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Article

Infection and Colonization pattern in children with chronic granulomatous disease: a prospective cohort study

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Abstract:

Backgrounds: Chronic granulomatous disease (CGD) is one of the most common primary immunodeficiency (PID) diseases characterized by an increased tendency to infection with catalase-positive organisms due to defective phagocytic killing mechanism. This study aimed to identify the microbiological colonization pattern in children with CGD and study the different characteristics encountered in different genetic subtypes. A prospective study was conducted on 18 CGD patients at Alexandria University Children's Hospital. *Methods:* Samples for cultures from the oropharynx, skin and nasal cavity were obtained when the patients were in an infection-free period. The children's characteristics and different manifestations were studied in relation to the CGD subtypes. *Results:* We found more normal flora colonizing p47 deficient patients (p= 0.015) while more pathogenic bacteria and Asperigellus fungal spp were detected in p91, X-linked CGD patients (p= 0.021, p=0.004 respectively). *Conclusions:* Different frequencies of normal flora and pathogenic species could be isolated in colonization cultures of different CGD subtypes which may affect the infection pattern in each specific group.

Keywords: Chronic granulomatous disease, NADPH oxidase, bacterial infections, normal flora, colonization, risk factors.

Introduction

Chronic granulomatous disease (CGD) is a primary immunodeficiency (PID) disease of phagocytic function with X-linked or autosomal recessive inheritance. CGD is caused by mutations in genes related to one of the five components of the NADPH oxidase enzymatic complex responsible for the production of superoxide (O2−) and other reactive oxygen species (ROS) that are crucial for the intracellular killing of catalase-positive microbes (1, 2).
Laboratory diagnosis of CGD can be made by dihydrorhodamine (DHR) assay, flow cytometric analysis of the NADPH components and molecular testing (3, 4). Patients with CGD are highly susceptible to recurrent and severe infections, such as pneumonia, adenitis, osteomyelitis, and abscesses either in the skin or internal organs like the liver and lung. In addition, they suffer different inflammatory manifestations with granuloma formation which can lead to gastrointestinal or genitourinary obstruction (1). Catalase-positive organisms such as Staphylococcus aureus, Enterobacteriaceae, candida and Aspergillus spp. are the most frequently isolated agents. (5) Long-term antibiotic and antifungal prophylaxis, interferon-gamma (IFN-γ) therapy, hematopoietic stem cell transplant (HSCT) and gene therapy are the available therapeutic options in CGD.(6)

Generally, infections occur when the pathogens can colonize the host then bypass the immune mechanisms and invade the body. Many host-related characteristics may contribute to the changes in colonization pattern and, consequently, in the pathogenesis of different infections such as the disease severity, antibiotic therapy, and recurrent hospital admissions. (7) Colonization of the skin, nasal and oropharynx by infection-causing microorganisms in adults has been discussed in some studies but few studies of colonization in the pediatric population are available, especially in the immunocompromised groups. As a result, it was necessary to study the patients' characteristics and microorganism colonizing patterns in different forms of CGD. In addition, the study describes the different infectious and inflammatory manifestations encountered in those patients.

Subjects

A prospective study was conducted in the Immunology Unit, Pediatric Department, Alexandria University from the first of January 2021 to the end of December 2021. Eighteen children with CGD were enrolled in the study according to the Pan-American Group for Immunodeficiency (PAGID) and the European Society for Immunodeficiency (ESID) diagnostic criteria. (8) The study protocol was approved by the ethics committee of the Faculty of Medicine, Alexandria University, Egypt and the ethics committee approval number was 0201276. Informed written consent was obtained from the children's guardians.

Methods

Eighteen patients with CGD were recruited in an infection-free state during the period of the study. Diagnosis of CGD was based on flow cytometry Dihy-drorhodmaine (DHR) 123 assay(9)

and assessment of the defective NADPH oxidase component with glycoprotein assay by flow cytometry.(3) Patients' characteristics and different infectious and inflammatory complications were recorded.

