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REVIEW ARTICLE

Multisystem inflammatory syndrome and COVID-19: a scoping review

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Abstract

Background: *Multisystem inflammatory syndrome temporally associated with COVID-19 presents with similar symptomatology and therapeutic approach to Kawasaki disease in the pediatric population. Given the novelty of the disease and the growing scientific literature on the subject, it is relevant to collect and report available scientific information. This review aimed to explore the medical evidence on multisystem inflammatory syndrome temporally associated with COVID-19 in a population under 18 years of age.* **Methods:** *We conducted a scoping review using Scopus and PubMed, including observational (cohort, case-control, and cross-sectional) studies and case series.* **Results:** *Of the total articles reviewed as of April 10, 2021, 45 articles met eligibility criteria: case series (n = 32), retrospective cohort studies (n = 6), prospective cohort studies (n = 4), case-control studies (n = 2), and cross-sectional studies (n = 1). Gastrointestinal and respiratory symptoms and myocardial dysfunction are the most commonly reported. The most relevant paraclinical markers were lymphopenia, thrombocytopenia, and elevated D-dimer levels.* **Conclusions:** *The multisystem inflammatory syndrome temporally associated with COVID-19 presents a broad spectrum of signs and symptoms. Aneurysms of the coronary arteries and myocarditis are usually present in the acute phases of the disease. The early diagnosis led by a multidisciplinary group of pediatric intensivists, infectious disease specialists, cardiologists, and rheumatologists allows adequate and effective medical management.*

Keywords: *Kawasaki Disease. Vasculitis. COVID-19. SARS-CoV-2. Systematic review.*

Síndrome inflamatorio multisistémico y COVID-19: una revisión exploratoria

Resumen

Introducción: *El síndrome inflamatorio multisistémico temporalmente asociado con COVID-19 se presenta con una sintomatología y un enfoque terapéutico similares a los de la enfermedad de Kawasaki en la población pediátrica. Dado lo novedoso de la enfermedad y la creciente literatura científica al respecto, resulta relevante recopilar y comunicar la información disponible. El objetivo fue explorar la evidencia médica sobre el síndrome inflamatorio multisistémico temporalmente asociado con COVID-19 en población menor de 18 años.* **Métodos:** *Se realizó una revisión exploratoria utilizando Scopus y PubMed, incluyendo estudios observacionales (estudios de cohorte, casos y controles, y transversales) y series de casos.* **Resultados:** *Del total de los artículos revisados hasta el 10 de abril de 2021, 45 cumplieron con los criterios de elegibilidad: series de casos (n = 32), estudios de cohorte retrospectiva (n = 6), estudios de cohorte prospectiva (n = 4), estudios de*

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casos y controles (n = 2) y estudios transversales (n = 1). Los síntomas gastrointestinales, respiratorios y de disfunción miocárdica son los que más se reportan en la literatura. Por su parte, los marcadores paraclínicos más relevantes fueron linfocitopenia, trombocitopenia y valores elevados de dímero D. **Conclusiones:** *El síndrome inflamatorio multisistémico temporalmente asociado con COVID-19 se presenta con un amplio espectro de signos y síntomas. Las complicaciones más graves son el compromiso aneurismático de las arterias coronarias y la miocarditis. El diagnóstico temprano liderado por un grupo multidisciplinario de pediatras intensivistas, infectólogos, cardiólogos y reumatólogos permite un manejo médico adecuado y eficaz.*

Palabras clave: *Enfermedad de Kawasaki. Vasculitis. COVID-19. SARS-CoV-2. Revisión sistemática.*

Introduction

Severe acute respiratory syndrome type 2 coronavirus (SARS-CoV-2) infection was identified in December 2019 in Hubei province, Wuhan, China[1]. In January 2020, the World Health Organization (WHO) named COVID-19 the new disease caused by SARS-CoV-2 and officially declared a pandemic on March 11, 2020[1,2]. The virus belongs to the *Coronaviridae* family and has a positive-sense single-stranded ribonucleic acid genome surrounded by an extracellular membrane[2,3]. The clinical presentation of COVID-19 varies from an asymptomatic clinical presentation to acute respiratory distress syndrome (ARDS) and multiorgan failure[4]. In a systematic review, Cui et al.[5] reported that in the pediatric population, clinical manifestations such as fever and cough are less frequent when compared to the adult population.

Throughout the pandemic, a multisystem inflammatory syndrome temporally associated with COVID-19 (MIS-C) began to be identified in children with previous SARS-CoV-2 infection. This syndrome's clinical and paraclinical features are similar to Kawasaki disease (KD)[6,7]. MIS-C presents with an acute, self-limited, systemic vasculitis affecting the pediatric population under 5 years of age[7,8]. Its pathophysiology is mediated by monocytes, macrophages, T cells, and proinflammatory cytokines such as interleukin 6 (IL-6), promoting an inflammatory phenomenon that weakens the vascular wall and can lead to coronary artery aneurysms[7,9].

Vasculitis pathophysiology seems to be explained by the interaction between the infection, a genetic component of the individual, and a disproportionate immune response, with a marked tropism for the endothelium and vascular wall[8]. Countries such as Italy are a clear example of the high number of cases of MIS-C in children with previous SARS-CoV-2 infection, where early diagnosis allowed adequate management of the disease, preventing possible short- and medium-term cardiac complications[10,11]. Given the novelty of the disease and the growing scientific literature on the subject, it is relevant to collect and communicate the available scientific information that will help health professionals to make decisions in their clinical practice[6,7,10]. Therefore, the present review explored the current medical evidence up to April 10, 2021, on MIS-C and SARS-CoV-2 infection in the population under 18 years of age.

Methods

We followed the steps proposed by Arksey and O'Malley[12] and refined by Levac et al.[13] for the review: i) definition of the research question; ii) search for and identification of relevant studies; iii) selection of studies; iv) data collection; v) summary and reporting of results, and vi) review by the expert team. The review adhered to the preferred reporting elements for systematic reviews and meta-analyses PRISMA-ScR[14] (Table 1).

Our research questions were as follows:

– What is the current medical evidence on MIS-C and SARS-CoV-2 infection in the pediatric population (< 18 years)?
– What are the research gaps in the literature on MIS-C in the pediatric population with previous SARS-CoV-2 infection?

Eligibility criteria

Due to a large amount of medical literature available on the topic and the need to concisely analyze and describe the impact of SARS-CoV-2 infection in the pediatric population, this exploratory review included only analytical and descriptive observational studies (cohort, case-control, case series, and cross-sectional studies) on MIS-C. Only articles published in English and Spanish between 2019 and 2021 were included. Case reports, theoretical publications, or publications with no available abstract or full text were excluded.

Table 1. Characteristics of the publications included in the review

Author, year [reference]	Type of document	Target population characteristics	Objective	Country	Main finding/contribution
Whittaker et al., 2020[47]	Case series	58 patients Mean age of 9 years	To describe and compare clinical and paraclinical characteristics in pediatric patients who met criteria for MIS-C	United Kingdom	A total of 58 patients with a median age of 9 years were evaluated, of which 13 met the definition of MIS-C
Feldstein et al., 2020[45]	Case series	186 patients Mean age of 8.3 years	To report patients with MIS-C from March 15 to May 20, 2020, of whom 74 (40%) documented KD-like features	United States	MIS-C caused severe disease involving damage to multiple organs and systems in previously healthy pediatric and adolescent patients
Toubiana et al., 2020[21]	Prospective cohort	21 patients Mean age of 7.9 years	To describe the characteristics of the pediatric patients affected by MIS and KD, evaluating a possible association with SARS-CoV-2 infection	France	MIS-C could be related to COVID-19 in the pediatric population; in addition, it is associated with gastrointestinal symptoms and shock
Dufort et al., 2020[29]	Case series	99 patients	To describe the clinical manifestations of patients hospitalized for MIS-C	United States	MIS-C in pediatric patients coincided with the widespread transmission of SARS-CoV-2, whose dermatological, mucocutaneous, and gastrointestinal manifestations were associated with cardiac dysfunction
Verdoni et al., 2020[11]	Retrospective cohort	19 patients with KD before the pandemic 10 patients with MIS-C after the pandemic	To evaluate the incidence and clinical characteristics of MIS-C patients diagnosed during the COVID-19 pandemic	Italy	A 30-fold higher incidence of MIS-C was found in the pediatric population; in addition, a higher rate of cardiac involvement of a severe form of KD was reported
Pouletty et al., 2020[46]	Retrospective cohort	10 patients with KD before the pandemic 16 patients with MIS-C	To analyze the clinical and paraclinical characteristics in patients with a confirmed diagnosis of COVID-19 and MIS-C	France	MIS-C represents a new inflammatory syndrome associated with high morbidity and mortality in the pediatric population; prospective studies are needed to characterize this syndrome better
Chiotos et al., 2020[52]	Case series	6 children with MIS-C Treated in the PICU	To describe the clinical and paraclinical features in six children with MIS-C seen in a pediatric intensive care unit	United States	Patients received Ig and methylprednisolone therapies, achieving the reduction of systemic inflammation, resolution of fever, and improvement of cardiac function
Lee et al., 2020[26]	Retrospective cohort	28 patients with MIS-C Mean age of 9 years	To describe the clinical and paraclinical manifestations of pediatric patients diagnosed with MIS-C	United States	MIS-C encompasses a broad phenotypic spectrum with clinical and laboratory features distinct from KD
Ramcharan et al., 2020[15]	Case series	15 patients Mean age of 8.8 years	Description of short-term cardiovascular manifestations in a pediatric population with MIS-C in a tertiary children's hospital	United Kingdom	Cardiovascular involvement was reported to be greater than in other published series; it is necessary to emphasize pediatric cardiology assessment

(continues)

Table 1. Characteristics of the publications included in the review *(continued)*

Author, year [reference]	Type of document	Target population characteristics	Objective	Country	Main finding/contribution
Cheung et al., 2020[65]	Case series	17 patients Mean age of 8.8 years	To describe the clinical manifestations and therapeutic approaches in a previously healthy pediatric population infected with SARS-CoV-2 with an inflammatory phenotype	United Kingdom	Cytokine elevation via IFN, TNF-α, and IL-13 and abnormal cardiac findings suggest the need for surveillance regarding MIS-C-associated complications
Blondiaux et al., 2020[17]	Case series	4 patients Mean age of 9 years	To evaluate cardiac magnetic resonance imaging findings in a pediatric population admitted to the intensive care unit for MIS-C	France	Diffuse myocardial edema was evident on T2 and T1 sequences, with no evidence of late gadolinium enhancement suggestive of replacement fibrosis or focal necrosis; these findings suggest post-infectious myocarditis
Labé et al., 2020[22]	Case series	2 patients aged 3 and 6 years	To describe two cases with a clinical picture of fever and cutaneous eruptions with involvement of mucous membranes (erythema multiforme) associated with COVID-19	France	SARS-CoV-2 infection is a trigger for MIS-C; respiratory symptoms are evident as a typical clinical picture
Rostad et al., 2020[39]	Prospective cohort	10 patients with MIS-C 10 patients with symptomatic COVID-19 5 patients with KD 4 healthy controls	To evaluate the diagnostic capability of serological tests for MIS-C	United States	Quantitative SARS-CoV-2 serology may have a role in diagnosing MIS-C, distinguishing it from similar clinical entities, and stratifying the risk of adverse outcome
Ouldali et al., 2020[36]	Case series	230 patients with KD	To determine if SARS-CoV-2 infection is associated with an increased incidence of KD	France	An increase in MIS-C cases was evident, particularly in countries where the peak of COVID-19 had recently been reached
Capone et al., 2020[49]	Case series	33 patients Mean age of 8.6 years	To describe the presentation and clinical course of 33 children with MIS concomitant with SARS-CoV-2 infection	United States	MIS-C was related to COVID-19; furthermore, a large proportion of patients developed shock requiring vasoactive agents and anti-inflammatory therapy
Waltuch et al., 2020[16]	Case series	4 patients with MIS-C	To describe four pediatric patients with confirmed SARS-CoV-2 infection who presented to the emergency department with features associated with MIS-C	United States	Patients presented with prolonged fever, gastrointestinal symptoms with or without rash; in addition, these patients may decompensate rapidly and require specialized care
Toubiana et al., 2020[56]	Case-control	23 patients with MIS-C 102 controls	To evaluate the association between severe SARS-CoV-2 infection and MIS-C in pediatric patients in France.	France	SARS-CoV-2 infection was confirmed in 17/23 cases vs. 11/102 controls (95% CI: 6.0-116.9); suggesting a strong association between COVID-19 and systemic proinflammatory state

(continues)

Table 1. Characteristics of the publications included in the review *(continued)*

Author, year [reference]	Type of document	Target population characteristics	Objective	Country	Main finding/contribution
Corwin et al., 2020[66]	Retrospective cohort	33 patients Mean age of 10.9 years	To compare the presentation of clinical and paraclinical features in a pediatric population with MIS-C	United States	The initial pattern of lymphopenia, thrombocytopenia, hyponatremia and abnormal creatinine can help recognize patients with MIS-C
Lima-Setta et al., 2021[18]	Prospective cohort	56 patients with MIS-C Mean age of 6.2 years	To describe the clinical, laboratory, and radiological characteristics in a pediatric population diagnosed with MIS-C	Brazil	Diagnostic chest images with bilateral diffuse interstitial infiltrate. On echocardiogram, mild pericardial effusion, left ventricular dysfunction, and signs of coronary dilation. Laboratory tests: anemia, leukocytosis, lymphopenia, and thrombocytopenia, altered C-reactive protein, ESR, and fibrinogen levels
Toubiana et al., 2021[19]	Case-control	30 patients with KD onset after the pandemic 59 patients with KD onset before the pandemic	To analyze the clinical manifestations, therapeutic approaches, and clinical outcomes in a pediatric population diagnosed with MIS-C	France	Specific characteristics of MIS-C and classic KD are recognized; therefore, it is necessary to differentiate both pathologies, allowing an early and effective diagnosis
Heidemann et al., 2020[53]	Case series	3 patients aged 5, 6, and 7 years	To describe 3 cases of vasculitis associated with SARS-CoV-2 infection and their therapeutic approaches	United States	Partial response to intravenous Ig and ECMO therapy was evidenced; arrhythmias may be related to inflammation and myocardial ischemia
Falah et al., 2020[24]	Case series	10 patients Mean age of 6 years	To describe the clinical manifestations, paraclinical features, therapeutic approaches, and clinical outcomes in a pediatric population with MIS-C	United States	MIS-C in the pediatric population manifests with fever, rash, seizures, cough, tachypnea, and gastrointestinal symptoms due to the associated hyperinflammatory state
Bordet et al., 2021[28]	Case series	32 patients	To analyze the clinical features of MIS-C as a new disease between a spectrum of KD and viral myocarditis	France	The pediatric population with COVID-19 presented with mild to severe myocarditis and fever plus two to three KD-like symptoms
Carbajal et al., 2021[40]	Case series	7 patients with MIS-C 40 patients with KD	To determine the relationship between COVID 19 and MIS-C and compare it with the main characteristics of KD	France	The clinical manifestations of MIS-C are associated with a hyperinflammatory state, and its clinical features are different from those presented in KD
Del Greco et al., 2020[50]	Case series	4 patients	To present four cases of MIS-C in the emergency department and describe their therapeutic approach	United States	Patients with MIS-C have a good recovery with medical management with Ig and corticosteroids, and a low mortality rate
Fouriki et al., 2021[34]	Case series	6 patients	To report six cases of MIS-C in pediatric patients in Switzerland and to describe the therapeutic approach	Switzerland	The use of anakinra could be an alternative to corticosteroid treatment

(continues)

Table 1. Characteristics of the publications included in the review *(continued)*

Author, year [reference]	Type of document	Target population characteristics	Objective	Country	Main finding/contribution
Grimaud et al., 2020[51]	Case series	20 patients	To describe the clinical characteristics in a pediatric population with cardiogenic shock secondary to acute myocarditis and suspected SARS-CoV-2 infection	France	Early recognition and referral to a specialized center are required in MIS-C patients
Gruber et al., 2020[41]	Case series	9 patients with MIS-C	To determine possible autoantibodies linked to target organs in SARS-CoV-2 and MIS-C	United States	The profile revealed known disease-associated autoantibodies (anti-La), which recognize endothelial, gastrointestinal, and immune cell antigens
Iio et al., 2021[27]	Case series	30 patients with KD onset before the pandemic 14 patients with KD onset after the pandemic	To analyze the clinical manifestations in a pediatric population with an initial diagnosis of COVID-19 in order to determine the relationship between the infection and a systemic inflammatory response	Japan	There was no evidence of an increase in the incidence of KD; instead, MIS-C with a different profile of clinical manifestations than KD was described
Matsubara et al., 2020[30]	Case series	28 patients with MIS-C 20 patients with KD 20 healthy controls	To describe the echocardiographic findings in MIS-C	United States	MIS-C presents aneurysmal dilatations in the coronary arteries, usually reverting to normal; the myocardial lesion is similar to that produced in KD
Ng et al., 2020[57]	Case series	3 patients	To describe the clinical presentations and outcomes of three adolescents with confirmed SARS-CoV-2 infection admitted to the pediatric intensive care unit	United Kingdom	Similarities were found between KD and MIS-C; the mechanism of MIS-C depends on macrophage activation. Further studies on MIS-C, including cytokine and immune profiles, are needed
Papadopoulou et al., 2021[58]	Case series	19 patients Mean age of 9.1 years	To describe the clinical presentation and therapeutic approach to MIS-C in the pediatric population and to highlight the role of the pediatric rheumatologist in this setting	United Kingdom	Nineteen children met MIS-C criteria, and nine also met diagnostic criteria for complete or incomplete KD; immunomodulatory therapy is necessary
Rekhtman et al., 2021[37]	Prospective cohort	31 patients with COVID-19 or MIS-C	To characterize the mucocutaneous disease and its relationship with the clinical course of hospitalized patients with MIS-C	United States	The mucocutaneous disease is common in children and adolescents with MIS-C
Sethurama et al., 2021[38]	Case series	34 patients with MIS-C Mean age of 8 years	To describe the clinical and paraclinical manifestations of 34 children with MIS-C who were evaluated within 12 weeks	United States	MIS-C starts at an older age than KD, with a predominance of gastrointestinal symptoms, presence of myocarditis, and shock
Shahbazneja et al., 2020[25]	Case series	10 patients Mean age of 5.37 years	To examine the association between exposure to COVID-19 and MIS-C	Iran	MIS-C in the pediatric population is present with fever, rash, seizures, cough, tachypnea, and gastrointestinal symptoms due to the associated hyperinflammatory state

(continues)

Table 1. Characteristics of the publications included in the review *(continued)*

Author, year [reference]	Type of document	Target population characteristics	Objective	Country	Main finding/contribution
To et al., 2020[42]	Case series	3 patients with KD	To describe three patients with KD and false-positive COVID-19 serology	China	Neutralizing antibody screening is recommended to confirm previous SARS-CoV-2 infection in patients who are positive by serology but negative for RT-PCR
Vergnano et al., 2020[55]	Case series	7 patients with MIS-C Younger than one year	To describe seven cases who presented MIS-C in five hospital centers	United Kingdom	Pediatricians must consider early and aggressive treatment and close cardiac monitoring in patients with MIS-C and SARS-CoV-2 infection
Fabi et al., 2021[20]	Cross-sectional study	8 patients with KD 1 patient with myocarditis 4 patients with MIS-C	To compare patients with diagnoses of KD, myocarditis, and MIS-C from February to April 2020 versus patients diagnosed before the pandemic	Italy	MIS-C and myocarditis responded rapidly to treatment without cardiac sequelae; it is necessary to differentiate KD and MIS-C despite their diagnostic and therapeutic similarities
Plebani et al., 2020[67]	Case series	9 patients Mean age of 8.9 years	To report nine previously healthy children (six males and three females) admitted for MIS-C and SARS-CoV-2 infection	Italy	*Mycoplasma pneumoniae* co-infection in pediatric patients with MIS-C may contribute to a more severe clinical course
Vukomanovic et al., 2020[70]	Case series	3 patients with MIS-C	To present three male adolescents with MIS-C and myocardial injury admitted to hospital.	Serbia	Clinical presentation, laboratory and echocardiographic findings pointing to MIS-C with a cardiac lesion
Shikhare et al., 2021[32]	Case series	6 patients with MIS-C with a mean age of 8 years	To report six children with MIS-C who were admitted to the hospital between May 5, 2020, and June 25, 2020.	United States	Two children with complete KD, three with incomplete KD, and one with terminal ileitis with late-onset circulatory shock were managed with Ig, corticosteroids, and aspirin.
Esteve et al., 2021[60]	Case-control	14 patients with MIS-C 9 patients with COVID-19 with no MIS-C 14 patients with pre-pandemic KD 37 healthy controls	Hypothesize that pre-pandemic MIS-C patient profiles are different from the clinical manifestations observed in MIS-C	Spain	An essential role for IFN-γ in MIS-C pathogenesis is evidenced, which may be relevant for therapeutic management
Cattalini et al., 2021[59]	Retrospective cohort	149 patients: 96 patients with KD and 53 patients with MIS-C	To collect data from patients diagnosed with MIS-C by surveying between February 1, 2020, and May 31, 2020	Italy	The clinical characteristics and treatment response of MIS-C and its relationship with KD were better characterized
Niño-Taravilla et al., 2021[54]	Case series	26 patients with MIS-C Mean age of 6.5 years	To describe pediatric population with MIS-C in the pediatric intensive care unit	Chile	Most patients had echocardiographic abnormalities, and half required treatment with vasoactive drugs and immunomodulatory therapy

(continues)

Table 1. Characteristics of the publications included in the review *(continued)*

Author, year [reference]	Type of document	Target population characteristics	Objective	Country	Main finding/contribution
Coll-Vela et al., 2020[63]	Case series	8 patients with MIS-C Mean age of 5.1 years	To present a series of 8 cases with clinical presentation of fever, acute gastrointestinal problems, and ocular and mucocutaneous involvement	Peru	All patients received Ig, corticosteroids, and aspirin. Only two cases received a second dose of Ig, and only one patient presented myocarditis, shock, and required ventilatory support

CI, confidence interval; ECMO, extracorporeal membrane oxygenation; ESR, erythrocyte sedimentation rate; IFN, interferon; Ig, immunoglobulin; IL, interleukin; KD, Kawasaki disease; MIS, multisystem inflammatory syndrome; MIS-C, multisystem inflammatory syndrome temporally associated with COVID-19; NT-proBNP, N-terminal brain natriuretic peptide; PICU, pediatric intensive care unit; RT-PCR, real-time polymerase chain reaction; TNF-α, tumor necrosis factor-alpha

Search strategy

The search strategy was developed with the guidance of a research librarian from the Universidad de La Sabana, Colombia, to identify relevant references. We used Boolean operators and key terms according to each electronic database: PubMed and Scopus were included (Table 2). References cited in the selected papers were added, and, additionally, papers provided by experts were incorporated if they met the inclusion criteria and had not been previously identified. The last update of the search was performed on April 10, 2021.

Selection of studies

The titles and abstracts of the references were reviewed independently by three authors (CM, GM, AR), following the eligibility criteria. At regular meetings of these three authors, a consensus was reached on the full-text documents included, which were finally reviewed independently by all the other authors. Publication data were extracted as follows: authors, country, type of document, type of study, number of participants, objective, and main findings.

Data extraction

Data from the included articles were extracted by four independent reviewers (ET, CM, AR, GM) in the scoping review. We present the results in a table with an overview of the studies, followed by a narrative synthesis of the most important findings.

Results

We selected 45 papers that met the eligibility criteria (Figure 1). We further divided them according to study type: case series (n = 32), retrospective cohort studies (n = 6), prospective cohort studies (n = 4), case-control studies (n = 2), and cross-sectional study (n = 1). The countries of origin of these studies were as follows: United States (n = 16), France (n = 10), United Kingdom (n = 6), Italy (n = 4), Brazil (n = 1), Iran (n = 1), Serbia (n = 1), Japan (n = 1), Spain (n = 1), China (n = 1), Chile (n = 1), Switzerland (n = 1), and Peru (n = 1). The general characteristics of the included studies are presented in Table 1.

Case series

Ramcharan et al.[15] described the short-term cardiovascular manifestations, therapeutic approaches, and clinical outcomes in 15 patients under 18 years of age with a confirmed diagnosis of MIS-C based on the Royal College of Paediatrics and Child Health definition. The patients were African, Afro-Caribbean, or South Asian, with a median age of 8.8 years (interquartile range (IR): 6.4-11.2 years). All patients presented with fever, 13 patients with gastrointestinal symptoms, and eight patients with features of Kawasaki disease (KD) that did not meet diagnostic criteria. Two patients manifested symptoms typical of SARS-CoV-2 infection, and three patients had family members with symptoms of COVID-19 in the two months prior to the study. Twelve patients were positive for enzyme-linked immunosorbent assay (ELISA) combined immunoglobulins (IgG, IgA, and IgM). In addition, elevated levels of C-reactive protein (CRP), ferritin, troponin I, and pro-B-type natriuretic peptide (proBNP) were found. At the structural level, seven patients had normal coronary arteries, six had ectatic dilated coronary arteries, and one showed a fusiform aneurysm. Ten patients had mitral insufficiency, eight had pericardial effusion, and

Table 2. Search strategy

PubMed
((“Mucocutaneous Lymph Node Syndrome”[Mesh] OR “Kawasaki Disease” OR “Kawasaki Syndrome” [Title]) AND (“pediatric multisystem inflammatory disease, COVID-19 related” [Supplementary Concept] OR “MIS-C”[Title] OR “PIMS-TS”[Title]) AND ((“coronavirus”[MeSH]) OR (“coronavirus infections”[MeSH Terms]) OR (“coronavirus”) OR (“covid 2019”) OR (“SARS2”) OR (“SARS-CoV-2”) OR (“SARS-CoV-19”) OR (“severe acute respiratory syndrome coronavirus 2”[supplementary concept]) OR (“severe acute respiratory” AND “pneumonia outbreak”) OR (“novel cov”) OR (2019ncov) OR (“sars cov2”) OR (cov2) OR (ncov) OR (“covid19”) OR (“coronaviridae”) OR (“corona virus”))
Scopus
(TITLE-ABS-KEY (kawasaki AND disease) OR ALL (kawasaki AND disease) AND ALL (mucocutaneous AND lymph AND node AND syndrome) AND ALL (pediatric AND multisystem AND inflammatory AND disease) OR TITLE-ABS-KEY (pims-ts) OR TITLE-ABS-KEY (mis-c) AND TITLE-ABS-KEY (sars-cov-2) OR TITLE-ABS-KEY (covid-19) OR ALL (covid-19) OR ALL (2019-ncov))

twelve had altered left ventricular ejection fraction. Ten patients required medical assistance in intensive care with inotropic and vasopressors for a median of 3 days, and only four patients were mechanically ventilated. Medical treatment with immunoglobulin was administered in ten patients, of whom only two required a second dose, and only five patients received a course of methylprednisolone. Hospital discharge occurred on day 12 (RI: 9-13 days) with normal clinical and paraclinical parameters. Although the diagnostic and therapeutic approach is similar to KD patients, the authors concluded that early medical treatment is necessary to reduce inflammation and associated complications.

