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MINIREVIEWS

Impacts of COVID-19 on children and adolescents: A systematic review analyzing its psychiatric effects

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Abstract

OBJECTIVE

To summarize the most relevant data from a systematic review on the impact of COVID-19 on children and adolescents, particularly analyzing its psychiatric effects.

METHODS

This review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) guidelines and included experimental studies (randomized-individually or pooled-and non-randomized controlled trials), observational studies with a group for internal comparison (cohort studies-prospective and retrospective-and case-control) and qualitative studies in the period from 2021 to 2022.

RESULTS

The search identified 325 articles; we removed 125 duplicates. We selected 200 manuscripts, chosen by title and selected abstracts. We excluded 50 records after screening titles and abstracts, as they did not meet the inclusion criteria. We retrieved 150 records selected for a full reading. We excluded 90 text articles and we selected 25 records for the (n) final. Limitations: Due to the short period of data collection, from 2021 to 2022, there is a possibility of lack of relevant studies related to the mental health care of children and adolescents. In addition, there is the possibility of publication bias, such as only significant findings being published.

CONCLUSION

The impact of COVID-19 on the mental health of children and adolescents is of great concern to child and youth psychiatry. Situations such as fear, anxiety, panic, depression, sleep and appetite disorders, as well as impairment in social interactions caused by psychic stress, are punctual markers of pain and psychic suffering, which have increasing impacts on the mental health panorama of children and adolescents globally, particularly in vulnerable and socially at-risk populations.

Key Words: Child psychiatry; Adolescent psychiatry; Mental health; COVID-19; Kids; Teens

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Core Tip: Fear, anxiety, panic, depression, sleep, and appetite disorders, as well as impairment in social interactions caused by psychic stress are punctual markers of pain and psychic suffering, which have increasing impacts on the mental health panorama of children and adolescents in the coronavirus disease 2019 pandemic.

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INTRODUCTION

The outbreak of coronavirus disease 2019 (COVID-19) has caused pain and psychological suffering in children and adolescents, particularly considering the new variants of the disease[1]. Psychologically stressful situations are the main effects caused to populations under the influence of COVID-19, which can contribute to the development of post-traumatic stress symptoms, especially for vulnerable children/adolescents (C-A) in critical developmental stages, with variable prevalence, risk factors, and severity[2]. Recent studies highlight that C-A are more likely to have high rates of depressive or anxiety disorders, impairing family, school, cultural, and social interactions, with multiple and adverse consequences to mental health in the medium and long term[3,4].

Current studies have observed that parental stress, co-parenting, emotional well-being, and children and adolescents' adjustment were impacts that acted unfavorably in the COVID-19 pandemic [5,6]. These findings highlight the psychic burden and stress faced by caregivers of C-A with disabilities and compromised psychiatric development during the pandemic.



In this context, C-A with neurodevelopmental disorders (NDD) have higher levels of distress compared to typically developing children. Distress levels may be heightened by restrictions associated with the COVID-19 pandemic [7,8]. Parents' perceptions of how the pandemic has mitigated their mental health have implications for their well-being and that of their children, with a stronger association for low-income families[9].

Although parenting is essential for positive development, increased parental distress interferes with children's well-being. Sesso et al[10] warn that internalization problems in C-A with NDD were among the strongest predictors of parental stress during the pandemic lockdown. The dysfunctional interactions of a child are usually mediated by their internalizing/externalizing problems[11,12]. In this context, parents of children with NDD should be valued groups in public policies to promote mental health in the post-pandemic period[13].

It is also important to highlight that the prevalence of anxiety generally varies from 19% to 64% and depression from 22.3% to 43.7% among adolescents. Among children aged 5 to 12 years, the prevalence of anxiety ranges from 19% to 78%, while depression among adolescents ranges from 6.3% to 22.6% [14]. Among preschool-age children, some studies have found that behavioral and emotional problems worsen during the pandemic[4,15].

This paper aims to summarize the most relevant data on the impact of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) pandemic on C-A through a systematic review, particularly analyzing its psychiatric effects.

METHODS

A systematic review was carried out using the Preferred Reporting Items for Systematic Reviews and Meta-analyses (PRISMA) protocol from 2021 to 2022. Qualitative studies, quantitative studies (e.g., prospective/retrospective cohorts, case-control studies), and experimental studies (randomized, pooled or individual, and non-randomized controlled trials) were included. Case reports, case studies, opinions, editorials, letters, and conference abstracts were excluded.

