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"Clinical Aspects of Chronic Granulomatous Disease in Upper Egypt"

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ABSTRACT

Chronic granulomatous disease (CGD) is a rare inherited primary immunodeficiency disorder that affects phagocytes and is characterized by a marked increased susceptibility to severe bacterial and fungal infections. We aimed to describe the clinical presentations of pediatric patients with CGD in Upper Egypt and to identify the defective component of NADPH oxidase. Pediatric patients diagnosed with CGD within one year from January 2018 to January 2019 were enrolled in the study. Patient history, clinical and laboratory investigations were carried out, including nitroblue tetrazolium test and flow cytometry DHR analysis. Infectious microorganisms were isolated from infected sites to identify the causative agents and their resistance profile. A total of 15 patients were diagnosed with CGD. Failure to thrive and lymphadenopathy were the most common presentations. The median age of clinical onset was 1.17 years of age. The most common gene mutations were observed in the CYBA gene. All cases showed pulmonary infections followed by abscesses. *Staphylococcus aureus* and *Klebsiella pneumoniae* were the most frequently isolated bacterial pathogens, *Aspergillus spp* and *Candida spp* were isolated from fungal infections. 4/15 (26.7%) children died due to severe serious infections. We concluded that CGD is common in Upper Egypt, and we recommend raising the awareness and testing for CGD in pediatric patients with recurrent or persistent infections, especially those with a familiar history of similar manifestations to avoid delays in proper diagnosis and deterioration of cases.

Abbreviations: CGD: chronic granulomatous disease; XL: X-linked; AR: autosomal recessive

KEYWORDS

Primary immunodeficiency; chronic granulomatous disease; genetic disorders

Introduction

Chronic Granulomatous Disease (CGD) was described for the first time by both B.H. Landing and R.A. Good in 1957 (Landing and Shirkey 1957). CGD comprises a rare group of genetically determined disorders affecting the immune system characterized by the inability of the body's phagocytic cells (neutrophil and monocyte granulocytes) to kill certain phagocytosed microorganisms. This defect in phagocytic cells is attributed to mutations in the gene coding for the NADPH oxidase (phox) enzyme complex, which is

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essential for the intracellular killing activity of phagocytic cells as a result of the inability to produce normal amounts of superoxide anion (Leusen et al. 1994). CGD is a rare disease, and the incidence is approximately 1/200,000 live births in the United States and Europe and 1/220,000 in Japan (van den Berg et al. 2009; Winkelstein et al. 2000).

CGD is caused by mutations in one of the five genes coding for NADPH oxidase subunits. Approximately 70% of cases are caused by mutations in the CYBB gene leading to X-linked CGD, which often causes a severe form of the disease (Ko et al. 2014; van den Berg et al. 2009). More than 700 pathogenic mutations in the CYBB gene encoding the gp91-phox protein have been documented. Other biallelic mutations in *CYBA, NCF1, NCF2*, and *NCF4* cause autosomal recessive CGD (Boonyawat et al. 2018). In addition to the previously described genes coding for NADPH oxidase, a novel ER-resident transmembrane protein called Eros (essential for reactive oxygen species) that is essential for the regulation of NADPH oxidase and controls the phagocyte respiratory burst was described. EROS is essential for the expression of gp91phox-p22phox heterodimer, which is one of the essential components of the phagocyte NADPH oxidase (Thomas et al. 2019, 2017). A homozygous CYBC1/EROS mutation was associated with the development of CGD (Monies et al. 2017).

Symptoms may appear in infancy or later in adult life. As a result of impairment in phagocytosis, patients usually suffer from repeated bacterial infections leading to pneumonia, lymphadenitis, liver, prostate or skin abscess and osteomyelitis (Agochukwu et al. 2012; Wakabayashi et al. 2019). In addition, CGD patients are more subjected to invasive life-threatening fungal infections that affect the lungs and bones (Falcone and Holland 2012). The defective generation of superoxide anions and reactive oxygen intermediates is also associated with excessive inflammatory reactions leading to granulomatous lesions that typically affects the bladder and gastrointestinal tract. To date, the only curative treatment for CGD is the bone marrow stem cell transplantation. Other strategies based on targeted gene therapy is currently under research and development (De Ravin and others, 2016; Kang et al. 2010)

Few studies have been conducted that describe the genetic diversity and clinical presentation of the disease in Africa (Ben-Farhat et al. 2016; El Hawary et al. 2016). In fact, no studies have been conducted on Egyptian CGD patients from Upper Egypt. Therefore, this study aimed to describe the clinical and genetic aspects of CGD in pediatric patients in Upper Egypt.

Patients and methods

Ethical statement

The study protocol was reviewed and approved by the Ethical Committee of Sohag University Hospital. Written informed consent was obtained from all parents of the participants.