Waltuch et al.[16] described four pediatric patients with MIS-C and associated SARS-CoV-2 infection confirmed by IgG testing serologic but negative nasopharyngeal reverse transcriptase-polymerase chain reaction (RT-PCR) swab. Two patients had no significant history, one had asthma, and the last patient had hypothyroidism. All patients had symptoms in common: fever, cough, fatigue, and rash; in addition, two patients reported gastrointestinal symptoms such as nausea, vomiting, and diarrhea. On physical examination, three patients showed a diffuse non-pruritic erythematous rash on the chest, abdomen, back, and extremities (including palms and soles) and conjunctival injection; one patient had pain on palpation in the epigastrium and both iliac fossae with no signs of peritoneal irritation. Medical management was performed in the intensive care unit (ICU) with hydration support and broad-spectrum antibiotics coverage. Three patients were treated with immunoglobulin and tocilizumab. One patient who presented ARDS required mechanical ventilation and administration of anakinra. After an echocardiogram, coronary dilatation and left ventricular ejection fraction (LVEF) of 47% were found. The authors reported that patients with MIS-C presented fever and gastrointestinal symptoms with or without exanthema and features similar to KD. However, it is necessary to differentiate this disease, manage it medically, and immediately admit patients to the ICU because they may deteriorate rapidly.

Blondiaux et al.[17] reported the most common findings on cardiac magnetic resonance imaging, and transthoracic echocardiography in the pediatric population admitted to the ICU for tachycardia and inflammatory shock syndrome with acute myocarditis. All patients had a diffuse non-pruritic erythematous rash, lymphopenia, and elevated brain natriuretic peptide levels, troponin I, and CRP. Transthoracic echocardiography showed LVEF < 30% in one patient and > 50% in three patients. Septal hypokinesia was found in three patients, mitral insufficiency in two patients, and diffuse myocardial hyperintensity of the left ventricle in T2 sequences suggestive of interstitial edema in three patients. No late gadolinium enhancement—suggestive of replacement fibrosis or focal necrosis, findings indicative of transient post-infective myocarditis—was observed in any patient.

Retrospective cohorts

Verdoni et al.[11] evaluated the incidence, clinical and paraclinical characteristics in a cohort of 19 patients diagnosed with Kawasaki-like disease before the onset of the COVID-19 pandemic (group 1) and ten patients diagnosed between February and April 2020 (group 2). The mean age of disease onset in group 1 was 3 years (standard deviation (SD):2.5) versus 7.5 years (SD:3.5) in group 2 (p = 0.003). Patients diagnosed with Kawasaki-like disease during the pandemic showed more pronounced leukopenia and thrombocytopenia than those in group 1 (p = 0.017 and p = 0.001, respectively). In addition, an abnormal echocardiogram with LVEF < 50% was observed in five patients, pericardial effusion in four patients, and coronary aneurysm > 4 mm in two patients in group 2 (p = 0.089). Elevated proBNP levels were observed in the ten patients diagnosed with Kawasaki-like disease during the pandemic, hypertriglyceridemia in seven, and eight

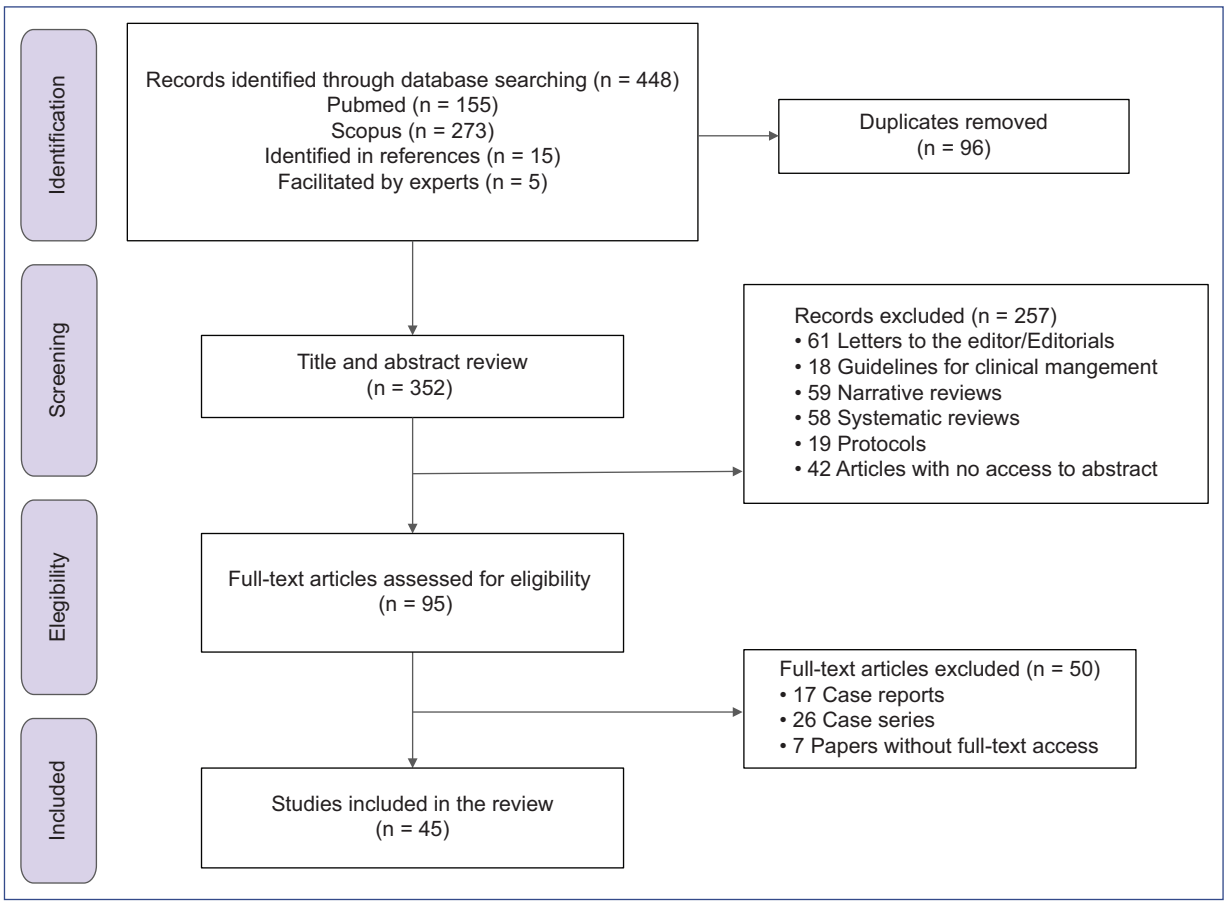

Figure 1. Flow chart for the scoping review process (PRISMA).

required supplemental corticosteroid therapy (p = 0.045). The authors reported a monthly incidence at least 30 times higher during the pandemic than the incidence prior to the first case of COVID-19, with positive seroconversion to the virus in most patients.

Prospective cohorts

Lima-Setta et al.[18] analyzed the clinical manifestations, inflammatory and respiratory markers, and diagnostic imaging of 56 patients younger than 18 years diagnosed with MIS-C in Brazil. The median age was 6.2 years, and all confirmed SARS-CoV-2 cases were positive by RT-PCR or serologic testing. Gastrointestinal symptoms, such as abdominal pain, diarrhea, and vomiting, were present in 71% of patients. Skin rash, headache, or irritability were also common symptoms. Inflammation markers (CRP) and cardiac dysfunction markers (troponin and proBNT peptide) were elevated in most patients. Among the diagnostic imaging findings, chest radiographs showed a bilateral diffuse interstitial infiltrate, computed tomography showed ground-glass opacities, and echocardiography showed mild pericardial effusion, left ventricular dysfunction, and signs of coronary dilatation. The most commonly used medical treatment was intravenous Ig in 89% of patients, broad-spectrum antibiotics in 59%, corticosteroids in more than 50%, and aspirin in 45%. Only 11% of patients required invasive mechanical ventilation with a mean duration of five days. The authors emphasized the importance of serology and clinical manifestations in establishing an early diagnosis and an effective therapeutic scheme for MIS-C.

Case-control studies

Toubiana et al.19 compared 30 patients with a suspected diagnosis of MIS-C versus a control group of 59 patients diagnosed with KD according to American Heart Association criteria before the pandemic in a pediatric population in France. Of the case group, 23 patients had positive SARS-CoV-2 IgG antibodies, and 9 had positive RT-PCR tests. The mean age in this group was 8.2 years versus 4 years in the control group (p < 0.001). Gastrointestinal symptoms such as abdominal pain,

vomiting, and diarrhea (odds ratio (OR): 84 [4.9-1456]), myocarditis (OR: 387 [38-3933]), and pericardial effusion (OR: 11.6 [3.7-36.5]) were frequent in patients with MIS-C, along with higher ICU admission (OR:196 [31-1257]). Higher CRP levels, lymphopenia, and severe anemia were observed in this group compared to the control group. Two KD patients developed coronary artery aneurysms. Both groups were treated with intravenous Ig with an adequate clinical and paraclinical response.

Cross-sectional study

Fabi et al.[20] described cardiovascular manifestations during the increase in cases of SARS-CoV-2 infections in the Emilia-Romagna region (Italy), including the pediatric population with diagnoses of KD, myocarditis, and MIS-C. Eight patients were diagnosed with KD, of whom three showed transient coronary lesions, and all were negative for SARS-CoV-2. One 5-year-old patient positive for parvovirus B19 and negative for SARS-CoV-2 was diagnosed with myocarditis. Lastly, four patients positive for SARS-CoV-2 were diagnosed with MIS-C, of whom three showed myocardial dysfunction and pericardial effusion, and one case developed multi-coronary aneurysms and mitral and aortic insufficiency. Finally, all responded to medical therapy with Ig with no cardiac sequelae.

Discussion

This study reviewed the available medical evidence on the clinical, paraclinical, and therapeutic aspects of MIS-C in the pediatric population. The most common clinical manifestations were fever, gastrointestinal symptoms (abdominal pain, diarrhea, and vomiting), diffuse non-pruritic erythematous rash on the chest, abdomen, back, and extremities (including the palmar and dorsal regions of the hands and feet), and conjunctival injection[15-18,21-28]. Cardiac manifestations are the most dangerous presentation of the disease. Due to transient post-infectious myocarditis, patients may present reduced LVEF, septal hypokinesia, atrioventricular or aortic insufficiency, and interstitial edema with no signs of fibrosis or focal necrosis[11,17,18,29]. Markers of inflammation and myocardial injury justify close cardiac and vascular structural follow-up to diagnose coronary artery aneurysms during the acute phase[30], the most severe complication. Most patients are treated with intravenous Ig, aspirin, corticosteroids, and immunomodulatory agents such as tocilizumab and anakinra[16,31-34]. Hydration support and broad-spectrum antibiotic therapy are also widely used[15,18].

WHO defined MIS-C as a syndrome in patients younger than 18 years characterized by a fever of three or more days and at least two of the following criteria: i) rash or bilateral nonpurulent conjunctivitis or signs of mucocutaneous inflammation; ii) hypotension or shock; iii) features of myocardial dysfunction, pericarditis, valvulitis, or coronary abnormalities (including echocardiographic signs or elevated troponin or proBNP values); iv) suggestive evidence of coagulopathy; v) acute gastrointestinal symptoms (diarrhea, vomiting, or abdominal pain); vi) elevated levels of inflammatory markers without an apparent microbial cause of inflammation in patients with previous SARS-CoV-2 infection[35-38].

The pathophysiologic mechanisms of MIS-C are still unknown. However, resembling systemic inflammatory diseases in pediatrics, a humoral and cellular immune response secondary to SARS-CoV-2 infection is suggested[39]. The recognition of autoantigens by antibodies or T cells results in autoantibodies and immune complexes by viral mimicry; these activate an inflammatory cascade that promotes tissue injury with tropism at the cardiovascular level[40-42]. Similarly, damage-associated molecular patterns and pathogen-associated molecular patterns lead to the formation of inflammasomes and precipitate cell death by pyroptosis and cytokine storm, mainly involving interleukin-1 (IL-1), which has inflammatory effects on the endothelial cells of the coronary arteries[43].

Unlike adult patients, the pediatric population experiences a transient type of myocardial and vascular injury, with troponin elevation in patients without underlying chronic pathologies, which decreases the associated mortality rate[44-46]. Inflammatory or cardiac injury markers are associated with the development of myocarditis, allowing the initial clinical suspicion. However, follow-up by echocardiography, CT angiography, magnetic resonance imaging, or electrocardiography (because some patients also develop arrhythmias) is essential in all cases of MIS-C[47,38]. In addition, the absence of paraclinical markers that identify the development of aneurysms requires strict follow-up during the hospital stay and for 2 to 6 weeks after discharge[17,48].

The conventional medical treatment for patients with MIS-C is based on the protocol used for KD, consisting of the administration of intravenous Ig with or without aspirin, corticosteroids, immunomodulatory agents such as infliximab (tumor necrosis factor-alpha neutralizer)[49,50], tocilizumab (IL-6 signal transduction inhibitor)[51], and anakinra (IL-1 receptor antagonist)[52-55], which are

effective in diseases with a similar systemic inflammatory load[56,57]. The use of early immunotherapy with tocilizumab in patients without cardiac complications or infliximab in patients with positive echocardiographic findings avoided the need for extracorporeal membrane support therapy, demonstrating that immunotherapy prevents further supportive interventions in patients with MIS-C[54]. However, clinical trials with a more extensive study population and study time are needed to elucidate the mechanisms of this therapy and its possible effects on survival[58-60].

Currently, antiviral (lopinavir and interferon) and non-antiviral (colchicine) treatments do not positively impact mortality, mechanical ventilation requirements, or length of hospital stay[61-63]; only remdesivir has shown clinical improvement in hospitalized adults with severe COVID-19 symptoms[64]. However, its use is limited in the pediatric population with clinical manifestations of MIS-C because the clinical picture presented in the acute phase of the disease is associated with undetectable viral loads by RT-PCR[35]. To date, the dose of anticoagulant and antiplatelet therapy in critically ill pediatric patients is guided by elevated D-dimer and fibrinogen, a decision made in conjunction with pediatric hematologists[65-67].

This review evaluated the available medical evidence on MIS-C in the pediatric population regarding clinical manifestations, treatments used, and overall prognosis, demonstrating the gaps that still exist in epidemiological, clinical, and immunological research on this new disease. Therefore, this review allows establishing new research questions and guiding the development of long-term follow-up clinical studies[68].

Strengths and limitations

A librarian guided our review strategy to ensure its adequacy. Only publications from PubMed and Scopus in English and Spanish were included. We did not perform a quality assessment of the included studies because this does not correspond to an objective of scoping reviews[12,13].

The small sample size of patients with MIS-C is an important limitation for studying this new and complex disease. More observational studies and clinical trials are needed to establish the pathophysiology and therapeutic schemes focused on immunomodulation, cardiovascular myocardial injury, mechanical ventilation, and renal replacement therapy[69,70].

In conclusion, patients with MIS-C experience a broad spectrum of signs and symptoms, including gastrointestinal, cutaneous, and conjunctival injection manifestations. Aneurysmal involvement of the coronary arteries and myocarditis are the most severe complications and frequently appear in the acute phases of the disease, along with electrocardiographic and imaging alterations. Early diagnosis allows the initiation of appropriate and effective medical treatment using Ig, corticosteroids, antiplatelet agents, anticoagulants, and other immune system modulators. The potential severity of MIS-C requires multidisciplinary care with pediatric intensivists, infectious disease specialists, cardiologists, and rheumatologists.

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 patient data publication.

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

Conflicts of interest

The authors declare no conflict of interest.

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REVIEW ARTICLE

Acne and diet: a review of pathogenic mechanisms

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Abstract

Acne is a chronic inflammatory disease of the pilosebaceous unit with multifactorial etiology. Abnormal proliferation of keratinocytes, altered sebum production, inflammation of the sebaceous follicle, and colonization by Cutibacterium acnes have been traditionally implicated. However, the diet has also been highlighted in the pathogenesis because of its direct relation with some biochemical markers and the transcription of specific genes associated with sebaceous gland activity, inflammation, and bacterial proliferation, which together promote the development of the disease, affect the severity of the condition, and modify its response to treatment.

Keywords: *Diet. Acne vulgaris. Glycemic index. Glycemic load.*

Acné y dieta: una revisión de los mecanismos patogénicos

Resumen

El acné es una enfermedad inflamatoria crónica de la unidad pilosebácea de etiología multifactorial, en la que clásicamente se han implicado la proliferación anormal de queratinocitos, la producción alterada de sebo, la inflamación del folículo sebáceo y la colonización por Cutibacterium acnes. Sin embargo, también destaca la dieta en la patogenia al relacionarse directamente con la alteración de algunos marcadores bioquímicos y transcripción de ciertos genes que se asocian con la actividad de la glándula sebácea, la inflamación y la proliferación bacteriana, que en conjunto promueven el desarrollo de la enfermedad, afectan la gravedad del cuadro y modifican su respuesta al tratamiento.

Palabras clave: *Dieta. Acné vulgar. Índice glicémico. Carga glicémica.*

Introduction

Acne is a chronic inflammatory disease of the pilosebaceous unit with multifactorial etiology[1]. It is considered one of the most frequent diseases in adolescents and young adults, affecting 85% of individuals between 12 and 24 years of age and compromising 9.4% of the population worldwide[2,3].

Besides those traditionally described, current evidence on the pathogenesis of acne shows that certain factors influence the development and exacerbation of lesions, such as genetics, stress, and, especially, diet[4].

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Historically, there has been controversy about the role of diet in the pathogenesis of acne. Early studies emerged at the beginning of the 20th century; however, the results were considered conflicting and inconclusive due to limitations such as inadequate sample size, lack of control groups, and blinding, among other factors[5].

The study of the relationship between diet and acne gained interest until the 1960s and 1970s due to the myth of worsening acne caused by chocolate consumption. Thus, Fulton et al., in 1969, published a study showing that the consumption of cocoa solids-based foods was not associated with increased acne severity, but rather the intake of the other ingredients added to commercial chocolate bars[6].

At the beginning of the 21st century, studies on the relationship between diet and acne reappeared. Notably, Smith et al. in 2007 compared two groups of patients with acne: the first group followed a diet rich in moderate/high glycemic index (GI) carbohydrates, and the second group followed a diet with a low glycemic load (GL). As a result, the second group showed a significant reduction in acne lesions and improved insulin sensitivity[7]. Furthermore, Tayel et al. in 2013 showed that the intake of a low GL diet in adolescents and young adults resulted in a significant improvement in acne severity[8]. Finally, Çerman et al. in 2016 demonstrated that a high GI and GL diet was directly related to acne, reaffirming studies conducted years earlier and establishing a starting point for future research and a potential target for treatments[9].

In 2014, Burris et al. studied the relationship of GI and consumption of certain foods in patients with acne of different severity, finding that patients with moderate to severe acne reported higher GI in their diet and increased intake of milk, saturated fats, and *trans* fats. Moreover, 58.1% of participants perceived that diet aggravated or influenced their acne[10]. The same authors published in 2017 a study in which they evaluated the relationship between GI, GL, and biological factors associated with acne, and reported that acne patients consumed foods with higher GL, which increased insulin and insulin-like growth factor-1 (IGF-1) concentrations[11].

Fabroccini et al., in 2016, conducted a study comparing two groups of patients with acne and an altered metabolic profile. One group received a hypocaloric diet and metformin, and the other did not. At the end of the follow-up, it was found that the patients who received treatment showed a significant improvement in acne severity, corroborating the relationship between insulin resistance and aggravation of the disease[12].

Pathogenesis

The pathogenesis of acne consists of four main aspects: 1) increased androgens that stimulate keratinocyte proliferation, sebum production, and sebaceous gland growth; 2) abnormal keratinocyte proliferation leading to the formation of comedones; 3) inflammation of the pilosebaceous follicle, and 4) bacterial colonization by *Cutibacterium acnes*. However, in recent years, the diet has gained importance, and some biochemical markers modified by the diet directly influence the severity of acne[11].

Biochemical markers

IGF-1

IGF-1 is a potent mitogen synthesized mainly in the liver that stimulates sebocyte growth and lipogenesis in the sebaceous glands[9,13,14]. It also induces androgen production in ovaries and testes and inhibits hepatic synthesis of sex hormone-binding globulin (SHBG) and IGF-binding proteins (IGFBP)-1 and IGFBP-3. SHBG is a glycoprotein that binds sex hormones (specifically testosterone and estradiol and has a high affinity for dihydrotestosterone), inhibiting their function and bioavailability. In addition, IGFBPs modulate the half-life of IGF and even modulate its interaction with its receptor. Therefore, inhibition of IGFBPs and SHBG increases the bioavailability of androgens and IGF-1, thereby increasing the severity of acne[13,15,16] (Figure 1).

Insulin, FOX O1, and mTORC-1

Insulin response to glucose increases during puberty and adolescence, while insulin sensitivity decreases significantly. These changes are essential, as insulin can completely modify the androgen axis, which is necessary during this stage of sexual maturation. Insulin targets the liver, where it inhibits SHBG production[17].

Moreover, hyperinsulinemia can promote the appearance of acne by two mechanisms: in the first, it inhibits hepatic production of IGFBP-1 and IGFBP-3 and increases androgen synthesis[17]; in the second, it activates the phosphatidylinositol-3-kinase/Akt pathway, which reduces FOX O1 (Forkhead box O1) protein expression at the nuclear level[11,18]. FOX O1 is a transcription factor that inhibits protein synthesis, cell

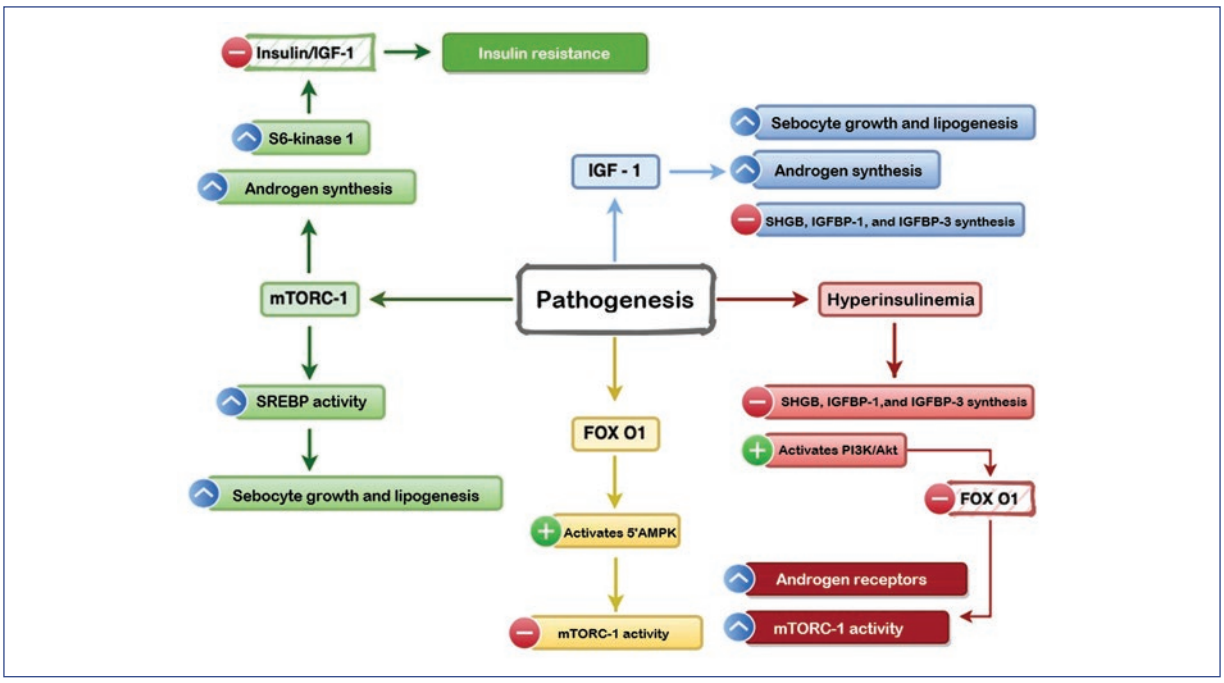

Figure 1. The actions of IGF-1 (insulin-like growth factor), FOX O1 (forkhead box O1), and mTORC-1 (nutrient-responsive mammalian target of rapamycin complex) markers in the pathogenesis of acne.
5'AMPK, 5'-adenosine monophosphate-activated protein kinase; IGFBP, IGF-binding protein; SHBG, sex hormone-binding globulin; SREBP, sterol regulatory element-binding protein.

growth, and lipid metabolism[17,18]. Under normal conditions, FOX O1 induces activation of the 5' adenosine monophosphate-activated protein kinase (5'AMPK) pathway, which is a critical negative regulator of the nutrient-responsive mammalian target of rapamycin kinase (mTORC)-1[15,17].

FOX O1 depletion causes an increase in androgen receptor activity and mTORC-1 activity in the pilosebaceous unit[11,12]. mTORC-1 is a serine/threonine kinase that functions as a regulator of cell growth, proliferation, lipid synthesis, and protein transcription; it is sensitive to growth factors (insulin, IGF-1), energy levels (glucose, AMP/ATP ratio), and some amino acids (leucine)[19,20].

Increasing androgen synthesis also increases androgen receptor activity, which inhibits the DEP domain of mTORC-1 (DEPTOR), activating mTORC-1. As a result, keratinocyte proliferation and differentiation are increased and lipid synthesis in the pilosebaceous unit[20,21].

When mTORC-1 activity is increased, transcription of the sterol regulatory element-binding protein (*SREBP*) gene also increases. This protein regulates the synthesis of cholesterol, fatty acids, triglycerides, and phospholipids and stimulates lipogenesis and sebaceous gland enlargement[9,22]. Subsequently, *C. acnes* triacylglycerol lipase converts the triacylglycerols present in normal sebum into free fatty acids, such as palmitic acid, sapienic acid, and oleic acid, stimulating the formation of biofilms[23].

Increased mTORC-1 activity and decreased FOX O1 also stimulate androgen and S6 kinase-1 secretion[12,13]. Furthermore, S6 kinase-1-mediated insulin receptor substrate-1 phosphorylation downregulates insulin/IGF-1 signaling and consequently induces insulin resistance (Figure 1)[14,20].

Glycemic index and glycemic load

The glycemic index (GI) measures the effect of carbohydrates from a given food on postprandial glucose concentration. Food with a high GI is characterized by rapidly digested and absorbed carbohydrates that can significantly increase blood glucose and insulin concentrations[17]. Conversely, glycemic load (GL) considers the quality and quantity of carbohydrates consumed and, therefore, estimates the overall glycemic effect of a standard portion of a given food[9,17].

