genetics.*

La patogenia del trastorno del espectro autista

Resumen

Este manuscrito revisa los mecanismos que contribuyen a la producción del trastorno del espectro autista (TEA), especialmente los componentes genéticos e inmunológicos. Conocer los elementos y mecanismos participantes es fundamental para establecer medidas preventivas y buscar marcadores tempranos. El TEA puede tener manifestaciones sutiles o devastadoras, y ejercer acciones inmunomoduladoras podría ser útil en el manejo de estos pacientes. Parece haber diferentes agresiones ambientales que pueden actuar como desencadenantes en sujetos genéticamente predispuestos; Estas agresiones pueden promover una respuesta inflamatoria en la que la interleucina-6 podría participar actuando activamente a nivel de células madre neurales y progenitores. El grado de implicación en la neurogénesis y la astrogénesis, y por tanto, el espectro clínico observado, dependerá de dos hechos que alteran los circuitos neuronales, incluida la región del cerebro que pierde la conectividad de entrada o salida adecuada debido a la migración anormal de un grupo de neuronas, y la supervivencia astrocítica.

Palabras clave: *Autismo. Patogenia. Factores de riesgo. Inmunología. Genética.*

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Introduction

The autism spectrum disorder (ASD) is a neurodevelopmental disorder characterized by repetitive, stereotypical behavior, deficiencies in communication and social interactions, including deficiencies in social reciprocity, in nonverbal communicative behaviors, and in the skills to develop, to maintain and understand social relationships[1]. Leo Kanner described autism in 1943; at that time, it was considered to affect 4-5/10,000 children; in 2011, the incidence was 1 per 110 children in the United States and one per 64 in the United Kingdom. At present, about 1, in 59 children under the age of eight, have been diagnosed with ASD according to the Developmental Disabilities Monitoring Network, and predominates in boys, with a ratio of 4:1[2]. ASD is multifactorial: a mother and an embryo with genetic susceptibility, probably exposed to external triggers such as infections, toxins, environmental pollutants, and nutritional deficiencies, and/or to internal factors (autoimmune diseases) that condition an inflammatory state in which the microglia and numerous cytokines, especially interleukin 6 (IL-6), exert actions that negatively affect the structure and functionality of the developing brain. Knowing the participating elements is essential to establish preventive measures and look for early markers. The ASD can have subtle or devastating manifestations, and exerting immunomodulatory actions could be useful in the management of these patients. Therefore, our objective is to present a current overview of the pathogenesis of the ASD, specially focused on the genetic factor and the immune system, for which a review of the literature was carried out using the terms, pathogenesis, immunology, and genetics of ASD.

Genetics

The genetic factor is important. If one identical twin is affected, the other will present the problem in 36-95% of cases. In non-identical twins, the other is affected about 0-31% of the time. And parents who have a child with ASD have a 2-18% chance of having a second child, affected[3]. Neurodevelopment is influenced by innate and environmental factors that can modify synapse plasticity, brain structures, cognition, and behavior[4]. There is an interaction between multiple genes to give the autistic phenotype. Heritability in ASD is about 50 or 55%[5]. Genes related to neurodevelopment such as *TEX 49* (*LINC00935)* and *CCNT1* are implicated in ASD and in reading disabilities[6]. Furthermore, genes that code for proteins related to the immune system are involved. It is important to have a general idea of the genes involved in autism, since, as will be seen later, in embryonic processes, the participation of cells of the immune system is important for tissue molding. A large exome sequencing study showed 102 genes involved in risk for ASD. Most of them are expressed in excitatory and inhibitory neuronal lineages, affect synapses, regulate other genes or are related to the immune system[7].

HLA system

The DR4 allele of the MHC is one of the susceptibility markers for certain autoimmune diseases, such as rheumatoid arthritis, hypothyroidism, and autoimmune diabetes. Compared to controls, children with ASD had significantly increased numbers of HLA-DR$^+$CD4$^+$, HLA-DR$^+$CD8$^+$, CD28$^+$HLA-DR$^+$, HLA-DR$^+$CXCR4$^+$, and HLA-DR$^+$CCR7$^+$ cells[8]. Boys with ASD and their mothers have a higher frequency of DR4 than subjects with normal development[8]. Increased relative risk for autism has been observed with HLA hypervariable region 3 of DRB1*0401 in children from North America and China[9]. Furthermore, ASD children have increased frequency of the null allele of the *C4B* gene. This gene encodes the basic form of complement factor 4, part of the classical activation pathway and is located in the major histocompatibility complex (MHC) Class III region, on chromosome 6[10].