Study subjects

This study was carried out on patients with CGD attending the Pediatric allergy, immunology and rheumatology unit, Faculty of Medicine, Sohag University Hospital, from January 2018 to January 2019 in a cross-sectional study. Diagnosis of CGD was

carried out by clinical examination and laboratory tests based on the diagnostic criteria for primary immunodeficiency by Pan-American Group for Immunodeficiency (PAGID) and European Society for Immunodeficiencies (Conley et al. 1999). Demographic characteristics, anthropometric measures, medical histories, data of laboratory investigations including complete blood count with differential WBC count, immunoglobulin assay, nitroblue tetrazolium reduction test and flowcytometric DHR analysis were collected from each subject. Absolute leukocyte numbers and immunoglobulin levels in studied CGD patients were compared to the normal ranges according to the different age groups as reported by (Shapiro and Greenfield 1987; Stiehm and Fudenberg 1966).

Nitroblue tetrazolium test (NBT)

NBT was used to identify neutrophil dysfunction. Blood was incubated with a buffered solution of nitroblue tetrazolium solution (Sigma Aldrich) and PMA as a stimulant. Smears were prepared and examined microscopically after staining with Wright stain to determine intracytoplasmic deposits of formazan in neutrophils. Neutrophils of CGD patients showed low NBT test values. The diagnosis was based on less than 5% normal neutrophil activity (Fernando et al. 2018).

Dihydrorhodamine (DHR) flow cytometry assay

DHR flow cytometry assays were performed using the PHAGOBURST kit (BD, USA). Heparinized blood samples were collected and cells were stimulated with *E coli* bacteria, phorbol-myristate-acetate (PMA) and fMLP as a low physiological stimulus in separate test tubes as recommended by the manufacturer. Tubes were incubated for 10 min at 37°C in a water bath, treated with dihydrorhodamine 123 and incubated for another 10 min at 37°C in the water bath. Then, erythrocytes were removed by the addition of the lysing solution which was incubated for 20 min at room temperature then washed. Finally, cells were analyzed by FACSCalibur Flow Cytometer (Becton Dickinson, USA) and the percentage of cells that produced reactive oxygen metabolites were determined using the Cell Quest software (Becton Dickinson, USA) compared to control non-stimulated samples.

Mutation analysis

Detection of mutations in reactive oxygen intermediate (ROI) genes was carried out as previously described (Wu et al. 2017). Genomic DNA was extracted from peripheral blood by using GeneJET Whole Blood Genomic DNA Purification (ThermoFisher Scientific, USA). Mutations in CYBB, CYBA, NCF1, NCF2, and NCF4 genes were analyzed by direct sequencing of the amplified PCR products. PCR amplification conditions involved 30 cycles of initial denaturation step at 94°C for 15 S, 58°C annealing step for 30 S, and extension step at 72°C for 1 min. Amplified products were detected by 1% agarose gel electrophoresis. Identification of gene mutations was carried out by aligning the sequencing results with the standard sequences published in NCBI.

Statistical analysis

Statistical analysis was carried out using SPSS 16.0 software (SPSS Inc, Chicago, IL, USA). Comparisons were determined by unpaired *t*-test. *P*-value of less than 0.05 was considered as statistically significant.

Results

The diagnosis of CGD was based on reduced nitroblue tetrazolium test and confirmed by DHR assay. Both tests produced results consistent with CGD cases. During the study period, 9 males and 6 females were diagnosed as CGD patients. Most of the analyzed cases had a history of consanguinity (11/15; 73.3%). Of these patients, 2 had reported the presence of a family history of the disease. Laboratory characteristics showed leucocytosis in about half of CGD cases (7/15) and these patients had marked neutrophilia. Most CGD patients had a marked increase in serum IgG levels (12/15) than the normal range while IgM results were increased in 2 patients. All patients had normal IgE levels. Hemoglobin level was within normal (Table 1). Importantly, there was a marked delay in the diagnosis of CGD cases that ranged between 4 and 16 months, which reflects the urgent need for suspecting CGD, especially in cases with a familiar history of similar manifestations to avoid delays in proper diagnosis and deterioration of cases. Moreover, death was recorded in 4 cases in which diagnosis was markedly delayed.

The mean age at the onset of symptoms and diagnosis was 1.17 years and 2.01 years, respectively. Most cases showed symptoms in the age range of 6–12 months, while the majority of cases were diagnosed in the age range of 12–24 months (Figure 1).

Failure to thrive was present in 9 cases (60%), lymphadenopathy in 9 cases (60%) followed by hepatomegaly in 6 cases (40%) and splenomegaly in 4 cases (26.6%). Some patients were presented with multiple infections. All patients were presented with lung infections; involving pneumonia (8/15, 53.3%), lung abscess (5/15, 33.3%) and tuberculosis (2/15, 13.3%) followed by lymphadenitis in 7/15 cases (46.7%), osteomyelitis in 6/15 (40%) and skin abscess in 4/15 (26.7%) (Table 2).

Organisms isolated from infected lesions included Staphylococci from 8 cases (53.3%), *Klebsiella pneumoniae* from 6 (40%) cases and *E. coli* from 4 cases (26.7%). Some pulmonary cases were infected with *Aspergillus spp*, and two cases were infected with *Candida spp*. All cases received BCG vaccination after birth, according to the national vaccination program in Egypt (Imam 1985). About half of the cases (7/15, 46.7%) suffered from BCG-related infections (Table 3). Of them, 4 patients were reported to have both regional and disseminated infections in lymph nodes.