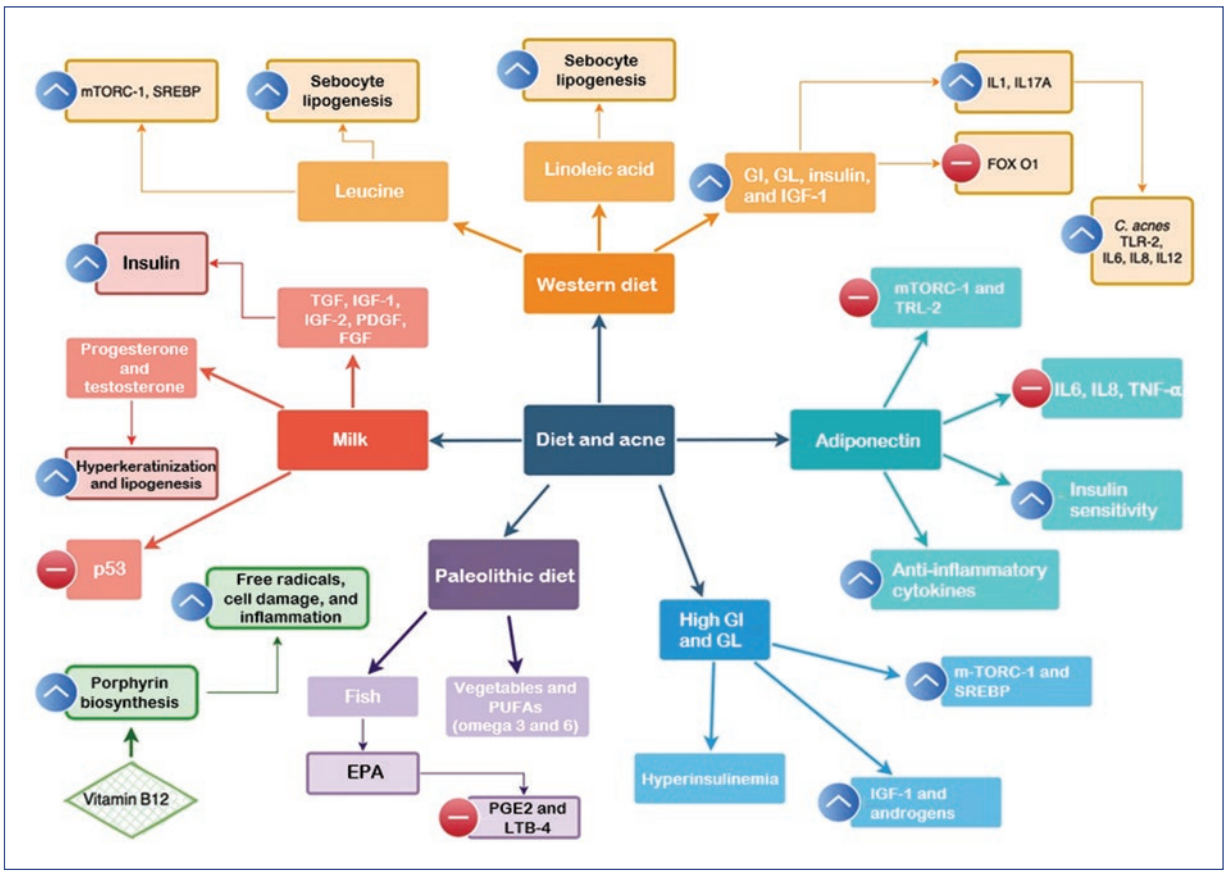

Figure 2. EPA, n-3 eicosapentaenoic acid; FGF, fibroblast-derived growth factor; FOXO1, forkhead box O1 protein; GI, glycemic index; GL, glycemic load; IGF, insulin-like growth factor; IL, interleukin; LTB4, leukotriene B4; PDGF, platelet-derived growth factor; PGE2, prostaglandin E2; PUFA, polyunsaturated fatty acids; SREBP, sterol regulatory element binding protein; TGF, tumor growth factor; TLR, Toll-like receptor; mTORC, nutrient-responsive mammalian target of rapamycin complex.

High GI and GL induce hyperinsulinemia, thus stimulating increased concentrations of IGF-1 and androgens[15,18], which can also activate the mTORC-1 receptor and SREBP, resulting in the amplification of pathways of acne pathogenesis[17] (Figure 2).

Adiponectin

Adiponectin, a hormone derived from adipocytes and produced mainly in subcutaneous fat, has anti-inflammatory, antioxidant, and anti-diabetic properties. It inhibits proinflammatory cytokines and induces anti-inflammatory cytokines, down-regulates the expression of Toll-like receptor (TLR)-2 ligands and receptors, and increases insulin sensitivity. Additionally, adiponectin inhibits mTORC-1 activity by activating 5'AMPK. GI, GL, body mass index, and obesity are inversely related to adiponectin concentrations[2,9,17] (Figure 2).

Western diet

The Western diet is characterized by high GI foods, refined grains, red meat, milk and dairy products, egg protein, and saturated fats[9,14,22].

This type of diet increases GL, insulin production, IGF-1, and leucine levels; in turn, upregulation of these factors decreases FOX O1 activity, thus losing the ability to inhibit androgen receptors and mTORC-1 activity. Moreover, these factors also stimulate basal keratinocytes to release interleukin-1 (IL-1) and other cytokeratins that result in hyperproliferation with hypercornification of the follicle wall, which is the precursor event for the formation of comedones[14,22].

The Western diet is also high in linoleic acid, a peroxisome proliferator-activated receptor-gamma (PPARγ) ligand that strongly stimulates lipogenesis in sebocytes and maturation of follicular keratinocytes[14,22].

Hypotheses on the influence of diet on acne pathogenesis are supported by observing low incidence of acne in cultures with "paleolithic diets"—composed of minimally processed foods, vegetables, low amounts of carbohydrates, and no dairy or its derivatives[18,22]. Furthermore, these diets contain high levels of omega-3 and omega-6 polyunsaturated fatty acids (PUFAs), which are essential mediators of inflammation and positively impact acne[24,25].

Regular fish intake has also been reported to reduce acne, as it contains high levels of n-3 eicosapentaenoic acid (EPA), which acts as a competitive inhibitor of the conversion of arachidonic acid (AA) into inflammatory mediators such as prostaglandin E2 (PGE2) and leukotriene B4 (LTB4), reducing acne-associated inflammation[13,26].

In contrast, saturated fatty acids in the Western diet increase TLR2/IL-1B receptor expression, promoting TH17 cell differentiation and increasing IL-17A secretion. Increased IL-1B and IL-17A can be found in all acne lesions. IL-17A contributes to keratinocyte hyperproliferation and decreases their differentiation[27,28].

Some studies have reported that low intake of vegetables and fruits can aggravate acne. In contrast, the Mediterranean diet—rich in vegetables, fruits, antioxidants, unsaturated fatty acids, and low GI foods—has a protective effect against developing this condition[13,29,30] (Figure 2).

Food and beverages

Milk and dairy products

Milk consumption increases the risk of acne and its severity. Milk is a complex fluid composed of several carbohydrates, proteins, and hormones[21,31]. In addition, dairy products contain high levels of branched-chain amino acids (BCAAs), such as leucine and palmitic acid, which increase insulin secretion[14,21]. Leucine also stimulates mTORC-1 and SREBP, increasing lipogenesis in the sebaceous glands[14,20]. Thus, elevated serum BCAA concentrations are related to oxidative stress and inflammation via mTORC-1[23,32].

Milk also contains and induces IGF-1, thus decreasing FOX O1 and increasing mTORC-1 activity[31,33]. Other studies have shown that milk consumption causes a disproportionate increase in insulin levels (despite having a low GI), producing an insulin response 3 to 6 times greater than its corresponding GI[31].

Depending on the level of fat, cow's milk is classified as whole milk (3.5%), low-fat milk (2%), and skim milk (fat-free), the latter being associated with higher plasma IGF-1 levels[13,31]. However, Ulvestad et al. demonstrated in 2016 a direct relationship between acne and high milk consumption regardless of milk fat content, which would reveal that the pro-acne effect of milk is associated more with its high content of hormones and bioactive molecules than with fat content[33,34].

Milk contains whey protein, which is highly acnegenic[22]. Extracts of this protein contain six growth factors: tumor growth factor (TGF), IGF-1 and IGF-2, platelet-derived growth factor (PDGF), fibroblast growth factor-1, and FGF-2, all of which are potent inducers of glucose-dependent insulinotropic polypeptides that stimulate insulin secretion in pancreatic β cells[13]. Natural milk contains 1% whey protein, compared to 2% in processed milk. In addition, reduced-fat milk often has added whey protein to balance the caloric content[22]. Whey protein is also a dietary supplement popular among athletes and can cause moderate to severe acne[14,20].

Also, milk comes from 75-90% of pregnant cows, which confers high progesterone, androstenedione, dehydroepiandrosterone (DHEA), and dihydrotestosterone (DHT) content. These hormones increase the expression of androgen receptors and, consequently, the activation of mTORC-1[13,21].

Finally, recent studies have shown that Western diet and milk intake reduce the activity of the transcription factor p53, implicated in the pathogenesis of acne and prostate cancer. In addition, epidemiological findings highlight a correlation between the onset of acne in late adolescence and an increased risk of prostate cancer[13] (Figure 2).

Alcohol

A significantly higher frequency of alcohol consumption has been reported in patients with acne. Alcohol has also been shown to increase testosterone levels and the production of proinflammatory cytokines. In addition, it suppresses the immune system in the long term, allowing bacterial proliferation with alteration of the skin microbiome and exacerbation of acne; when excreted in sweat, it acts as a nutrient for *C. acnes*[13,35].

Tea, coffee, and chocolate

To date, there is no consistent data to suggest that tea, coffee, or chocolate can induce or aggravate acne. The factor that triggers acne is the sugar added to

these beverages. Contrary to popular belief, studies show that the polyphenols in green tea have antimicrobial activities and can reduce sebum secretion, thus benefiting acne[13,36].

Vongraviopap and Asawnonda, and other authors have reported that 99% of dark chocolate exacerbates acne due to its content of saturated fatty acids, sugar, and milk[13,21,37].

Supplements

Vitamins A, D, and B12

Oral supplementation with vitamin A and D (1,25D3) has been related to their immunomodulatory capacity since the consumption of these vitamins inhibits the differentiation of Th17 cells, preventing the production of IL-17A. In addition, vitamin D can inhibit mTORC-1 activation and increase the production of cathelicidins against *C. acnes*. Findings have shown vitamin D deficiency in up to 48% of acne patients, demonstrating an inverse relationship between vitamin D concentrations and disease severity[21].

Regarding vitamin B12 (hydroxocobalamin), Kang et al. conducted a longitudinal study in 2017. They found that the biosynthesis pathway of this vitamin was negatively regulated in acne patients and healthy subjects receiving vitamin B12 supplementation. In healthy skin, when the levels of vitamin B12 are normal, the biosynthesis pathway of this molecule in *C. acnes* is adequate, and porphyrin biosynthesis is low[38]. As vitamin B12 levels in the host increase, transcriptional changes are induced in *C. acnes*, which diverts the metabolic flux of 2-oxoglutarate and L-glutamate to porphyrin biosynthesis and decreases vitamin B12 biosynthesis, demonstrating an inverse relationship[37,39].

Excess porphyrins in the pilosebaceous follicle interact with molecular oxygen, generating free radicals that damage adjacent keratinocytes and stimulating the production of inflammatory mediators; thus, inducing an inflammatory response that subsequently results in the development of acne[37,38].

Karadag et al. in 2011 and Johnson et al. in 2016 reported that vitamin B12 serum levels and porphyrins in the pilosebaceous unit decrease significantly after acne treatment[40,41] (Figure 2).

Zinc

This micronutrient has demonstrated a bacteriostatic effect against *C. acnes* by inhibiting chemotaxis and decreasing the production of proinflammatory cytokines. It has been shown that acne patients have lower serum zinc levels and that zinc supplementation would reduce the inflammatory lesion count without significantly increasing the incidence of treatment-associated adverse effects[42,43].

Skin-gut axis

The *skin-gut axis* concept proposes a relationship between alterations of the gastrointestinal microbiome with increased intestinal permeability, systemic inflammation, and acne onset. The gut microbiome influences oxidative stress, glycemic control, and adipose tissue metabolism. The literature estimates that 40% of acne patients have hypochlorhydria, which can cause migration of bacteria from the colon to the small intestine, leading to alterations of the microbiome and colonic bacterial overgrowth, causing direct damage to the epithelium, systemic inflammation, and possible cutaneous manifestations[22].

Oral supplementation with probiotics (lactobacilli, bifidobacteria, and enterococci) has been associated with clinical improvement of acne, mediated by the production of antibacterial proteins and bacteriocin-like substances with immunomodulatory effect on keratinocytes, as well as by the reduction of proinflammatory cytokines and induction of CD8 cell recruitment, thus regulating the intestinal microbiome. Studies to date, although scarce, have shown that administration of *Lactobacillus rhamnosus GG* for 12 weeks significantly improved acne and that treated patients had lower IGF-1 expression and higher FOX O1 expression in skin biopsies, which has led to consider oral administration of probiotics as an adjuvant in the management of acne[21,44-46].

Lifestyle

Modern lifestyles that include passive leisure, such as watching TV, playing video games, and working for hours in front of the computer, can lead to uncontrolled food intake, especially hypercaloric, high GI, or GL foods[13]. This type of diet leads to obesity and high body mass index, which have been linked to acne[47].

Treatment

Patients should be asked about their eating habits, lifestyle, and family history of acne or eating disorders during the first medical visit to verify whether nutrition can influence acne. If deemed necessary, body mass index

should be calculated to verify if the patient is overweight or obese, which would imply an increased risk of acne[13].

Several randomized clinical trials demonstrate that low GI/GL diets can decrease acne severity[25]. Jung et al. conducted a randomized, double-blind trial in 2014, in which they showed improvement of inflammatory and non-inflammatory lesions by dietary supplementation of omega-3 or omega-6 for at least 10 weeks[48]. Omega-3 is found naturally in fish and seafood, and omega-6 is found naturally in sunflower, corn, and safflower oil[21].

If the problem were the type of diet, patients should be advised to change their eating habits or even request the support of a nutritionist[13]. A review article reported the usefulness of metformin as an adjunctive treatment for acne therapy: its use significantly reduced the number of lesions, especially inflammatory lesions, with minimal side effects (diarrhea and flatulence)[25]. The usefulness of metformin is due to its activation of the 5'AMPK pathway, which is an indirect inhibitor of mTORC-1[15]. Therefore, using metformin combined with a low-GL diet can be considered in patients who do not respond to treatment or present a rapid relapse[13,25]. The patient should also be reminded of the importance of a balanced diet: dairy products, insulinotropic cereals, fatty diets, fast food, and other foods with high GI should be reduced as much as possible. Instead, the patient should be encouraged to opt for antioxidants such as fruits, vegetables, and omega-3, change lifestyle, and increase physical activity[49].

These measures act in conjunction with the known acne treatment, depending on its severity and each patient's unique conditions: anti-androgenic agents, topical or systemic retinoids, topical or systemic antibiotics, and keratolytic agents[15].

Understanding the multifactorial etiology of acne is key to providing a comprehensive approach to the patient. It is necessary to update the training and knowledge of dermatologists continuously, as new factors related to the severity and evolution of this disease are discovered every day. By knowing the primary molecular markers altered by diet and which are somehow involved in the pathogenesis of acne, a specialized treatment with behavioral education and individual counseling for therapeutic choice can be achieved.

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.

Conflicts of interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

COVID-19 in the pediatric population of the state of Jalisco: spatiotemporal analysis of 1,515 cases

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Abstract

Background: *Initial publications of COVID-19 (2019 coronavirus disease) focused on the adult population until March 2020, when the first series in children was reported. Our objective was to analyze the spatiotemporal behavior of the pediatric population with COVID-19 in the state of Jalisco.* **Methods:** *We conducted a cross-sectional study including subjects < 18 years of age with SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infection confirmed by reverse transcription-polymerase chain reaction, registered in the RADAR platform. We investigated the prevalence, incidence rate, age, sex, outpatient or inpatient status, distribution of cases by time, municipality of residence, and geographical region. Descriptive statistics were used for data analysis.* **Results:** *Of 58,231 subjects studied, 1,515 were children (3%): 768 males (51%) and 747 females (49%). The mean age was 12 ± 5 years; outpatients predominated (94%). The Central region concentrated the largest cases with 1,257 (82%) and was the second-highest incidence rate, behind the Occidental Coastal-Mountain region. The most affected municipality was Guadalajara. The distribution of new cases increased proportionally to mobility: after the holiday weekend in May, it rose from 28 to 161 cases; after the opening of beaches and recreational sites in June and July, to 539; and after the opening of movie theaters in August, to 673 cases.* **Conclusions:** *Although with a lower incidence, the pediatric population is not exempt from SARS-CoV-2 infection. We observed an increase in cases as restrictions on social activities diminished.*

Keywords: *COVID-19. SARS-CoV-2. Mexico. Pediatric. Epidemiology. Spatiotemporal analysis.*

COVID-19 en población pediátrica del estado de Jalisco: análisis espacio-temporal de 1,515 casos

Resumen

Introducción: *Las publicaciones iniciales de COVID-19 (enfermedad por coronavirus de 2019) se enfocaron en población adulta, hasta marzo de 2020, cuando se informó la primera serie en niños. Nuestro objetivo fue analizar el comportamiento espacio-temporal de la población pediátrica con COVID-19 en el estado de Jalisco.* **Métodos:** *Se llevó a cabo un estudio*

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transversal en el que se incluyeron sujetos < 18 años con infección por SARS-CoV-2 (coronavirus tipo 2 del síndrome respiratorio agudo grave) confirmada por reacción en cadena de la polimerasa con retrotranscriptasa, registrados en la plataforma RADAR. Se investigó la prevalencia, tasa de incidencia, edad, sexo, paciente ambulatorio u hospitalizado, distribución de casos por tiempo, municipio de residencia y región geográfica. Se utilizó estadística descriptiva para el análisis de los datos. **Resultados:** *De 58,231 sujetos estudiados, se encontraron 1,515 pacientes pediátricos (3%): 768 de sexo masculino (51%) y 747 de sexo femenino (49%). La media de edad fue de 12 ± 5 años; predominaron los pacientes ambulatorios (94%). La región Centro concentró la mayor cantidad de casos con 1,257 (82%) y fue la segunda con mayor tasa de incidencia, detrás de la región Costa-Sierra Occidental. El municipio más afectado fue Guadalajara. La distribución de nuevos casos incrementó al aumentar la movilidad: después del puente vacacional de mayo subió de 28 a 161 casos; tras la apertura de playas y sitios de recreación en junio y julio, a 539 casos, y posterior a la apertura de cines en agosto, a 673 casos.* **Conclusiones:** *Aunque con una incidencia menor, la población pediátrica no está exenta de la infección por SARS-CoV-2. Se observó un incremento de los casos a medida que disminuyeron las restricciones para las actividades sociales.*

Palabras clave: *COVID-19. SARS-CoV-2. México. Pediátrico. Epidemiología. Análisis espacio-temporal.*

Introduction

The COVID-19 (coronavirus 2 disease) pandemic has established itself worldwide as the most significant public health challenge of the last 100 years. Although the first reports were made in adult populations, the novel SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) also affects the pediatric population, including newborns[1-3].

Although children with COVID-19 generally do not present with severe symptoms, and up to 90% may be asymptomatic[3-6], it is essential to investigate the behavior of the disease in this population. Based on available data, the pediatric population could play a key role in transmitting the virus, mainly due to its prolonged presence in nasal secretions and feces, which may facilitate its community dissemination in daycare centers, schools, and homes[3,7,8].

No information was found on the epidemiological characteristics of patients < 18 years of age with confirmed SARS-CoV-2 infection in Jalisco nor data on the distribution by geographic region or monthly behavior during the pandemic. Therefore, the present study aimed to analyze the spatiotemporal behavior in pediatric patients with COVID-19 in Jalisco, Mexico.

Methods

Study design, source of information, and study period

We conducted a descriptive cross-sectional study from March 1 to September 27, 2020. Data on subjects with confirmed SARS-CoV-2 infection registered in the state of Jalisco, Mexico, were obtained from the official website of the Ministry of Health, Jalisco, through the Sistema RADAR Jalisco (Jalisco RADAR System) platform, which is an active epidemiological system for COVID-19 detection implemented by the state government in conjunction with the University of Guadalajara. Incidence rates were calculated per 100,000 inhabitants according to the National Institute of Statistics and Geography (INEGI for its Spanish acronym) latest census[9].

Selection criteria

We included individuals ≤ 18 years of age, residents of Jalisco, who met the definition of a confirmed case of SARS-CoV-2 infection. A confirmed case was established as a subject of any age who 7 days prior had presented at least two of the following signs or symptoms: cough, fever, headache (in children < 5 years of age, irritability instead); and at least one of the following signs or symptoms: respiratory distress, arthralgia, muscle pain, odynophagia, rhinorrhea, conjunctivitis, chest pain. Also, a positive result in the real-time retrotranscription polymerase chain reaction (RT-PCR) test for SARS-CoV-2, performed in the National Network of Public Health Laboratories, which are recognized by the Institute of Epidemiological Diagnosis and Reference (InDRE for its Spanish acronym)[10-12].

Study variables and definitions

Health status

Patient health status was divided into ambulatory (outpatient) and hospitalized (inpatient) when the confirmed case was captured.

Spatial distribution

The distribution of cases in the 12 geographic regions that make up the state of Jalisco: North, Highlands North, Highlands South, Marshland, Southeast, South, Amula Mountains, Coastal South, Occidental Coastal-Mountain, Valleys, Lagoons, and Central, including 125 municipalities.

Temporal distribution

The information of each individual captured in the Sistema RADAR Jalisco platform was divided by month.

Statistical analysis

Data capture and processing were performed using Microsoft Office Excel® software 2013 (Microsoft Corp., Redmond, WA) IBM®SPSS Statistics for Windows v22 (IBM Corp., Armonk, NY). The Kolmogorov-Smirnov test was used to determine the type of distribution of the groups analyzed. Descriptive statistics were used to determine averages and percentages according to every statistical variable. The χ^2 test was used to compare qualitative variables, and the Student's t-test was used for the quantitative variables. A value of $p < 0.05$ was considered statistically significant.

Temporary milestones and control measures

The first case of COVID-19 in Mexico was detected on February 27, 2020, in Mexico City. On February 28, two additional cases were confirmed, resulting in the declaration of COVID Phase 1; that is, the scenario in which the cases were imported from abroad, and there was no local contagion or spread. On March 11, the World Health Organization declared the COVID-19 pandemic. On March 14, 2020, the Ministry of Public Education (Secretaría de Educación, Mexico) changed the Easter vacations and extended them to one month (from March 23 to April 20) for all educational institutions nationwide[13].

Following the first local infections, the federal government declared Phase 2 on March 24. Some economic activities were suspended; large gatherings were restricted, and it was recommended to stay at home, especially for individuals > 60 years. As of March 26, all non-essential federal government activities were suspended, except those related to security, health, energy, and sanitation. Sneezing into the elbow was recommended, along with frequent hand washing and continuous cleaning and disinfecting of high-use public areas. Persons with symptoms and confirmation of COVID-19 should wear facial masks[13].

Due to the evolution of confirmed cases and deaths, a national public health emergency was declared on March 30. The immediate suspension of all non-essential activities in all economic sectors of the country was proclaimed for one month (until April 30). Subsequently, due to evidence of active outbreaks and spread of the virus with more than 1000 cases, phase 3 was initiated on April 21, 2020, which brought with it measures such as the suspension of non-essential activities in the public, private, and social sectors, and the extension of the National Safe Healthy Distance Guidelines until May 30[13,14].

Ethical aspects

The local Research and Ethics Committee approved the present study (Instituto Mexicano del Seguro Social registration number CLIS R-2020-1302-031). The study was conducted based on the Ethical Standards and Guidelines for Research Involving Human Beings established by the Declaration of Helsinki (Fortaleza, Brazil, 2013). Following good clinical practice, the confidentiality of the participants was protected: at no time were they identified by name, and they were assigned a code to protect their anonymity.

Results

From March 1 to September 27, 2020, 58,231 positive cases of SARS-CoV-2 infection were detected in Jalisco. Among them, the pediatric population corresponded to 1,515 patients (prevalence of 3%): the mean age was 12 ± 5 years (range 0-18 years), with a predominance of males (n = 768; 51%), and a higher prevalence of ambulatory patients (n = 1,427; 94%). When comparing the epidemiological characteristics and health status of the pediatric population with the adult population, we found a statistically significant difference in age (12 vs. 43 years, $p = 0.001$, Student *t*-test) and in the percentage of hospitalizations (6% vs. 14%, $p = 0.001$, χ^2 test).

According to age distribution in individuals ≤ 18 years, we found that SARS-CoV-2 infection predominated in adolescents aged 15 to 18 years (n = 738; 49%). The prevalence gradually decreased the younger the age of the patients, so that the least affected group was infants < 1 year of age (n = 48; 3%). In this group, we

Table 1. Epidemiological characteristics of patients with SARS-CoV-2 infection in Jalisco distributed by age (n = 58,231)

Variable	< 1 year (n = 48)	1-4 years (n = 156)	5-9 years (n = 205)	10-14 years (n = 368)	15-18 years (n = 738)	> 18 years (n = 56,716)
Sex						
Male, n (%)	27 (56)	89 (57)	112 (55)	178 (48)	362 (49)	28,923 (51)
Female, n (%)	21 (44)	67 (43)	93 (45)	190 (51)	376 (51)	27,793 (49)
Ratio M:F	1.28:1	1.32:1	1.20:1	0.93:1	0.96:1	1.04:1
Age in years						
Mean ± SD	—	2.2 ± 1.1	6.9 ± 1.4	12.4 ± 1.3	16.8 ± 1.1	43 ± 15
Health status						
Ambulatory, n (%)	28 (58.3)	140 (89.7)	188 (91.7)	349 (94.8)	722 (97.8)	48,864 (86)
Hospitalized, n (%)	20 (41.6)	16 (10.2)	17 (8.2)	19 (5.1)	16 (2.1)	7,852 (14)
Ratio A:H	1.4:1	8.75:1	11.05:1	18.36:1	45.12:1	6.22:1

A, ambulatory; F, female; H, hospitalized; M, male; SD, standard deviation.

found a slight predominance of the males with a male-female ratio of 1.28:1. This ratio decreased in the older age groups since both sexes were affected almost equally in the 15 to 18 years group, with a male-female ratio of 0.96:1. The percentage of ambulatory to hospitalized patients was lower in the < 1 year of age group than the 15-18 years of age group (1.4:1 vs. 45.12:1) (Table 1).

The spatial distribution of pediatric patients with SARS-CoV-2 (n = 1,434; 95%) was concentrated in five geographic regions: Central (n = 1,257; 83%), Occidental Coastal-Mountain (n = 58; 4%), Highlands South (n = 50; 3%), Highlands North (n = 37; 2.4%), and Marshland (n = 32; 2.1%); the Coastal South region recorded no cases. This spatial distribution by geographic area was similar to that observed in the > 18 years age group, where four of the five regions had a higher number of cases (n = 51,350; 91%) (Table 2). When evaluating the location of the five geographic regions with the most pediatric patients, we found that they were predominately located in the northern part of Jalisco. The Northern region, which is not among the areas with the highest frequency of cases, ranked third in incidence rate, with 148 cases per 100,000 inhabitants, and is located in the northern part of Jalisco (Figure 1).

The greatest number of cases was concentrated in the central region, both in the pediatric (n = 1,257; 83%) and adult (n = 46,078; 81%) populations. This region had the second-highest incidence rate in the entire state: 236 cases per 100,000 inhabitants in the pediatric population and 8,647 cases per 100,000 inhabitants in the adult population; it ranked only behind the Occidental Coastal-Mountain region where incidence rates were 942 cases per 100,000 inhabitants and 49,502 cases per 100,000 inhabitants, respectively (Table 2). Of the 12 municipalities that comprise the Central region, the five with the highest numbers of individuals infected with SARS-CoV-2 were Guadalajara, Zapopan, Tlaquepaque, Tlajomulco de Zúñiga, and Tonalá, where we detected 1,224 (81%) patients ≤ 18 years of age and 44,774 (79%) > 18 years (Table 3). Other municipalities with a high number of pediatric cases were Puerto Vallarta, with 53 cases (3%) and 18 cases per 100,000 inhabitants; Tepatitlán, with 30 cases (2%) and 20 cases per 100,000 inhabitants; and San Juan de los Lagos, with 18 cases (1%) and 25 cases per 100,000 inhabitants.

When addressing the temporal distribution of pediatric patients with COVID-19 in these five municipalities, we observed an increase in the number of cases starting in the second two-month period of the study since 17 cases were detected between March-April. In contrast, the number increased to 311 in May-June. Finally, in the last quarter of the study period, 896 cases were identified; the highest number of cases occurred in August (353 patients) (Table 4).