The microbiological colonization pattern was assessed twice three month apart during the infection-free period. Infection and inflammation free state was assessed clinically and laboratory by normal white cell count and negative acute phase reactants (CRP and ESR). Systemic antibiotics were not given for at least 2 weeks before swabbing then microbiological swabs were done. Swabs were soaked first in sterile saline and then applied to the area to be swabbed. Swabs were inoculated onto blood agar, macConkey's agar and sabouraud's dextrose agar plates. Blood and MacConkey's agar plates were incubated at 37 and read at 24 and 48 hours. Sabouraud's dextrose agar plates were left at room temperature and growth of fungi was observed for up to 7 days. Bacteria or fungi were considered either absent or present for all statistical analyses.

The following specimens were collected:

1- Oropharyngeal swab: samples were collected by rolling a swab in the oral cavity, tonsilar and posterior pharynx areas. All collections were performed in the morning.
2- Nasal swabs: an anterior nasal swab in one of the nares was performed in the morning.
3- Axillary and groin swabs: swabs were collected from the right axillary fossa and groin area not heavily exposed to faeces, wipes, or emollients (e.g., diaper area). Skin swabs were gently rolled on a 2 cm^2 surface for 5 seconds.

Statistical analysis of the data

Data were fed to the computer and analyzed using IBM SPSS software package version 20.0. (Armonk, NY: IBM Corp). Qualitative data were described using numbers and percentages. The Shapiro-Wilk test was used to verify the normality of distribution. Quantitative data were described using range (minimum and maximum), mean, standard deviation and median. A Chi-square test for categorical variables was performed to compare different groups. Monte Carlo correction for chi-square was done when more than 20% of the cells have an expected count of less than 5. F-test (ANOVA) for normally distributed quantitative variables was performed to compare between more than two groups. The significance of the obtained results was judged at the 5% level.

Results

Table (1): Comparison between the different groups according to demographic data:

Demographic data	All cases (n = 18)	X-CGD (gp91) (n = 4)	AR-CGD (n = 14)			p
			Total AR-CGD (n = 14)	gp22 (n = 10)	gp47 (n = 4)	
Sex						
Male	13 (52.2%)	4 (100%)	9 (64.3%)	7 (70%)	2 (50%)	
Female	5 (27.8%)	0 (0%)	5 (35.7%)	3 (30%)	2 (50%)	
Age at first symptom (months)						
Min. – Max.	0.0 – 24.0	1.0 – 24.0	0.0 – 18.0	0.0 – 6.0	0.60 – 18.0	
Mean ± SD.	5.95 ± 7.77	12.75 ± 13.0	4.01 ± 4.65	2.55 ± 2.22	7.65 ± 7.35	p= 0.066
Median	3.0	13.0	3.0	2.0	6.0	
Age at diagnosis (months)						
Min. – Max.	1.0 – 144.0	1.0 – 144.0	4.0 – 120.0	4.0 – 27.0	24.0 – 120.0	
Mean ± SD.	41.1 ± 47.26	65.5 ± 71.5	34.1 ± 38.81	14.70 ± 7.57	82.5 ± 44.46	p= 0.015*
Median	18.50	58.50	18.50	14.50	93.0	
Age, at last, follow up (months)						
Min. – Max.	9.0 – 180.0	20.0 – 180.0	9.0 – 144.0	9.0 – 144.0	72.0 – 144.0	
Mean ± SD.	70.5 ± 56.94	104.0 ± 76.3	60.93 ± 49.5	40.90 ± 40.2	111.0 ± 33.1	p= 0.035*
Median	54.0	108.0	45.0	23.0	114.0	
Consanguinity	16 (88.9%)	4 (100%)	12 (85.7%)	8 (80%)	4 (100%)	
Family history	15 (83.3%)	4 (100%)	11 (78.6%)	8 (80%)	3 (75%)	

p: p-value for comparing the studied subgroups (**p91, p22 and p 47**)