Antibiotic sensitivity testing was carried out to determine the level of antibiotic resistance of the isolated pathogens. Generally, bacterial isolates were sensitive to many of tested antibiotics; Staphylococci were mostly sensitive to vancomycin, imipenem and amikacin, while Gram-negative isolates were mostly sensitive to imipenem (Table 4).

Of note, Genetic analysis revealed that the autosomal recessive form of CGD disease was observed in 14/15 (93.3%) of the CGD cases while only one case was of the X-linked type (Table 5). The most common gene mutations were observed in CYBA (autosomal recessive), found in 11 patients (73.3% of cases) followed by NCF1 in 2 patients (13.3%), one case with mutated NCF2 (6.7%) and finally one case with mutated CYBB (6.7%).

Table 1. Demographic and laboratory characteristics of CGD patients.

Patient no.	Sex	Presence of Consanguinity	Present of family history	Age at onset of symptoms (months)	Age at admission to hospital (months)	Age at diagnosis (months)	Delay time for diagnosis (months)	Death	WBC x10^3/ ul	Neutrophil %	IgG mg/ dl	IgM mg/ dl	Hg g/dl
#01	m	Yes	No	4	6	8	4	No	7.1	4520	1310*	55	12.5
#02	m	Yes	No	2	4	10	8	No	5.8	4260	710	62	13
#03	m	No	No	3	5	13	10	No	16.3*	9440*	1980*	150*	12
#04	f	Yes	Yes	5	10	16	11	No	14.2*	7920*	1420*	71	12.1
#05	f	No	No	8	10	14	6	No	5.2	5490	850	62	12.3
#06	m	Yes	No	9	12	18	9	No	4.6	6220	760	58	12
#07	f	Yes	No	10	14	22	12	yes	6.1	7180	1420*	50	11.9
#08	f	Yes	No	11	15	22	11	No	4.2	5390	1690*	52	12.7
#09	m	Yes	No	14	18	30	16	yes	5.9	5240	1620*	63	12.6
#10	f	No	No	20	26	34	14	yes	15.2*	8350*	1650*	65	12.5
#11	m	Yes	Yes	20	24	36	16	yes	15.4*	9960*	1350*	60	12.5
#12	m	Yes	No	16	20	25	9	No	13.2*	8240*	1760*	67	12.4
#13	m	Yes	No	30	36	38	8	No	14.2*	8550*	1650*	56	13
#14	m	No	No	30	36	38	8	No	5.9	5560	1760*	63	13.1
#15	f	Yes	No	30	36	38	8	No	15.1*	8110*	2350*	110*	13.2

* Asterix refers to values above the normal range.

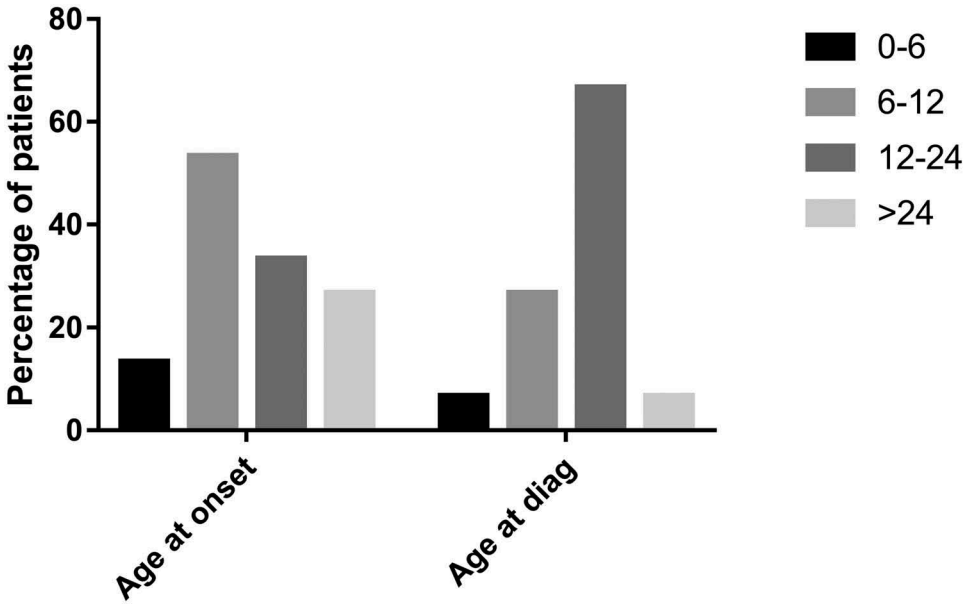

Figure 1. Age of patients with CGD in months.

Table 2. Summary of infections in CGD patients.

Type of infection	No. (%) of patients
Lung	15 (100%)
(a) Pneumonia	8/15 (53.3%)
(b) Tuberculosis	2/15 13.3%)
(c) Lung abscess	5/15 (33.3%)
Liver abscess	6/15 (40%)
Cutaneous infection (Skin abscess)	4/15 (26.7%)
Lymphadenitis	7/15 (46.7%)
Bone (Osteomyelitis)	6/15 (40%)
BCG-related infections	7/15 (46.7%)

Table 3. Isolated microorganisms from CGD patients.