When evaluating the temporal distribution of pediatric cases according to the health measures adopted by the state government, we observed that social isolation and the suspension of in-person classes decreased the number of cases from seven to two and that the closure of non-essential commercial centers and the mandatory use of facial masks allowed a slight increase of only 19 patients in one month. In contrast, with the decrease in the strictness of these measures due to festivities or holidays, the number of cases increased exponentially: in May, after the Labor Day long weekend, the number of patients increased to 161 (eight times more than those recorded the previous month); in June, the opening of beaches, hot

Table 2. Spatial distribution by geographical region of patients with SARS-CoV-2 infection in Jalisco (n = 58,231)

Number	Region	≤ 18 years (n = 1,515)		> 18 years (n = 56,716)	
		n (%)	Incidence rate*	n (%)	Incidence rate*
1	North	12 (1)	148	157 (0.2)	1,930
2	Highlands North	37 (2.4)	89	958 (2)	2,293
3	Highlands South	50 (3)	122	915 (2)	2,224
4	Marshland	32 (2.1)	94	1,265 (2)	3,731
5	Southeast	9 (0.5)	77	288 (0.5)	2,469
6	South	30 (2)	81	1,753 (3)	4,715
7	Amula Mountains	9 (0.5)	90	498 (0.8)	5,003
8	Coastal South	0 (0)	0	317 (0.5)	1,775
9	Occidental Coastal-Mountain	58 (4)	942	3,049 (5)	49,502
10	Valleys	13 (1)	34	909 (2)	2,365
11	Lagoons	8 (0.5)	14	529 (1)	955
12	Central	1,257 (83)	236	46,078 (81)	8,647

*Incidence rate per 100,000 inhabitants.
In the < 18 years age group, four of the five geographic regions with the highest number of cases corresponded to those with the highest incidence rates: Occidental Coastal-Mountain, Central, Highlands South, and Marshland. Similarly, in the > 18 years age group, four regions with the highest frequency of cases also had the highest incidence rates: Occidental Coastal-Mountain, Central, Marshland, and South.

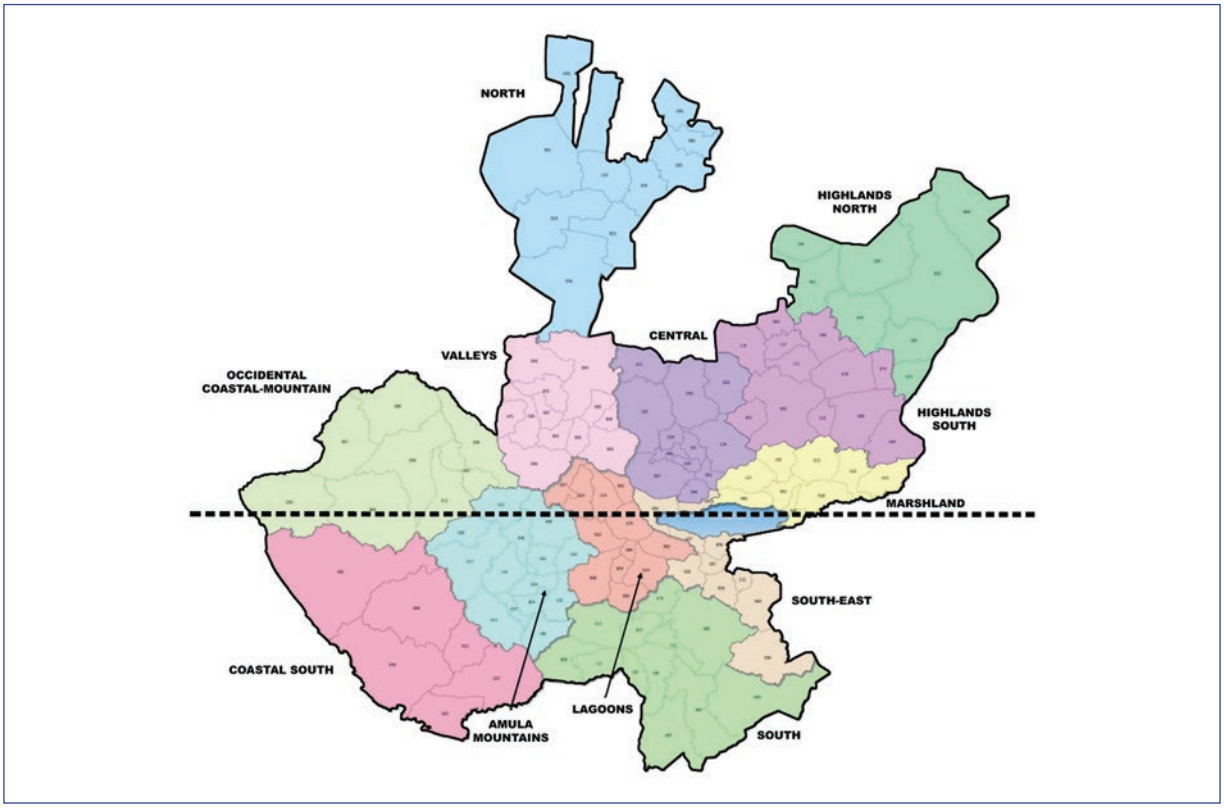

Figure 1. The Central, Occidental Coastal-Mountain, Highlands North, and Marshland regions (top half of the map) are among the top five locations with the highest cases in both age groups.

Table 3. Five municipalities with highest numbers of cases with SARS-CoV-2 infection in Jalisco distributed by age groups (n = 58,231)

Region	≤ 18 years (n = 1,515)		> 18 years (n = 56,716)	
	n (%)	Incidence rate*	n (%)	Incidence rate*
Guadalajara	486 (32)	35	20,851 (37)	1,505
Zapopan	453 (30)	31	13,225 (23)	896
Tlaquepaque	112 (7)	16	4,300 (7.5)	626
Tlajomulco de Zúñiga	100 (7)	14	3,267 (6)	449
Tonalá	73 (5)	13	3,131 (5.5)	549

*Incidence rate per 100,000 inhabitants.

Table 4. Temporal behavior of the numbers of cases ≤ 18 years of age with SARS-CoV-2 infection in the five most affected municipalities in Jalisco (n = 1,224)

Municipality	March	April	May	June	July	August	September	Total
Guadalajara	3	8	44	100	113	133	85	486
Zapopan	0	1	26	65	114	149	98	453
Tlaquepaque	1	1	16	14	40	24	16	112
Tlajomulco	0	0	12	17	31	29	11	100
Tonalá	1	2	9	8	18	18	17	73
Total	5	12	107	204	316	353	227	1,224

springs, water parks, and zoos was associated with an increase of 539 cases in the 50 days (Table 5).

Discussion

Research related to the epidemiological behavior of SARS-CoV-2, especially in pediatric age groups, is scarce. In our study, the prevalence of SARS-CoV-2 infection in children and adolescents was 3%, similar to the 3.9% reported by the Dirección de Información Epidemiológica in subjects under 20 years of age nationwide[15], but higher than that reported in China—with 2% of positive cases in individuals under 19 years of age—and Italy with 1.2%[3,4]. However, these rates could be higher, as asymptomatic infections are known to occur in the pediatric population. In China, for example, up to 13% of virologically confirmed cases are considered to have an asymptomatic infection; that is, many children without symptoms are not evaluated[4].

Regarding age at presentation, we found a mean age of 12 years. In contrast, Dong et al. found a mean age of 7 years[4]. Other countries also report lower mean ages than the pediatric population of Jalisco: 6 years in India, 7 years in Morocco, and 8 years in the United States[16-18]. While in these other countries, school-age children predominate, we found in our population that almost half of the subjects were adolescents (15-18 years of age) with a prevalence of 49%. This difference could be explained because we included ambulatory and hospitalized patients, whereas the approach in other countries considered only patients requiring hospitalization. In our study, 94% of the pediatric population was ambulatory.

Furthermore, we found a slight predominance of males (51% of cases), a lower prevalence than that reported in the pediatric population in China (56.6% males) and Mexico City (59% males). However, in both studies, the difference regarding sex was not significant[4,19].

Regarding the health status of the subjects included in the present study, we found 16.2 ambulatory patients for every hospitalized one patient in the group ≤ 18 years of age, whereas this ratio diminished to 6.2 ambulatory patients for every hospitalized one patient in the group >18 years, which was a statistically

Table 5. Patients with SARS-CoV-2 infection distributed according to measures adopted by the government of the state of Jalisco (n = 58,231)

Period	Actions	≤ 18 years (n = 1,515)	> 18 years (n = 56,716)
01/03 to 13/03	Day zero Before the onset of measures	7	217
14/03 to 01/04	March 13 Social isolation	2	121
	March 17 Suspension of in-person classes		
2/04 to 20/04	April 01 Closure of non-essential commercial centers	9	390
21/04 to 01/05	April 20 Obligatory use of facial masks	10	359
2/05 to 31/05	May 03 Preventive measures are decreased (long-weekend?)	161*	4,011**
1/06 to 11/06	June 01 Opening of non-essential businesses at 50% capacity	87	4,066
12/06 to 15/06	June 11 Declaration of Individual Responsibility	27	1,439
16/06 to 20/07	June 15 Opening of beaches	353***	15,441***
21/07 to 04/08	July 20 Opening of hot springs, water parks, zoos	186***	7,683***
05/08 to 27/09	August 06 Open cinemas and casinos	673	22,989

*In individuals ≤ 18 years, there were 55 cases from May 2-14 and 106 from May 15-31 (14 days after the May long weekend).
**In individuals > 18 years, there were 915 cases from May 2-14 and 3,096 from May 15-31.
***The opening of beaches, hot springs, water parks, and zoos brought about 539 new cases in individuals ≤ 18 years and 23,124 new cases in those > 18 years of age.

significant difference. This finding supports what has already been observed: the pediatric population presents less severe clinical conditions that allow ambulatory treatment[3], while the prognosis is worse in the adult population, partly due to comorbidities that make them more susceptible to developing complications and being hospitalized[20].

The ratio of pediatric ambulatory patients to hospitalized patients varied among age groups. We found that most hospitalizations occurred in the < 1-year-old group, where for every 1.4 ambulatory individuals, one was hospitalized (almost a 1:1 ratio). Conversely, this ratio increased proportionally with age, so that in the 15-18 years-old group, there were 45.1 ambulatory patients for every hospitalized one.

Regarding spatial distribution, it was interesting to note that four of the five geographic regions with the highest number of cases (Central, Occidental Coastal-Mountain, Highlands North, and Marshland) are located in northern Jalisco. We do not know whether environmental conditions in these areas, such as air pollution, environmental degradation, climate, or temperature factors, may facilitate infection or modify virus survival, as mentioned in a previous publication[21]. In any case, this finding could be the basis for future research.

When analyzing the number of individuals with COVID-19 by geographic region, we found that most of the confirmed cases were concentrated in the Central area: 83% of patients in the pediatric population and 81% in the adult population. This is an expected finding if we consider that the Central region includes the municipalities of Guadalajara, Zapopan, Tlaquepaque, Tlajomulco de Zúñiga, and Tonalá where 81% of our pediatric patients were concentrated. These high percentages could be attributed to the higher population density than other municipalities in the state. According to the latest INEGI[9] census, Guadalajara has 10,361

inhabitants/km^2, while the mean population density for the state is 100 inhabitants/km^2. Therefore, the higher the population density, the greater transmissibility of the virus.

The Occidental Coastal-Mountain (4%) and Highlands South (3%) regions followed the Central area in positive cases in the pediatric population. Although they do not have the population density of the Guadalajara metropolitan area, these regions include the municipalities of Puerto Vallarta, Tepatitlán de Morelos, and San Juan de los Lagos. These municipalities have a high urban concentration during part of the year due to recreational, commercial, and religious tourism[22].

As expected, the number of cases increased exponentially at the onset of the community spread phase regarding the temporal distribution of cases. However, we should emphasize that the infection peaks were related to reducing sanitation measures to prevent it[23]. For example, between May 2 and May 14, 55 cases were confirmed in ≤ 18 years, and 915 in > 18 years; however, between May 15 and May 31, 14 days after the Labor Day holiday (May 1), rates rose to 106 cases (almost double) in < 18 years, while they tripled in those >18 years of age with 3,096 patients.

Similarly, on June 1, non-essential commercial activities returned to 50% of their standard capacity in the state of Jalisco[23]. Unfortunately, this opening resulted in the reporting of 5,619 new cases in the first 15 days of the month alone, when the total number of positive cases reported in March, April, and May were 5,287. Although this increase was mainly at the expense of the adult population, pediatric patients were not exempt since, in those 15 days, 114 new cases were registered; that is, 60.3% more than previously recorded in this population.

Finally, in July, following the reopening of recreational aereas[23] such as beaches, hot springs, water parks, and zoos, 539 cases were recorded in <18-year-old and 23,124 in those >18 years; while in August, with the opening of cinema theatres and casinos[23], the highest rates observed in our study were registered, with 673 new cases in the pediatric population and 22,989 in adults.

Due to the nature of the database contained in the Sistema RADAR Jalisco platform, this study has several limitations: the clinical presentation of the disease is unknown; the patients included belong to an anonymous database, so neither their treatments nor their results were available; there is no follow-up of the individuals included, so the clinical evolution, vital status, prognosis, and variables that may affect them are unknown; finally, the information is from a single state, so the results cannot be generalized to the rest of the country. Despite these limitations, our series is more extensive than that reported for the state of Sinaloa (51 patients)[24] and for Mexico City (510 patients)[19], which allows us to contribute essential data of interest that have not been published previously, and that can guide us on the epidemiological and spatiotemporal behavior of pediatric patients with COVID-19 in the state of Jalisco.

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 has this document.

Conflicts of interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

Pediatric hospitalization due to COVID-19: experience in a regional hospital

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Abstract

Background: *SARS-CoV-2 (severe acute respiratory syndrome coronavirus 2) infection is usually mild in children, although it can become severe in some cases. Initially, doubts arose due to vertical perinatal transmission in infected mothers. Therefore, the first recommendations were very restrictive, suggesting mother-newborn separation. This study aimed to describe the clinical behavior of newborns born to mothers with SARS-CoV-2 infection and of children admitted to hospital due to COVID-19 (coronavirus-2 disease).* **Methods:** *We conducted a retrospective descriptive study of pediatric patients hospitalized between May 1, 2020, and April 30, 2021.* **Results:** *We included 19 patients: 47.4% were neonates born to mothers infected with SARS-CoV-2 (1.63% of deliveries), and 52.6% were pediatric patients aged 2 months to 12 years with confirmed COVID-19 infection (3.43% of all pediatric admissions). All patients presented mild symptomatology and remained isolated with a family member in the room. Vertical transmission was not found, although a positivity rate of 88.89% was detected in fathers.* **Conclusions:** *Pediatric admissions for COVID-19 did not represent an overload of care. No patient developed complications or required specific treatment. The incidence of COVID-19 deliveries was low, and vertical perinatal transmission was not observed. Admission with a companion facilitated pediatric care, which was favorable for the patient and the healthcare staff.*

Keywords: *COVID-19. Coronavirus. Emerging epidemic diseases. SARS-CoV-2. Pediatrics. Hospitalization.*

Ingresos por COVID-19 en pediatría: experiencia en un hospital comarcal

Resumen

Introducción: *La infección por SARS-CoV-2 (coronavirus tipo 2 del síndrome respiratorio agudo grave) es habitualmente leve en niños, aunque llega a evolucionar de forma grave en algunos casos. Inicialmente surgieron dudas por la transmisión perinatal vertical en madres infectadas, por lo que las primeras recomendaciones fueron muy restrictivas, ya que sugerían la separación madre-hijo. El objetivo de este estudio fue describir el comportamiento clínico de los recién nacidos de madres con infección por SARS-CoV-2 y de los niños ingresados al hospital por COVID-19 (enfermedad por coronavirus 2).* **Métodos:** *Se llevó a cabo un estudio descriptivo retrospectivo de pacientes pediátricos hospitalizados entre el 1 de mayo de 2020 y el 30 de abril de 2021.* **Resultados:** *Se incluyeron 19 pacientes: el 47.4% eran neonatos hijos de madres infectadas con SARS-CoV-2 (1.63% de los partos) y el 52.6%, pacientes pediátricos de entre 2 meses y 12 años de edad con COVID-19 (3.43% de los ingresos pediátricos). Todos los pacientes presentaron sintomatología leve y permanecieron aislados en la habitación con un familiar. No se constató la transmisión vertical, aunque se detectó una tasa de positividad en el*

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padre del 88.89%. **Conclusiones:** *Los ingresos pediátricos por COVID-19 no supusieron sobrecarga asistencial. Ningún paciente desarrolló complicaciones ni precisó tratamiento específico. La incidencia de partos COVID-19 fue baja y no se constató la transmisión vertical. El ingreso con un acompañante facilitó los cuidados pediátricos, lo que resultó favorable para el paciente y para el personal del servicio.*

Palabras clave: *COVID-19. Coronavirus. Enfermedades epidémicas emergentes. SARS-CoV-2. Pediatría. Hospitalización.*

Introduction

Coronaviruses (CoVs) were not considered highly pathogenic agents for humans before the 2003 outbreak of severe acute respiratory syndrome. Subsequently, this epidemic highlighted the virulence of this group of viruses and included them among the causative agents of emerging epidemic diseases[1].

Later, in 2012, a new CoV responsible for Middle East respiratory syndrome was identified. Both infections were considered a threat to global health security. We are currently experiencing the third epidemic caused by a new CoV, called SARS-CoV-2 (severe acute respiratory syndrome coronavirus type 2), which emerged in Wuhan (China) and has triggered an unprecedented pandemic.

There are already countless publications and studies related to the clinical features, diagnosis, treatment, prognosis, and evolution of SARS-CoV-2 infection (COVID-19). However, most of them focus on adult patients, whereas the literature on disease in the pediatric population is scarce and heterogeneous.

Progressive advances in the knowledge of COVID-19 have resulted in constant changes in all types of recommendations over the last year, which has made it difficult to unify and standardize clinical practice.

Pediatric patients have been a population group with markedly different behavior towards COVID-19[2]; within the pediatric population, neonates have constituted a particularly protected subgroup[3].

At the beginning of the pandemic, many doubts arose about the risk of perinatal transmission from mother to newborn among mothers infected with SARS-CoV-2. The first recommendations on the management of pregnant women and newborns issued by the Ministry of Health were very restrictive, recommending separation of the newborn from the mother at the time of birth due to the potential risk of transmission. After the initial uncertainty, most scientific societies recommended that breastfeeding continue and be maintained along with respiratory isolation measures[4,5]. Based on these recommendations, the protocol of our hospital contemplates these aspects to preserve the humanization of the birth process and breastfeeding[6,7].

Published data suggest that the clinical course of infection is usually mild in pediatric patients. However, a small percentage may progress to a severe disease requiring admission to Intensive Care Units (ICU)[8].

The objectives of the present study were to describe the clinical behavior of newborns born to mothers with SARS-CoV-2 infection and infants admitted to the hospital for COVID-19.

Methods

We conducted a retrospective descriptive study of patients with a confirmed diagnosis of active SARS-CoV-2 infection by PCR (polymerase chain reaction positive for SARS-CoV-2 on admission) hospitalized in the pediatric ward of the Hospital de Barbastro from May 1, 2020, to April 30, 2021. Patients admitted to the pediatric ward were those aged 0-13 years. Criteria for hospitalization were as follows: age < 3 months, children with risk factors (immunocompromised, with heart disease, or chronic respiratory pathology), hypoxemia (SaO2 < 92%), or moderate/severe respiratory distress (score > 4 on the Wood-Downes scale), poor general condition, refusal of food, apneas, suspected PMIS-TS (pediatric multisystem inflammatory syndrome linked to SARS-CoV-2). The social motive was also included: family admission in the same room was agreed upon for three infected patients whose parents also required hospitalization due to symptoms.

Various data were collected, such as the reason for and duration of admission (in days), associated symptomatology, severity, treatment administered, need for mechanical ventilation or transfer to the pediatric ICU, and the age and sex of the patients. All patients remained in the room with one of their parents, maintaining strict isolation during the entire admission. A contact study was performed in all cases, and the family member accompanying the patient was also positive.

In the case of newborns, the inclusion criteria were mothers with positive PCR for SARS-CoV-2 at the time of delivery at the Hospital de Barbastro from May 1, 2020, to April 30, 2021. At our center, PCR for SARS-CoV-2 is performed on all women admitted to the hospital to give

birth, regardless of whether or not they have symptoms suspicious for COVID-19. We also perform a PCR test for the father in case of a positive maternal PCR result.

Data were collected on maternal and neonatal relatedness, symptoms, gestational age, type of delivery, birth weight, performance, and results of two PCRs for SARS-CoV-2 in the newborn in the first 48-72 hours of life, and feeding at discharge. All newborns born to mothers with SARS-CoV-2 infection underwent a PCR at birth and another before discharge, with a minimum interval of 48 hours from the first test in our hospital. Families were also oriented on caring for the newborn to minimize the risk of infection. They were advised to wash their hands frequently, especially before touching the newborn, and wear a mask, especially when less than 2 m away from the newborn.

A contact study was initiated in all admitted patients, and at least one of their parents was allowed to accompany them.

The Hospital de Barbastro is an IHAN (Initiative for the Humanization of Birth and Breastfeeding Assistance) or BFH (Baby-Friendly Hospital) hospital, a maternal and infant health reference.

Results

Between May 1, 2020, and April 30, 2021, a total of 19 pediatric patients who met the inclusion criteria were admitted to the pediatric ward of the Hospital de Barbastro. Of these, 47.4% were newborns born to mothers with SARS-CoV-2 infection; the remaining 52.6% were pediatric patients aged 2 months to 12 years with positive PCR for SARS-CoV-2.

Newborns born to mothers with SARS-CoV-2 infection

During the study period, there were a total of 549 deliveries, of which nine were to mothers with SARS-CoV-2 infection (1.63% of deliveries). Although 66% (6/9) were asymptomatic, they were positive by PCR on admission, and the remaining 33% (3/9) had symptomatology of COVID-19 infection. One of the mothers had a mild fever, and two had a high fever and respiratory symptoms. Regarding the type of birth, 77.77% were vaginal deliveries, and 22.22% were cesarean sections, which corresponded to the two mothers with severe symptoms due to their clinical situation.

There was no difference in the distribution by sex: 55.55% of the newborns were male, and 44.44% were female.

All newborns were hospitalized together and kept isolated in the family room with the mother and father during the entire hospitalization. The PCR positivity rate of the fathers who remained in the room with the mother and newborn was 88.89%.

Before hospital discharge, all newborns underwent two PCRs for SARS-CoV-2: one at birth and one at 48-72 hours of life. All PCRs performed on the newborns were negative. None of the newborns presented complications, and all were discharged at 48-72 hours of life. Of the newborns, 88.89% were exclusively breastfed at discharge, and one newborn was artificially breastfed (born by cesarean section at 36 weeks of gestation to a mother with symptoms) (Table 1).

Children admitted for COVID-19

Regarding pediatric patients admitted with positive PCR for SARS-CoV-2 (excluding the neonatal period), no differences in sex distribution were observed; ages ranged from 2 months to 12 years, with a mean age of 4.05 years. It should be noted that the first pediatric patient with this diagnosis was admitted in July 2020. In the Pediatrics service, there were a total of 262 admissions during the study period, so patients with COVID-19 accounted for 3.43% of pediatric admissions. During this period, a total of 802 patients with a diagnosis of COVID-19 were admitted to the Hospital de Barbastro. Pediatric patients represented 1.12% of the total number of admissions for COVID-19.

The duration of pediatric admissions for COVID-19 ranged from 1 to 5 days, with a mean stay of 2.88 days.

The accompaniment of these patients did not imply an overload of work for the nursing staff or an increase in the risk of contagion, but rather the opposite, since family members collaborated in the care of the patient, minimizing the need for the staff to enter the room (i.e., they took the patient's temperature and transmitted it by telephone, cleaning, and hygiene, among other activities).

All patients had mild symptoms associated with SARS-CoV-2 infection, and none required referral to another center. None of the patients received specific treatment for SARS-CoV-2. Patients receiving therapy during admission were treated for symptoms (antipyretics, analgesics, antiemetics, and intravenous glucose perfusion) or related to the intercurrent process (intravenous antibiotics in infants with febrile urinary tract infection). All patients evolved favorably without developing complications of SARS-CoV-2 disease and did not require respiratory support or transfer to the PICU.

Table 1. Characteristics of newborns and their mothers

	Sex	Gestational age (w+d)	Weight at birth	PCR at birth	PCR > 48 hours	NB clinical picture	Mother clinical picture	Type of delivery	Type of feeding
NB 1	F	38+3	2740 g	Negative	Negative	Asymptomatic	Asymptomatic	Normal delivery	Breastfeeding
NB 2	M	36+1	2580 g	Negative	Negative	Asymptomatic	COVID-19 pneumonia	Cesarean section	Breastfeeding
NB 3	M	38+3	3695 g	Negative	Negative	Asymptomatic	Asymptomatic	Normal delivery	Breastfeeding
NB 4	F	37+2	2600 g	Negative	Negative	Asymptomatic	Asymptomatic	Normal delivery	Breastfeeding
NB 5	F	39+4	3050 g	Negative	Negative	Asymptomatic	Asymptomatic	Instrumented delivery	Breastfeeding
NB 6	F	39+6	3570 g	Negative	Negative	Asymptomatic	Asymptomatic	Instrumented delivery	Breastfeeding
NB 7	M	39+4	3300 g	Negative	Negative	Asymptomatic	Mild symptoms	Normal delivery	Breastfeeding
NB 8	F	39+2	4300 g	Negative	Negative	Asymptomatic	COVID-19 pneumonia	Cesarean section	Breastfeeding
NB 9	M	37+1	2935 g	Negative	Negative	Asymptomatic	Asymptomatic	Normal delivery	Breastfeeding

COVID-19, coronavirus disease 2019; F, female; M, male; NB, newborn; PCR, polymerase chain reaction test for SARS-CoV-2.

Furthermore, none of the patients required a chest X-ray since they showed no complications. Pneumonia was not suspected in any patient, as they remained with adequate baseline oxygen saturation (SaO2) and no signs of respiratory distress. Blood tests were not performed in all patients but only in those whose age or clinical situation required it.

The most frequent reason for admission was for observation of clinical or analytical evolution (5/10, 50%), followed by social reasons (3/10, 30%) and intolerance to oral feeding (2/10, 20%).

Three asymptomatic patients were admitted for social reasons; i.e., as their parents were also admitted, they requested joint isolation, and this measure was allowed. One 2-month-old infant was admitted for high fever but remained stable with no other complications. This patient's blood test results showed slightly elevated C-reactive protein, with normal PCT (procalcitonin) (6.58 mg/L and 0.12 μg/L, respectively). The complete blood count (CBC) showed lymphocytosis and monocytosis (lymphocytes, 6050/mm^3; monocytes, 1680/mm^3).