The following descriptors were used with the respective Boolean operators: "2019 nCoV" OR # 2019 nCoV OR "2019 novel coronavirus" OR "COVID 19" OR "COVID19" OR "new coronavirus" OR "novel coronavirus" OR "SARS CoV-2" OR "Mental health" OR "depression" OR "Anxiety" OR "Child Psychiatry" OR "Adolescent Psychiatry".

Search strategy

We searched the Web of Science Index Medicus, MEDLINE, WHO COVID-19 databases, EMBASE, Scopus, and Cochrane Library. Non-indexed databases, including MedRxiv preprint and Google Scholar, were also used. To identify missing documents, all systematic reviews and relevant comments were manually searched.

Types of participants

Studies on children and adolescents aged 3 to 19 years from 2021 to 2022, and which focused on psychiatric interventions in children and adolescents during the SARS-CoV-2 pandemic were included.

Selection of studies

Articles were included only if the study exclusively examined the mental health impacts of COVID-19 on children and adolescents from 2021 to 2022. Detailed inclusion and exclusion criteria are shown in Table 1. Using Covidence, a web-based tool that helps to identify studies and involves data extraction processes, two reviewers (MLRN and JPP) independently examined all potential articles. In the case of disagreement, both reviewers read the article and discussed it until a consensus was reached.

Data extraction

Relevant data were extracted from each study, including year and country of publication, study design, target population, pandemic exposure, interventions, and outcomes (Table 2). One reviewer (NNRL) used a form that the research team developed to extract the data. A second reviewer (AOAR) verified the entire data extraction activity and verified its accuracy and completeness. Disagreements were resolved through discussion.

Quality assessment

The methodological quality of the studies was assessed using the Mixed Methods Assessment Tool.

Data analysis/synthesis

Data were aggregated and analyzed according to the results and objectives of the study. Therefore, the results were summarized according to the reported results and the study design (Table 2).

Table 1 Inclusion and exclusion criteria							
Inclusion criteria	Exclusion criteria						
Types of studies: Quantitative, qualitative, mixed methods, experimental and observational studies, human studies	Articles that were not in English; studies that did not report age; studies that included participants with mental health issues prior to COVID-19						
Types of Participants: Studies carried out with children and adolescents (3 to 19 years old) from 2021 to 2022							
Interventions: Children and adolescents impacted by COVID-19 and its repercussions on mental health							
Types of results: Rates of psychiatric disorders in children and adolescents in times of COVID-19							
Secondary outcomes: Fear, anguish, pain and psychic suffering related to the pandemic							

COVID-19: Coronavirus disease 2019.

Risk of publication bias

The likelihood of a treatment effect reported in systematic reviews resembling the truth depends on the validity of the studies included in the analysis as certain methodological characteristics may be associated with effect sizes. Therefore, it was important to determine in the systematic reviews whether the sample of studies obtained was representative of all the research carried out on depression in childhood and adolescence in times of COVID-19. The possibility of bias resulting from a trend of only positive findings being published-known as the "file drawer effect"-was addressed using two methods: Calculating the fail-safe N and the p-curve approach.

The fail-safe N is determined by calculating the number of studies with a mean null result needed to make the overall results insignificant. The p-curve was introduced to account for "p-hacking", a theory stating that researchers may be able to get most studies to find positive results across different reviews. The p-curve assesses the slope of the reported p-values to determine whether p-hacking has occurred.

The most significant findings of depression in children and adolescents impacted by COVID-19 were found in 24 studies, which required the p-value to be set at > 0.05. In addition, quarantine, sleep disturbances, post-traumatic stress symptoms, and the prevalence of anxiety were findings that validated the results. The p-curve was applied to explain p-hacking-to guarantee positive results. When calculating the p-curve, only 13 studies were included that examined the psychiatric impact on adolescents and children during the COVID-19 pandemic[2,3,6,7,15-23]. The studies existing in the literature (P = 0.5328) indicating depression among children and adolescents have sufficient evidence in their findings, particularly because there were 11 studies on potential interventions to improve the mental health of children and adolescents[1,4,5,8,9-13,23,24].

Clearly, solutions to the file drawer problem present an irritating and challenging issue for metaanalytic research and it will likely take a paradigm shift to truly address this problem, as authors who submit their literature reviews and methods only, abandoning conventional inferential statistics in favor of Bayesian Approaches, or the registration of studies and protocols online before conducting a study.