*: Statistically significant at $p \leq 0.05$

Eighteen patients with CGD from15 different families were studied; 13/18 (52.2%) were males and 5/18 (27.8%) were females. Four patients with CGD had p91 phox protein deficiency (X CGD) and 14 patients had autosomal recessive forms; 10 patients with p22 phox deficiency and 4 patients with p47 phox deficiency). The four male patients with X-linked CGD were from two different families while the four patients with defective p47 were from 3 families. Consanguinity was high (88.9%) and a history of an affected family member was noticed in 15/18 patients (83.3%). The median age at onset of symptoms of all cases was 3 months (range from 0– 24 months) as some patients were diagnosed at birth due to the presence of a positive family history of CGD. The

diagnosis of all cases was made at a median age of 18.5 months (range from 1 – 144 months). Although the AR CGD patients started the manifestation earlier than the X CGD (median 3 and 13 months respectively), there was no statistically significant difference between the three different forms of CGD (p91, p22 and p47). On the other hand, there was a statistically significant difference in the age of diagnosis between the different groups with significantly lower age observed in p22 deficient patients than that in the X CGD (p91) and p47 phox protein group (median 14.5, 58.5 and 93 months respectively, p=0.015). The age at last follow up was significantly low in the p22 subtype compared with other groups reflecting a worse outcome and lower survival in that group (p= 0.035).

Table (2): Comparison between the different groups according to clinical data

	All cases (n = 18)	X-CGD (gp91) (n = 4)	AR-CGD (n = 14)			p
			Total AR-CGD (n = 14)	gp22 (n = 10)	gp47 (n = 4)	
No infectious episodes						
Min. – Max.	2.0 – 11.0	2.0 – 9.0	4.0 – 11.0	4.0 – 10.0	5.0 – 11.0	
Mean ± SD.	7.0 ± 2.28	5.75 ± 2.87	7.36 ± 2.06	7.50 ± 1.90	7.0 ± 2.71	^{F}p=0.456
Median	6.50	6.0	7.0	7.50	6.0	
No hospital infections						
Min. – Max.	1.0 – 9.0	1.0 – 2.0	1.0 – 9.0	2.0 – 5.0	1.0 – 9.0	
Mean ± SD.	3.0 ± 1.88	1.75 ±0.50	3.36 ± 1.98	3.20 ±1.14	3.75 ±3.59	^{F}p=0.300
Median	2.50	2.0	3.0	3.0	2.50	
Infectious manifestations						
Pneumonia	17 (94.4%)	4 (100%)	13 (92.9%)	9 (90%)	4 (100%)	^{MC}p=1.000
OM	8 (44.4%)	3 (75%)	5 (35.7%)	2 (20%)	3 (75%)	^{MC}p=0.088
LND abscess	9 (50%)	1 (25%)	8 (57.1%)	6 (60%)	2 (50%)	^{MC}p=0.812
Splenic abscess	3 (16.7%)	1 (25%)	2 (14.3%)	2 (20%)	0 (0%)	^{MC}p=1.000
Liver abscess	4 (22.2%)	1 (25%)	3 (21.4%)	2 (20%)	1 (25%)	^{MC}p=1.000
Lung abscess	2 (11.1%)	0 (0%)	2 (14.3%)	1 (10%)	1 (25%)	^{MC}p=0.715
Sup skin abscesses	13 (72.2%)	1 (25%)	2 (85.7%)	9 (90%)	3 (75%)	^{MC}p=0.045*
Perianal abscess	7 (38.9%)	1 (25%)	6 (42.9%)	6 (60%)	0 (0%)	^{MC}p=0.140
Lymphadenitis	9 (50%)	2 (50%)	7 (50%)	4 (40%)	3 (75%)	^{MC}p=0.812
Gastroenteritis	10 (55.6%)	2 (50%)	8 (57.1%)	7 (70%)	1 (25%)	^{MC}p=0.331
PUO	14 (77.8%)	3 (75%)	11 (78.6%)	8 (80%)	3 (75%)	^{MC}p=1.000
UTI	2 (11.1%)	1 (25%)	1 (7.1%)	1 (10%)	0 (0%)	^{MC}p=0.715
Osteomyelitis	4 (22.2%)	0 (0%)	4 (28.6%)	3 (30%)	1 (25%)	^{MC}p=0.765
Septic arthritis	2 (11.1%)	0 (0%)	2 (14.3%)	2 (20%)	0 (0%)	^{MC}p=1.000
Sepsis and septic shock	5 (27.8%)	0 (0%)	5 (35.7%)	3 (30%)	2 (50%)	^{MC}p=0.326
Thrush stomatitis	2 (11.1%)	1 (25%)	1 (7.1%)	1 (10%)	0 (0%)	^{MC}p=0.715
Inflammatory manifestations						