		Specimens		
Pathogen	No. (%) of patients	Sputum (n)	Pus (n)	Blood (n)
Staphylococcus aureus	8/15 (53.3%)	0	7	1
Mycobacterium tuberculosis	2/15 (13.3%)	2	0	0
Klebsiella pneumoniae	6/15 (40%)	4	1	1
Escherichia coli	4/15 (26.7%)	0	4	0
Pseudomonas aeruginosa	3/15 (20%)	0	3	0
Aspergillus spp	3/15 (20%)	3	0	0
Candida spp	2/15 (13.3%)	2	0	0

Mutations in CYBA gene were associated with alteration in p22-phox. In all autosomal recessive-CGD patients with a defective p22-phox, the common mutation p.Val99Pro fs*90 was detected, while in patients with p47-phox deficiency, p.Tyr26His fs*26 mutation was observed. One patient had defective p67-phox with p.Gln92* mutation and the only X-linked CGD patient had p.Arg91* mutation.

Table 4. Antibiotic resistance profile for the microorganisms isolated from different sites.

	Staphylococcus aureus (n = 8)		Klebsiella pneumoniae (n = 6)		E coli (n = 4)		Pseudomonas aeroginosa (n = 3)	
Antibiotic	n	%	n	%	n	%	n	%
Ampicillin	6	75	3	50	2	33.3	3	100
Amoxicillin/clavulanic acid	3	37.5	3	50	3	50	3	100
Ceftriaxone	1	12.5	0	0	1	16.7	3	100
Cefotaxime	1	12.5	1	16.7	0	0	2	66.7
Ceftazidime	1	12.5	1	16.7	0	0	2	66.7
Cefpodoxime	1	12.5	1	16.7	1	16.7	2	66.7
Imipenem	0	0	0	0	0	0	0	0
Amikacin	0	0	0	0	0	0	1	33.3
Gentamicin	1	12.5	0	0	1	16.7	2	66.7
Tetracycline	1	12.5	1	16.7	0	0	2	66.7
Ciprofloxacin	4	50	1	16.7	1	16.7	3	100
Norfloxacin	4	50	0	0	0	0	2	66.7
Chloramphenicol	2	25	2	33.3	2	33.3	3	100
Trimethoprim/sulfamethoxazole	1	12.5	2	33.3	1	16.7	3	100
Vancomycin	0	0	ND	ND	ND	ND	ND	ND

ND; not determined.

Table 5. Subtype and genetic characters of the CGD patients.

Patient no.	CGD subtype	Gene affected	Affected protein	Nucleotide change	Amino acid change
#01	AR	NCF1	p47-phox	c.75_76delGT	p.Tyr26His fs*26
#02	AR	CYBA	p22-phox	c.295_301delGTGCCCG	p.Val99Pro fs*90
#03	AR	CYBA	p22-phox	c.295_301delGTGCCCG	p.Val99Pro fs*90
#04	AR	CYBA	p22-phox	c.295_301delGTGCCCG	p.Val99Pro fs*90
#05	AR	CYBA	p22-phox	c.295_301delGTGCCCG	p.Val99Pro fs*90
#06	AR	NCF1	p47-phox	c.75_76delGT	p.Tyr26His fs*26
#07	AR	CYBA	p22-phox	c.295_301delGTGCCCG	p.Val99Pro fs*90
#08	AR	CYBA	p22-phox	c.295_301delGTGCCCG	p.Val99Pro fs*90
#09	AR	CYBA	p22-phox	c.295_301delGTGCCCG	p.Val99Pro fs*90
#10	AR	CYBA	p22-phox	c.295_301delGTGCCCG	p.Val99Pro fs*90
#11	AR	CYBA	p22-phox	c.295_301delGTGCCCG	p.Val99Pro fs*90
#12	AR	NCF2	p67-phox	c.574C>T	p.Gln92*
#13	AR	CYBA	p22-phox	c.295_301delGTGCCCG	p.Val99Pro fs*90
#14	X-linked	CYBB	gp91-phox	c.271C>T	p.Arg91*
#15	AR	CYBA	p22-phox	c.295_301delGTGCCCG	p.Val99Pro fs*90

AR; Autosomal recessive.

Following diagnosis, all patients received a prophylactic treatment with cotrimoxazole and itraconazole to prevent bacterial and fungal infections. None of the patients received the IFNγ therapy because it is not available in Egypt. By the end of this study, 4/15 (26.6%) CGD patients died because of serious infections. Other patients were under preparation for hematopoietic stem cell transplantation.

Discussion

This is the first cohort of CGD patients described in Upper Egypt. It describes the clinical and laboratory data of 15 children diagnosed with CGD, which provides further insight into the clinical course of CGD in North African countries. It is to be noted that most cases were diagnosed around 12 months after the appearance of the symptoms. That could be attributed to the lack of diagnostic tools in peripheral hospitals and the absence of

enough knowledge about the disease among medical staff. From such a high frequency of observed cases, we concluded that CGD is common in this area of Upper Egypt (Sohag governorate) which may be attributed to the high rate of consanguineous marriages or high incidence of marriages between close-knit population groups in this area (Rawat et al. 2014). In other countries, the prevalence rates differ according to geographical, social, and cultural factors that affect birth rates and frequency of consanguinity (Holland 2013).

