

Kinetic of SARS-Cov2 shedding among COVID-19 treated children in Morocco

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Abstract

Background: Diagnosis of COVID-19 is based on real-time polymerase chain reaction (RT-PCR) and possibly thoracic computed tomography (CT) scan. Samples are usually nasopharyngeal or oropharyngeal.

In Morocco, all children diagnosed with COVID-19 have so far been hospitalized in an isolation unit and managed according the national protocol established by the Ministry of Health. They receive a tight medical surveillance, radiological and biological explorations.

Material and Methods: Children (N= 60) aged from 1 year to 18 years were recruited in the pediatrics' Hospital at the University Hospital Center Ibn Sina, Rabat. All children were confirmed with COVID-19 between March 24, 2020 to June 5, 2020 based on the national case definition derived from the criterion of the World Health Organization (WHO) interim guidance

Demographic, epidemiological and clinical data were collected from medical records.

Result: The average age of the patients was 8.7 years +/- 6.2, ranging from 4 months to 18 years. All age groups

were affected. In addition, a male predominance is noted, with 53.3% (32/60) of males compared to 46.6% (28/60) of females, and an M/F ratio of 1.1.

All patients were in contact with a patient of Covid-19, 85% of which belonged to a family cluster.

In our series 91.1% (56/60) of the cases were asymptomatic and 8% (4/60) of the cases were in a mild to moderate form, the symptoms presented were; cough (3/60) and respiratory distress (1/60). Infants with a moderate form, presented a ground of immune deficiency .

All the patients were declared cured and did not receive any specific treatments. However, infants in a moderate form received a combination of hydroxychloroquine, azithromycin and lopinavir/ritonavir.

The average molecular RT-PCR negativation time is 14 days with an extreme reaching 33 days. 48% of cases negate their RT-PCR on the 9th day; 10% of cases negate their RT-PCR on the 14th day; 7% of cases negate their RT-PCR on the 15th day; 17% of cases negate their RT-PCR on the 19th day and 18% of cases negate their RT-PCR after 20 days.

The mean RT-PCR negativation as a function of clinical expression is identical (14 days) in symptomatic (4 cases/60 cases) and asymptomatic (56 cases/60 cases) patients.

The mean RT-PCR negativation is 13.7 days (+/- 6) for females compared to 14 days (+/- 6.5) for males.

Conclusion: the average negativation is 14 days. Gender and clinical expression do not seem to be a determining factor in the kinetics of RT-PCR, while age appears to be the only determining factor.

Keywords: RT-PCR, Covid -19, SARS- CoV-2, ACE2.

Introduction

The new Coronavirus, henceforth named SARS-CoV-2, has emerged in Wuhan, Hubei province, China. It has caused a series of acute and atypical respiratory diseases. In December 2019. This viral infection has been named COVID-19 [1]. The virus's high transmissibility from human-to-human has provoked a widespread pandemic in 216 countries, regions, or territories, with more than 6 million cases confirmed until June 2, 2020 [2].

Children seem to be the less affected category, but data regarding the epidemiological and clinical features of COVID-19 in pediatric are scarce. Most of them are mainly based on limited case series.

Among the huge number of laboratory-confirmed cases in Italy, children accounted for 1,2% of the overall cases. The case-fatality rate was estimated to 7,2 % versus 2,3 % in China, but deaths amongst pediatric age group were not notified, stating that no specific risk factor could be identified. [3,4] As described in a case study in Shenzhen, (China), the roots of transmission of SARS-CoV-2 are similar among children and adults. However, most children show less severe symptoms [3]. Larger epidemiological and clinical studies could speculate on the protection of children against Covid-19 versus adults. One

the stated hypothesis, is that SARS-CoV-2 Spike (S) protein has been shown to bind to the angiotensin-2 converting enzyme (ACE2) [5,6] and children may be protected because this enzyme is less mature at a younger age [7].

Moreover, the diversity of alpha-coronavirus to which they were exposed in their prime-childhood could induce a cross-immunity [8].

There is also 3growing evidence that defensive barrier in children against SARS-CoV2 could be explained also by the innate ability of the pediatric immune system to react and adapt to new pathogens [9,10,11].

Another theory is the protective role of the Bacillus Calmette-Guérin (BCG) vaccine ~~in~~ against COVID-19. In fact, BCG vaccination was associated with heterologous immunity to other pathogens, potentially through a phenomenon called "trained immunity" involving innate cells such as macrophages, monocytes and epithelia [12].

Diagnosis of COVID-19 is based on real-time polymerase chain reaction (RT-PCR) and possibly thoracic computed tomography (CT) scan. Samples are usually nasopharyngeal or oropharyngeal [13].