A 3-month-old infant was admitted for mild respiratory symptoms that progressively improved. A 4-year-old patient with hereditary spherocytosis was admitted for a hemolytic crisis secondary to SARS-CoV-2 infection. Blood tests showed C-reactive protein of 32.48 mg/L, PCT of 0.86 μg/L, and lymphopenia of 590/mm^3. Furthermore, due to the underlying disease, the patient showed significant hemolysis triggered by SARS-CoV-2 infection, with a total bilirubin of 5.93 mg/dL from indirect bilirubin, lactate dehydrogenase (LDH) of 1835 IU/L, and hemoglobin (Hb) of 9.2 g/dL, with a subsequent decrease to 7.4 g/dL, after which there was a progressive improvement. A 7-month-old infant was admitted for hypoglycemia secondary to feeding refusal caused by COVID-19. Blood tests showed blood glucose values of 47 mg/dL; CBC, C-reactive protein and PCT were within normal range. A 6-year-old female patient admitted for vomiting showed C-reactive protein, PCT, and CBC within normal values. A 6-month-old infant was admitted for intravenous antibiotic therapy for a urinary tract infection. During admission, PCR detected SARS-CoV-2 infection. Blood tests showed C-reactive protein values of 250.55 mg/L and PCT of 2.84 μg/L, an increase attributed to the urinary tract infection and not to COVID-19. The CBC showed leukocytosis (22,400/mm^3), 8890/mm^3 neutrophils, 9700/mm^3 lymphocytes, and 3670/mm^3 monocytes, most likely secondary to the urinary tract infection. Finally, a 6-year-old patient was

Table 2. Characteristics of pediatric patients admitted for SARS-CoV-2 (severe acute respiratory syndrome coronavirus type 2) infection

	Sex	Age at admission	Length of stay (days)	Reason for admission	Severity	Need for oxygen	Need for intensive care
Patient 1	F	3 months	1	Surveillance for RF	Mild	No	No
Patient 2	M	6 months	3	Surveillance for RF	Mild	No	No
Patient 3	F	6 years	1	Food intolerance via the oral route	Mild	No	No
Patient 4	F	2 months	1	Surveillance for RF	Mild	No	No
Patient 5	M	12 years	5	Social	Mild	No	No
Patient 6	M	5 years	5	Social	Mild	No	No
Patient 7	M	7 months	1	Food intolerance via the oral route	Moderate	No	No
Patient 8	F	4 years	5	Surveillance for RF	Moderate	No	No
Patient 9	F	6 years	4	Social	Mild	No	No
Patient 10	M	6 years	2	Surveillance for RF	Moderate	No	No

F, female; M, male; RF, risk factor.

admitted for fever, altered general condition, vomiting, and abdominal pain related to SARS-CoV-2. The patient also showed alterations in blood tests: pro-B-type natriuretic peptide (proBNP) values of 273 ng/L, D-dimer of 1144 ng/mL, C-reactive protein 100.52 mg/L, and PCT 0.88 ng/L. The CBC showed lymphocytosis and monocytosis (lymphocytes 6050/mm^3, monocytes 1680/mm^3), and no other findings. The patient evolved favorably and progressively with symptomatic antipyretic treatment and normalization of laboratory tests before discharge (Table 2).

Discussion

In contrast to adults, COVID-19-related admissions in pediatric patients did not represent a work overload in our unit since they only represented a small percentage of the number of admissions to the pediatric ward (1.12%). These data are similar to those published in other studies[2,8,9], in which children admitted presented mild SARS-CoV-2 infections more frequently. In these studies, the most frequent reason for admission in most cases was monitoring[8], similar to that observed in our study.

As for blood test findings, these were diverse in our patients. We did not always find elevation of acute phase parameters or alterations in the CBC. However, the small sample size was one of the main limitations of the study. The studies available to date conclude that there are no specific blood alterations in children affected by COVID-19, although lymphopenia is frequent, especially in severe forms of the disease[10]. This finding was observed in only one of our patients.

Co-admission and accompanied admissions have facilitated the care of pediatric patients, which has been favorable for both the patient and the healthcare staff.

In our sector, the incidence of deliveries of patients with SARS-CoV-2 infection has been low, and most of the mothers were asymptomatic, detected by PCR testing on admission. Two pregnant women were previously diagnosed with mild respiratory symptoms but no complications. However, those with moderate-severe symptoms (COVID-19 pneumonia) required cesarean section due to their clinical situation. All symptomatic and asymptomatic mothers were admitted with the father and the newborn in our study. All newborns remained asymptomatic, and no vertical transmission was observed in any case. These data are similar to those published in other series, in which most of the newborns had negative PCR and remained asymptomatic[2]. With the available data, we can conclude that—considering the appropriate measures—mother-to-child transmission is low[11,12].

Co-hospitalization, together with the support offered by our healthcare staff, has facilitated the successful establishment of breastfeeding with very satisfactory results, both for the healthcare personnel and for the families.

Admissions with COVID-19 during this period, contrary to what occurred in adults, did not imply an overload of work in our unit since they represented a small percentage of the total number of admissions to the pediatric ward. Furthermore, none of the patients presented complications derived from SARS-CoV2 infection or required specific treatment or transfer to the ICU.

Vertical transmission was excluded in all newborns born to mothers with SARS-CoV2 infection, regardless of maternal symptoms or type of delivery. Co-admission with a companion facilitated maternal and infant care and allowed breastfeeding to be established in almost all cases.

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 has this document.

Conflicts of interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

Description of the initial approach to patients with suspected child abuse in the emergency department of a pediatric hospital

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Abstract

Background: *Child abuse is one of the leading causes of morbidity and mortality worldwide. This study aimed to identify whether the approach to the diagnosis of child abuse was comprehensive in a tertiary care hospital.* **Methods:** *We conducted a retrospective study of patients with a final diagnosis of child abuse admitted through the emergency department.* **Results:** *A total of 73 confirmed cases were analyzed. We observed a predominance of female patients (65.8%). Physical abuse was the most common type of abuse (80.8%). Complete blood count and a nutritional status analysis were recorded in 100% of the patients and coagulation times in 43%; however, no patient had a thorough bone series study.* **Conclusions:** *According to the evidence found in medical records, gaps were detected in the approach to patients with suspected child abuse upon arrival at the emergency department. For this reason, all areas where pediatric medical care is provided should have tools that offer agility and ease of diagnosis.*

Keywords: *Child abuse. Diagnosis. Management. Approach. Mexico.*

Descripción del abordaje inicial en pacientes con sospecha de maltrato infantil en el área de urgencias de un hospital pediátrico

Resumen

Introducción: *El maltrato infantil es una de las principales causas de morbilidad y mortalidad en todo el mundo. El objetivo de este estudio fue identificar si el abordaje del diagnóstico de maltrato infantil se realizó de manera completa en un hospital de tercer nivel de atención.* **Métodos:** *Se llevó a cabo un estudio retrospectivo de pacientes con el diagnóstico final de maltrato infantil que ingresaron a través del servicio de urgencias.* **Resultados:** *Se analizaron 73 expedientes. Se observó un predominio de pacientes del sexo femenino (65.8%). El maltrato físico fue el tipo de maltrato más común (80.8%). Se registraron los datos de hemograma y estado nutricional en el 100% de los pacientes y de tiempos de coagulación en el 43%; sin embargo, no se registró en ningún paciente el dato de una serie ósea completa.* **Conclusiones:** *Según las evidencias encontradas en los expedientes clínicos, se detectaron lagunas en el abordaje de los pacientes con sospecha de maltrato infantil a su llegada al servicio de Urgencias. Por este motivo, todas las áreas donde se brinda atención médica pediátrica deberían contar con herramientas que ofrezcan agilidad y facilidad para realizar el diagnóstico.*

Palabras clave: *Maltrato infantil. Diagnóstico. Manejo. Abordaje. México.*

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Introduction

The World Health Organization (WHO) defines child abuse as the maltreatment and neglect of children under 18 years of age that includes all types of abuse—physical or psychological, sexual, and commercial or other types of exploitation—and causes or has the potential to cause harm to health, developmental problems or endangers their survival or dignity of children in the context of a relationship of responsibility, trust or power[1]. However, each country may have its definition of child abuse. Even in the United States, each state has its way of classifying child abuse[2].

Since a child or adolescent can be abused in different ways, the diagnosis of this condition is complex and challenging. Children may present with injuries that are the reason for the medical visit, or during the physical examination, the physician may detect damage that indicates suspicion of child abuse[3]. Being legal matters, physicians are responsible for studying the case and performing a complete diagnosis of abuse for notification to the prosecutor[4], which is sometimes a difficult criterion to meet due to the uncertainty and difficulty of diagnosis, coupled with lack of training to perform the diagnosis and treatment, no time to complete the necessary studies, and the desire of medical staff not to be involved in a case of child maltreatment or medicolegal case[5]. However, health care professionals should be alert to physical injuries that are unexplained or inconsistent with the parents' or other caregivers' explanation or the child's developmental status[6].

Child maltreatment is a leading cause of morbidity and mortality worldwide that knows no cultural, social, ideological, or geographical boundaries. No country or community is immune, as this situation occurs in both developed and developing countries.

Each year, Child Protective Service agencies investigate more than 2 million reports of suspected child abuse in the United States[2,7]. The Pan American Health Organization (2017) ranks Mexico ninth in Latin America and the Caribbean in physical abuse. According to UNICEF 2010, Mexico ranks first in physical violence, sexual abuse, and homicides of children under 14 inflicted by their parents[8,9]. SAVE THE CHILDREN reports that seven out of every 10 children are victims of some type of violence in Mexico, and it is estimated that three children die every day due to violence[10].

In 2015, at least six in 10 children aged 1-14 years experienced some violent method of discipline, and one in two children < 18 years experienced psychological aggression[11].

Worldwide, an estimated 40,150 homicide deaths of children < 18 years of age occur each year, some of which are likely due to child maltreatment. However, this figure almost certainly underestimates the true magnitude of the problem, as a significant proportion of deaths due to child maltreatment are incorrectly attributed to falls, burns, drowning, and other causes[12].

Based on the United Nations Convention on the Rights of the Child and other human rights standards, the following general principles need to be observed when caring for children and adolescents who may have been exposed to maltreatment, including emotional, physical, and sexual abuse and neglect[13].

- Attention to the best interests of children and adolescents by promoting and protecting the safety, providing sensitive care, and protecting and promoting privacy and confidentiality.
- Address the evolving capacities of children and adolescents by providing age-appropriate information, seeking informed consent and assent, as appropriate, respecting their autonomy and wishes, and offering choices in the course of their medical care.
- Promote and protect nondiscrimination in care provision, regardless of sex, race, ethnicity, religion, sexual orientation, gender identity, ability, disability, and socioeconomic status.
- Ensure the participation of children and adolescents in decisions that have implications for their lives by soliciting and considering their opinions and involving them in the design and delivery of care.
- Demonstrate respect for caregivers to support their engagement in caregiving—when safe and appropriate—including interventions that promote nurturing and responsive caregiving.

Physical abuse consists of hitting, shaking, poisoning, burning, scalding, choking, suffocating, or other physical harm to the child. Physical damage can be caused when a parent or caregiver deliberately fabricates symptoms by inducing illness in the child[14].

Healthcare professionals should consider exposure to child maltreatment when assessing children with conditions that may be caused or complicated by maltreatment to improve diagnosis/identification and subsequent care without putting the child at greater risk. Healthcare professionals should consider the following[13]:

- Be alert to an implausible, inadequate, or inconsistent explanation of any warning characteristics. All of these can be a sign of child maltreatment; however, none of them is sufficient evidence of child maltreatment.

- Consider child maltreatment when maltreatment is one of the possible explanations for the warning feature or is included in the differential diagnosis.
- Child maltreatment should be suspected when there is a severe level of concern about the possibility of child maltreatment.
- Exclude maltreatment when an adequate explanation for the warning features is found.

Abused children can present various injuries, from minor to life-threatening, whether parents, caregivers, or others report witnessing the act or, more importantly, the physician identifying a suspicious injury[15].

In its latest review (2015), the American Academy of Pediatrics mentioned that the patient should be stabilized before further evaluation, as in any case of a child with trauma. In some cases, the patient requires a trauma response team and pediatric specialists in surgery, emergency medicine, and intensive care, depending on the severity of the trauma. After a severe life-threatening injury has been ruled out and the patient has been treated at the primary evaluation, a thorough physical examination should be performed to look for specific personal injuries and certain injury patterns that may be suggestive of abuse. However, few personal injuries are known to be pathognomonic[16].

The guidelines for the evaluation of suspected child physical abuse propose several steps to complete the review of these cases, including laboratory and image tests, some of them mandatory, such as complete blood count (CBC) and nutritional status assessment, and others specific to the patient's injury, such as head or abdominal computed tomography[17].

Studies have reported that most cases of child abuse remain undiagnosed. Only 10% of them are diagnosed in emergency departments, and 64% are not diagnosed at the first consultation. If the abuse remains undetected, the risk of recurrence increases by 50% and the possibility of death by 30%[18-24]. The different types of abuse should be prevented, and late intervention *a posteriori* should be avoided so as not to allow physical and psychological distress. Another reason for abuse prevention is the immense morbidity and significant mortality. Furthermore, prevention could reduce multiple long-term cognitive, physical, behavioral, social, and emotional consequences, such as brain damage, learning problems, aggressiveness, juvenile delinquency, criminal behavior in adulthood, depression, and difficulties in work, social, and personal life[25]. Hence the importance of early detection; to this end, we must constantly look for sentinel lesions and have standardized and validated guidelines for detecting and following abuse[5,21].

The purpose of this study was to identify whether the approach to the diagnosis of child abuse is entirely performed in a tertiary-level children's hospital in Mexico City.

Methods

We conducted a retrospective review of the clinical records of patients with a final diagnosis of child abuse (diagnosis determined by the public prosecutor's office) according to the ICD-10 classification. We considered those clinical archives of patients admitted to the emergency department of the children's hospital in Mexico City between 2000 and 2016. In some of these patients, there was a suspicion of possible child abuse upon arrival; however, some were admitted to the emergency department for other reasons (e.g., pneumonia or traumatic brain injury). The following demographic variables were analyzed: sex, age, history of previous illness, disability, the reason for emergency department admission, type of abuse (sexual, physical, emotional, neglect), type of family (nuclear or single-parent), presence of dysfunctional family (referred to by the social services team in the clinical history as a family whose interrelationships serve to detract from, rather than promote, the emotional and physical health and well-being of its members), socioeconomic status (high, low, middle, referred to by the social services team in the clinical history), malnutrition, types of injuries (skin, skeletal, abdominal trauma, chest trauma, traumatic brain injury), the identity of the aggressor, history of parental child abuse, presence of domestic violence, parental substance abuse, the person suspecting abuse, follow-up, and death. It was also recorded whether the specialists performed different studies and assessments for diagnosis, including CBC, coagulation tests, nutritional status evaluation, fundus examination, consultation to ophthalmology or other departments (if necessary), liver function tests, cranial computed tomography (CT), or fontanel ultrasonography (USG), amylase and lipase, general urinalysis, FAST (focused assessment with sonography in trauma) ultrasound, abdominal CT, surgical consultation, and complete bone series.

The IBM SPSS (20.0) statistical package was used. Variables measured on a nominal scale are presented in absolute and relative frequencies. For variables measured on a numerical scale, the mean and median were used as measures of central tendency.

Informed consent was requested from the Hospital directors to review the medical records, guaranteeing the privacy of the information. The internal committees of the institution approved the study.

Results

Data from medical and social work notes obtained from the files of patients suspected of child abuse after admission are described. The initial emergency department assessment and notification were provided to the prosecutor's office, who confirmed the diagnosis of child abuse. Seventy-three patients were found between 2000 and 2016. Maltreatment occurred in a higher percentage of females (65.8%). The most affected category by age was infants (67%), followed by school-age children (19.2%) (Table 1). The most frequent type of maltreatment was physical (80%); however, other types of abuse were also found (38.3%).

The factors frequently predisposed to child abuse in the population studied were chronic disease (34.2%) and disability (13.7%). Of the 73 patients, 46.6% were first-born children.

The frequency of other variables was as follows: dysfunctional family (73.2%), history of family violence (35.6%), low socioeconomic level (86.3%), parental alcoholism (30.6%), and history of child abuse during childhood (24.7%) (Table 1).

In most cases (49), the mother brought the child for consultation. Unfortunately, the medical records did not report in 41 patients (56%) who the aggressor was, while in 13% of the cases, the father was the perpetrator, as documented in the records.

In most patients, physicians were responsible for identifying or suspecting child maltreatment, followed by mothers in 16% of cases (Table 2).

Regarding the person who brought the child to the consultation for examination after the maltreatment, in 67% of the cases was the mother, followed by uncles and aunts (9.6%), grandparents (6.8%), both parents (4.1%), and, less frequently, the father (2.7%). Other persons who identified or suspected child abuse included the house staff (6.8%) and paramedics (2.7%).

As for a reason for consultation on admission to the emergency department, *suspected child abuse* was found in 21.9% of medical records, followed by *traumatic brain injury* (16.4%), *sexual abuse* (11%), *bone fractures* (11%), *malnutrition* (11%), *altered mental status* (9.6%), *polytrauma* (4.1%), *suspected poisoning* (2.7%), *community-acquired pneumonia* (1.4%),

Table 1. Demographic characteristics and reason for consultation

Variable	n = 73 n (%)
Sex	
Male	25 (34.2)
Female	48 (65.8)
Age group	
Neonate	2 (2.7)
Infant	49 (67.1)
Scholar	14 (19.2)
Adolescent	8 (11)
Factors that can condition abuse	
Disability	10 (13.7)
Chronic disease	25 (34.2)
Family characteristics	
Non-integrated family	42 (57.5)
Dysfunctional family role	52 (73.2)
Classification of socioeconomic level	
I-II	72 (98.6)
III	1 (1.4)
Parental drug abuse	
None	18 (25)
Alcoholism	22 (30.6)
Smoking (Tobacco)	14 (19.4)
Both	17 (23.6)
Other drugs	1 (1.4)
Family history of violence	26 (35.6)
History of parental child abuse	18 (24.7)

Table 2. Distribution of responsibilities

Variable	n = 73 n (%)
Person who brought the child for consultation	
Mother	49 (67.1)
Uncles	7 (9.6)
Grandparents	5 (6.8)
Orphanages	5 (6.8)
Both parents	3 (4.1)
Paramedics	2 (2.7)
Father	2 (2.7)
Person who suspected the abuse	
Medics	49 (67,1)
Mother	12 (16.4)
Other relatives	4 (5.5)
Psychology	4 (5.5)
Father	3 (4.2)
Teacher	1 (1.4)
Person responsible for the aggression	
Unidentified	39 (53.4)
Mother	12 (16.4)
Father	10 (13.7)
A third person	7 (9.6)
Other members of the family	3 (4.1)
Stepfather	2 (2.7)

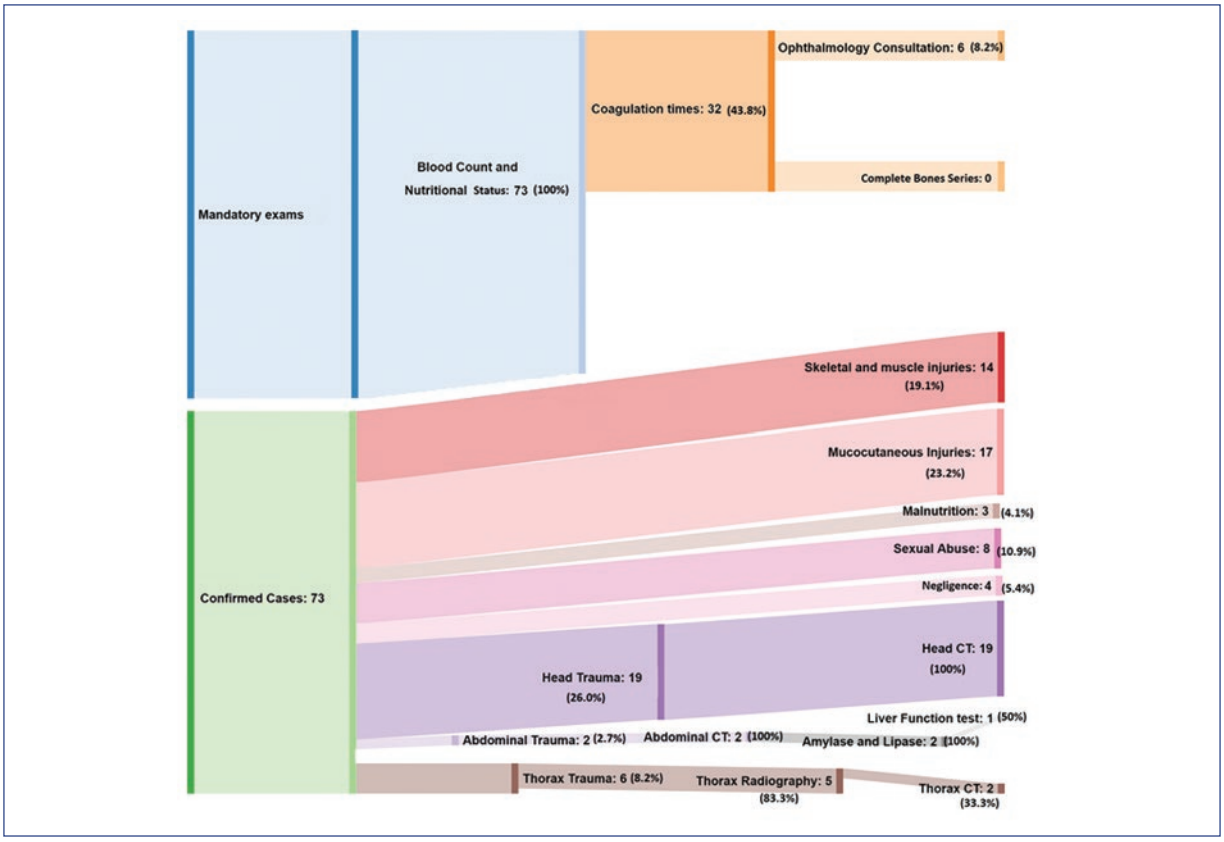

Figure 1. The mandatory exams have been incomplete regarding patient evaluation due to the lack of coagulation tests, ophthalmological consultations, and X-rays. When abdominal and cranial CT scans were required, they were performed in 100% of the cases. On the contrary, this was not the case for thoracic lesions since only one-third of CT scans were performed. CT, computed tomography.

cardiorespiratory arrest (1.4%) and *other causes* (16.4%) including sepsis, soft tissue infection, tracheal injury, urinary tract infection, foreign body ingestion, or myopathy under study.

Regarding mandatory studies in cases of child abuse, we found in medical records that 100% of the patients underwent CBC and nutritional status study, 43% underwent coagulation tests [thromboplastin time (aPTT), prothrombin time (PT)], and 8% underwent ophthalmological consultation; however, no patient underwent complete bone series (Figure 1).

According to the written medical records, the hospital followed up in 67% of the 73 cases after diagnosis. Unfortunately, in 4% of the cases, the injuries were so severe that they resulted in death.

Discussion

Our findings show that more than half of the patients were female. According to previous literature, boys experience slightly higher rates of physical abuse than girls, and, overall, adolescents are more likely to be physically abused than other children[2]. Moreover, in this study, infants were the most affected age group. International studies reveal that approximately 3 out of 4 children aged 2 to 4 years regularly suffer physical punishment or psychological violence from their parents and caregivers. Furthermore, 1 in 5 women and 1 in 13 men report being sexually abused as children[12].

Previous studies have reported that physicians miss the opportunity to perform abuse identification and intervention up to 60% of the time[2]. The medical records included in this study show that 83% of the patients had a history of prior consultation for a violent injury, which may reflect the lack of suspicion and protocol initiation in these patients in previous visits to a medical service, resulting in delayed management and treatment.

In the reform of the Ley General de los Derechos de Niñas, Niños y Adolescentes (General Law on the

Rights of Children and Adolescents)—published in the Diario Oficial de la Federación (Official Gazette of the Federation) in January 2021—Article 12 stipulates that "It is the obligation of any person who knows cases of children and adolescents who suffer or have suffered, in any way, a violation of their rights, to immediately inform the competent authorities, so that the corresponding investigation can be followed and, if necessary, the appropriate precautionary, protection and comprehensive restitution measures can be implemented in terms of the applicable provisions"[26].

This study shows that the most frequent type of child abuse was physical (80.8%), followed by sexual abuse. It is imperative to consider these data, as previous studies show that physical and sexual abuse are the two types of abuse with the most severe long-term adverse effects. For example, Mass et al. (2008) reported that physical abuse in childhood is the type of violence most strongly associated with aggressive and violent behaviors in adulthood[27]. This type of phenomenon will result in a generational transmission of violence that can be prevented.

The literature reports the presence of sentinel injuries (hematomas, oral cavity injuries, or fractures) in 25% of patients and one-third of patients with head injuries due to maltreatment[28]. In this study, half of the patients had injuries not explained by the kinematics of the trauma or condition at the time of consultation. Consistent with other studies, dermal lesions were the most frequent in our population. Hematomas, bites, burns, and injuries with specific pattern objects were the most frequent injuries. Craniocerebral trauma was present in 23% of the patients, the first cause of death after physical abuse[29]. Fractures were present in 19% of the cases at consultation.

Rib fractures are strongly associated with physical abuse. A positive predictive value for abuse of 85% has been reported from the combination of any three of the following: rib fractures, subdural hematoma, brain parenchymal injury, or retinal hemorrhages[29]. Thoracic contusions are common, although they were recorded in only six patients in this study. Although abdominal injuries are a severe form of physical abuse and usually represent the second leading cause of death[30], injuries to internal organs were infrequent in this study.

Given the wide variety of presentations of physical abuse, a systematic approach to the arrival of a patient with sentinel lesions or suspected child abuse in the emergency department is essential. Unfortunately, there are no precise algorithms in the emergency department defining the steps to follow in managing patients with suspected physical abuse, or at least there are none formally.

For example, suppose a patient arrives with polytrauma or is unstable. In that case, this patient should be treated according to the critical condition as any other patient with polytrauma, without neglecting the specific studies for suspected child abuse.

Any first contact area or emergency department should have an algorithm that includes the studies to be performed on all patients suspected of abuse: CBC, coagulation tests, complete bone series, fundus examination, nutritional status, and the mandatory initial ophthalmology and psychology evaluations. Also, the case should be reported to the maltreatment clinic if the institution has one, to the social work department, and the Public Prosecutor's Office (MP, for its Spanish acronym). It should be considered that the MP in Mexico determines the diagnosis of child abuse and, therefore, is in charge of deciding whether or not the child is returned to the custody of the family.

No written evidence was found in this study that all the studies mentioned above were performed. Only the CBC and nutritional status assessment were documented in writing in 100% of the cases.

In cases with head trauma due to maltreatment, cranial CT (mandatory study) or USG of the fontanel was reported in 19 patients, although the diagnosis of head trauma at admission was only reported in 12 patients. Unfortunately, there is only a written record of the performance of fundus examination in six patients who received ophthalmologic consultation.

There are clear indications for bone series[30], including obvious or suspicious injuries in children under two years of age, such as bruises or contusions, oral injuries, injuries not consistent with the history provided or the mechanism of injury, infants with a sudden unexplained death, infants with intracranial injuries, and in siblings under two years of age and twins of infants and toddlers who have been abused[2]. The projections included in the bone series are anteroposterior (AP) and lateral radiographs of the skull with optional Towne projection, AP and oblique of the thorax, AP of the abdomen, AP and lateral of the cervical, thoracic, and thoracolumbar spine, AP of the arms, forearms, femur, and lower extremities, posteroanterior (PA) of the hands and dorsoplantar (DP) of the feet, all of which should be repeated two weeks after the first bone series, as this will increase the identification of fractures by 25%. In the review of these records, reference was made to the performance of a chest X-ray in five patients; however, no written evidence was found of the complete

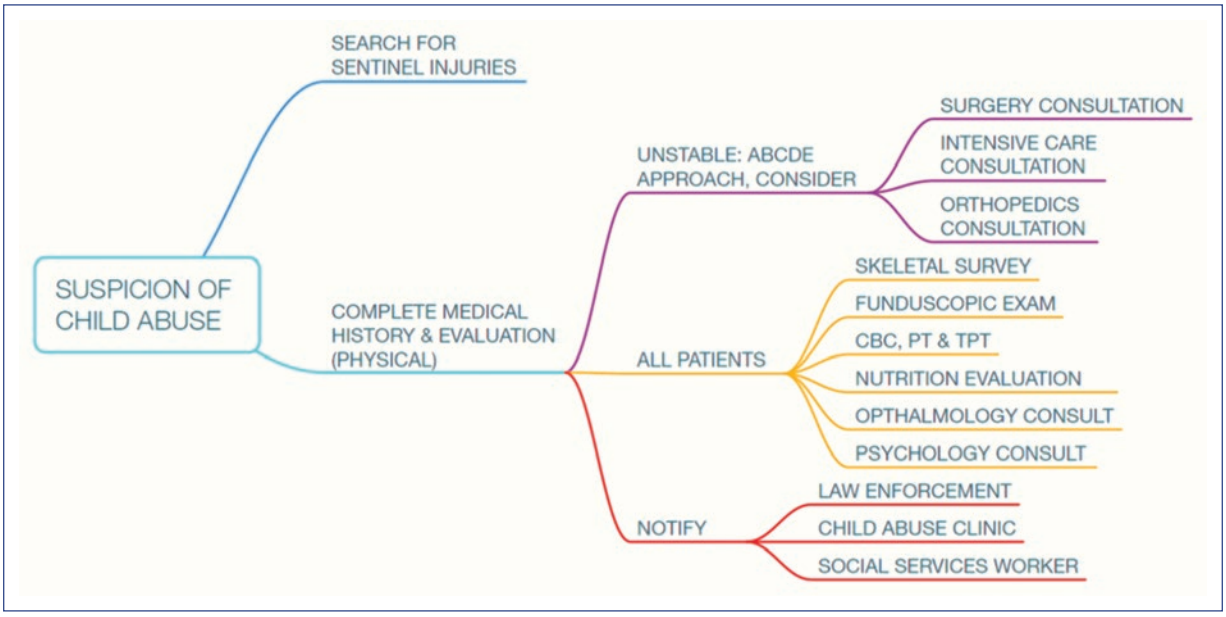

Figure 2. Initial steps for the evaluation of patients with suspected child physical abuse. These initial studies and steps are mandatory.

bone series in the rest of the patients, much less of its repetition.