RESULTS

The search identified 325 articles, but 125 duplicates were removed. Therefore, 200 articles were selected, chosen by the title and abstract. Fifty articles were excluded after screening the titles and abstracts, as they did not meet the inclusion criteria. Consequently, a total of 150 articles were selected to be read in full. After that, 91 text articles were excluded, with 24 being selected for the final (n) (Figure 1).

Study results

We analyzed the studies thematically and divided them into two categories: (1) Psychiatric impact on children and adolescents in times of COVID-19; and (2) potential interventions to improve the mental health of children and adolescents.

Psychiatric impact on children and adolescents in times of COVID-19

Among the studies included, 13 examined the psychiatric impact on children and adolescents in times of COVID-19[2,3,6,7,14-22].

A research study by Demaria and Vicari[2] and Sayed et al[3] showed that quarantine is a psychologically stressful experience. For children, missing school and interruptions in daily routines can have a negative impact on their physical and mental health. In this perspective, they pointed out that parents



Table 2 Characteristics of included studies (n = 24)

Ref.	Country	Study design	Target population	Total participants	Exposure	Outcomes
Barros et al[19], 2022	Brazil	Cross-sectional- electronic questionnaire	12-17 years	9470 adolescents	COVID-19	The data showed that factors such as: Family problems, female gender, age 15- 17 years, learning disabilities, relatives infected with COVID-19, and death of close friends from COVID-19 were factors associated with worsening mental health
Okuyama <i>et al</i> [1], 2021	Japan	Review	Children under 18 years	Studies included (<i>n</i> = 28)	COVID-19	Studies have shown correlation between physical activity and psychological health and sedentary time leading to mood disorders. Some studies on adolescents reported a correlation between physical activity and psychological health and others did not
Demaria and Vicari[2], 2021	Italy	Commentary	NA	NA	COVID-19	The pandemic context, with regard to quarantine, proved to be a psychologically stressful experience
Sayed <i>et al</i> [3], 2021	Saudi Arabia	Cross-sectional- online <i>via</i> social media	12.25 ± 3.77 years	537 children (263 boys and 275 girls)	COVID-19	The data showed that Post-traumatic stress disorder symptoms were not correlated with school grade, sex, age or having a close relative working with people infected by COVID-19
Meherali <i>et al</i> [4], 2021	Canada, Pakistan, Australia	Systematic reviews	5-19 years	Studies included (<i>n</i> = 18)	COVID-19	These studies reported that pandemics cause stress, worry, helplessness, and social and risky behavioral problems among children and adolescents
Bussières <i>et al</i> [5], 2021	Canada	Meta-analysis	5-13 years	Studies included (n = 28)	COVID-19	During the COVID-19 pandemic, the restriction measures imposed had an impact on children's mental health. During this period, there was also a change in sleep habits. Even so, the results do not show significant differences in relation to the general population
Bentenuto <i>et al</i> [6], 2021	Italy	Retrospective	Children with NDD and TD	Total 164 (NND 82 and TD 82)	COVID-19	Quantitative analyzes demonstrated an increase in children's externalizing behaviors and parental stress. However, they also showed that parents enjoyed spending more time with their children and strengthening the parent-child relationship. Furthermore, in children with NDD, the reduction in therapeutic measures predisposes to high externalizing behaviors
Burnett <i>et al</i> [7], 2021	Sweden, Australia, Italy	Cross-sectional- online self- reported survey	Parents of children aged 3-18 years	Australia ($n = 196$); Italy ($n = 200$)	COVID-19	When compared to other developmental disorders among parents in Australia and Italy, intellectual or learning disorders are the ones that bring them the most suffering
Raffagnato <i>et al</i> [8], 2021	Italy	Longitudinal	Psychiatric patients age between 6 and 18 years and their parents	39 patients and their parents (25 girls and 14 boys)	COVID-19	Patients with behavioral disorders were more impacted when compared to patients with internalizing disorders, who were shown to have adapted better to the pandemic context. In parents, it was possible to observe a protective factor against psychological maladjustment. A decrease in mothers' anxiety and fathers' stress over time was also observed
Kerr et al[9], 2021	United States	Cross-sectional- online survey	Parents with at least one child 12 years old or younger	1000 participants	COVID-19	As for the psychological impacts, the data show high levels of stress and low levels of positive behavior in children, and a high rate of parental exhaustion. Still, there is an indirect association between parental behavior and the psychological impacts of COVID-19 and children's behaviors. The data also showed that the difference in income is a factor that can increase this indirect