Previous studies on Coronavirus 2019 (COVID-19) have mainly focused on the epidemiological, clinical, and radiological characteristics of patients with confirmed infections [13, 14, 15, 16]. Little attention has been paid to monitoring the RT-PCR kinetics of these patients.

In Morocco, all children diagnosed with COVID-19 have so far been hospitalized in an isolation unit and managed according the national protocol established by the Ministry of Health. They receive a tight medical surveillance, radiological and biological explorations, Nine days after their admission, these patients are declared cured and can be safely discharged from the hospital, only if they are meeting the following criteria:

- Improvement in clinical signs.
- Apyrexia for three days.
- Two negative RT-PCRs at 24-hour intervals [17].

Due to the lack of consistent information regarding the shedding of SARS-CoV2 RNA during the course of the disease among child, the aim the present research is to set the evolution pattern of SARS-CoV2 detection among the pediatric population during the natural history of the disease.

Material and Methods

Patients: Children (N= 60) aged from 1 year to 18 years were recruited in the pediatrics' Hospital at the University Hospital Center Ibn Sina, Rabat. All children were confirmed with COVID-19 between March 24, 2020 to June 5, 2020 based on the national case definition derived from the criterion of the World Health Organization (WHO) interim guidance [18].

The enrollment of the children was set during the investigation of their family members as a COVID-19 case index or a close contact to a COVID-19 confirmed case.

Demographic, epidemiological and clinical data were collected from medical records.

Clinical samples

The nasopharyngeal sample was performed by swabbing into the nostril following the floor of the nasal cavities. A slow rotation was applied and the swab was then placed immediately into a sterile transport tube containing 2 mL either of viral transport medium (VTM) or sterile saline. All collected samples were stored at +4°C until the shipment to the virology laboratories.

SARS-CoV2 RNA extraction

RNA was extracted from 200 µL clinical specimens, and eluted into 60 µL elution buffer by using a commercial

extraction kit (MagaBio plus Virus RNA purification kit, Bioer, China), yielding high purified Viral RNA. Extraction was performed using a GenePure Pro, fully automatic Nucleic Acid purification System (Bioer, China).

SARS-CoV qRT-PCR

5µL of extracted RNA (from the final eluted material at the end of the automated viral RNA extraction) was used as input material for the multiplex single-step PCR for three genes using the Gene finder One-Step qRT-QPCR kit (COVID-19 Plus RealAmp Kit (OSANG, Korea). The said SARS-CoV-2 qRT-QPCR test provided positive results when a signals for the N, RdRp and/or E genes were detected with a Ct within the valid range (<40 Ct) and endpoint above the minimum setting, as per manufacturer protocol. The amplification process and detection were performed using CFX96TM Real Time PCR detection system with analysis on CFX Manager Version 3.1. (Bio Rad, USA)

The qRT-PCR was then performed for all children included in the study on the 9th day of their hospitalization. The child was declared cured if there is a disappearance of clinical signs related to the virus with apyrexia for more than 3 days and two negative molecular samples at D9 and D10. If there is no negativation of the RT PCR, it is necessary to repeat two other samples at D14 and D15.

Results

Between March 24 and June 05, 2020, information about 60 children infected with SARS-CoV-2 was collected at the Rabat Children's Hospital.

The average age of the patients was 8.7 years +/- 6.2, ranging from 4 months to 18 years. All age groups were affected (Fig. 1). Approximately 75% of all cases were

over 5 years of age, while infants accounted for only 8% of cases.

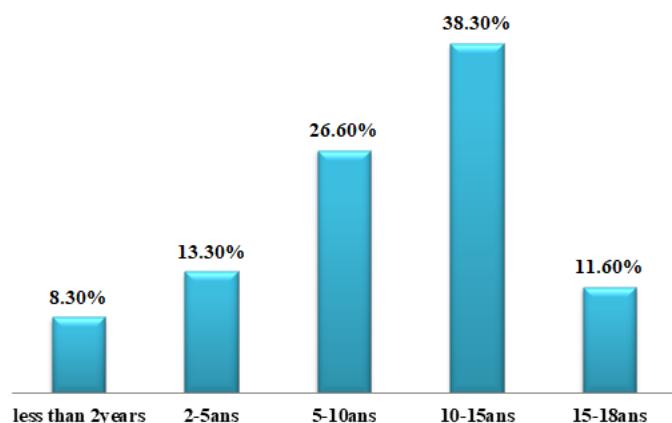


Fig. 1: Distribution of confirmed cases of COVID-19 by age group.

