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Early acquisition of *Pseudomonas aeruginosa* in Mexican children with cystic fibrosis

Infección temprana por *Pseudomonas aeruginosa* en niños mexicanos con fibrosis quística

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Abstract

INTRODUCTION: Chronic infection with *Pseudomonas aeruginosa (P. aeruginosa*) is associated with a greater deterioration of pulmonary function, increase in hospitalizations and shorter survival in patients with cystic fibrosis.

OBJECTIVES: To evaluate the age of first detection of *P. aeruginosa*, effectiveness of the eradication treatment and prevalence of other pathogens in airway cultures.

MATERIALS AND METHODS: A cohort of patients with CF diagnosed within first year of life, who had follow-up at University Hospital was studied. Respiratory cultures were obtained quarterly. Patients with infection by *P. aeruginosa* received eradication treatment.

RESULTS: Thirty-five patients were included. During follow-up, 608 cultures were performed. In 34.7%, normal flora was detected, *S. aureus* 32.8%, *P. aeruginosa* 20.4%. Median age at first positive culture was 9 months. Eradication was achieved in 81.8%.

CONCLUSIONS: In this cohort of patients with cystic fibrosis, the acquisition of *P. aeruginosa* is earlier than in other groups reported in literature.

KEYWORDS: Chronic infection; *Pseudomonas aeruginosa*; Cystic fibrosis; Eradication.

Resumen

INTRODUCCIÓN: La infección con *Pseudomonas aeruginosa* (*P. aeruginosa*) en pacientes con fibrosis quística se asocia con deterioro de la función pulmonar, incremento de hospitalizaciones y menor supervivencia.

OBJETIVOS: Evaluar la edad de la primera infección por *P. aeruginosa*, la efectividad del tratamiento y la prevalencia de otros patógenos en cultivos de la vía aérea obtenidos por expectoración espontánea o aspirado nasofaríngeo.

MATERIALES Y MÉTODOS: Estudio retrospectivo, de cohorte, en el que se incluyeron pacientes con fibrosis quística diagnosticados en el primer año de vida. Se obtuvieron cultivos respiratorios (exudado faríngeo o expectoración espontánea) al momento del diagnóstico. Los pacientes con *P. aeruginosa* recibieron tratamiento de erradicación.

RESULTADOS: Se registraron 35 pacientes, a quienes se efectuaron 608 cultivos. En 34.7% se detectó flora normal, en 32.8% *S. aureus* y en 20.4% *P. aeruginosa*. La mediana de edad al primer cultivo positivo fue de 9 meses.

CONCLUSIONES: La infección por *P. aeruginosa* fue más temprana en pacientes con fibrosis quística que en otros grupos reportados en la bibliografía.

PALABRAS CLAVES: Infección crónica; *Pseudomonas aeruginosa*; fibrosis quística; erradicación de la infección.

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INTRODUCTION

Cystic fibrosis is a genetic, multisystemic disease, characterized by thick airway secretion, bacterial infection, and neutrophilic chronic inflammation, which leads to progressive lung damage and chronic respiratory failure.

Pseudomonas aeruginosa (*P. aeruginosa*) is the most important bacteria in the airway of these patients. Reports estimated that by age eighteen 80% of patients are infected with this microorganism. It is associated with earlier deterioration of pulmonary function, increase in hospitalizations and lower survival.[1,2,3]

For this reason, clinical practice guidelines recommend detection and identification of *P. aeruginosa*, as well as eradication treatment once detected.[4] Currently, there are no studies that evaluate the clinical course, age of first positive culture for *P. aeruginosa* and prevalence of other bacteria in children less than 6 years with cystic fibrosis in Mexico. The main objective of this study was to evaluate, in a cohort of pediatric patients with cystic fibrosis diagnosed within the first year of life, age of first detection of *P. aeruginosa*, effectiveness of eradication treatment, time to first recurrence and prevalence of different bacteria.

MATERIALS AND METHODS

A retrospective study of a cohort of patients who attended the cystic fibrosis clinic at University Hospital in Monterrey, Mexico was conducted. Patients diagnosed, within the first year of life, by neonatal screening or clinically, born between January 2004 and October 2017, who were followed regularly in the clinic until December 2018, were included. Diagnosis was established with a sweat chloride value ≥60 mmol/L obtained by quantitative pilocarpine iontophoresis.[5]

Patients were followed in the cystic fibrosis clinic in accordance with international guidelines.[4]

A respiratory sample was obtained by spontaneous expectoration or oropharyngeal swab (OP), at initial visit, repeated every three months and after eradication treatment. Additional samples were obtained if patients had respiratory symptoms. Age of initial infection by *P. aeruginosa* was defined as the age in months in which patients had the first positive culture for this bacterium. Both first detection of *P. aeruginosa* and recurrences during longitudinal follow-up were included in the analysis.