RELN gene

The *RELN* gene codifies a protein called reelin that activates a signaling pathway that triggers neurons to migrate to their proper locations. After birth, reelin participates in the extension of axons and dendrites, the regulation of synaptic plasticity, and the release of neurotransmitters. The homozygous mutations lead to brain hypoplasia, developmental delay, and epilepsy. However, heterozygous mutations in RELN are related with the ASD[11].

SHANK3 gene

It encodes a protein that acts as a scaffold that supports the connections between neurons, ensuring communication between them; the protein participates in the formation and maturation of dendritic spines. Alterations in this gene, including the 22q13.3 deletion, appear to be related to ASD. Lymphoblasts of individuals with ASD show reduced expression of this gene[12].

MET gene

MET gene encodes a member of the receptor tyrosine kinase family of proteins. When this receptor binds to the hepatocyte growth factor, it dimerizes and has a role in cellular survival, embryogenesis, and cellular migration and invasion. In the human brain, this gene is highly expressed in temporal, occipital, and medial portions of the parietal lobes. At least, two common risk alleles have been identified. Postmortem analysis of temporal cortex of ASD patients showed 50% reduced levels of MET protein in the superior temporal gyrus. MET signaling has a role not only in neocortical and cerebellar growth and maturation, but also in gastrointestinal repair, and immunological competence, functions that have been reported as abnormal in children with autism[13].

CNTNAP2 gene

The *CNTNAP2* gene (contactin associated protein 2), located in chromosome 7q35-q36.1, encodes a member of the neurexin family which functions as cell adhesion molecule and receptor in the central nervous system (CNS). It is also involved in localization of potassium channels within differentiating axons. *FOXP2* encodes a transcription factor involved in the regulation of numerous genes, including *CNTNAP2*. Both genes have been related to autism and language disorders although some authors have not confirmed these findings[14]. On the other hand, *FOXP1* gene has elevated expression in ASD subjects and functions as a transcription repressor, forming a heterodimer with *FOXP2*[14].

SLC6A4 gene (5-HTTLPR gene)

Solute carrier family 6, member 4 gene, encodes a membrane protein that transports serotonin from synaptic spaces into presynaptic neurons. Autism patients have shown linkage/association with S/L alleles of *5- HTTLPR* locus, with over transmission of S alleles. In animal models, mothers under prenatal stress having the low activity allele had an increased risk for autism-like characteristics in the offspring[15].

Activation of the immune system

Nervous and immune systems are in constant communication. Reactive antibodies have been isolated from the CNS and there is evidence of intraplacental transmission of mother to fetus antibodies resulting in ASD. There appears to be an immune dysfunction in ASD; inflammatory mediators, including serum and brain antibodies, inflammatory serum cytokines, chemokines, and adhesion molecules have been documented. Autoimmune phenomena such as hyperactivity of monocytes, rapid responses of NK, decrease of both, regulatory cells, and IL-10 production have also been found[16].

Cells of the immune system

Phagocytic cells/Microglia

The microglia cells located in CNS cell, when activated, become macrophages. It is known that androgen-induced increases in endocannabinoid tone, promote microglia phagocytosis during a critical period of amygdala development. Phagocytic microglia engulfs more viable astrocytic newborn cells in males; in females, less phagocytosis allows more astrocytes to survive to the juvenile age[17]. Therefore, microglia have an important role in neurodevelopment and ASD is precisely, a neurodevelopmental disorder. Microglial cells responds to type-2 alarmin, and to IL-33, derived from astrocytes, to promote synaptic pruning in the reticular thalamic nucleus, as well as the hippocampus[18]. Microglia are able to regulate programmed neuronal death, participate in promotion of synaptogenesis, and strip excess synapses from developing neurons allowing the integration of functional neuronal circuits. Deficits in synaptic pruning play a role in ASD where either hyperconnectivity and/or hypoconnectivity are observed across the amygdala, pre-frontal cortex, and components of the default-mode network[19].