Compared with other countries, the mean age of presentation of symptoms in our study was early (1.17 years). In India, most cases showed signs at 20 months and in Latin America at 23.9 months (Fernando et al. 2018; Rawat et al. 2014). Infections usually become evident before five years of age (Martire et al. 2008). However, some studies reported cases of CGD have been diagnosed in adolescence or adulthood which was explained by the detection of mild cases that have been treated successfully with the newly developed antimicrobial agents (Godoy et al. 2008; Liese et al. 1996; Margolis et al. 2017; Thomsen et al. 2016). The delay in diagnosis of CGD ranged between 4–16 months. This delay in the accurate diagnosis is due to the low awareness of the disease in Upper Egypt and the low availability of improved laboratory diagnostic tools. In fact, many patients were managed in peripheral hospitals, where the immunological diagnosis was lacking. These cases were identified as CGD cases only when referred to the main tertiary hospital in Sohag governorate.

CGD patients are characterized by severe bacterial and fungal infections due to the dysfunctional immune cells. The majority of infections were in the lung and liver. Hepatic abscesses are a common complication in patients with CGD and are commonly caused by the catalase-positive *Staphylococcus aureus* organism. These abscesses are usually recurrent and multiple and are difficult to treat compared to abscesses in patients without CGD (Johnston and Newman 1977; Marciano et al. 2015; van den Berg et al. 2009). Lublin et al. (2002) reported that approximately 35% of CGD patients develop liver abscesses, of which about 50% experience recurrence. Clinical data from 429 European CGD patients showed that 32% of patients suffered from liver disease (van den Berg et al. 2009). Moreover, osteomyelitis was also frequently observed in our patient group. The high prevalence of bone infection may result from the primary pulmonary disease which was reported in 100% of CGD patients (Ben-Ari et al. 2012). Possible explanations for these complications of the CGD disease include the defective apoptosis of the neutrophils, excessive activation of NF-κB signaling, upregulation in the gene expression of TNFα, IL17, IL6, and granulocyte colony-stimulating, dysregulation of IL-8 production and defective Nrf2 activation (Brown et al. 2008; Bylund et al. 2007; Fernandez-Boyanapalli et al. 2010; Kobayashi et al. 2004; Lekstrom-Himes et al. 2005; Segal et al. 2010, 2002).

In our study, *Staphylococcus aureus, Klebsiella pneumoniae*, and *E coli* were the most frequently isolated pathogens. The fact that bacterial isolates were not characterized by a high level of resistance is consistent with the impaired function of the immune cells in these patients. In line with other studies, about half of CGD cases suffered from BCG-related infections (Conti et al. 2016). Sites of infection and the spectrum of microorganisms may differ according to geographical factors, sanitation, and food handling, but infections and granulomatous lesions remain the first clinical presentation that lead the clinicians to the diagnosis of CGD (Lee et al. 2008; Song et al. 2011). The overall mortality (26.6%) was similar to the cohort from Europe (20%) and lower than in Sri Lanka and

India (35% and 38% respectively) (Fernando et al. 2018; Rawat et al. 2014; van den Berg et al. 2009).

The majority of the Egyptian patients studied were autosomal recessive CGD cases (93.3%), which is similar to other studies in Asian countries as Turkey, Iran and India (Koker et al. 2013; Movahedi et al. 2004; Rawat et al. 2014). A previous cohort of 28 Egyptian patients also reported a predominance of the autosomal recessive form (82%), mainly due to mutations in CYBA (El Hawary et al. 2016). Another study from the Mediterranean region was carried out on 11 Tunisian patients reported a unique mutation in NCF2 (Monies et al. 2017). Even immigrants to Europe from Arab/North African showed a predominance of autosomal recessive forms (72% and 67% respectively). Similar results have been found in populations in Oman where only one patient had X-linked CGD (Al-Zadjali et al. 2015). In Israel, autosomal recessive-CGD was the most prevalent type in consanguineous Arab and Jewish populations (Wolach et al. 2017). In all these countries and in this study, the remarkable high prevalence of the autosomal recessive form of CGD could be attributed to the consanguineous marriage practice which is common within these communities. Of note, the consanguinity among the studied cohort families in our study was 73.3% and all the reported AR-CGD cases had consanguineous parents. Our data are different from those reported in Europe, Latin America and Sri Lanka (de Oliveira-junior et al. 2015; Fernando et al. 2018; van den Berg et al. 2009) where X-lined-CGD was predominantly reported.