Hepatosplenomegaly	9 (50%)	1 (25%)	8 (57.1%)	8 (80%)	0 (0%)	^{MC}p=0.016*
Lymphadenopathy	9 (50%)	1 (25%)	8 (57.1%)	8 (80%)	0 (0%)	^{MC}p=0.016*
Failure to thrive	4 (22.2%)	0 (0%)	4 (28.6%)	4 (40%)	0 (0%)	^{MC}p=0.121
Gingivitis	2 (11.1%)	1 (25%)	1 (7.1%)	0 (0%)	1 (25%)	^{MC}p=0.190
Colitis	4 (22.2%)	1 (25%)	3 (21.4%)	2 (20%)	1 (25%)	^{MC}p=1.000
HLH	3 (16.7%)	1 (25%)	2 (14.3%)	1 (10%)	1 (25%)	^{MC}p=0.565
Pneumonitis/ ILD	2 (11.1%)	0 (0%)	2 (14.3%)	1 (10%)	1 (25%)	^{MC}p=0.715
Fate (number of deaths)	4 (22.2%)	0 (0%)	4 (28.6%)	2 (20%)	2 (50%)	^{MC}p= 0.456

χ^2: Chi square test , MC: Monte Carlo, F: F for One way ANOVA test

p: p-value for comparing the studied subgroups (**X-CGD, gp22 and gp 47**)

*: Statistically significant at p ≤ 0.05

Almost the same mean number of infectious episodes and hospital admissions was observed in different CGD groups. Pneumonia, skin abscesses and PUO were the most frequent infections in our patients but some infections occurred more frequently in certain subtypes, for example; severe infections like osteomyelitis and septicemia were present only in AR CGD patients. X-linked CGD and p47 deficient patients suffered more attacks of pneumonia (100%) and otitis media (75%) while p22 patients suffered more frequent attacks of gastroenteritis (70%) and lymph node and perianal abscesses (60% for each) but, only skin abscesses were statistically more frequent in p22 group compared with other subtypes (90%, p=0.045). As regards inflammatory manifestations, although failure to thrive and pneumonitis were found in AR CGD patients, only hepatosplenomegaly and lymphadenopathy were significantly more frequent in the p22 subtype than in X CGD (25% and 80% respectively, p=0.016).

Table (3): Normal and pathogenic flora in the colonization cultures of all studied cases. (n = 36 from both screens)

	Oropharyngeal samples (n=36)	**Axillary samples (n=36)**	**Nasal samples (n=36)**	**Groin samples (n=36)**
Normal flora:				
Moraxella	17 (47.2)	2 (5.5)	4 (11.1)	0 (0.0)
CONS	7 (19.4)	17 (47.2)	14 (38.9)	13 (36.1)
Streptococcus	9 (25)	6 (16.7)	15 (41.7)	4 (11.1)
Diphtheroid	4 (11.1)	0 (0.0)	6 (16.7)	5 (13.9)
Micrococci	2 (5.5)	17 (47.2)	4 (11.1)	7 (19.4)
Pathogenic bacteria:				
Klebsiella spp.	17 (47.2)	15 (41.7)	15 (41.7)	18 (50)
Acinetobacter spp.	23 (63.9)	21 (58.3)	19 (52.8)	11 (30.5)
Proteus spp.	0 (0.0)	0 (0.0)	0 (0.0)	4 (11.1)
E.coli	3 (8.3)	0 (0.0)	0 (0.0)	6 (16.7)

Pseudomonas spp.	0 (0.0)	0 (0.0)	0 (0.0)	2 (5.5)
MRSA	0 (0.0)	1 (2.8)	2 (5.5)	4 (11.1)
Spore bearer bacilli	1 (2.8)	0 (0.0)	0 (0.0)	2 (5.5)
Fungal				
Candida spp	15 (41.7)	8 (22.2)	5 (13.9)	16 (44.4)
Aspergillus spp	0 (0.0)	1 (2.8)	4 (11.1)	2 (5.5)

Table 3 shows the microbiological colonization pattern of all studied cases. Two swabs from each site were cultured and revealed one to multiple bacterial and/ or fungal isolates (Min-Max: 1 – 5) with an almost fixed pattern of colonization noticed in each patient through different swabs. In all patients, 32/36 (88.9%) of oropharyngeal cultures, 16/36 (44.4%) of axillary and groin cultures and 17/36 (47.2%) of nasal cultures grew pathogenic microorganisms. Acinetobacter spp. was the predominant isolated pathogenic bacteria in all samples except groin samples in which Klebsiella spp. was the most frequent (50%). As regards fungal isolates, Candida spp was the most frequent in all samples.