In addition, a male predominance is noted, with 53.3% (32/60) of males compared to 46.6% (28/60) of females, and an M/F ratio of 1.1.

All patients were in contact with a patient of Covid-19, 85% of which belonged to a family cluster.

In our series 91.1% (56/60) of the cases were asymptomatic and 8% (4/60) of the cases were in a mild to moderate form, the symptoms presented were; cough (3/60) and respiratory distress (1/60). Infants with a moderate form, presented a ground of immune deficiency (co-infection of cytomegalovirus and human immunodeficiency virus). All the patients were declared cured and did not receive any specific treatments. However, infants in a moderate form received a combination of hydroxychloroquine (10 mg/kg/day for 10 days), azithromycin (20 mg/kg/day on the 1st day, then 10 mg/kg/day from the 2nd to the 4th day as a single dose) and lopinavir/ritonavir (0.2 ml/kg twice daily).

The average molecular RT-PCR negativation time is 14 days with an extreme reaching 33 days. 48% of cases negate their RT-PCR on the 9th day; 10% of cases negate their RT-PCR on the 14th day; 7% of cases negate their

RT-PCR on the 15th day; 17% of cases negate their RT-PCR on the 19th day and 18% of cases negate their RT-PCR after 20 days. (Fig. 2)

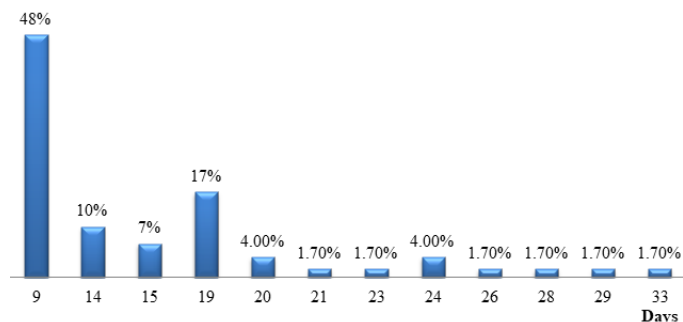


Fig. 2: RT-PCR negativation rate as a function of time
The average negativation of RT-PCR as a function of age is as follows (Fig. 3):

- In infants under 2 years of age, the average is 11.2 +/- 3 days.
- In children between 2 and 5 years of age, the average is about 14.8 +/- 7.5 days.
- For children between 5 and 10 years old, the average is 13 +/- 4.8 days.
- For children between 10 and 15 years of age, the average is about 14.9 +/- 7.4 days.
- For children between 15 and 18 years of age, the average is 16 +/- 5.6 days.

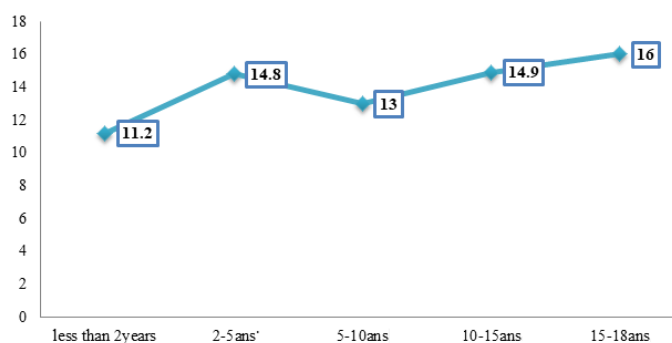


Fig. 3: The COVID-19 RT-PCR negativation kinetics as a function of age

The mean RT-PCR negativation as a function of clinical expression is identical (14 days) in symptomatic (4

cases/60 cases) and asymptomatic (56 cases/60 cases) patients.

The mean RT-PCR negativation is 13.7 days (+/- 6) for females compared to 14 days (+/- 6.5) for males. (Fig. 4)

- The rate of RT-PCR negativation at 9th day was 25% (15 cases/60 cases) for males versus 26% (16 cases/60 cases) of total cases for females.

- The rate of RT-PCR negativation at 14th day was 6.6% (4 cases/60 cases) for males versus 5% (3 cases/60 cases) for females.

- The rate of RT-PCR negativation at 15th was 5% (3 cases/60 cases) for males versus 1.6% (1 case/60 cases) for females.

- The RT-PCR negativation rate at 19th was 8.3% (5 cases/60 cases) for males versus 5% (3 cases/60 cases) of total cases for females.

- The rate of RT-PCR negativation beyond 20 days was 8.3% (5 cases/60 cases) in males versus 8.3% (5 cases/60cases) of total cases in females.