Our study showed that defects in the p22-phox were the most common autosomal recessive-CGD among Egyptian patients in Upper Egypt (73%). Similarly, a study in Cairo, Egypt reported that the most common defective protein was p22-phox (46.4%). Interestingly, all mutations described in our study were also reported by the previous Egyptian study (El Hawary et al. 2016). On the other hand, other investigators in Jordon, Tunisai and Morocco reported that the p47-phox deficiency was the commonest cause of AR-CGD (Bakri et al. 2009; El Kares et al. 2006; Yu et al. 2008). The X-linked CGD patient reported in the present study showed a defective gp91-phox, which is the most common described defect in X-linked CGD cases (Lutzkanin et al. 2019; Rae et al. 1998; Rider et al. 2018; van den Berg et al. 2009).

Conclusion and future directions

CGD patients in Upper Egypt represented a classical course of manifestations and causative pathogens where pulmonary infections and Staphylococci were prevalent. We believe that a percentage of children remained unreported and undiagnosed due to the lack of resources in peripheral hospitals and the absence of enough knowledge about the disease among medical staff. Children with repeated infections should be routinely screened to enable the early administration of prophylactic antimicrobial therapy that interferes with the life-threatening complications associated with CGD. Generally speaking, one may consider that BCG vaccination to be avoided in these children. However, this seems to unpractical since BCG is usually administered in Egypt in the first month of life, and according to our study, the mean age of diagnosis of CGD is two years of age. But at least BCG-associated complications should be considered in these children.

Disclosure of interest

Authors have no potential conflict of interest related to this manuscript.

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References

Agochukwu NQ, Rastinehad AR, Richter LA, Barak S, Zerbe CS, Holland SM, Pinto PA. 2012. Prostatic abscess in a pediatric patient with chronic granulomatous disease: report of a unique case and review of the literature. J Pediatr Surg. 47(2):400–03.

Al-Zadjali S, Al-Tamemi S, Elnour I, AlKindi S, Lapoumeroulie C, Al-Maamari S, Pathare A, Dennison D, Krishnamoorthy R. 2015. Clinical and molecular findings of chronic granulomatous disease in Oman: family studies. Clin Genet. 87(2):185–89.

Bakri FG, Martel C, Khuri-Bulos N, Mahafzah A, El-Khateeb MS, Al-Wahadneh AM, Hayajneh WA, Hamamy HA, Maquet E, Molin M, et al. 2009. First report of clinical, functional, and molecular investigation of chronic granulomatous disease in nine Jordanian families. J Clin Immunol. 29(2):215–30.

Ben-Ari J, Wolach O, Gavrieli R, Wolach B. 2012. Infections associated with chronic granulomatous disease: linking genetics to phenotypic expression. Expert Rev Anti Infect Ther. 10(8):881–94.

Ben-Farhat K, Ben-Mustapha I, Ben-Ali M, Rouault K, Hamami S, Mekki N, Ben-Chehida A, Largueche B, Fitouri Z, Abdelmoula S, et al. 2016. A founder effect of c.257 + 2T > C mutation in NCF2 gene underlies severe chronic granulomatous disease in eleven patients. J Clin Immunol. 36(6):547–54.

Boonyawat B, Suksawat Y, Pacharn P, Suwanpakdee P, Traivaree C. 2018. X-linked chronic granulomatous disease: initial presentation with intracranial hemorrhage from vitamin K deficiency in infant. Case Rep Pediatr. 2018:7041204.

Brown KL, Bylund J, MacDonald KL, Song-Zhao GX, Elliott MR, Falsafi R, Hancock RE, Speert DP. 2008. ROS-deficient monocytes have aberrant gene expression that correlates with inflammatory disorders of chronic granulomatous disease. Clin Immunol. 129(1):90–102.

Bylund J, MacDonald KL, Brown KL, Mydel P, Collins LV, Hancock RE, Speert DP. 2007. Enhanced inflammatory responses of chronic granulomatous disease leukocytes involve ROS-independent activation of NF-kappa B. Eur J Immunol. 37(4):1087–96.

Conley ME, Notarangelo LD, Etzioni A. 1999. Diagnostic criteria for primary immunodeficiencies. Representing PAGID (Pan-American Group for Immunodeficiency) and ESID (European Society for Immunodeficiencies). Clin Immunol. 93(3):190–97.

Conti F, Lugo-Reyes SO, Blancas Galicia L, He J, Aksu G, Borges de Oliveira E Jr., Deswarte C, Hubeau M, Karaca N, de Suremain M, et al. 2016. Mycobacterial disease in patients with chronic granulomatous disease: A retrospective analysis of 71 cases. J Allergy Clin Immunol. 138(1):241–248 e243.

de Oliveira-junior EB, Zurro NB, Prando C, Cabral-Marques O, Pereira PV, Schimke LF, Klaver S, Buzolin M, Blancas-Galicia L, Santos-Argumedo L, et al. 2015. Clinical and genotypic spectrum of chronic granulomatous disease in 71 Latin american patients: first report from the LASID registry. Pediatr Blood Cancer. 62(12):2101–07.

De Ravin SS, Reik A, Liu PQ, Li L, Wu X, Su L, Raley C, Theobald N, Choi U, Song AH, et al. 2016. Targeted gene addition in human CD34(+) hematopoietic cells for correction of X-linked chronic granulomatous disease. Nat Biotechnol. 34(4):424–29.