According to the American Academy of Pediatrics, the extent of diagnostic studies depends on several factors, including the severity of the injury, the type of injury, the age and developmental status of the patient. In general, a more comprehensive diagnostic approach is necessary for more severe injuries and at younger ages. There are different management algorithms or guidelines in the case of patients with suspected maltreatment, among which are the NICE guidelines, the Clinical Report of the American Academy of Pediatrics elaborated by the report of the Commission to Eliminate Child Abuse and Neglect Fatalities, or in Mexico, the CENETEC clinical practice guidelines. Unfortunately, these guidelines have not been updated since 2009, have 11 algorithms, and are not standardized. Therefore, given these findings and after reviewing the current literature, we propose an algorithm for managing and diagnosing patients with suspected child abuse in the emergency department (Figures 2 and 3) to reduce the high percentage of undiagnosed and untreated cases.

In this study, the population of children diagnosed with child abuse seen in the emergency department over ten years was 73 patients. Despite being one of the most frequent pathologies in the country, the number of cases found and reviewed was small. One of the study's limitations is a bias in the number of patients evaluated for child abuse—because our institution is a tertiary level health care center with strict selection criteria for admission, with a chronic population with high comorbidities. Therefore, we consider that child abuse is more frequent in other first and second-level hospitals.

As this was a retrospective study, specific information was impossible to obtain, leading to other biases, implying another study limitation. In addition, in years after those referred to in this study, paper medical records were changed to electronic. Finally, retrospective follow-up is difficult as there are not always notes or reports of the assessments or interpretations made, as in the case of radiographs. Ultimately, the law establishes that the protection of physical medical records is only five years, which prevents an accurate assessment of whether a complete approach to patients was made after they arrived at the emergency department.

Child maltreatment is a frequent and severe problem that leaves short- and long-term sequelae and affects the future health of the individual and the offspring. Healthcare professionals are privileged to identify patients with risk factors and detect signs of maltreatment.

Therefore, tools that offer speed and simplicity in addressing child abuse are needed in all settings where pediatric medical care and consultation are provided to

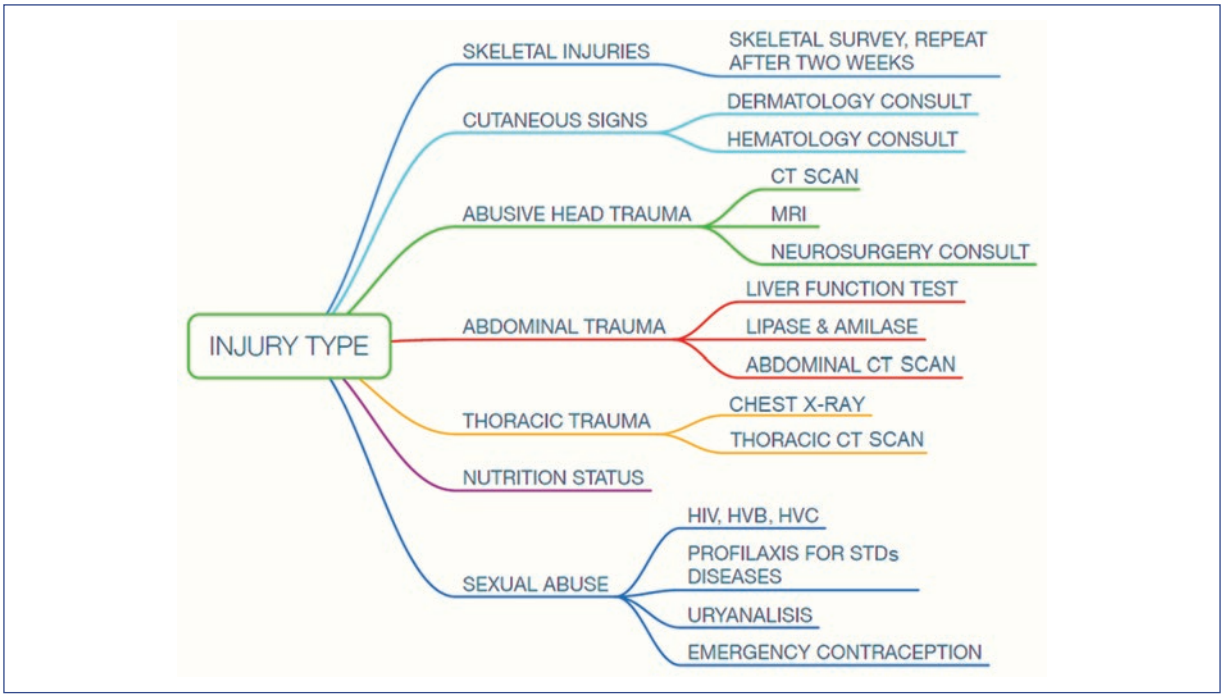

Figure 3. Assessment and studies to be expanded according to the clinical presentation data of the patient with suspected child physical abuse.CT, computed tomography; MRI, magnetic resonance imaging; HIV, human immunodeficiency virus; HVB, hepatitis B virus; HCV, hepatitis C virus; STD, sexually transmitted diseases.

significantly decrease the number of missed diagnostic opportunities in the future.

The correct and timely intervention of physicians is necessary in child abuse cases. As these are legal cases, it is essential to know the elements of the laws of each country to avoid obstacles in the management of each abused patient.

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. This study involved a retrospective review of medical records, for which approval was obtained from a formally constituted review board (Institutional Review Board or Institutional Ethics Committee). The corresponding author has this document.

Conflicts of interest

The authors declare no conflict of interest.

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RESEARCH ARTICLE

Cytotoxic activity of *Staphylococcus aureus* isolates from a cohort of Mexican children with cystic fibrosis

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Abstract

Background: *Cystic fibrosis (CF) is a genetic disease in which thick, sticky mucus is produced in the lungs (and other organs) that impairs ciliary clearance, leading to respiratory problems, increased chronic bacterial infections, and decreased lung function. Staphylococcus aureus is one of the primary bacterial pathogens colonizing the lungs of CF patients. This study aimed to characterize the genetic relatedness of S. aureus, its presence in children with CF, and its cytotoxic activity in THP1 cell-derived macrophages (THP1m).* **Methods:** *Genetic relatedness of S. aureus isolates from a cohort of 50 children with CF was determined by pulsed-field gel electrophoresis (PFGE). The VITEK® 2 automated system was used to determine antimicrobial susceptibility, and methicillin-resistance S. aureus (MRSA) was determined by diffusion testing using cefoxitin disk. The presence of mecA and lukPV genes was determined by the polymerase chain reaction and cytotoxic activity of S. aureus on THP1m by CytoTox 96® assay.* **Results:** *From 51 S. aureus isolates from 50 children with CF, we identified 34 pulsotypes by PFGE. Of the 50 children, 12 (24%) were colonized by more than one pulsotype, and 5/34 identified pulsotypes (14.7%) were shared between unrelated children. In addition, 3/34 pulsotypes (8.8%) were multidrug-resistant (MDR), and 2/34 (5.9%) were MRSA. Notably, 30/34 pulsotypes (88.2%) exhibited cytotoxicity on THP1m cells and 14/34 (41.2%) altered THP1m monolayers. No isolate carried the lukPV gene.* **Conclusions:** *Although a low frequency of MRSA and MDR was found among clinical isolates, most of the S. aureus pulsotypes identified were cytotoxic on THP1m.*

Keywords: *Staphylococcus aureus. Cytotoxicity. Macrophages. Methicillin-resistant Staphylococcus aureus (MRSA). Multidrug resistance.*

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Actividad citotóxica de Staphylococcus aureus provenientes de una cohorte de niños mexicanos con fibrosis quística

Resumen

Introducción: *La fibrosis quística (FQ) es una enfermedad genética en la que se produce moco espeso y pegajoso en los pulmones (y otros órganos), lo que conduce a problemas respiratorios, incremento de las infecciones bacterianas crónicas y disminución de la función pulmonar. Staphylococcus aureus es uno de los principales patógenos que colonizan los pulmones de los pacientes con FQ. El objetivo de este trabajo fue caracterizar la relación genética de S. aureus, su presencia en niños con FQ y su actividad citotóxica en macrófagos derivados de células THP1 (THP1m).* **Métodos:** *La relación genética de los aislados de S. aureus provenientes de una cohorte de 50 pacientes con FQ fue determinada por electroforesis en gel de campo pulsado (PFGE). La sensibilidad a los antimicrobianos se determinó mediante el sistema automatizado VITEK® 2, y la resistencia a la meticilina (SARM) mediante la prueba de difusión utilizando discos de cefoxitina. La presencia de los genes mecA y lukPV se determinó mediante reacción en cadena de la polimerasa, y la actividad citotóxica de S. aureus sobre células THP1m mediante el ensayo CytoTox96®.* **Resultados:** *A partir de 51 aislados de S. aureus provenientes de 50 niños con FQ se identificaron 34 pulsotipos por PFGE. De los 50 niños, 12 (24%) estaban colonizados por más de un pulsotipo y 5 de los 34 pulsotipos (14.7%) los compartían niños que no estaban relacionados. De los 34 pulsotipos, 3 (8.8%) presentaron multirresistencia (MDR) y 2 (5.9%) fueron SARM. Además, 30 pulsotipos (88.2%) fueron citotóxicos sobre células THP1m y 14 (41.2%) alteraron su monocapa. Ninguno de los pulsotipos presentó el gen lukPV.* **Conclusiones:** *Aunque se encontró una baja frecuencia de SARM y MDR en los aislados, la mayoría de los pulsotipos de S. aureus identificados fueron citotóxicos para células THP1m.*

Palabras clave: *Staphylococcus aureus. Citotoxicidad. Macrófagos. Staphylococcus aureus resistente a la meticilina (SARM). Multirresistencia.*

Introduction

Cystic fibrosis (CF) is a genetic disorder that mainly affects the lungs but also the pancreas, liver, kidney, and intestine. This disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator gene (*CFTR*)[1]. Mutations in *CFTR* alter the transport of chloride and sodium ions, HCO_3^- and water across the cell membrane in the airways.

Lung epithelia with impaired *CFTR* gene function produce thick, sticky mucus that clogs the airways and traps opportunistic bacteria, producing infections that cause inflammation, leading to decreased lung function, respiratory distress, and eventually respiratory failure[2].

During the first years of life, the airways of children with CF are rapidly colonized by non-typeable *Haemophilus influenzae* and *Staphylococcus aureus*[3], and progressively by *Pseudomonas aeruginosa* and *Burkholderia cepacia* complex, which are the primary opportunistic pathogens associated with chronic infection and decreased pulmonary function[3]. In Mexico, most children with CF are colonized by *P. aeruginosa* and *S. aureus*[4]. Worldwide, airway colonization by *S. aureus* ranges between 30-50%[5].

S. aureus is a Gram-positive bacterium associated with establishing an inflammatory process in the lower respiratory tract, reducing lung function and contributing significantly to lung tissue damage[6]. The ability of these bacteria to produce a biofilm is associated with increased resistance to antibiotics *in vitro*[7]. In particular, methicillin-resistant *S. aureus* (MRSA) has been associated with accelerated deterioration of lung function and increased mortality[8]. Methicillin resistance is attributed to an alternate penicillin-binding protein (PBP2a or PBP2') encoded by the *mecA* gene[9]. *S. aureus* is a versatile bacterium with an arsenal of virulence factors, including Panton-Valentine leukocidin (PVL), which facilitates tissue adhesion and host cell injury[10]. PVL is often related to community-associated MRSA (CA-MRSA)[11], and its expression has been associated with severe infections, bacteremia, osteomyelitis, and necrotizing pneumonia[12]. PVL is a bicomponent pore-forming cytotoxin that causes leukocyte lysis[13]. This study aimed to characterize the genetic relatedness, presence of MRSA, and macrophage cytotoxic activity in clinical isolates of *S. aureus* from a cohort of children with CF in Mexico.

Methods

We conducted a descriptive study derived from a study published in 2020[4]. This study was approved by the Institutional Review Board of the Faculty of Medicine

of the Universidad Nacional Autónoma de México (Protocol FMED/CI/RGG/022/2016).

Bacterial isolates

Bacterial isolates were obtained from sputum samples collected from 50 pediatric patients attending the CF clinic of the Hospital Infantil de México Federico Gómez (HIMFG), Mexico City, Mexico, from August 2016 to January 2018[4]. Parents of children with CF who agreed to participate in this study signed a consent form authorizing the collection of sputum samples to identify bacterial pathogens. Patient samples were collected as part of routine hospital care. Each patient had appointments scheduled every 3 to 6 months, although some patients and their parents do not always attend their scheduled appointments at HIMFG.

Sputum samples or cough swabs were transported to our laboratory for processing. Sputum samples were dissolved weight/volume (1:1) in sputolysin (Merk-Millipore, Darmstadt, Germany) for 30 minutes at 37ºC. Dissolved sputum samples and cough swabs were used to inoculate salt and mannitol, chocolate, blood, MacConkey (DIBICO, State of Mexico, Mexico), and cetrimide (Becton Dickinson, New Jersey) agar media plates. Plates were incubated at 37ºC for 24 hours. Chocolate agar and blood agar plates were also incubated at 37ºC for 24 hours under microaerophilic conditions. Bacterial isolates were identified by standard microbiological methods[4]. All samples were stored at –70ºC until analysis.

Pulsed-field gel electrophoresis

The bacterial relatedness of 50 clinical isolates of *S. aureus* was determined by pulsed-field gel electrophoresis (PFGE). Bacterial genomic DNAs were purified and prepared as described elsewere[14]. Genomic DNAs were digested with *Sma*I (Invitrogen) for 24 h and resolved by PFGE using a Gene Path system (BioRad® USA). Bacterial relatedness among *S. aureus* clinical isolates was determined according to the Tenover criteria and the use of the Dice coefficient, as previously described[4]. An isolate was considered a member of the same pulsotype when it had a > 85% correlation.

Antibiotic susceptibility testing

S. aureus isolates were tested for antimicrobial susceptibility to ciprofloxacin, levofloxacin, moxifloxacin, gentamicin, tigecycline, trimethoprim/sulfamethoxazole, oxacillin, erythromycin, clindamycin, linezolid, vancomycin, tetracycline, and rifampicin. The minimum inhibitory concentrations (MICs) were determined using the VITEK®2 system (bioMérieux®SA). Methicillin resistance was determined using the cefoxitin test by the disk diffusion method (Kirby-Bauer). Quality control and interpretation of results were performed according to Clinical and Laboratory Standards Institute (2019) guidelines[15]. *S. aureus* ATCC 43300, 25923, and USA300 were used as standard quality controls. The multidrug resistance (MDR) phenotype is defined as non-susceptibility to ≥ 1 agent from ≥ 3 antimicrobial categories[16].

DNA extraction and identification of *mecA* and *lukPV* genes by polymerase chain reaction (PCR)

S. aureus isolates were grown on salt and mannitol agar plates for 24 hours at 37ºC. An isolated colony was resuspended in 100 µL of MilliQ water and boiled for 10 minutes. The bacterial suspension was centrifuged at 10,000 rpm at 4ºC for 10 minutes. Supernatants containing genomic DNA were used for PCR reactions. Primers *mecA*(F): 5'-TGGCTATCGTGTCACAATCG-3' and *MecA*(R): 5'-CTGGAACTTGTTGAGCAGAG-3' were used for *mecA* amplification, whose amplification product is 310pb[17]. The *lukPV* gene was amplified with primer *LukPV*(F): 5'-ATCATTAGGTAAAATGTCTGGACATGATCCA-3' and *LukPV*(R): 5'-GCATCAACTGTATTGGATAGCAAAAGC-3', with an amplification product of 433pb[18].

PCR was performed as follows: we mixed 10 mM of each primer, 1X GoTaq® Green Master Mix, and 3 µL of DNA template extracted by boiling. The conditions for gene amplification were one cycle at 94ºC for 5 min, 30 cycles at 94ºC for 30 s, 55ºC for 30 s, and 72ºC for 90 s with a final extension step at 72ºC for 5 min. PCR products were resolved on a 1% (w/v) agarose gel for one hour at 100 volts. The gel was stained with ethidium bromide (1 µg/mL) for 5 min and washed twice in deionized water. The gel was analyzed on a UV light transilluminator using Quantity One software (BioRad® USA).

Reagents, cells, and growth conditions

Fetal bovine serum (FBS) and RPMI-1640 cell culture media were obtained from Invitrogen, PBS and Luria-Bertani (LB) broth from Sigma-Aldrich, and salt and mannitol agar from DIBICO (State of Mexico, Mexico). Human THP1 cells were obtained from ATCC®

TIB-202™; 3x10^5 human THP1 cells were differentiated into macrophages (THP1m) using PMA 100 ng/mL (Sigma-Aldrich) for 24 hours[19]. Prior to infection, the medium was changed to RPMI medium with no antibiotics. THP1m cells monolayers were infected with *S. aureus* at an MOI of 50. To synchronize the infection, we centrifuged the cells at 1,200 rpm for 1 min and then incubated the plates for one hour. After infection, the cells were washed three times with PBS to remove extracellular bacteria; infected cells were returned to the incubator for an additional 24 h in RPMI medium supplemented with gentamicin (100 μg/mL).

Cytotoxicity assays

Supernatants from THP1m and THP1m colonized with *S. aureus* were used to quantify cytosolic enzyme activity of lactate dehydrogenase (LDH; Promega, Madison WI, USA). The following formula determined the percentage of LDH activity:

$$\% \text{ of release} = \frac{(\text{experimental LDH activity} - \text{spontaneous LDH activity})}{(\text{maximal LDH activity} - \text{spontaneous LDH activity})} \times 100\%$$

Results

Presence and genotyping of S. aureus isolates from CF pediatric patients

Of the 50 pediatric patients studied, the most frequently isolated bacterial pathogen was *P. aeruginosa,* followed by *S. aureus*[4]. *S. aureus* was isolated from 26/50 patients, from whom we obtained 51 isolates (Figure 1). Chromosomal analysis by PFGE yielded 34 patterns (pulsotypes) (Table 1). With the 34 patterns, we were able to identify nine clusters (designed as I-IX), which differed by approximately 85% in PFGE band similarity (Figure 1). The cluster with more members (thirteen) was cluster II. The results showed that nine patients (CF001, CF010, CF011, CF013, CF014, CF016, CF019, CF022, and CF029) were chronically infected (the same pulsotype was identified in two or more samples for two or more months) (Figure 2). Twelve patients were colonized with different pulsotypes: eleven with two different pulsotypes and one with three different pulsotypes (Table 1). Patients CF008 (colonized with *Sau*25) and CF014 (colonized with *Sau*06 and *Sau*14) died during the study period.

Table 1. *S. aureus* pulsotypes isolated from Mexican children with cystic fibrosis

Patient	Sample			
	1st	2nd	3rd	4th
CF001	*Sau*01	*Sau*01	*Sau*01	—
CF003	x	*Sau*12	*Sau*29	—
CF004	x	Sau02	—	—
CF006	*Sau*03	*Sau*17	*Sau*32	—
CF007	*Sau*04	x	—	—
CF008[a]	x	*Sau*25	—	—
CF009	x	*Sau*15	x	x
CF010	*Sau*05	*Sau*05	*Sau*31	—
CF011	x	*Sau*19	*Sau*19	—
CF012	x	x	*Sau*22	—
CF013	x	*Sau*04	*Sau*04	*Sau*28
CF014[a]	*Sau*06	*Sau*14	*Sau*14	—
CF016	*Sau*07	*Sau*07	*Sau*07	—
CF019	*Sau*08	*Sau*16	*Sau*16	*Sau*16
CF022	*Sau*09	*Sau*09	*Sau*31	—
CF025	x	*Sau*18	—	—
CF028	x	*Sau*20	*Sau*26	—
CF029	*Sau*11	*Sau*11	—	—
CF030	x	*Sau*21	*Sau*30	—
CF032	*Sau*13	—	—	—
CF034	*Sau*01	*Sau*14	—	—
CF036	*Sau*17	—	—	—
CF041	x	*Sau*24	*Sau*27	—
CF042	*Sau*23	—	—	—
CF048	*Sau*10	*Sau*33	—	—
CF049	x	*Sau*34	—	—

[a]Patient died.
X, sample with no S. *aureus* isolate; —, no sample available.

Antimicrobial susceptibility of S. aureus pulsotypes isolated from CF children

One of the 34 *S. aureus* pulsotypes identified was used to determine the antimicrobial susceptibility pattern to ten different classes of antibiotics: glycylcyclines, oxazolidinones, glycopeptides, folate pathway

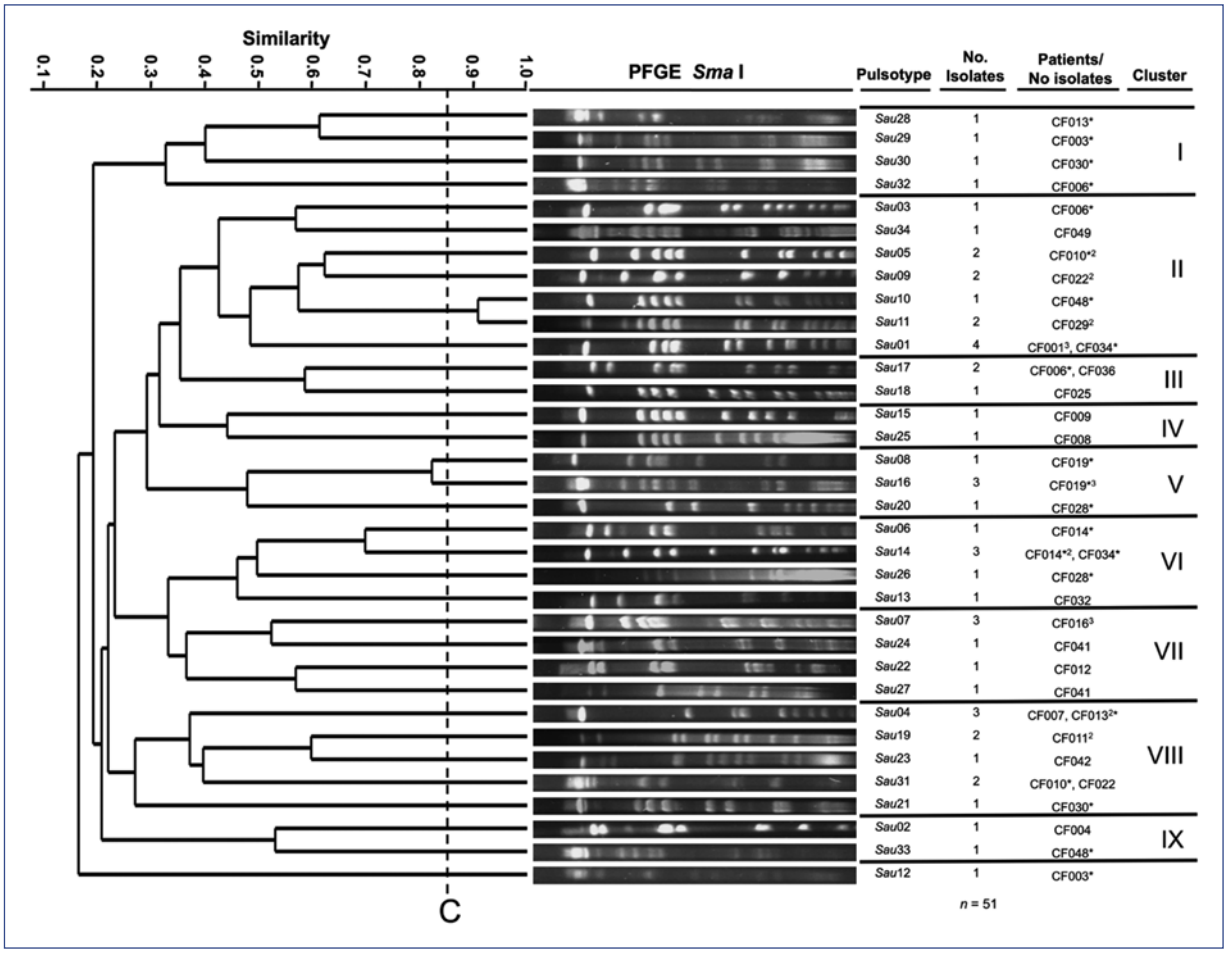

Figure 1. Dendrogram generated from PFGE analysis of 51 *S. aureus* isolates from pediatric CF patients. A representative PFGE profile of each pulsotype was used to construct the dendrogram. Each pulsotype frequency (number of isolates) is indicated, and each row indicates the patient in which the pulsotype was identified. Patients colonized by more than one pulsotype are indicated (*). A superscript indicates the frequency of each pulsotype identified per patient. The dotted line indicating 85% similarity was used to determine the cluster designation (I-IX).

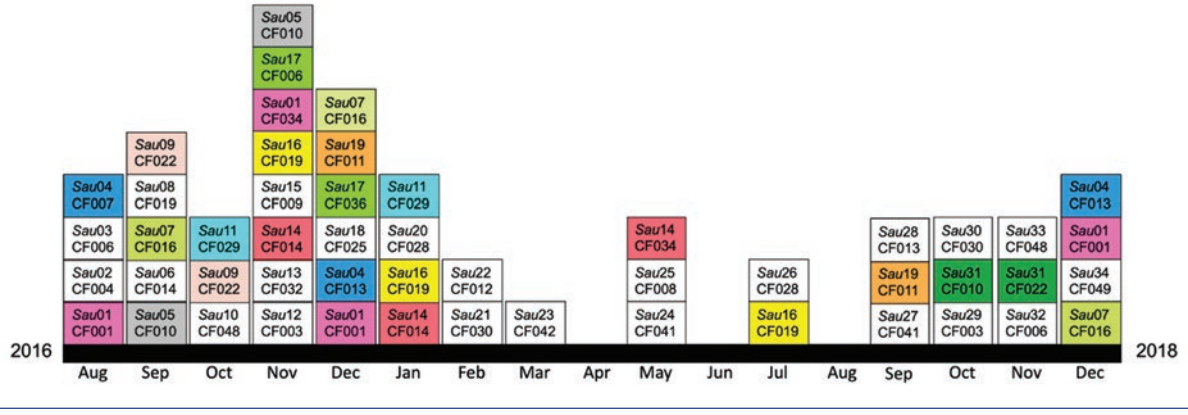

Figure 2. *S. aureus* pulsotypes were identified from 2016 to 2018. White boxes indicate a single pulsotype; colored boxes indicate a clone detected twice or more times.