						association
Sesso <i>et al</i> [10], 2021	Italy	Cross-sectional- online questionnaire	Parents of children 6.62 ± 3.12 years with neuropsy- chiatric disorders	77 participants	COVID-19	Internalizing problems in children during quarantine were the strongest predictor of parental stress
Li and Zhou[11], 2021	China	Cross-sectional- online questionnaire	5-8 years: 647 children; 9-13 years: 245 adolescents	892 valid question- naires (mothers 662 and fathers 230)	COVID-19	Concerning the data, it was possible to observe that parents are worried about their children's internalization and externalization problems. It was observed that, in elementary school, significant and negative relationships were observed between family-based disaster education and internalizing and externalizing problems
Bate <i>et al</i> [12], 2021	United States	Cross-sectional- online <i>via</i> social media	Parents of children (6-12 years)	158 parents of children (151 mothers and 7 fathers)	COVID-19	It was observed that the biggest EH problems of parents were due to the impact of COVID-19. Parents' EH was a positive predictor of children's EBH
Kim et al[13], 2021	Suwon, South Korea	Cross-sectional- web based questionnaire	Parents of children aged 7-12 years	217 parents	COVID-19	With schools closed, children had body weight gain, spent less time doing physical activities and more time using the media. In addition, an association can be observed between parental depression and children's sleep problems, TV time, tablet time and behavior problems
Minozzi <i>et al</i> [14], 2021	Italy	Systematic review	Pre-school children, children 5-12 years and adolescents	Studies included (<i>n</i> = 64)	COVID-19	Studies have reported an increase in suicides, reduced access to psychiatric emergency services, reduction in allegations of maltreatment. The prevalence of anxiety among adolescents varied considerably, as did depression, although in a lower percentage
Backer <i>et al</i> [15], 2021	Netherlands	Cross-sectional- questionnaire	0-4, 5-9, 10-19, 20-29, 30-39, 40-49, 50-59, 60-69, 70-79, 80-89 and ≥ 90 years	7250 participants	COVID-19	During the physical distancing restriction measures, it is possible to observe that community contacts in all age groups were restricted to an average of 5 contacts. After relaxation, it was observed that the children returned to maintain their normal contact number, while the elderly maintained their restricted contact numbers
Qin et al[16], 2021	Guangdong province, China	Cross-sectional- electronic questionnaire	School-aged students [12.04 (3.01) years]	1 199 320 children and adolescents	COVID-19	Among those who reported psychological distress, the risk of psychological distress was analyzed among high school and elementary school students, among students who never used a mask and those who did, and among students who spent less than 0.5 h exercising and those who spent more than 1 h
Lu et al[17], 2021	China, United Kingdom	Systematic review and meta- analysis	children and adolescents (0-18 years)	Studies included (<i>n</i> = 23)	COVID-19	Studies show a combined prevalence of depression, anxiety, sleep disorders, and post-traumatic stress symptoms
Ma et al[18], 2021	China, United States	Cross-sectional- online self-report questionnaires	6-8 years	17740 children and adolescents	COVID-19	The data reported that depressive, anxiety, compulsive, inattentive and sleep-related problems were more expressive when compared to before the COVID-19 outbreak
Spencer <i>et al</i> [23], 2021	United States	Cohort study	5-11 years	Caregivers of 168 children (54% non- Hispanic black, 29% Hispanic, and 22% non-English speaking)	COVID-19	Children had significantly higher emotional and behavioral symptoms mid-pandemic <i>vs</i> pre-pandemic in all scenarios
Han and Song [20], 2021	South Korea	Retrospective	Middle and high school students	54948 students	COVID-19	The data showed, through multivariate logistic regression, that there was a correlation between the perception of the economic situation of the family and the prevalence of depressive symptoms and suicidal ideation