El Hawary R, Meshaal S, Deswarte C, Galal N, Abdelkawy M, Alkady R, Elaziz DA, Freiberger T, Ravcukova B, Litzman J, et al. 2016. Role of flow cytometry in the diagnosis of chronic granulomatous disease: the egyptian experience. J Clin Immunol. 36(6):610–18.

El Kares R, Barbouche MR, Elloumi-Zghal H, Bejaoui M, Chemli J, Mellouli F, Tebib N, Abdelmoula MS, Boukthir S, Fitouri Z, et al. 2006. Genetic and mutational heterogeneity of autosomal recessive chronic granulomatous disease in Tunisia. J Hum Genet. 51(10):887–95.
Falcone EL, Holland SM. 2012. Invasive fungal infection in chronic granulomatous disease: insights into pathogenesis and management. Curr Opin Infect Dis. 25(6):658–69.
Fernandez-Boyanapalli R, Frasch SC, Riches DW, Vandivier RW, Henson PM, Bratton DL. 2010. PPARgamma activation normalizes resolution of acute sterile inflammation in murine chronic granulomatous disease. Blood. 116(22):4512–22.
Fernando SJA, Faiz NM, Handunnetti SM, De Silva AD, Dasanayake W, Wickramasinghe GD, Karunatilake R, de Silva NR. 2018. Preliminary study on chronic granulomatous disease in Sri Lanka. Allergy Asthma Clin Immunol. 14:37.
Godoy MC, Vos PM, Cooperberg PL, Lydell CP, Phillips P, Muller NL. 2008. Chest radiographic and CT manifestations of chronic granulomatous disease in adults. AJR Am J Roentgenol. 191 (5):1570–75.
Holland SM. 2013. Chronic granulomatous disease. Hematol Oncol Clin North Am. 27(1):89–99, viii.
Imam IZ. 1985. Immunization and primary health care in Egypt. Trop Geogr Med. 37(3):S65–S66.
Johnston RB, Newman SL. 1977. Chronic granulomatous disease. Pediatr Clin North Am. 24 (2):365–76.
Kang EM, Choi U, Theobald N, Linton G, Long Priel DA, Kuhns D, Malech HL. 2010. Retrovirus gene therapy for X-linked chronic granulomatous disease can achieve stable long-term correction of oxidase activity in peripheral blood neutrophils. Blood. 115(4):783–91.
Ko SH, Rhim JW, Shin KS, Hahn YS, Lee SY, Kim JG. 2014. Genetic analysis of CYBB gene in 26 korean families with X-linked chronic granulomatous disease. Immunol Invest. 43(6):585–94.
Kobayashi SD, Voyich JM, Braughton KR, Whitney AR, Nauseef WM, Malech HL, DeLeo FR. 2004. Gene expression profiling provides insight into the pathophysiology of chronic granulomatous disease. J Immunol. 172(1):636–43.
Koker MY, Camcioglu Y, van Leeuwen K, Kilic SS, Barlan I, Yilmaz M, Metin A, de Boer M, Avcilar H, Patiroglu T, et al. 2013. Clinical, functional, and genetic characterization of chronic granulomatous disease in 89 Turkish patients. J Allergy Clin Immunol. 132(5):1156–1163 e1155.
Landing BH, Shirkey HS. 1957. A syndrome of recurrent infection and infiltration of viscera by pigmented lipid histiocytes. Pediatrics. 20(3):431–38.
Lee PP, Chan KW, Jiang L, Chen T, Li C, Lee TL, Mak PH, Fok SF, Yang X, Lau YL. 2008. Susceptibility to mycobacterial infections in children with X-linked chronic granulomatous disease: a review of 17 patients living in a region endemic for tuberculosis. Pediatr Infect Dis J. 27(3):224–30.
Lekstrom-Himes JA, Kuhns DB, Alvord WG, Gallin JI. 2005. Inhibition of human neutrophil IL-8 production by hydrogen peroxide and dysregulation in chronic granulomatous disease. J Immunol. 174(1):411–17.
Leusen JH, de Boer M, Bolscher BG, Hilarius PM, Weening RS, Ochs HD, Roos D, Verhoeven AJ. 1994. A point mutation in gp91-phox of cytochrome b558 of the human NADPH oxidase leading to defective translocation of the cytosolic proteins p47-phox and p67-phox. J Clin Invest. 93 (5):2120–26.
Liese JG, Jendrossek V, Jansson A, Petropoulou T, Kloos S, Gahr M, Belohradsky BH. 1996. Chronic granulomatous disease in adults. Lancet. 347(8996):220–23.
Lublin M, Bartlett DL, Danforth DN, Kauffman H, Gallin JI, Malech HL, Shawker T, Choyke P, Kleiner DE, Schwartzentruber DJ, et al. 2002. Hepatic abscess in patients with chronic granulomatous disease. Ann Surg. 235(3):383–91.
Lutzkanin K, McKeone DJ, Greiner R, Andreae DA. 2019. A novel mutation in chronic granulomatous disease: treating the family, not just the patient. Front Pediatr. 7:107.
Marciano BE, Spalding C, Fitzgerald A, Mann D, Brown T, Osgood S, Yockey L, Darnell DN, Barnhart L, Daub J, et al. 2015. Common severe infections in chronic granulomatous disease. Clin Infect Dis. 60(8):1176–83.