Table 2. Antibiotic susceptibility for 34 *Staphylococcus aureus* pulsotypes of pediatric patients with cystic fibrosis

Antibiotic family	Antibiotic	Breakpoints (µg/mL)			Susceptible n (%)	Intermediate n (%)	Resistant n (%)
		S	I	R			
Aminoglycosides Lincosamides	Gentamicin	≤ 4	8	≥ 16	30 (88.2)	2 (5.9)	2 (5.9)
	Clindamycin	≤ 0.5	1-2	≥ 4	27 (79.4)	1 (2.9)	6 (17.7)
Penicillins Ansamycins	Oxacillin (MRSA)	≤ 2	—	≥ 4	30 (88.2)	0 (0.0)	4 (11.8)
	Rifampicin	≤ 1	2	≥ 4	31 (91.2)	0 (0.0)	3 (8.8)
Macrolides	Erythromycin	≤ 0.5	1-4	≥ 8	27 (79.4)	0 (0.0)	7 (20.6)
Tetracyclines	Tetracycline	≤ 4	8	≥ 16	27 (79.4)	0 (0.0)	7 (20.6)
Fluoroquinolones	Ciprofloxacin	≤ 1	2	≥ 4	29 (85.3)	2 (5.9)	3 (8.8)
	Levofloxacin	≤ 1	2	≥ 4	31 (91.2)	2 (5.9)	1 (2.9)
	Moxifloxacin	≤ 0.5	1	≥ 2	33 (97.1)	0 (0.0)	1 (2.9)
Folate pathway inhibitors	Trimethoprim/ sulfamethoxazole	≤ 2/38	—	≥ 4/76	34 (100.0)	0 (0.0)	0 (0.0)
Glycopeptides Oxazolidones	Vancomycin	≤ 2	4-8	≥ 16	31 (91.2)	0 (0.0)	3 (8.8)
	Linezolid	≤ 4	—	≥ 8	34 (100.0)	0 (0.0)	0 (0.0)
Glycylcyclines	Tigecycline	≤ 0.25	—	—	34 (100.0)	0 (0.0)	0 (0.0)

MRSA, methicillin-resistant *Staphylococcus aureus* S, susceptible; I, intermediate; R, resistant.
*Breakpoints were obtained from the Clinical and Laboratory Standards Institute (2019).

antagonists, fluoroquinolones, tetracyclines, macrolides, ansamycins, penicillins, lincosamides, and aminoglycosides (Table 2). We detected 3/34 (8.8%) MDR pulsotypes, and a low rate of resistance to vancomycin (8.8%), moderate resistance to tetracycline and erythromycin (20.6%), and clindamycin (17.7%). All the pulsotypes were susceptible to tigecycline, linezolid, and trimethoprim/sulfamethoxazole (Table 2). We found only two MRSA pulsotypes (5.9%): *Sau*08 and *Sau*16, both isolated from patient CF019. *Sau*16 was isolated three times at different times during sample collection (Figure 1 and Table 1). We identified both pulsotypes (*Sau*08 and *Sau*16) carrying the *mecA* gene by PCR (data not shown). These results also revealed that the two pulsotypes were MRSA with an MDR phenotype.

Pulsotypes of S. aureus induced cytotoxicity in human THP1-derived macrophages

The ability of *S. aureus* to induce cytotoxicity in human THP1 monocytes differentiated into macrophages (THP1m) was evaluated. The results showed that 30/34 (88.2%) pulsotypes were cytotoxic (> 10% of cytotoxicity) (Figure 3), 8/34 (23.5%) were highly cytotoxic (> 50% of cytotoxicity), and 14/34 (41.2%) were able to disrupt the THP1m monolayers (data not shown). To determine whether the cytotoxic effect was associated with pulsotypes carrying the *PVL* gene, we amplified the *lukPV* gene by PCR. The results showed that none of the analyzed pulsotypes carried the *lukPV* gene (data not shown).

Discussion

The lower airways of children with CF are rapidly colonized by *S. aureus* and non-typeable *H. influenzae*[5]. However, the pulmonary bacterial microbiome gradually changes with the emergence and persistence of *P. aeruginosa* during adolescence and adulthood[5]. The eradication of *S. aureus* in the lower respiratory tract has been compromised by the emergence of MRSA[20]. The World Health Organization (WHO) has declared priority level 2 in identifying new antibiotics to combat MRSA. To date, MRSA is usually associated with the community- and hospital-acquired infections. In CF, the occurrence of MRSA has been associated with a more rapid decline in lung function[21]. In a previous study, 44% of children with CF (22/50) were

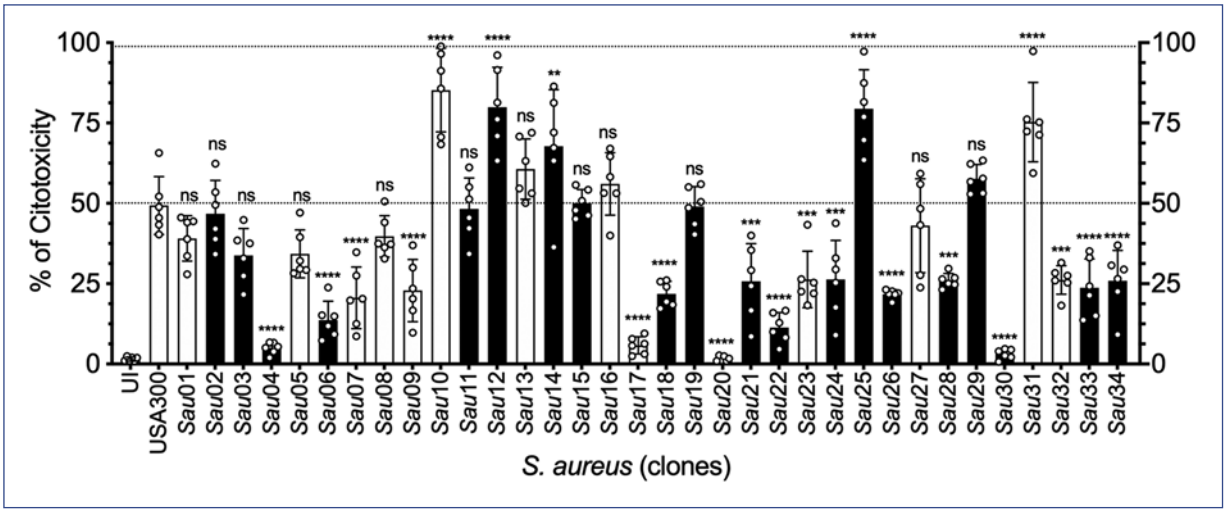

Figure 3. Induction of cytotoxicity by *S. aureus* isolates in THP1macrophages: 5 x 10^5 THP1 monocytes were differentiated into macrophages with 100 ng/mL of PMA for 24 h. Cells were infected with *S. aureus* isolates at MOI of 100 for 30 min. Once infected, cells were washed and incubated for 24 h. Supernatants were used to quantify macrophage cell death (cytotoxicity). Results were obtained from three independent experiments, each in duplicate (n = 6). Data were plotted as the mean ± SD and analyzed by one-way ANOVA and Dunnett's multiple comparisons in relation to *S. aureus* -USA300. **p < 0.01; ***p < 0.001; ****p< 0.0001; ns: non-significant.

colonized by *P. aeruginosa* and *S. aureus*[4]. In the present study, we identified 34 unrelated pulsotypes, of which 2/34 were MRSA. In particular, the *Sau*16 pulsotype was consistently isolated three times in patient CF019 during one year. We also isolated the genetically related *Sau*08 pulsotype (group V) in this patient, suggesting a genetic evolution. Analysis of clinical isolates of *S. aureus* by whole-genome sequencing suggests possible genetic evolution and spread[22]. In this study, we identified that pulsotypes *Sau*08 and *Sau*16 are MRSA with a consistent MDR phenotype. We also identified the persistence of *S. aureus* in eight patients. Persistence and long-term carriage of *S. aureus* are often associated with specific phenotypes, including small colony variants, increased antimicrobial resistance, and biofilm-formation[23]. Furthermore, MRSA colonization is more frequently associated with individuals carrying the ΔF508 mutation[24]. The patient CF019 from whom we isolated both MRSA pulsotypes carried the ΔF508 mutation[4].

S. aureus contains several virulence factors that promote host tissue damage[25-27]. Toxic shock syndrome toxin (TSST-1) and PVL are two important secreted virulence factors[12]. Our results showed that none of the *S. aureus* pulsotypes tested carried the *lukPV* gene. It has been demonstrated that PVL expression is not sufficient to induce cell death[28], suggesting the presence of additional virulence factors that contribute to pathogenesis[27].

We determined that 88.2% of the isolates induced cell death in THP1-derived macrophages and that 14/34 (41.2%) of the tested pulsotypes could alter the integrity of the THP1m monolayers. *S. aureus* α-toxin is a secreted virulence factor involved in the disruption of epithelial cell monolayers[29], suggesting that this virulence factor could alter THP1m monolayers, a hypothesis that we will address in the future.

In conclusion, in this study, we demonstrated a low presence of MRSA and MDR and a high frequency of *S. aureus* pulsotypes with the ability to induce cytotoxicity in THP1-derived macrophages.

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 has this document.

Conflicts of interest

The authors declare no conflict of interest.

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CLINICAL CASE

Thrombotic thrombocytopenic purpura associated with COVID-19 in a critically ill child: a Peruvian case report

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Abstract

Background: *Acquired thrombotic thrombocytopenic purpura (TTP) is a rare disease. In middle and low-income countries, specific resources are required for its diagnosis due to the lack of diagnostic tests and the variable response to plasma exchange, especially in the context of the new SARS-CoV-2 pandemic.* **Case report:** *We report the case of a 9-year-old male Hispanic patient with SARS-CoV-2 infection, atypical presentation, and multisystem involvement, thrombotic microangiopathy with dermal manifestations, hematologic, renal, and neurologic involvement. The patient was followed up after SARS-CoV-2 infection, the PLASMIC score was applied, and a genetic study was performed. Ventilation and hemodynamic support, corticotherapy, immunoglobulins, plasma exchange, renal replacement therapy, and monoclonal antibodies were given without favorable response.* **Conclusions:** *TTP associated with SARS-CoV-2 in the pediatric population is rare. However, resources for the diagnosis, support, and management of patients with TTP are required to avoid fatal outcomes.*

Keywords: *Thrombotic thrombocytopenic purpura. COVID-19. SARS-CoV-2. Child. Peru.*

Púrpura trombocitopénica trombótica asociada con COVID-19 en un niño críticamente enfermo: reporte de un caso peruano

Resumen

Introducción: *La púrpura trombocitopénica trombótica (PTT) adquirida es una enfermedad poco frecuente. En los países de mediano y bajo estatus económico se requieren recursos para el diagnóstico de la PTT, debido a la falta de pruebas diagnósticas y a la respuesta variable al recambio plasmático, especialmente en el contexto de la pandemia por el nuevo SARS-CoV-2.* **Caso clínico:** *Paciente de sexo masculino, de 9 años, hispano, con infección por SARS-CoV-2, presentación atípica y afectación multisistémica, microangiopatía trombótica con manifestaciones dérmicas, y compromiso hematológico, renal y neurológico. Se dio seguimiento posinfección por SARS-CoV-2, se aplicó la escala PLASMIC y se realizó un estudio genético. Se aplicaron soporte ventilatorio y hemodinámico, corticoterapia, inmunoglobulinas, recambio plasmático, terapia de reemplazo renal y anticuerpos monoclonales, sin respuesta favorable.* **Conclusiones:** *La PTT asociada al SARS-CoV-2*

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en la población pediátrica es poco frecuente. Aun así, se requieren recursos para el diagnóstico, el soporte y el manejo de los pacientes con PTT para evitar desenlaces fatales.

Palabras clave: *Púrpura trombocitopénica trombótica. COVID-19. SARS-CoV-2. Niño. Perú.*

Introduction

Thrombotic microangiopathy (TMA) is characterized by microthrombus formation associated with subsequent thrombocytopenia, microangiopathic hemolytic anemia (MAHA), and target organ injury[1]. Thrombotic microangiopathies are a group of disorders primarily related to endothelial dysfunction. This category of endothelial dysfunction results from various imbalances between platelets, the endothelial and immune systems, and cytokine production[2]. Thrombotic thrombocytopenic purpura (TTP) is a fatal condition, rare among hematological diseases, characterized by microvascular thrombosis with platelet aggregation in patients with severe functional deficiency of ADAMTS13 (activity < 10%)[3].

Furthermore, COVID-19 is a new disease with different clinical manifestations observed in children[4,5]. During SARS-CoV-2 infection, hematological diseases such as immune thrombocytopenia and TTP were reported in adults[2,6]. However, information in children is still limited. Hidalgo et al. reported a 14-year-old patient with COVID-19-associated TTP, with a favorable outcome after treatment with plasma exchange therapy (PEX)[7]. Verma et al. described another case of COVID-19 complicated with hemophagocytic lymphohistiocytosis and TTP in a 21-year-old male patient, who died despite treatment[8]. This study aimed to describe the unusual presentation of TTP associated with COVID-19 in a pediatric patient with a fatal outcome.

Clinical case

We describe the case of a 9-year-old male Hispanic patient who presented with abdominal pain and fever of 14 days of evolution to the pediatric emergency department. Five weeks earlier, the mother was positive for SARS-CoV-2 by RT-PCR (reverse transcription-polymerase chain reaction) test; on admission to the hospital, the patient's serological test (IgG) for SARS-CoV-2 antibodies was positive. He had no previous hospitalizations or any report of illness or surgical interventions and had complete immunizations. Vital functions on admission were the following: heart rate 120 beats/min, respiratory rate 22/min, temperature 38°C, weight 30 kg, height 128 cm. On clinical examination, we detected pallor of the skin and mucous membranes, chapped lips, erythematous macular lesions symmetrically distributed on the neck, forehead, and inguinal area. Abdominal pain was present on palpation of the mesogastrium. The results of clinical tests showed hemoglobin, 13.3 g/dL; leukocytes, 18.9 x 10^3/μL; platelets, 528 x 10^3/μL; C-reactive protein, 4.6 mg/dL; normal coagulation profile; fibrinogen, 581.3 mg/dL; lactate dehydrogenase, 1317 U/L; D-dimer, 1.6 mg/L; normal complement C3 and C4; urea, 18 mg/dL; creatinine, 0.4 mg/dL; albumin, 3.7 g/dL; triglycerides, 130 mg/dL; creatine kinase, 25 mg/dL; ferritin, 1016 ng/mL; serum electrolytes with normal values and urinalysis showed hematuria. Abdominal ultrasound showed hepatomegaly, and echocardiography showed no alterations. He received exogenous human immunoglobulin 2 g/kg/day, acetylsalicylic acid 3 mg/kg/day for 7 days, prednisone 2 mg/kg/day for 5 days to treat the probable multisystem inflammatory syndrome. On the second day of treatment, he was afebrile, and on the fourth day, the erythematous lesions decreased; also, there was no hematuria, and he presented a decrease in acute phase reactants, so he was discharged. Five days after discharge, abdominal pain and fever persisted. Clinical laboratory findings were as follows: hemoglobin, 11.3 g/dL; leukocytes, 10 x 10^3/μL; eosinophils, 1305/μL; absolute neutrophil count, 5321/μL; lymphocytes, 2710/μL; platelets, 606 x 10^3/μL; C-reactive protein, 8.2 mg/dL; fibrinogen, 664 mg/dL; D-dimer, 2 μg/mL; ferritin, 1030 ng/mL; lactate dehydrogenase, 890 U/L; coagulation profile, urea, creatinine, and serum electrolytes were normal. Immunological tests were performed: negative antinuclear antibodies (ANA), negative anti-dsDNA, negative antineutrophil cytoplasmic antibodies (ANCA), negative anti-cyclic citrullinated peptide antibodies (anti-CCP), negative rheumatoid factor, negative lupus antibodies, negative antiphospholipid profile, and negative IgG antibodies against *Toxocara*. The patient was treated with methylprednisolone at a dose of 2 mg/kg/day for 7 days, then at a dose of 30 mg/kg for 3 days, and then with prednisone at a dose of 2 mg/kg/day for 15 days. During hospitalization, he presented decreased abdominal pain and absence of fever, so he was discharged with an indication of follow-up control by rheumatology.

Four months later, the patient was readmitted to the pediatric emergency room with 12 days of evolution characterized by fever, abdominal pain, and generalized edema. On admission, he presented a heart rate

of 140 beats/min, respiratory rate of 29/min, blood pressure (p90-95), and edema of the face, genitals, hands, and feet. The skin was cold, turgid, with erythematous macular lesions of urticarial appearance (positive dermographism) associated with diffuse hematic crusts and fine desquamation on the face, neck, thorax, arms, and legs. Examinations found the following values: hemoglobin, 11.6 g/dL; platelet count, 170 x 10^3/μL; prothrombin time (PT), 13.1 s; INR (international normalized ratio), 1.1; activated partial thromboplastin time (aPTT), 34 s; fibrinogen, 432 mg/dL; urea, 16 mg/dL; creatinine, 0.34 mg/dL; D-dimer, 4.32 mg/L; lactate dehydrogenase, 1862 U/L; serum calcium, 7.2 mg/dL; complement C3, 120 mg/dL, and C4, 28 mg/dL, and hemoglobinuria. The patient was transferred to the dermatology department due to the dermatological lesions described. Three days later, the dermal lesions increased (Figure 1), so a skin biopsy of the lesions was performed. The biopsy results described an acute spongiotic dermatitis with intraepidermal vesicles and numerous necrotic keratinocytes in the epidermis, associated with a superficial and deep perivascular and periadnexal inflammatory infiltrate, with numerous eosinophils and extravasation of red blood cells. Histochemical study with periodic acid-Schiff and Alcian blue was negative. He presented with oliguria and generalized tonic-clonic seizures associated with 86% desaturation (fraction of inspired oxygen (FiO_2) 21%) on three occasions. Due to unstable hemodynamic compromise, it was decided to perform endotracheal intubation and transfer him to the Pediatric Intensive Care Unit (PICU) for ventilation and hemodynamic management. The following results were obtained in the PICU: hemoglobin, 10.6 g/dL; schistocytes, 6+/field (blood); positive direct Coombs test; leukocytes, 13.4 x 10^3/μL; platelets, 19 x10^3/μL; haptoglobin not detectable; triglycerides, 280 mg/dL; ferritin, 3442 ng/mL; lactate dehydrogenase, 1862 U/L; D-dimer, 4 mg/L; complement C3, 120 mg/dL, and C4, 28 mg/dL; creatinine, 1.23 mg/dL; creatine kinase, 248 mg/dL; erythrocyte sedimentation rate, 19 s, and ADAMTS13 activity (von Willebrand factor-cleaving protease) in 54% (RV: 40-130%) after transfusion support of blood products. Flow cytometry showed a decrease in T lymphocytes, CD4+ lymphocytes, CD8+ lymphocytes, an increase in CD4/CD8 ratio for age, and a decrease in natural killer (NK) cell count. Viral load for Epstein-Barr virus, cytomegalovirus, and parvovirus B19 was negative.

Renal ultrasound showed the left kidney with asymmetry compared to the contralateral one; in the Doppler study, a decreased wave depth was identified, suggesting left renal hypoperfusion. With the clinical and laboratory manifestations described, the patient was diagnosed with TTP. The PLASMIC score was 7 (Table 1). He received fresh frozen plasma, renal replacement therapy, methylprednisolone cycles, human immunoglobulin, and rituximab (Table 1). Due to persistent severe thrombocytopenia, it was decided to initiate plasma exchange (PEX), and the patient received 14 sessions. Although the patient received platelet transfusion to compensate for the platelet deficit, he did not respond well.

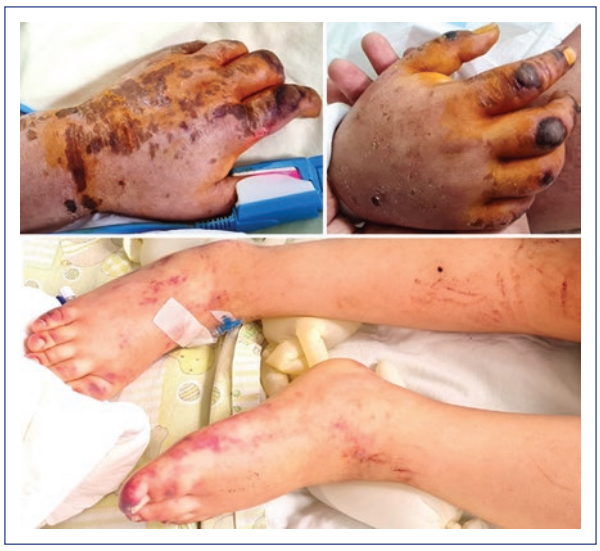

Figure 1. Erythematous macular lesions with excoriated hematic scabs in the hands associated with purpuric dermatosis in the patient's lower limbs on the Pediatric Intensive Care Unit admission day.

The patient presented sensorium deterioration during the PICU stay, and an intracerebral hemorrhage was detected one month after the last PEX session in a CT scan. The patient evolved unfavorably with a fatal outcome in the second month of hospitalization. Gene sequence analysis and deletion/duplication test of 13 genes (ADAMTS13, C3, CD46, CD55, CD59, CFB, CFH, CFI, DGKE, INF2, MMACHC, PLG, THBD) for atypical hemolytic uremic syndrome and thrombotic microangiopathy were negative.

Discussion

Acquired TTP has been described as a clinical presentation associated with COVID-19 in adults[6]. However, since TTP in children is rare[3], only a few cases have been reported in children[7].

Table 1. Laboratory test results during hospitalization

Hospital admissions	1st	2nd	3rd							
Department	Medicine	Medicine	Dermatology	PICU						
Hospitalization day	1	1	1	1*	3+	11	16	23	30	37
Treatment					MTP+Ig/ Rtmb/ PD	MTP/ PEX/ PD	MTP/ Rtmb/ PEX/ HD	PDN/ Rtmb/ PEX/ HD	PDN/ Rtmb/ HD	PDN/ HD
Hematology results										
Hemoglobin (g/dL)	13.3	11.3	11.6	10.6	8	10	7.8	9.1	7.8	8.4
Leukocytes ($10^3/\mu L$)	18.9	10	16.4	13.4	9.2	17.4	11.6	5.4	2.2	5.3
Platelets ($10^3/\mu L$)	528	606	170	19	14	68	40	32	99	51
Prothrombin time (s)	12.8	12.4	13	16	13	12.4	13.1	13.5	—	13.8
INR	1.1	1.0	1.1	1.4	1.1	1	1.1	1.1	—	1.2
Thromboplastin time (s)	29.6	28.5	34	44	29	33.5	37.2	43.7	—	42.7
Thrombin time (s)	17	17	18.7	27	23	21	19.8	25.8	—	24
Fibrinogen (mg/dL)	581.3	664	432	300	335	230	253.4	226.6	—	164.1
D-dimer (mg/L)	1.6	2.0	4.32	6.8	12.2	—	—	3.6	—	
Biochemical results										
Ferritin (ng/mL)	1016	1030	—	3442	23943	3254	—	—	—	—
C3 (mg/dL)	101		120	—		—	—	—	—	—
C4 (mg/dL)	24	32	28	—		—	—	—	—	—
Lactate dehydrogenase (U/L)	1317	890	1862	—	10,562	2279	1780	754	494	—
Urea (mg/dL)	18	23	16	49	173	177	245	84	54	57
Creatinine (mg/dL)	0.4	0.32	0.34	1.23	4	3.6	4.14	1.9	1.8	1.8
GOT (U/L)	34	50	48	233	360	—	—	32	—	—
GPT (U/L)	17	49	9	61	—	—	—	42	—	—
CPK (mg/dL)		44	248	—	1883	—	—	31	—	—
CPK-MB (U/L)	25	14	26	—	102	—	—	12	—	—
C-reactive protein (mg/dL)	4.6	8.17	4.76	5.9	6	2.6	—	2.2	—	12.8

(*) PLASMIC score: platelet count < 30,000/μL: 1; hemolysis: 1; no active cancer: 1; no solid organ or stem cell transplantation: 1; no diarrhea: 1; INR < 1.5: 1; creatinine < 2.0 mg/dL: 1 (score 7). Corticosteroid onset: MTP (1 g) + Ig 5% (30 g) for 5 days. Then it was decreased to MTP (125 mg) every 6 hours for 5 days and MTP (125 mg) every 8 hours for 5 days, then moved to PDN (1 mg/kg) equivalent dose in dexamethasone. Initiation of Rtmb (375 mg/m^2/dose each week*4 doses). (+) Indication of fresh frozen plasma (10 cc/kg/dose every 8 hours).
CPK, creatine phosphokinase; GOT, aspartate aminotransferase; GPT, alanine aminotransferase; HD, hemodialysis; Ig, immunoglobulin; MTP, methylprednisolone; PD, peritoneal dialysis; PDN, prednisone; PEX, plasma exchange: received 14 cycles; Rtmb, rituximab.

On our patient's first hospital admission, he was diagnosed with the multisystem inflammatory syndrome (MIS-C), for which he received MIS-C therapy. He was readmitted to the hospital four months later with multiple organ damage, probably of autoimmune origin, triggered by SARS-COV-2 post-infection.

After PEX, our patient underwent ADAMTS13 dosing. This procedure delivers the deficient enzyme (ADAMTS13) and eliminates autoantibodies that inhibit ADAMTS13 activity. In some cases of acquired TTP with ADAMTS13 deficiency, antibodies may be undetectable, probably related to the lack of sensitivity of the available methods[9] and the sensitivity of anti-ADAMTS13 antibodies of IgM or IgA type[10], or due to altered synthesis or secretion of ADAMTS13[11]. Therefore, the result was interpreted in normal ranges.

Additionally, the patient presented features of MAHA associated with TMA in the clinical context of anemia, reticulocytosis, schistocytosis, elevated lactate dehydrogenase, undetectable haptoglobin, hemoglobinuria, and severe thrombocytopenia.

The PLASMIC score discriminates between PTT and other TMA, such as atypical uremic syndrome, reliably assesses the likelihood of a severe deficit of ADAMTS13 activity in patients with TMA and could help improve the accuracy of clinical assessment and be beneficial when ADAMTS13 testing is unavailable[12,13]. Gupta et al. reported that the PLASMIC score could also be a good predictor to identify pediatric patients with an ADAMTS13 activity less than 10%[14].

On admission to the PICU, a PLASMIC score of 7 we obtained in the patient (platelet count < 30,000/μL, hemolysis, no active cancer, no solid organ or stem

cell transplantation, no diarrhea, INR < 1.5, creatinine < 2.0 mg/dL). Therefore, the PLASMIC score predicted ADAMST13 deficiency and high suspicion of TTP and guided the management of PEX according to its score.

RT-PCR for SARS-CoV-2 was negative on readmission, but serologic testing was positive for SARS-CoV-2 IgG, similar to a 14-year-old male with a previous infection with SARS-COV-2 who developed a TTP with a favorable evolution after PEX sessions[7].

The association of other viruses, such as human immunodeficiency virus, human lymphotropic lymphoma virus-1, hepatitis C, dengue, influenza, cytomegalovirus, human herpesvirus 8, human herpesvirus 6, and parvovirus B19, with the acute development of TTP has been described[15]. The late development of this complication, however, is unclear. Oka and Nohgawa described a case of acquired TTP associated with EBV reactivation in a 37-year-old healthy male who recovered spontaneously without any treatment[16]. As the mechanism is still under study, further clinical reports and studies are needed.