Giannakopoulos et al[21], 2021	Greece	Quality study- interviews	12-17 years	09 psychiatric inpatients	COVID-19	Patients identified that the state of quarantine caused negative changes in personal freedom and social life, as well as excessive contact with family members during social isolation
Almhizai <i>et al</i> [22], 2021	Saudi Arabia	Cross sectional study-online self- administered questionnaire	0-17 years	1141 respondents, 454 were < 18 years old and 688 children's parents	COVID-19	Among the data presented, age was a factor for sleep disorders, nervousness and malaise; aggressive behaviors were also associated with an increase in negative behaviors during the pandemic compared to the previous period
Maunula <i>et al</i> [24], 2021	Northern prairie communities, Canada	Multi-method study, focus groups, and interviews	Children grade 4-6 and their parents	31 patients (16 children and 15 parents)	COVID-19	Children were subjected to sudden and stressful changes in their routines. In addition, loneliness and increased screen time were a result of limited social interaction

NDD: Neurodevelopmental disorder; TD: Typical developing; EBH: Emotional and behavioral health; EH: Emotional health; COVID-19: Coronavirus disease 2019.

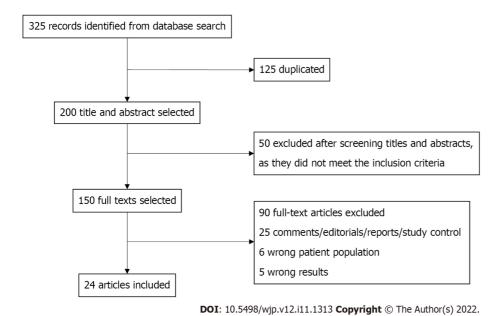


Figure 1 Diagram of preferred reporting items for systematic reviews and meta-analyses (PRISMA).

could also pass on their psychological suffering to children and parent them inappropriately, contributing to the development of post-traumatic stress symptoms. In addition, if the C-A has a mental disorder, the psychic suffering of the parents tends to be greater and depends on the way children externalize their emotions[6,7].

Minozzi et al[14] highlight high rates of anxiety and depression among C-A. Among preschool children, they found aggravation of behavioral and emotional problems, while others did not. They found that psychological well-being had significantly worsened, especially among adolescents. Backer et al[15] demonstrate that the reduced number of social contacts associated with strict social distancing measures contributes to inflicting pain and psychic suffering in children and adolescents. The authors also point out that not wearing a mask; being a high school student[15] and spending less than 0.5 h exercising were positively associated with increased psychological distress[16].

A meta-analysis of 23 studies (n = 57927 children and adolescents from Turkey and China) showed combined prevalence of anxiety, post-traumatic stress symptoms, sleep disorders and depression. In addition, female sex and adolescents were more associated with depressive and/or anxious symptoms when compared to male sex or children, respectively [18].

Barros et al[19] showed high rates of nervousness (48.7%) and sadness (32.4%) among Brazilian adolescents. Individuals aged between 15-17 years; being female; having learning difficulties during the pandemic; having a family that faces financial difficulties; and individuals who previously had trouble sleeping or poor health were the most affected. In the study by Han and Song[20] economic difficulties during the pandemic were correlated with depression and suicidal ideation. Concerning their emotions, adolescents recognized anxiety about self-harm and harm to their loved ones, as well as mood swings in the family nucleus[21].

Globally, the increase in drug abuse has also been mapped in the literature, with alcohol and marijuana being the most used[7]. Almhizai et al[22] showed that the older age of children and adolescents was a risk factor for sleep disorders, malaise, and nervousness. The presence of a relative infected with COVID-19 was also associated with higher rates of anxiety, irritability, sadness, and sleep disorders. Finally, physical punishment and verbal threats had a more negative impact on the mental health promotion of C-A when compared to the pre-pandemic period.

Impact of control measures to contain the effect on the mental health of children and adolescents

Eleven studies reported potential interventions to improve the mental health of children and adolescents[1,4,5,8-13,23,24].

Bussières et al[5] showed no association between the presence of previous chronic diseases (including NDD) and negative symptoms during the pandemic. Raffagnato et al[8] highlight that patients with internalizing disorders had better adaptation and lower rates of psychological distress when compared to patients with psychological distress.

In addition, the worsening of parents' mental health[10], school-age children belonging to urban racial and ethnical minorities[23], and physical inactivity[1,17] had a negative impact on the health of children and adolescents. Data from Li and Zhou[11] suggest that children less exposed to parental concerns (e.g., about finances, health and education) were less likely to have internalizing and externalizing problems[11]. It is crucial to promote family well-being through political practices and initiatives, including providing financial and care assistance to parents and supporting the mental and behavioral health of families[9]. In addition to focusing on symptom management, families can benefit from support aimed at the parent-child relationship. Insights and implications for practitioners are discussed[12]. Finally, promoting coping strategies for children and adolescents to deal with extreme situations (e.g., pandemics, wars, and natural disasters) is fundamental. Especially if the strategies encompass the communities/schools the children/adolescents attend[24].