T cells

Th17, Th1, Th2, and Th regulatory (T_{reg}) cells participate in the progress and development of neurological disorders. T_{reg} cells prevent the development of immune diseases and inhibit self-reactivity. Children with ASD have significantly fewer T_{reg} cells, but also a larger number of activated Th17 and myeloid dendritic cells (mDCs) compared to children without autism. There is an imbalance in the Th17/T_{reg} relationship with marked deviation towards Th17; furthermore, the number of Th17 cells correlates positively with the severity of the disease, while T_{reg} cells have a negative correlation[20]. Signal transducer and activator of transcription 3 (STAT3) and GATA-3 pathways appear to be primarily involved. Th17 is regulated by the translocation of STAT

to the nucleus where it promotes the transcription of related orphan receptor gamma (RORyt); the regulation of mRNA and expression of STAT3 are increased in patients with ASD[20]. In murine models, activation of nuclear factor erythroid 2 related factor (Nrf2) ameliorated autism-like symptoms through suppression of Th17 related signaling and rectification of oxidant-antioxidant imbalance in both, periphery, and brain. The transcription factor Nrf2 is related to antioxidant and anti-inflammatory genes; sulforaphane is an activator of Nrf2. In mice, the treatment with sulforaphane corrected Th17 immune dysfunction and oxidant-antioxidant imbalance in neutrophils and cerebellum[21].

Forkhead Box P3 (Foxp3) is the most important transcription factor in the proliferation and function of T_{reg} lymphocytes. Foxp3 is also expressed in glial cells and inhibits inflammation-induced neuronal excitability by attenuation of TLR4 signaling and inflammation; T_{reg} cell suppresses pro-inflammatory T cell responses directed against "self" antigens and favors the resolution of immune responses that can harm the body. In fact, the genetic variant rs2232365 is associated with ASD in dominant inheritance model[22].

T cell immunoglobulin and mucin domain-3 (TIM-3) or T helper (Th)-specific type I membrane protein, and its ligand, galectin-9 have an important role in Th1 immunity and tolerance induction because downregulate Th1 responses. TIM-3 has regulatory functions extended to Th17 cells, CD4 (+) CD25 (+) Treg, CD8 (+) T cells, and certain innate immune cells. In fact, Increased numbers of $CD3^+TIM\text{-}3^+$, $CD4^+TIM\text{-}3^+$, $CD8^{+-}TIM\text{-}3^+$, CD11a,b$^+$TIM-3$^+$, $CD14^+TIM\text{-}3^+$, $CD62P^+TIM\text{-}3^+$, and $CXCR5^+TIM\text{-}3^+$ cells have been documented in children with ASD as compared with controls. These children also have increased production of IL-1β^+TIM-3$^+$, IFN-γ^+TIM-3$^+$, and IL-17$^+$TIM-3$^+$, and decreased production of $Foxp3^+TIM\text{-}3^+$ compared with controls. In this way, TIM3 could be considered as an early marker of ASD[23]. In the other hand, histopathological and immunohistochemical studies in the ASD brains have shown perivascular cuffs of predominantly CD3+T lymphocytes with less proportion of $CD20^+$ B lymphocytes and $CD8^+$ over $CD4^+$ T lymphocytes. The number of lymphocytes in those cuffs correlates with the quantity of astrocyte-derived round membranous blebs. Membranous blebs are cytotoxic reaction to lymphocyte attack and produce damage to the BBB with increased vascular permeability at the at periventricular white matter, and in the subpial region of the cerebral cortex[24].

B cells

A systemic mitochondrial dysfunction has been reported in ASD and a subset of ASD patients could have a genetic predisposition to mitochondrial/antioxidant insults; B-lymphocytes in these patients show less growth suppression and less mitochondrial proliferation when exposed to dichlorodiphenyldichloroethylene, estradiol, and dihydrotestosterone[25]. In addition, CT genotype in rs10001565 of *CD157*/*BST1* gene, also referred to as bone marrow stromal cell antigen-1 (BST-1) confers susceptibility to ASDs, at least in Japanese patients. CD157 is a glycosylphosphatidylinositol-anchored molecule that promotes pre-B-cell growth[26].