In the PICU, management was performed with hemodynamic support, corticosteroids, immunoglobulin, rituximab, and fresh frozen plasma. The patient showed a gradual but ineffective response while waiting for the initiation of PEX, which began 11 days after the diagnosis of TTP. It has been shown that the use of immunosuppressive therapy together with PEX improves the therapeutic response and decreases the days of PEX, avoiding the complications of this procedure[17].

During PEX, a partial response was evidenced with a decrease in MAHA and partial clinical improvement of the patient. Unfortunately, due to the lack of resources needed to maintain PEX, the patient only received 14 cycles, and the target platelet count > 150 x 10^3/μL on two control measurements, or more days as a cut-off point for completion of this procedure, was not achieved (Table 1). PEX is used in patients with a presumptive or confirmed clinical diagnosis of TTP. It is used in all patients because, if left untreated, they progress to neurologic deterioration or renal failure. Mintz et al. have described the use of up to two cycles of PEX in patients with TTP in a clinical trial. In this study, of seven patients with a second cycle of PEX, four achieved remission[18]. Another report described up to 25 daily sessions of PEX without complications and with a favorable evolution[7]. A recent study on the use of PEX in a PICU in Chile reported complications related to the apheresis circuit, hypotension, anaphylaxis, or transfusion-related acute lung injury[19]. Although the mean number of PEX sessions in this study was lower than that in our patient because the pathologies evaluated were different, no hemorrhagic complications were reported[19].

The delay in the initiation of treatment, the premature interruption of PEX, and the suspicion of recurrent acquired TTP were the possible causes of the patient's poor response to treatment, with severe renal and neurological compromise. Analysis of the disease course suggested TMA diagnosis that was partially treated with corticosteroids, immunomodulatory therapy with immunoglobulin, rituximab, and PEX. The genetic panel to rule out hereditary TTP, complement-associated hemolytic uremic syndrome, and alterations of vitamin B12 metabolism tested negative, confirming acquired TTP.

One of the limitations of this report is the insufficient evidence in the literature to conclude that the immune response to SARS-CoV-2 caused acquired TTP. Further studies are required to confirm a causal relationship between these two conditions.

In conclusion, the presentation and clinical course of acquired TTP probably associated with SARS-CoV-2 was severe. Early diagnosis and prompt initiation of combination therapy are essential for a better prognosis.

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 has this document.

Conflicts of interest

The authors declare no conflict of interest.

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CASO CLÍNICO

Leucodistrofia megalencefálica con quistes subcorticales: la importancia del diagnóstico temprano

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Resumen

Introducción: *La leucoencefalopatía megalencefálica con quistes subcorticales es una leucodistrofia poco frecuente, asociada con mutaciones en los genes MLC1 y GlialCAM. La forma clásica se caracteriza por macrocefalia, neurodesarrollo temprano normal o con retraso seguido por un periodo de pérdida lenta de habilidades motoras, con ataxia cerebelosa y espasticidad; algunos pacientes desarrollan trastornos del movimiento y crisis convulsivas. La resonancia magnética muestra afección difusa generalizada de la sustancia blanca con edema y quistes subcorticales.* **Caso clínico:** *Se presenta el caso de dos hermanas de 6 y 10 años con historia de retraso psicomotor y macrocefalia, hijas de padres consanguíneos. La mayor inició con crisis convulsivas a los 4 años y espasticidad sin pérdida de la marcha autónoma; la menor presentó un cuadro clínico similar. La resonancia magnética mostró una alteración difusa de la sustancia blanca y quistes subcorticales en los lóbulos temporales. El electroencefalograma detectó actividad epileptiforme focal. Se logró el control de las crisis convulsivas al iniciar el tratamiento con carbamazepina. Por secuenciación, se encontró una variante homocigota del gen MLC1 en el exón 3: c.255T>G (p.Cys85Trp).* **Conclusiones:** *Las leucodistrofias son enfermedades raras que representan un desafío para su diagnóstico. Los hallazgos clínicos, radiológicos y moleculares permiten la certeza del diagnóstico, la dirección adecuada de las intervenciones y el ajuste al pronóstico de cada una. La mutación c.255T>G fue descrita previamente en pacientes sudamericanos, lo que sugiere que podría tratarse de una variante específica de poblaciones latinas.*

Palabras clave: *Leucodistrofia. Leucoencefalopatía megaloencefálica. Quistes subcorticales. MLC1.*

Megalencephalic leukoencephalopathy with subcortical cysts: the importance of early diagnosis

Abstract

Background: *Megalencephalic leukoencephalopathy with subcortical cysts is a rare type of leukodystrophy associated with mutations in the MLC1 and GlialCAM genes. The classic form is characterized by macrocephaly, early or delayed normal neurodevelopment followed by a period of slow motor skill loss, with cerebellar ataxia and spasticity; some patients develop movement disorders and seizures. Magnetic resonance imaging shows widespread diffuse white matter involvement with*

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edema and subcortical cysts. **Case report:** *We describe the case of two sisters aged 6 and 10 years, consanguineous parents, with a history of psychomotor delay and macrocephaly. The older sister presented with seizures at the age of 4 years and spasticity without loss of gait; the younger sister had a similar clinical picture. Magnetic resonance imaging showed diffuse alteration of the white matter and subcortical cysts in the temporal lobes. Electroencephalogram detected focal epileptiform activity. Seizure control was achieved upon initiation of carbamazepine treatment. By sequencing, a homozygous variant of the MLC1 gene was found in exon 3: c.255T>G (p.Cys85Trp).* **Conclusions:** *Leukodystrophies are rare diseases that represent a diagnostic challenge. Clinical, radiological, and molecular findings allow diagnostic certainty, the appropriate direction of interventions, and adjustment to the prognosis of each entity. The c.255T>G mutation was previously described in a South American patients, suggesting that it is a specific variant to Latin populations.*

Keywords: *Leukodystrophy. Megalencephalic leukoencephalopathy. Subcortical cysts. MLC1.*

Introducción

Aunque la leucodistrofia es una enfermedad que puede presentarse a cualquier edad, se presenta con mayor frecuencia durante la infancia y la adolescencia. La incidencia de la leucodistrofia es de 1 en 7000 a 50,000 nacidos vivos. En 1995, van der Knaap et al.[1] describieron por primera vez la leucoencefalopatía megalencefálica con quistes subcorticales (MLC, *megalencephalic leukoencephalopathy with subcortical cysts*). Se trata de un trastorno neurológico raro de incidencia desconocida, aunque se han reportado casos en casi todas las áreas geográficas. Exhibe mayor frecuencia en áreas con alta consanguinidad, como los países mediterráneos, la India, Turquía y China[1-3].

La presentación clínica abarca desde el retraso del desarrollo psicomotor hasta un cuadro de regresión neurológica. En la mayoría de los casos, los pacientes inician con síntomas neurológicos, principalmente motores, con hipotonía o espasticidad, alteraciones en la marcha y caídas frecuentes. Algunas leucodistrofias pueden manifestar distonía o ataxia, crisis convulsivas, trastornos de conducta, hiperactividad, falla escolar, sordera y alteraciones en el perímetro cefálico, principalmente macrocefalia. Otras manifestaciones no neurológicas incluyen alteraciones oculares, como cataratas, retinitis pigmentosa y mancha rojo cereza, problemas endocrinológicos (como hipotiroidismo o falla suprarrenal), problemas dentales (como hipodontia), anormalidades esqueléticas, organomegalias y alteraciones dérmicas[4].

La manifestación más común en la forma clásica de MLC es la macrocefalia; esta característica, en la mayoría de los casos, se observa desde el nacimiento o antes del primer año de vida. El neurodesarrollo temprano puede ser normal o con retraso leve, seguido por un periodo de pérdida paulatina de habilidades motoras, con ataxia cerebelosa, espasticidad y problemas de deglución; la mayoría de los afectados pierden la marcha en la adolescencia. El deterioro cognitivo ocurre más tardíamente que la afección motora. No es frecuente, pero se pueden observar problemas de lenguaje, conducta y bajo rendimiento escolar. Es común que los pacientes desarrollen epilepsia y, ocasionalmente, estado epiléptico; algunos presentan trastornos del movimiento, como distonía, atetosis y tics. Los traumatismos craneoencefálicos leves pueden provocar un deterioro transitorio en algunos pacientes, con crisis convulsivas, pérdida prolongada del estado de alerta y deterioro motor con una recuperación gradual[5-8].

Un segundo fenotipo menos frecuente se caracteriza por una evolución benigna, donde la macrocefalia está presente desde el nacimiento, aunque frecuentemente ocurre durante el primer año de vida. En algunos pacientes el perímetro cefálico puede normalizarse. El neurodesarrollo es normal o con ligera discapacidad intelectual y algunos signos de autismo, hipotonía y torpeza motora, sin datos de regresión. Algunos pacientes presentan crisis convulsivas, cuya intensidad varía desde muy leve hasta el estado epiléptico[9].

La resonancia magnética (RM) de cráneo es útil en el abordaje de las leucodistrofias y fundamental en el caso de la MLC. La sustancia blanca hemisférica cerebral muestra una alteración difusa con edema leve; la sustancia blanca central, incluyendo el cuerpo calloso, la cápsula interna y el tallo, se encuentran mejor preservadas, aunque no son completamente normales. La sustancia blanca cerebelosa, en general, muestra una intensidad de señal levemente incrementada, sin edema. Los quistes subcorticales, casi invariablemente, se ubican en la región temporal anterior y, con frecuencia, en la frontoparietal. Estos quistes pueden aumentar de tamaño y en número; incluso, en algunos individuos se vuelven de gran volumen, ocupando gran parte de la sustancia blanca frontoparietal. Con el tiempo, el edema de la sustancia blanca disminuye, al igual que la hiperintensidad, pero se observa atrofia cerebral. Las imágenes ponderadas por difusión revelan una mayor difusividad de la sustancia blanca anormal[10,11].

En este trabajo se describe el caso de una familia de padres consanguíneos con dos hijas afectadas por una mutación en *MLC1*, que fue descrita anteriormente en una familia de Sudamérica, lo que destaca la importancia de realizar estudios diagnósticos en forma temprana.

Caso clínico

Padres primos hermanos. El primer producto, de sexo femenino, sin antecedentes perinatales ni neonatales, sostén cefálico a los 9 meses, sedestación a los 14 meses, deambulación a los 24 meses, bisílabos a los 12 meses, control de esfínteres a los 4 años. Fue valorada por primera vez a los 4 años por caídas repetidas de su propia altura y crisis convulsivas focales motoras, por lo que inició tratamiento con fenitoína. A la exploración física se observó los siguiente: perímetro cefálico de 54 cm (p98), pobre desarrollo de lenguaje expresivo, receptivo normal, pares craneales, fondo de ojo y fuerza normales, espasticidad Ashworth 1, hiperreflexia, clonus agotable bilateral, Babinsky y sucedáneos presentes, sensibilidad normal, sin signos cerebelosos ni marcha atáxica, pero con amplia base de sustentación. Electroencefalograma epileptogénico en la región frontal izquierda. La RM a los 6 años mostró desmielinización de toda la sustancia blanca; en las regiones temporales se identificaron quistes subcorticales y adelgazamiento de la corteza cerebral (Figs. 1 y 2). La última evaluación se realizó a los 10 años y se observó un perímetro cefálico de 55 cm (p98), sin crisis convulsivas, aún con marcha autónoma, pero con espasticidad de grado 2 de Ashworth en los miembros inferiores y de grado 1 en los miembros superiores, y mejoría leve en lenguaje expresivo.

La hermana menor fue valorada a los 2 años de edad. Sin antecedentes perinatales ni neonatales, sostén cefálico a los 8 meses, sedestación a los 12 meses, deambulación a los 22 meses, primeros bisílabos a los 12 meses y retraso importante en el lenguaje expresivo. A la exploración física se encontró perímetro cefálico de 51 cm (p98), adecuado lenguaje receptivo, pares craneales normales, fondo de ojo normal, fuerza 5/5, tono normal, reflejos de estiramiento +++/++++, sin Babinsky ni signos cerebelosos. A los 3 años de edad inició con crisis convulsivas focales; a la exploración se encontró perímetro cefálico de 52 cm (p98), espasticidad Ashworth 1 en los miembros inferiores, marcha autónoma, mejoría en el lenguaje expresivo, electroencefalograma epileptogénico focal y RM con las mismas características que su hermana, por lo que se inició tratamiento con carbamazepina. La última evaluación se realizó a los 6 años de edad: se encontró libre de crisis, macrocefalia, incremento de la espasticidad en los miembros inferiores sin pérdida de la marcha, problemas de lenguaje y retraso en el aprendizaje.

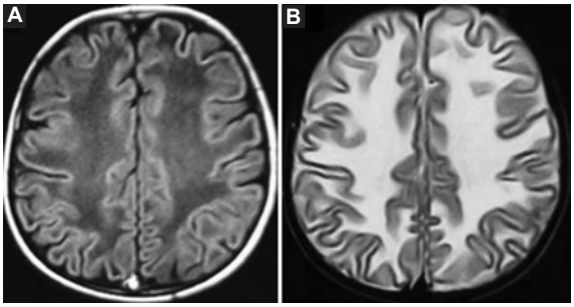

Figura 1. Resonancia magnética de cráneo en secuencias T1 y T2. **A:** imagen axial potenciada en T1 *spin-echo*. **B:** imagen axial potenciada en T2 *fast spin-echo*, del encéfalo, a nivel de los centros semiovales frontales. Se observa una importante desmielinización de la materia blanca en forma generalizada y adelgazamiento de la corteza cerebral.

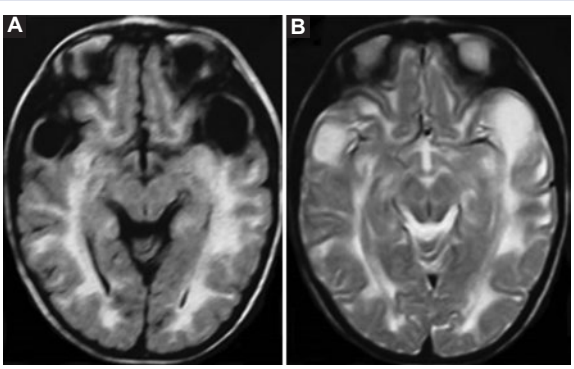

Figura 2. Resonancia magnética. Imágenes en FLAIR T2 y T2 *fast spin-echo*. **A:** imagen axial potenciada en densidad protónica o FLAIR T2. **B:** imagen axial potenciada en T2 *fast spin-echo* a nivel del mesencéfalo y los lóbulos temporooccipitales que muestra importante desmielinización de la materia blanca bilateralmente, excepto en el mesencéfalo. Se observan lesiones quísticas temporales y adelgazamiento de la corteza cerebral.

De acuerdo con los hallazgos en la RM, se realizó un estudio molecular en busca de mutaciones en el gen *MLC* en las dos pacientes. Se reportó una mutación ya descrita en el exón 3: c.255T>G (p.Cys85Trp) (Fig. 3).

Discusión

El reconocimiento temprano de las enfermedades de la sustancia blanca representa un desafío para el pediatra y

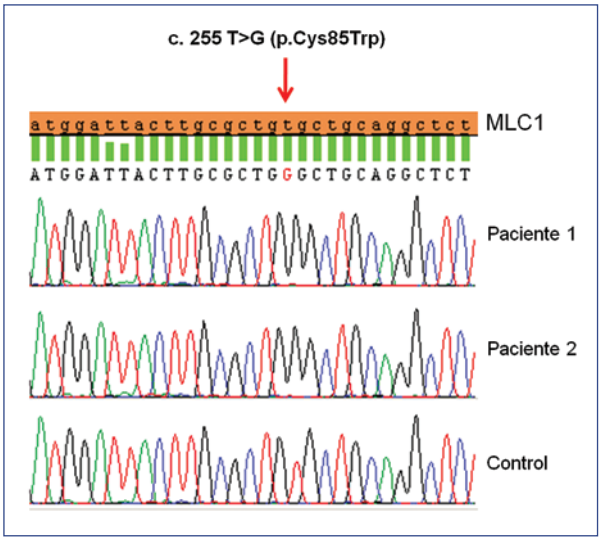

Figura 3. Secuenciación del gen *MLC*. En el exón 3 se identificó la presencia de guanina en lugar de timina en el residuo 255 de la secuencia original.

el neurólogo pediatra. Una vez que se sospecha una leucodistrofia, la RM es esencial para el diagnóstico diferencial. Por ejemplo, en la enfermedad de Alexander, las alteraciones son de predominio frontal, captan gadolinio y la degeneración quística es rara. En la enfermedad de Canavan, típicamente se ven afectados los tálamos y el globo pálido, algo no observado en la MLC; no es habitual la formación de quistes en la sustancia blanca y en la espectroscopía hay elevación de N-acetil aspartato. En los pacientes con deficiencia de laminina alfa-2, la afección de la sustancia blanca se asemeja a lo observado en la MLC, pero sin quistes subcorticales; en el cuadro clínico predominan las manifestaciones de distrofia muscular congénita, principalmente debilidad e hipotonía, algo no característico en la MLC. Otro diagnóstico por considerar es la gangliosidosis-GM2, en la que predomina la afección de los núcleos de la base y los tálamos[1].

En conjunto con el genetista, el abordaje se vuelve esencial y se puede confirmar con la secuenciación única de *MLC1,* aprovechando la ventaja de que este gen forma parte de diversos paneles genéticos, como el de la leucodistrofia, cuando el intento diagnóstico parte del hallazgo de imagen con anormalidad en la sustancia blanca, o de paneles de sobrecrecimiento, cuando parte de descartar causas genéticas de macrocefalia, acelerando el momento diagnóstico. La detección temprana asegura el asesoramiento genético para las familias y permite prevenir la ocurrencia de casos mediante el diagnóstico prenatal, la preimplantación y la detección de portadores sanos.

La MLC se ha clasificado en tres tipos: MLC1 (#604004), por mutaciones en el gen *MLC1* localizado en el cromosoma 22q13.33; MLC2A (#613925), por mutaciones en el gen *GlialCAM* localizado en el cromosoma 11q24.2 (estas dos formas tienen un patrón de herencia autosómico recesivo); y MLC2B (#613926), que se asocia con un patrón de herencia autosómico dominante por mutación en *GlialCAM.* El 75% de los casos se asocian con variantes patogénicas en MLC1 y el 25% en HEPACAM; el 5% podrían explicarse por un tercer gen[1,12,13].

El gen *MLC1* (OMIM) abarca 12 exones y codifica una proteína de 377 aminoácidos con ocho dominios transmembrana. Se expresa mayormente en la membrana de los astrocitos del cerebelo, el tracto olfatorio, el tallo cerebral y el tálamo; su expresión es baja en la corteza cerebral, el estriado y el hipocampo. El segundo gen involucrado en este padecimiento codifica la proteína de adhesión celular GlialCAM, altamente expresada en el hígado y en el sistema nervioso central, en concreto en las neuronas y las células gliales, donde se une a MLC1. También se conoce como HEPACAM, ya que originalmente se encontró en el carcinoma hepatocelular[14-17]. Ambas proteínas interactúan como moduladores funcionales de la homeostasis del agua y de diferentes iones, incluyendo el canal de cloro 2 (CLC-2), la Na,K-ATPasa, el canal de potasio Kir4.1, el canal permeable de calcio TRPV4, el canal de agua AQP4, las conexinas 30 y 43, la ATPasa vacuolar, la bomba de protones reguladora de la acidez, el complejo distrofina-glucoproteína, la sintrofina, la distrobrevina, la caveolina, la zónula *occludens* 1 (ZO-1) y el canal aniónico regulado por volumen (VRAC), lo que llevó a clasificar a la MLC como la primera leucodistrofia producida por una canalopatía cerebral[17-19].

Los estudios con ratones *knock-out* Mlc1 y GlialCAM han mostrado una deficiencia en la liberación de cloruro y otros aniones orgánicos, como taurina, glutamato e, incluso, adenosín trifosfato, lo que conduce al edema crónico en el astrocito que precede a la vacuolización de mielina en un proceso mediado por la retención de agua[20], que se manifiesta como quistes.

La anormalidad en la absorción y la dispersión del potasio extracelular conduce a la deficiencia de este ion, provocando actividad epileptiforme por un umbral convulsivo alterado[21,22]. Lo anterior explica que el 70% de los pacientes presenten crisis convulsivas antes de los 20 años y el 15-20% desarrollen un evento de estado epiléptico[1,22]. Las crisis convulsivas son de fácil control en la mayoría de los casos[9]. En ambas pacientes descritas en este reporte se ha logrado el control completo de las crisis con monoterapia con carbamazepina. Este

fármaco tiende a estabilizar a los pacientes con canalopatías dependientes de potasio. Una mejor comprensión de la forma en que MLC1/GlialCAM influyen en los procesos de transducción de señales podría ayudar a identificar fármacos que mejoren los síntomas de los pacientes con MLC.

Se han descrito alrededor de 90 variantes en *MLC1*. En China se ha descrito la tasa más alta de mutaciones nuevas (50-70%), en comparación con la población turca (38%) y la india (5%)[23-25]. Existen también mutaciones recurrentes, como la c.136delT en Irán. En India se ha reportado la c.135_136insC en alrededor del 73% de los pacientes, y en China se ha reportado una mutación en c.772-1G>C en el 23.7%[23,24]. Tsujino et al.[26] informaron que c.278C>T es una mutación común en el 85.7% de los pacientes japoneses con MLC, y también ha sido previamente descrita en pacientes italianos, turcos y finlandeses. En familias judías libanesas predomina la mutación c.176G>A, y en el este de la India predomina la variante c.135dupC[26-28]. Resulta interesante que la mutación c.255T>C, encontrada en ambas pacientes aquí estudiadas, fue descrita por primera vez por Leegwater et al.[28] en un par de hermanos procedentes de Sudamérica. La consanguinidad, el efecto fundador y la existencia de puntos calientes en la secuencia de los genes son algunas de las explicaciones para la alta frecuencia de algunas mutaciones en ciertas áreas geográficas. La consanguinidad es un factor de riesgo importante, documentado en nueve de las 11 familias que se estudiaron inicialmente en el camino hacia la identificación del gen causal de la MLC1[29].

La terapia génica con vectores virales comienza a ser una posibilidad de tratamiento, como se ha intentado en otros trastornos cerebrales[5,30], aunque se debe tener especial cuidado para controlar los niveles de expresión de *MLC1*, ya que su elevada expresión podría ser perjudicial. Otra opción es el uso de chaperones farmacológicos para restaurar la expresión de MLC1 en la membrana plasmática, tal como se ha probado en otras enfermedades, como la fibrosis quística[31]. Sin embargo, al no existir una opción de este tipo a la fecha, el tratamiento, por lo pronto, se basa en la terapia física, de lenguaje, educación especial, uso de antiepilépticos y ciertas medidas para evitar factores como la fiebre y los traumas craneales.

Responsabilidades éticas

Protección de personas y animales. Los autores declaran que para esta investigación no se han realizado experimentos en seres humanos ni en animales.

Confidencialidad de los datos. Los autores declaran que han seguido los protocolos de su centro de trabajo sobre la publicación de datos de pacientes.

Derecho a la privacidad y consentimiento informado. Los autores han obtenido el consentimiento informado de los pacientes o sujetos referidos en el artículo. Este documento obra en poder del autor de correspondencia.

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Conflicto de intereses

Los autores declaran no tener ningún conflicto de intereses.

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LETTER TO THE EDITOR

Nebulizations: are they a safe practice?

¿Son las nebulizaciones una práctica segura?

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Dear Editor,

We have recently reviewed articles on inhalation devices, such as the article by Madrid et al. published in the October issue in the *Boletín Médico del Hospital Infantil de México*. In that article, the authors report exhaled nitric oxide measurement by analyzing different devices[1]. Although the management of respiratory infections by respiratory devices has been performed since 1828 with liquid atomizers[2], the first pressurized inhaler was introduced to the market in the 1950s for epinephrine delivery. Since then, new and improved devices have been developed with differences in design, construction, sound, output, and particle size.

The effectiveness of nebulization depends on several factors, such as the compressor-nebulizer system used, its maintenance, the characteristics of the drug to be nebulized, and the proper inhalation technique used by the patient[3]. In this regard, there is a minimum deposition of the drugs in the upper airways since the speed of the inhaled nebulizer droplets is similar to that of the child's respiratory flow, which minimizes the impact on the oropharynx. Therefore, in the best of cases, it will vary from 5 to 10% of the inhaled dose[3]. The minimum inspiratory flow required for the aerosol produced by a nebulizer to reach the lungs is 6-8 L/min. However, significant drug losses occur as much of the medication is retained in the nebulizer as dead space or is lost to the ambient air during exhalation[2,3]. Lung scintigraphy studies similar to those reported by Madrid et al.[1] have shown that only 10% of the dose initially placed in the nebulizer will be deposited in the lungs.

With the emergence of the COVID-19 pandemic, the CDC (Centers for Disease Control and Prevention) have recommended limiting the use of nebulizers and have even contraindicated their use, arguing that "nebulization causes saline droplets in contact with the respiratory tract to break up and produce a fine mist that becomes a vapor that transmits disease" and that "airborne transmission of the COVID-19 virus may be possible in specific circumstances, and in settings where procedures are performed or treatments are administered that may generate aerosols (e.g., administration of a drug by nebulization)." In addition, studies have shown that particles contaminated with SARS-CoV-2 can remain in the environment for up to one hour[4].

The Society of Critical Care Medicine recommends using ventilators when nebulizing a patient in a negative pressure room, as it is considered a high-risk procedure for contagion[5]. The GINA 2020 Guidelines recommend avoiding their use during the pandemic or substituting them for other devices[6].

In contrast, the British National Institute for Health and Care Excellence (NICE) recommends using nebulizers, arguing that SARS-CoV-2 remains in the nebulizer mask in liquid form and not in aerosol form with the potential for contagion[7]. Furthermore, a recent systematic review by pulmonologists concluded that it is not possible to define nebulization as a source of contamination by aerosol particle dispersion[8].

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The American College of Chest Physicians and the American College of Asthma, Allergy, and Immunology recommend that drugs, such as bronchodilators and steroids be administered with other types of inhalation devices, such as pressurized devices, fine mist inhalers, and dry powder devices with inhalation chambers. These devices are not only more effective in drug delivery but are also more hygienic, more practical, and prevent the dispersion of infectious particles not only from COVID-19 but from respiratory viruses and bacteria[9,10].

A study published in the *Pediatrics* journal concluded that parents prefer to use nebulized medications due to their perception of inhalation drug delivery, even when inhalation devices are portable, easy to use, and less expensive[11].

There is controversy regarding the possible dispersion of contaminating viral particles in the environment using nebulizers. Some authors suggest that the particles generated may remain in the environment for more than 10 minutes and, therefore, may contribute to the contagion of other individuals in the same room[2-4]. Other authors mention that when the mask is correctly positioned and fixed to the face, the viral particles will not be dispersed in the environment since they will remain adhered to the mask itself[4,10]. Unfortunately, correct mask placement in pediatrics is often a challenge because patients do not accept it attached to the face.

Considering that inhaled drugs with spacers or chambers have a good effect and that there is no evidence that the effect of nebulizer administration is comparatively better, we believe that nebulized drug therapy should be reserved only for a few cases in which no other method of delivery is available. This is especially the case in patients with acute respiratory disorders, regardless of the virus involved, and mainly in pediatric patients, in whom it is often difficult to achieve a complete seal between the mask and the face.

On this basis, the question arises as to whether nebulizers are indispensable or already obsolete.

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.

Conflicts of interest

The authors declare no conflict of interest.

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