Table (4): Distribution of the colonizing microbe according to the CGD subtype.

	Normal flora	Pathogenic flora	Fungal		Total
			Candida	Asperigellus	
X-CGD (gp91)	24 (28.9%)	47 (56.6%)	6 (7.2%)	6 (7.2%)	83 (100%)
AR-CGD	**132 (46%)**	**116 (40.4%)**	**38 (13.2%)**	**1 (0.35%)**	**287 (100%)**
gp22	93 (44.5%)	88 (42.1%)	27 (12.9%)	1 (0.48%)	209 (100%)
gp47	39 (50%)	28 (35.9%)	11(14.1%)	0 (0.0%)	78 (100%)
χ^2p	0.015*	7. 0.021*	0.317	MCp=0.004*	

χ^2: Chi-square test, MC: Monte Carlo

p: p-value for comparing the studied categories

*: Statistically significant at $p \leq 0.05$

When comparing the three CGD subtypes, it was found that there was a statistically significant high percentage of normal flora colonizing p47 deficient patients (50% of isolated microbes in that group and p= 0.015) while a higher percentage of pathogenic organisms was detected in X CGD (56.6%, p= 0.021). Regarding fungal isolates, pathogenic Asperigellus was significantly higher in p91, X CGD patients (six isolates, 7.2% and p=0.004).

Discussion

One of the important data to judge the severity of CGD is the genetic subtype. Eighteen patients with CGD (13 male and 5 female patients) were followed for one year; 4/18 (22.2%) were X CGD (p91 phox deficiency) and 14/18 (77.8%) were AR CGD with the p 22 phox deficiency was the most frequent (10/18, 55.5%). This high frequency of autosomal recessive forms especially p22 deficiency was reported in previous Egyptian studies (3, 10, 11) and could be attributed to the high rate of consanguinity (88.9% in this study). The same in Turkey, Köker et al found that most patients have an AR genotype (56.2%) with p22 deficiency was also the most frequent AR form (22.5%).(12) AR CGD was also common in countries around Egypt but defects in NCF1 and NCF2 genes coding for p47phox and p67 phox respectively were more frequent.(13-16) On the other hand, in Western countries, the X-linked type (p91) was more common.(17-20)

Another important piece of data to judge the severity of CGD is the age of presentation and diagnosis. In all cases, the median age at onset of symptoms and that of diagnosis were 3 months and 18.5 months respectively. The AR CGD patients started the manifestation earlier than the X CGD (median 3 and 13 months respectively) but there was no statistically significant difference between the three different forms of CGD. While, significantly lower age of diagnosis was observed in p22 deficient patients than that in the X CGD (p91) and p47 group (median 14.5, 58.5 and 93 months respectively, p=0.015).

Older ages of presentation and diagnosis were observed in other Egyptian studies; in Meshal et al study the mean ages of presentation and diagnosis were 18.3 ± 26 months and 55.9± 50 months respectively(10) while in El-Mokhtar M et al the median ages (1.17 years and 2.01years respectively).(11) Older ages of onset and diagnosis were also reported in Egypt by El Hawary et al (median: 8.5 months and 33 months respectively). But unlike this study, they reported that in XCGD, the presentation and diagnosis were at a younger age than in AR CGD; median: 2 months and 18 months respectively in XCGD and 8 and 36 months in AR CGD however there was no statistically significant difference obtained when comparing the age at diagnosis among different subgroups (p= 0.058) with the p47-phox-deficient patients being diagnosed at an older age than our study (median: 102, 93 months respectively)(3). The same result as El Hawary et al study was reported in many countries with X CGD started and diagnosed significantly earlier than the AR CGD.(12, 14, 16, 17, 20, 21) This could be explained by the more frequent p47 AR CGD in these countries which have a milder phenotype and tend to present and diagnosed at an older age, the same observation for p47 patients was found in our study.