Natural killer cells (NK)

NK cells are innate lymphocytes activated by infected cells, foreign cells, and neoplastic cells. Many patients with autism have a reduction of a mature lymphocyte subpopulation of natural killer cells CD57(+)CD3(-). $CD57^+$ NK cells differentiate from $CD56^{dim}CD57^-$; acquisition of CD57 represents a shift toward a higher cytotoxic capacity. Reduced number of circulating $CD57^+$ NK cells and/or impaired NK cell cytotoxicity is associated with autoimmune disease, suggesting that cytotoxic $CD57^+$ NK cells may play a regulatory role, preventing, or suppressing autoimmune disease (AD). A high frequency of AD in children with autism has been described[27].

In high-functioning ASD adult patients, a high level of NK cell activation has been observed with spontaneous degranulation and interferon-gamma production when compared with healthy controls, whereas these cells become exhausted after *in vitro* stimulations[28]. The profile $HLA\text{-}DR^+KIR2DL1^+NKG2C^+$ NK-cell was observed in ASD patients. This overexpression of NKG2C is indicative of viral infections, and it was inversely correlated with the NKp46 receptor level[28]. On the other hand, NK cells are activated by the interaction between killer-cell immune globulin-like receptor (KIR) and the HLA ligands. Class I alleles (HLA-A2 and HLA-G 14 bp-indel) and three activating KIR genes: 3DS1, 2DS1, and 2DS2 have increased frequencies in autism populations[29].

Dendritic cells

Dendritic cells are antigen-presenting cells; children with ASD have significantly higher percentages of mDCs and plasmacytoid dendritic cells (pDCs) than

controls. Amygdala volume and repetitive behavior in children with ASD are related to the increase in the frequency of mDCs[30].

Mast cells

In children, ASD and atopy have increased in incidence in recent years. Mast cells participate in type I hypersensitivity reactions; stress and environmental stimuli activate mast cells, to produce immunomodulators that alter the functionality of BBB and cause microglia activation, leading to abnormal synaptic pruning and dysfunctional neuronal connectivity, that in combination with the corticotropin-releasing hormone secreted under stress, may contribute to the pathogenesis of ASD[31].

Humoral components

The relationship between the immune system and the nervous system is complex; there are cytokines that directly affect the function and development of neural tissue, for example, IL 1, 6, and 12. In addition, immune dysfunctions in this disease lead to the generation of antibodies.

Cytokines

An imbalance of Th1- Th2 cytokines in the cerebrospinal fluid (CSF) of children with ASD has been demonstrated. Furthermore, lower concentration of anti-inflammatory cytokines IL-10 and IL-1Ra[32], and higher plasma levels of IL-1β, IL-6, IL-8, and IL-12p40 have been observed in ASD patients compared to controls[33]. Some ASD children show excessive innate immune responses with higher concentration of TNF-α in plasma and CSF compared to controls[34]. In murine models, maternal immune activation induced by pathogens during pregnancy changed the cytokine expression profile in maternal and fetal organs and correlated with TNFα and IL-18 dysregulation[35]. In addition, cortical neurons of frontal and temporal lobes involved in learning and memory are more susceptible to cytokine-induced inflammation through the NF-κB signaling pathway. There is evidence of aberrant expression of NF-κB in the orbitofrontal cortex in autopsy material in humans[36]. In mice models, high levels of IL-6 in the brain are related to alterations in excitatory and inhibitory synaptic formations and to abnormal dendritic spines in the cerebellum; IL-6 over-expression promoted the formation of granule cell excitatory synapses, without affecting inhibitory synapses[37]. High levels of IL-6 have been documented in maternal serum and amniotic fluid of children that later on, developed autism. In animal models, it was found that circulating IL-6 levels, two-fold above baseline, increased the platelet-derived growth factor (PDGF)-responsive multipotent progenitor, the phosphorylated STAT3, and the Fbxo15 expression, and decreased Dnmt1 and Tlx expression; the evident consequence was decreased astrogliogenesis in the frontal cortex, proving that inflammation is able to alter neural stem cells and progenitors[37]