Family socioeconomic level was evaluated following national guidelines. Families were classified as high income (AB, C+ and C) or low income (C-, D and E).[6]

Pulmonary function tests were performed by spirometry in the Pulmonary Function Laboratory of the Center for the Prevention and Rehabilitation of Chronic Pulmonary Diseases (CEPREP), following the guidelines of the American Thoracic Society (ATS).[7]

The tests were performed on the Platinum Elite Series plethysmograph (MGC Diagnostics) with Breeze 7.2.B software (MGC Diagnostics). The results were expressed as a percentage of the predicted values for forced expiratory volume in the first second (FEV1) and for forced vital capacity (FVC) according to sex, age and height.

For chest tomography evaluation, the Bhalla score was used.[8]

Eradication treatment *of P. aeruginosa*

All patients with *P. aeruginosa* infection received eradication treatment with inhaled tobramycin

300 mg every 12 hours for 28 days plus oral ciprofloxacin 30 mg/kg/day every 12 hours for 14 days.

Statistical Analysis

Continuous variables were reported as mean and standard deviation or median and range, as appropriate, categorical variables as absolute numbers and percentages. For the analysis of categorical variables, contingency tables and chi-square test were used. For comparison of medians, the Mann-Whitney *U* test was used. A value of $p < 0.05$ was considered statistically significant. Data was analyzed using SSPS version 20.0 (IBM Corp, Armonk, NY).

The study's protocol was approved by the institutional Committee of Ethics and Research and patients and their families signed informed consent.

RESULTS

Thirty-five patients were included, with a median follow-up of 6 years (1-6). Median age at diagnosis was 31.5 days (11-180) for screened patients and 214 days (62-331) for unscreened patients ($p < 0.001$). Demographic characteristics are summarized in **Table 1**.

Microbiologic outcome

Across all clinical visits, 608 cultures were obtained. In 77 (12.6%) of them two or more bacteria were detected, with a total of 685 bacterial isolations. Bacteriological findings are summarized in **Table 2**.

Median age at first positive culture for *P. aeruginosa* was 9 months (2-57). For unscreened patients median age was 8 months (2-57) and for screened patients, 10.5 months (2-20) ($p = 0.96$). For female patients, median age was 10.5 months (4-33) while for males, 8 months (2-57) ($p = 0.46$). No significant difference was observed between median age of *P. aeruginosa* acquisition in different socioeconomic status: for high income it was 8 months (2-25) while for low income, 11 months (4-57) ($p = 0.32$).

In the study, 22 patients (62%) had their first positive culture for *P. aeruginosa* before the first year of life. Fourteen (40%) cultures were positive in the first appointment to the clinic.

Table 1. Demographic characteristics of 35 patients with cystic fibrosis in Northeast México

Variables	(n = 35)	%
Sex		
Female	15	42.9%
Male	20	57.1%
Mutation		
Phe508del Homozygous	7	20.0%
Phe508del Heterozygous	14	40.0%
Other	7	20.0%
Not studied	7	20.0%
Diagnosis		
Neonatal screening	12	34.3%
Signs and symptons	23	65.7%
Socioeconomic status		
High	21	60.0%
Low	14	40.0%

Table 2. Bacterial epidemiology in cystic fibrosis patients in Northeast México

Bacteria	Isolates (n = 685)	%
Normal flora	239	34.8%
P. aeruginosa	140	20.4%
S. aureus	217	31.8%
Enterobacteria	34	4.9%
H. influenzae	12	1.8%
K. pneumoniae	16	2.4%
MRSA	7	1.0%
Other	20	2.9%

Eradication of first isolation was achieved in 27 cases (81.8%) of 33 patients who received eradication treatment. There was no difference in time to recurrence of *P. aeruginosa* between screened and unscreened patients. For both groups, median time to first recurrence was 15 months (3-47) (p = 0.78).