DISCUSSION

The rapid spread of COVID-19 has significantly influenced the psychological state of children and adolescents. It is clear that poverty[19,20], hunger, housing insecurity, domestic violence, and sexual abuse[19], black children and adolescents, and homeless people living in favelas, especially older adolescents, need urgent mental health support. The physical restrictions of the COVID-19 pandemic and the social distancing measures have affected all domains of life. Anxiety, depression, drug abuse, sleep and appetite disorders, as well as impaired social interactions, are the most common presentations [4,13].

The frequency of mask use and time spent on schoolwork were factors associated with good mental health[16]. The prevalence of depression ranges from 13.5% to 81.0%. Analysis by age indicated that the prevalence of depression is higher in children aged 5-9 years and adolescents aged 12-18 years. Analysis by gender showed that the prevalence of depression in females was higher than in males. The prevalence of anxiety among children and adolescents was 45.6%. The prevalence of post-traumatic stress symptoms is statistically higher in vulnerable and/or socially at-risk children and adolescents. The prevalence of sleep disorders varies according to the stressor involved in family ties and the way they face COVID-19, as well as the economic situation and the healthcare system, which vary greatly between countries[17]. Parental anxiety has the greatest influence on a child's psychological symptoms, explaining about 33% of the variation in a child's overall symptoms[18,23].

Most studies point to negative symptoms being caused by social distancing in children and adolescents of vulnerable families, including restrictions on social life and personal freedom, as well as excessive contact with family members during stay-at-home periods[1,2,21].

It is important to highlight that children and adolescents in extreme poverty report a wide range of negative thoughts associated with the pandemic (for example, abandonment, helplessness, sadness, anguish, anxiety, and feelings of panic). The thoughts and feelings of such teenagers can be triggered by the fact that their survival is threatened[4,5].

Special populations, especially lesbian, gay, bisexual, transgender, and queer (LGBTQ) adolescents, have higher rates of pain and psychological distress that lead to anxiety, depression, compulsion, and post-traumatic stress disorder (PTSD). Additionally, coming into conflicts with parents due to gender issues is observed in the literature as a factor that worsens mental health in this population[7,22].

LIMITATIONS

Due to the short data collection period, from 2021 to 2022, relevant studies on how to care for the mental health of children and adolescents may be lacking. In addition, there is the possibility of publication bias, *i.e.*, only significant findings being published.



CONCLUSION

Fear, anxiety, panic, depression, insomnia and appetite disorders, as well as impaired routine caused by psychic stress, are individual markers of pain and psychic suffering, which have increasing impacts on the mental health panorama of children and adolescents. A better understanding of the psychological pathways available is necessary to help clinicians, researchers, and decision makers prevent the deterioration of mental and general functioning disorders, as well as other stress-related disorders in children and adolescents[2,4,6,13].

Agreeing with Giannakopoulos *et al*[21] and Barros *et al*[19] professionals should continue to provide strategies to mitigate the impact of the pandemic on the mental health of children, adolescents and their families, aiming at improving the quality of life and rehabilitation in the post-pandemic period. It is necessary to emphasize the need to build resilience and promote strategies to manage negative feelings during crises (environmental, social, political, and economic)[24].

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FOOTNOTES

Author contributions: Gabriel IWM, Lima DGS, Pires JP, Gonçalves Júnior J, Vieira NB, Brasil AAGM, Pereira YTG, and Rolim-Neto ML designed the review, developed the inclusion criteria, screened titles and abstracts, appraised the quality of included papers, and drafted the manuscript; Oliveira EG, Menezes HL, Lima NNR, Reis AOA, Alves RNP, Silva UPD and Rolim-Neto ML reviewed the study protocol and inclusion criteria and provided substantial input to the manuscript; Pires JP, Reis AOA, Gonçalves Júnior J, Lima NNR and Rolim-Neto ML reviewed the study protocol; Rolim-Neto ML read and screened articles for inclusion; all authors critically reviewed drafts and approved the final manuscript.

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