Antibodies

Maternal autoimmunity is a risk factor for having a child with autism (rheumatoid arthritis, celiac disease, psoriasis, systemic lupus erythematosus, and autoimmune thyroid disease); also, a family history of type I diabetes increases the risk. Children born to mothers with autoimmune disease are 34% more likely to develop ASD[38]. During critical windows of fetal life, maternal immune response has a long-lasting impact on neurodevelopment. Mothers with autoantibodies to the 37 and 73 kDa fetal brain bands (found only in ASD) have in 95% of the cases, a functional variant in the 5′ promoter of the gene encoding the MET receptor tyrosine kinase. The MET promoter variant, rs1858830MET is a G-to-C single-nucleotide polymorphism (SNP) called the 'C' allele that confers susceptibility for the production of those autoantibodies. Furthermore, this allelic variant is associated with decreased levels of IL-10, a crucial anti-inflammatory cytokine[39]. Antibodies to the 37 and 73 kDa fetal brain bands recognize several developmentally regulated proteins in the fetal brain. These proteins are lactate dehydrogenase A and B; stress-induced phosphoprotein 1, that in combination with cellular prion protein participate in neuritogenesis and neuronal survival; Guanine Deaminase (GDA) with an important role in dendritic branching of hippocampal neurons; collapsin response mediator proteins 1 and 2 that are required for proper growth cone collapse and adequate cell migration and axon-dendrite specification: and Y-box binding protein 1(YBX1). These antibodies are associated with lower adaptive and cognitive function as well as core behaviors associated with autism[40].

Allergic reactions

Mostafa et al. observed allergic manifestations (bronchial asthma, atopic dermatitis, and/or allergic rhinitis)

in 52% of autistic patients, significantly higher than in controls[41]. Some children with ASD have gluten and casein intolerance. IgE-mediated allergic diseases can aggravate behavioral symptoms and may be under-diagnosed in part due to the impaired communication skills of these children. Allergy is associated with activation of mast cells, innate lymphoid cells, and Th2 cells, and production of type-2 cytokines (IL4 and IL13), which favor the M2A phenotype in microglia and macrophages. These cells produce brain-derived neurotrophic factor (BDNF) and insulin-like growth factor-1. In neurons, these growth factors activate the enzyme mammalian Target of Rapamycin (mTOR), and inhibit autophagy. Redundant synapses are removed by autophagy[42].

Neuroinflammation and brain alterations

As mentioned, pro-inflammatory cytokines profile (NF-κB, TNF -α, IFN-γ, MCP-1, TGF-β1, IL-6, IL-1β, and IL-17) is a key component of autism[16]. Furthermore, there is evidence of structural changes in the CNS of individuals with autism. Head circumference is significantly larger in autistic patients compared to control individuals, and around 16 % of autistic individuals had macrocephaly[43]. Several studies have shown cortical abnormalities like dysplasia and heterotopia, as well as reduced neuronal, and cytoplasmic volumes in the majority of examined areas compared to age-matched controls; also, thickening in the subependymal layer and significant increase in neuropil, in the frontopolar region and the anterior cingulate; slower pruning of spines in the temporal lobe with greater spine density in adolescence has been shown. Other alterations include: abnormal persistence of vascular remodeling in the superior temporal cortex; increased diffuse density of microglia; abnormal overgrowth of neurons in prefrontal cortex; significantly smaller pyramidal neurons in the inferior frontal cortex, and alterations at the cellular level in specific areas of the brain that are associated with ASD behaviors, such as fusiform gyrus, frontoinsular and cingulate cortex, hippocampus, amygdala, cerebellum, and brainstem[44]. Controlled neuroinflammation plays a role in the normal development and maintenance of the dendritic spines involved in glutamatergic and GABAergic neurotransmission, and also influences blood-brain permeability. Cytokines released from microglia can impact the length, location or organization of dendritic spines on excitatory and inhibitory cells as well as recruit and impact glial cell function around the neurons. However, uncontrolled, dysfunctional neuroinflammation may have negative effects on brain development. It seems that the symptoms of ASD are the consequence of abnormal circuit wiring during embryonic development[45].