Of the 35 patients, 21 (60%) completed 6 years of follow-up. For this group of patients, the mean of the Bhalla score was 21.3 points (±3.4). The spirometry performed at school admission, the forced expiratory volume in the first second (FEV1) average was 100% (±17.19), the forced vital capacity (FVC) average was 106.71% (±16.7) and the CVF/FEV1 ratio was 90.7% (±7.6). Regarding the clinical and functional status of the patients, there were no significant differences between screened and unscreened. **Table 3**

DISCUSSION

P. aeruginosa is a microorganism that is widely distributed in nature and its optimum growth temperature is 37º C. In patients with cystic fibrosis, it is the main pathogen responsible for morbidity and mortality.[8]

Studies support a relationship between the first isolation of *P. aeruginosa* and clinical deterioration in cystic fibrosis patients. Pitman *et al* found a strong association between early age of acquisition of *P. aeruginosa* and severity of lung disease.[2]

Age of acquisition of *P. aeruginosa* is related to interactions between environmental and host-dependent factors. Host-dependent risk factors for early acquisition are genetic mutation, female sex, history of meconium ileus, among others.[9,10] In respect to environmental factors, geographic variability in the prevalence of lower airway infections in patients with cystic fibrosis has been reported.[11,12]

Other factors, such as high temperature, can cause higher prevalence of *P. aeruginosa* in the environment, and therefore stimulating the acquisition of infection.

Epidemiological information about first detection of *P. aeruginosa* in Latin American countries is scarce. In Argentina mean age at first isolation has been reported at 1.18 months.[13]

In a study conducted in Australia median age of first detection was 30.5 months. Also, mean duration of reported eradication was 19 months, comparable to 15 months in this study.[14] On the contrary, Psoter *et al* reported a mean age of 1.3 years in the USA.[15] This study showed that,

Table 3. Characteristics of clinical and functional status of 21 patients at 6 years old

Characteristics	Cohort	Screened	Unscreened	p
Bhalla Score	21.3 (± 3.4)	22.3 (± 2.81)	20.7 (± 3.59)	0.24
FVC (6 a)	106 (± 16.7)	111.6 (± 25.64)	104.7 (± 12.18)	0.40
FEV1(6 a)	100 (±17.1)	108 (± 28.55)	96.8 (± 9.6)	0.18
FEV1/FVC	90.7 (±7.6)	93.8 (± 9.5)	89.5 (± 6.8)	0.30
BMI	15.7 (±1.67)	15.5 (± 2.16)	15.8 (± 1.37)	0.41

FVC: forced vital capacity; FEV1: forced expiratory volume 1; BMI: body mass index.

compared to patients who remained without *P. aeruginosa,* those who acquired it were more likely to reside in places with higher average daily temperatures (13.8 *vs* 12.9 ° C) and higher precipitation (0.14 *vs* 0.12 mm).[15]

In this study, one of the factors that can cause early acquisition of the bacteria is probably the high average annual temperature in the region, as the climate in Northeast Mexico is semi-arid and average annual temperature is around 24 - 25 °C.[16] This association has been reported in previous studies.[14,17]

Low socio-economic level has significant negative effects on cystic fibrosis, as it's associated with worse nutritional status and poorer outcomes. In Mexico it has been reported that low socioeconomic level is the most detrimental factor in the outcome of patients with cystic fibrosis,[18] although it was not statistically significant in our group.

Moreover, a study showed that Hispanic patients with cystic fibrosis residing in the United States had lower survival compared to non-Hispanic patients and one of the factors suggested by the authors to explain this ethnic difference were genetic polymorphisms not currently known.[19] In another study, after adjusting for socioeconomic status and clinical severity, Hispanic patients had 2.81 the mortality rate compared to non-Hispanic patients, which underscores the importance of intrinsic and extrinsic ethnic factors related to survival.[20]

This study has certain limitations, including a small sample size and that all patients were recruited from a single center. Other factors associated with respiratory exacerbations and with early acquisition of *P. aeruginosa* are air pollution, in particular PM10 and PM 2.5, as well as exposure to tobacco smoke.[21] These factors were not studied in our cohort and should be investigated.

However, the same medical team followed patients longitudinally, using standardized protocols, which enhances consistency among patient management.

This is one of the scarce reports that investigates prevalence of pathogens in young cystic fibrosis patients in Mexico and demonstrates that in these patients acquisition of *P. aeruginosa* is earlier than in other cohorts, potentially leading to a poorer quality and length of life, thus alerting on the need of quick detection and intensive standardized eradication therapy.

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