Similarly, In Jordon, they reported that p22 and p67 AR CGD were diagnosed at the earliest ages (mostly below one year and 1-5 years respectively) while the p47 patients at the oldest (11-20 years). In Mexico, the same median age of onset but older than that of diagnosis was reported (3 and 30 months respectively).(19) Older age of diagnosis were also reported in Morocco (mean age: 5.1 years, range: 0.16–13 years) while in Brazil younger age at onset of symptoms but older age at diagnosis was reported (45 days and 23 months respectively).(22, 23) The younger ages reported in this study high lightened the importance of the high index of suspicion and diagnosis of suspected infants especially when there is suggestive family history.

An important criterion which can affect microbial colonization is the frequency of hospital

admissions. In this study, there was no significant difference among different CGD subtypes regarding the frequency of infections and hospital admissions. The same result was reported by El Hawary et al in Egypt with almost the same mean number of hospitalization in different groups.(3)

As regards manifestations, severe infections like osteomyelitis and septicemia occurred only in AR CGD patients. Pneumonia and otitis media occurred more frequently in X-linked CGD and p47 deficient patients while p22 patients suffered more gastroenteritis (70%) and lymph node and perianal abscesses (60% in each), yet only skin abscesses were statistically more frequent in the p22 group compared with other subtypes (90%, p=0.045). Also, hepatosplenomegaly and lymphadenopathy were significantly more frequent in the p22 subtype than in X CGD (25% and 80% respectively, p=0.016). Unlike this study, El Hawary et al in Egypt found that pneumonia was more frequent in AR-CGD followed by recurrent abscesses, while in X-CGD, the most common presentations were recurrent abscesses, anemia, and lymphadenopathy but there was no correlation between different manifestations and the deficient proteins. (3) In Iran Fattahi et al found that severe infections were more frequent in the patients with XL-CGD with pneumonia and pulmonary abscess being the most significant. (14) In a large cohort in India, the results were similar to ours; they found that more frequent attacks of pneumonia encountered in p91 X CGD patients and skin abscesses were more common in the p22 subtype while deep abscesses were more common in the p91 subtype. (24) In the UK, there was no difference in the frequency of pneumonia and suppurative adenitis between the X CGD and AR CGD but like our study, septicemia and osteomyelitis were more frequent in AR CGD.(17)

Few studies reported the microbiological colonization pattern in children but none was done in immunocompromised groups. In this study, 88.9% of oropharyngeal cultures, 44.4% of axillary and groin cultures and 47.2% of nasal cultures grew pathogenic microorganisms. Acinetobacter spp. was the predominant isolated pathogenic bacteria in all samples except nasal samples in which Klebsiella spp. was the most frequent while candida spp was the most frequent isolated fungus in all samples. In comparison with the CGD subtypes, it was found that there was a high percentage of normal flora colonizing p47 deficient patients while a higher percentage of pathogenic bacteria and pathogenic Asperigellus was detected in p91, X CGD patients. Some researchers tried to study the effect of the patients' characteristics on the colonization pattern in children. Kusahara et al studied the oropharyngeal colonization in children admitted to the pediatric intensive care unit (PICU) and found that 41.8% of studied patients had pathogenic microorganisms and most of them had chronic diseases and showed a longer hospitalization time. (7) The higher percentage of pathogenic species in the oropharynx reported in our study (88.9%) is attributed to the different patients' characteristics like the patients' age, the disease itself, its severity and frequent infections and hospitalizations. In addition, our patients received long term prophylactic antimicrobials which add to the alteration of colonizing microbes.

In addition, there was a difference in the type of pathogenic organisms isolated between the two studies; in Kushara et al study in addition to methicillin-resistant staphylococcus aureus (MRSA) and Klebsiella spp, Citrobacter spp, Enterobacter spp, Serratia spp and Providencia spp were identified in oropharyngeal cultures and no fungal isolates could be reported. (7)

Regarding nasal colonization, Gesualdo F et al reviewed the prevalence and risk factors of MRSA nasal colonization in children and found increased MRSA prevalence in children with chronic diseases and frequent hospitalizations and antibiotic use. (25) In another study, he found that most MRSA-nasal colonized patients had been hospitalized previously. (26) Regarding skin colonization, Meylan P et al studied staph aureus as a predisposing factor for the development of atopic dermatitis and found that infants testing positive for S. aureus skin colonization were younger than uncolonized subjects. (27)

To the best of our knowledge, this study is one of few studies conducted on pediatric CGD patients in Egypt and the first one to study the microbiological colonization pattern specific for those patients. However the study is limited by the short duration and the small number of patients. The small number of patients was caused by the fact that some patients had severe infections and died before studying their colonization pattern and others were not free of infection during the study duration.