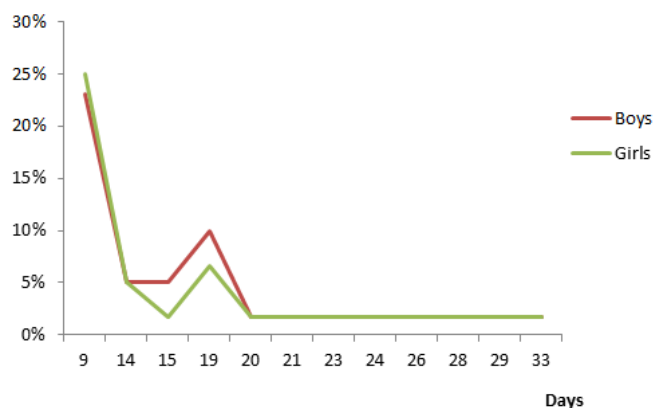


Fig. 4: The COVID-19 RT-PCR negativation kinetics as a function of gender

Discussion

The number of papers about the current global pandemic has been increasing dramatically since the COVID-19 outbreak. However, only very few studies have been

carried out to study the RT-PCR kinetics of COVID-19 throughout the course of the disease in children.

In our series, the average age is 8.7 years +/- 4.8

A large study of 171 patients under 16 years of age found a median age of 6.7 years [19], another study found that the age of onset of the disease ranged from 1 day to 18 years, [20] which is consistent with our study.

In our series all cases had close contact with a patient with Covid-19 disease, of which 85% were among an intrafamilial cluster.

In a study conducted by Xiaoxia Lu and colleagues, the contamination was mainly of intra-family origin (90%) [21], of which 76% had close contact with confirmed cases of COVID-19.

Xia et al reported that 13% of their cases had a history of close contact with a confirmed COVID-19 case [21]. However, in two other studies, the rate of close contact with a confirmed COVID-19 case is 90% [21,22], which is consistent with our study.

A male predominance of 53.3% is noted in our study with a M / F ratio of 1.1.

Dong and colleagues reported that 56.6% of the 2,143 patients in their study were boys [24]. Xiaoxia Lu and colleagues found male predominance in 60% of the 171 cases in their study [19], which is consistent with our study.

Some authors suggest that the male predominance is attributed to increased expressions of ACE2 in men [25].

Other authors have found no significant difference in the expression of ACE2 between males and females [26].

Another study suggests that estrogen could have a protective effect against the coronavirus [27] in our Moroccan context, this could be related to a male predominance in the population.

The clinical study showed that 91% of children with COVID 19 had an asymptomatic to mild form (Cough 5%), and only one infant had a moderate form (respiratory distress), no severe forms were recorded.

A study by Dong and colleagues showed that over 90% of all children were asymptomatic or had mild or moderate forms. Corresponding thus with the results of our series [24]

In our series, the average negativation time of RT-PCR molecular tests is 14 days +/- 6.2 with a maximum of 33 days. RT-PCR became negative on day 9 in 51.6% of cases (31 cases/60 cases).

In a study of 10 children under 10 years old, RT-PCR became negative after 12 days on average, with a maximum of 22 days [28].

In a study carried out by Qiu and his colleagues on 36 children, the average duration of RT-PCR negativation is 14 days [22] which is consistent with our results.

In our study we note a shorter duration of RT-PCR negativation in infants, compared to the rest of the age group with an average of 11 days, Kai-qian Kam and colleagues reported the case of an infant whose RT-PCR became negative on the 17th day of admission [29], which is not consistent with our results.

This rapid viral clearance in infants may be due to healthier respiratory tracts due to lower exposure to pollution and cigarette smoke [12].

In infants under 1 year of age, the production of specific antibodies against viral antigens by plasma cells is maximal, [30] which may be a protective factor for infants against COVID-19.

In our study, the mean of RT-PCR negativation in symptomatic (4 cases / 60 cases) and asymptomatic (56 cases / 60 cases) patients is identical (14 days), which

means that the clinical expression is unlikely to affect the kinetics of RT-PCR.

In our study, the average negativation is slightly short for females 13.7 days, compared to 14 days for males.

Ai Tang Xiao and colleagues found that the mean of negative RT-PCR tests was shorter in the female sex. However, the difference was not statistically significant. [31] It appears that gender is not a determining factor in the kinetics of COVID-19 molecular tests.

In conclusion, the average negativation is 14 days. Gender and clinical expression do not seem to be a determining factor in the kinetics of RT-PCR, while age appears to be the only determining factor.

In the light of these data, we can say that it would be more practical to perform the first RT-PCR control from day 14 onwards regardless of age, gender or clinical expression in order to rationalize resources.

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