Gestational influence

Gestation is a state of risk for the mother and the product. Bacteria and viruses are able to produce an immune-inflammatory response during pregnancy that could be a risk factor for neurodevelopmental disorders including ASD and schizophrenia; pro-inflammatory cytokines are able to cross the placenta and cause damage in the CNS. Mice studies suggest that Th17 cells (RORγt-expressing) and IL-17 are required in the maternal immune activation (MIA) model for induction of ASD-like phenotypes in offspring. In MIA offspring, abnormal expression of TNFα, and a reduction in the expression of the synaptic organizing proteins cerebellin-1 and GluRδ2 have been described. Alteration in synaptic proteins is associated with a deficit in the ability of Purkinje cells to form synapses; in these models, male offspring is more affected[20]. In a murine-model, early dietary supplementation with Vitamin D, due to its immunomodulatory and neuroprotective effects, was able to mitigate or prevent neurodevelopmental disorders following maternal inflammation[46].

Intestinal microbiota

There is a microbiota-gut-brain axis. Microbiota are able to influence on brain function via microglial-induced synaptic pruning. The newborn has an immature brain and to have the right intestinal flora at early postnatal stages is very important. There is a dysbiosis in ASD children, which may influence the development and severity of ASD symptomatology. The microbiota of these children are composed of the phyla Bacteroidetes, Firmicutes, and Actinobacteria. Among Egyptian children, those with ASD had two types of Clostridium (*Clostridium diffiicile* and *Clostridium clostridiioforme*) not found in neurotypical children, whereas neurotypical children yielded only one species (Clostridium tertium) not found in the ASD children[47]. The metagenome is the set of microbial genes present in a given environment or ecosystem. Investigations of both, metagenome and the effect of the microbiota on the functionality of the nervous system are ongoing.

Associated pathogens

Influenza viruses, herpes simplex virus and rubella, urinary tract bacteria, and toxoplasma have been linked to the development of ASD. Human endogenous retroviruses (HERVs) are assumed to be remnants of retroviruses infections resulting from ancestral infections that were integrated into the genome and transmitted to offspring. These HERVs respond to external stimuli and can somehow modulate the immune response if they are located in strategic places; in fact, high expression levels of HERV-H in blood of autistic patients have been demonstrated[48].

Vaccination

A Nationwide Cohort Study in Denmark found no significant difference between vaccinated (Measles, Mumps, Rubella) and unvaccinated children in relation to the frequency of autism[49].

Discussion

ASD is a multifactorial pathology, characterized by dysregulation of various components, including gene expression and the immune system. It is important to recognize which genes are involved in the disorder and how their altered expressions contribute to the problem. Alterations in immune responses mediated by cytokines, pro-inflammatory factors, and the cellular component, have been associated with neurodevelopmental disruption. Allergies, maternal immune activation, viral infections, and exposure to toxic substances converge in the inflammatory process. The marked increase in the incidence of autism in the last decades is probably related to environmental phenomena that deregulate a precisely controlled inflammatory process throughout fetal neurodevelopment, conditioning permanent changes in the structure of CNS. Inflammation is capable of altering neural stem cells and progenitors, thereby altering neurodevelopment and the proper functioning of neural circuits. A critical situation with ASD is that although several pathophysiological mechanisms involved are known, there are very few that can be targeted by drugs. Therefore, therapeutic efforts have focused on the reestablishment of the excitatory/inhibitory balance described in this pathological spectrum. Besides glutamate and GABA receptors, serotonergic, oxytocinergic, dopaminergic, and cannabinoid systems have been implicated in autism, and more recently, G protein-coupled receptor heteromers have been described (mGlu2-5HT2A, mGlu5-D2-A2A, D2-OXT, CB1-D2, D2-5HT2A, D1-D2, D2-D3, and OXT-5HT2A)[50]. There is still a long way to go in the therapeutic research of ASD.

Conclusion

Several environmental insults may act as triggers in genetically predisposed subjects; these insults can promote an inflammatory response in which IL-6 could participate acting at the level of neural stem cells and progenitors. The moment of appearance of the insult and its intensity will determine the degree of damage to neurogenesis and astrogenesis and to neuronal circuits and therefore, the clinical spectrum of the disease.

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Conflicts of interest

None.

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Ethical disclosures

Protection of human and animal subjects. The authors declare that no experiments were performed on humans or animals for this study.

Confidentiality of data. The authors declare that no patient data appear in this article.

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