From this, we **conclude** that different patients' characteristics could be risk factors for the change in the pattern of their colonizing microbes emphasizing the importance of the patient's age, state of health, frequency of hospitalization and antibiotic use in determining these changes. All these risk factors were present in our patients in addition to the peculiar immunocompromised state of CGD. Added to this was the effect of different CGD subtypes on the frequency and type of colonized pathogenic organisms which was related not only to the genetic subtype itself but also to the different patients' characteristics in each type. Further longer duration and wider scale multicenter studies are mandatory to determine the risk factors and differences in colonization patterns during infection-free periods in CGD patients. This may help to determine the causative organisms when bacteremia happens and help to decide the prophylactic antimicrobials for clearance before the occurrence of infection.

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Ethics approval and consent to participate

This study was approved by the ethics committee of the Faculty of Medicine, Alexandria University, Egypt and the ethics committee approval number was 0201276. The IRB number is 00012098 and the FWA number is 00018699. The parents of all participants provided written consent.

Conflict of interest

None

References

1. Arnold DE, Heimall JR. A Review of Chronic Granulomatous Disease. Advances in therapy. 2017;34(12):2543-57.
2. Anjani G, Vignesh P, Joshi V, Shandilya JK, Bhattarai D, Sharma J, et al. Recent advances in chronic granulomatous disease. Genes & Diseases. 2020;7(1):84-92.
3. El Hawary R, Meshaal S, Deswarte C, Galal N, Abdelkawy M, Alkady R, et al. Role of Flow Cytometry in the Diagnosis of Chronic Granulomatous Disease: the Egyptian Experience. Journal of clinical immunology. 2016;36(6):610-8.
4. Elloumi HZ, Holland SM. Diagnostic assays for chronic granulomatous disease and other neutrophil disorders. Methods in molecular biology (Clifton, NJ). 2014;1124:517-35.
5. Bennett N, Maglione PJ, Wright BL, Zerbe C. Infectious Complications in Patients With Chronic Granulomatous Disease. Journal of the Pediatric Infectious Diseases Society. 2018;7(suppl_1):S12-S7.
6. Thomsen IP, Smith MA, Holland SM, Creech CB. A Comprehensive Approach to the Management of Children and Adults with Chronic Granulomatous Disease. The journal of allergy and clinical immunology In practice. 2016;4(6):1082-8.
7. Kusahara D, Peterlini M, Pedreira M. Children's oropharyngeal colonization upon admission at a pediatric intensive care unit. Acta Paulista de Enfermagem. 2007;20(4):421-7.
8. Conley ME, Notarangelo LD, Etzioni A. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clinical immunology (Orlando, Fla). 1999;93(3):190-7.
9. Richardson MP, Ayliffe MJ, Helbert M, Davies EG. A simple flow cytometry assay using dihydrorhodamine for the measurement of the neutrophil respiratory burst in whole blood: comparison with the quantitative nitrobluetetrazolium test. Journal of immunological methods. 1998;219(1-2):187-93.
10. Meshaal S, El Hawary R, Abd Elaziz D, Alkady R, Galal N, Boutros J, et al. Chronic granulomatous disease: Review of a cohort of Egyptian patients. Allergologia et immunopathologia. 2015;43(3):279-85.
11. El-Mokhtar MA, Salama EH, Fahmy EM, Mohamed ME. Clinical aspects of chronic granulomatous disease in upper Egypt. Immunological investigations. 2021;50(2-3):139-51.
12. Köker MY, Camcıoğlu Y, van Leeuwen K, Kılıç S, Barlan I, Yılmaz M, et al. Clinical, functional, and genetic characterization of chronic granulomatous disease in 89 Turkish patients. The Journal of allergy and clinical immunology. 2013;132(5):1156-63.e5.
13. El Kares R, Barbouche MR, Elloumi-Zghal H, Bejaoui M, Chemli J, Mellouli F, et al. Genetic and mutational heterogeneity of autosomal recessive chronic granulomatous disease in Tunisia. Journal of human genetics. 2006;51(10):887-95.
14. Fattahi F, Badalzadeh M, Sedighipour L, Movahedi M, Fazlollahi MR, Mansouri SD, et al. Inheritance pattern and clinical aspects of 93 Iranian patients with chronic granulomatous disease. Journal of clinical immunology. 2011;31(5):792-801.

15. Bakri FG, Mollin M, Beaumel S, Vigne B, Roux-Buisson N, Al-Wahadneh AM, et al. Second Report of Chronic Granulomatous Disease in Jordan: Clinical and Genetic Description of 31 Patients From 21 Different Families, Including Families From Lybia and Iraq. Frontiers in immunology. 2021;12:639226-.
16. Wolach B, Gavrieli R, de Boer M, van Leeuwen K, Berger-Achituv S, Stauber T, et al. Chronic granulomatous disease: Clinical, functional, molecular, and genetic studies. The Israeli experience with 84 patients. American journal of hematology. 2017;92(1):28-36.
17. Jones LBKR, McGrogan P, Flood TJ, Gennery AR, Morton L, Thrasher A, et al. Special article: chronic granulomatous disease in the United Kingdom and Ireland: a comprehensive national patient-based registry. Clinical and experimental immunology. 2008;152(2):211-8.
18. Wu J, Wang WF, Zhang YD, Chen TX. Clinical Features and Genetic Analysis of 48 Patients with Chronic Granulomatous Disease in a Single Center Study from Shanghai, China (2005-2015): New Studies and a Literature Review. Journal of immunology research. 2017;2017:8745254.
19. Blancas-Galicia L, Santos-Chávez E, Deswarte C, Mignac Q, Medina-Vera I, León-Lara X, et al. Genetic, Immunological, and Clinical Features of the First Mexican Cohort of Patients with Chronic Granulomatous Disease. Journal of clinical immunology. 2020;40(3):475-93
20. van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, et al. Chronic granulomatous disease: the European experience. PloS one. 2009;4(4):e5234.
21. Rawat A, Singh S, Suri D, Gupta A, Saikia B, Minz RW, et al. Chronic granulomatous disease: two decades of experience from a tertiary care centre in North West India. Journal of clinical immunology. 2014;34(1):58-67.
22. Baba LA, Ailal F, El Hafidi N, Hubeau M, Jabot-Hanin F, Benajiba N, et al. Chronic granulomatous disease in Morocco: genetic, immunological, and clinical features of 12 patients from 10 kindreds. Journal of clinical immunology. 2014;34(4):452-8.
23. Oliveira AFB, Pastorino AC, Dorna MB, Castro A, Pegler JRM, Morgenstern B, et al. Microbiological profile in chronic granulomatous disease patients in a single Brazilian primary immunodeficiency center. Allergologia et immunopathologia. 2021;49(2):217-24.
24. Rawat A, Vignesh P, Sudhakar M, Sharma M, Suri D, Jindal A, et al. Clinical, Immunological, and Molecular Profile of Chronic Granulomatous Disease: A Multi-Centric Study of 236 Patients From India. Frontiers in immunology. 2021;12:625320.
25. Gesualdo F, Onori M, Bongiorno D, Campanile F, Carloni E, Mancinelli L, et al. Methicillin-resistant Staphylococcus aureus nasal colonization in a department of pediatrics: a cross-sectional study. Italian journal of pediatrics. 2014;40:3.
26. Gesualdo F, Bongiorno D, Rizzo C, Bella A, Menichella D, Stefani S, et al. MRSA nasal colonization in children: prevalence meta-analysis, review of risk factors and molecular genetics. The Pediatric infectious disease journal. 2013;32(5):479-85.
27. Meylan P, Lang C, Mermoud S, Johannsen A, Norrenberg S, Hohl D, et al. Skin Colonization by Staphylococcus aureus Precedes the Clinical Diagnosis of Atopic Dermatitis in Infancy. The Journal of investigative dermatology. 2017;137(12):2497-504.