Margolis R, Wiener L, Pao M, Malech HL, Holland SM, Driscoll P. 2017. Transition from pediatric to adult care by young adults with chronic granulomatous disease: the patient's viewpoint. J Adolesc Health. 61(6):716–21.

Martire B, Rondelli R, Soresina A, Pignata C, Broccoletti T, Finocchi A, Rossi P, Gattorno M, Rabusin M, Azzari C, et al. 2008. Clinical features, long-term follow-up and outcome of a large cohort of patients with chronic granulomatous disease: an Italian multicenter study. Clin Immunol. 126(2):155–64.

Monies D, Abouelhoda M, AlSayed M, Alhassnan Z, Alotaibi M, Kayyali H, Al-Owain M, Shah A, Rahbeeni Z, Al-Muhaizea MA, et al. 2017. The landscape of genetic diseases in Saudi Arabia based on the first 1000 diagnostic panels and exomes. Hum Genet. 136(8):921–39.

Movahedi M, Aghamohammadi A, Rezaei N, Shahnavaz N, Jandaghi AB, Farhoudi A, Pourpak Z, Moin M, Gharagozlou M, Mansouri D. 2004. Chronic granulomatous disease: a clinical survey of 41 patients from the Iranian primary immunodeficiency registry. Int Arch Allergy Immunol. 134 (3):253–59.

Rae J, Newburger PE, Dinauer MC, Noack D, Hopkins PJ, Kuruto R, Curnutte JT. 1998. X-Linked chronic granulomatous disease: mutations in the CYBB gene encoding the gp91-phox component of respiratory-burst oxidase. Am J Hum Genet. 62(6):1320–31.

Rawat A, Singh S, Suri D, Gupta A, Saikia B, Minz RW, Sehgal S, Vaiphei K, Kamae C, Honma K, et al. 2014. Chronic granulomatous disease: two decades of experience from a tertiary care centre in North West India. J Clin Immunol. 34(1):58–67.

Rider NL, Jameson MB, Creech CB. 2018. Chronic granulomatous disease: epidemiology, pathophysiology, and genetic basis of disease. J Pediatric Infect Dis Soc. 7(suppl_1):S2–S5.

Segal BH, Han W, Bushey JJ, Joo M, Bhatti Z, Feminella J, Dennis CG, Vethanayagam RR, Yull FE, Capitano M, et al. 2010. NADPH oxidase limits innate immune responses in the lungs in mice. PLoS One. 5(3):e9631.

Segal BH, Kuhns DB, Ding L, Gallin JI, Holland SM. 2002. Thioglycollate peritonitis in mice lacking C5, 5-lipoxygenase, or p47(phox): complement, leukotrienes, and reactive oxidants in acute inflammation. J Leukoc Biol. 71(3):410–16.

Shapiro MF, Greenfield S. 1987. The complete blood count and leukocyte differential count. An approach to their rational application. Ann Intern Med. 106(1):65–74.

Song E, Jaishankar GB, Saleh H, Jithpratuck W, Sahni R, Krishnaswamy G. 2011. Chronic granulomatous disease: a review of the infectious and inflammatory complications. Clin Mol Allergy. 9(1):10.

Stiehm ER, Fudenberg HH. 1966. Serum levels of immune globulins in health and disease: a survey. Pediatrics. 37(5):715–27.

Thomas DC, Charbonnier LM, Schejtman A, Aldhekri H, Coomber EL, Dufficy ER, Beenken AE, Lee JC, Clare S, Speak AO, et al. 2019. EROS/CYBC1 mutations: decreased NADPH oxidase function and chronic granulomatous disease. J Allergy Clin Immunol. 143(2):782–785 e781.

Thomas DC, Clare S, Sowerby JM, Pardo M, Juss JK, Goulding DA, van der Weyden L, Storisteanu D, Prakash A, Espeli M, et al. 2017. Eros is a novel transmembrane protein that controls the phagocyte respiratory burst and is essential for innate immunity. J Exp Med. 214 (4):1111–28.

Thomsen IP, Smith MA, Holland SM, Creech CB. 2016. A comprehensive approach to the management of children and adults with chronic granulomatous disease. J Allergy Clin Immunol Pract. 4 (6):1082–88.

van den Berg JM, van Koppen E, Ahlin A, Belohradsky BH, Bernatowska E, Corbeel L, Espanol T, Fischer A, Kurenko-Deptuch M, Mouy R, et al. 2009. Chronic granulomatous disease: the European experience. PLoS One. 4(4):e5234.

Wakabayashi Y, Jubishi D, Okamoto K, Ikeda M, Tatsuno K, Mizoguchi M, Sato T, Okugawa S, Moriya K. 2019. A rare case of a prostatic abscess, bacteremia and chronic granulomatous disease associated with Klebsiella pneumoniae. J Infect Chemother. 25(5):365–67.

Winkelstein JA, Marino MC, Johnston RB Jr., Boyle J, Curnutte J, Gallin JI, Malech HL, Holland SM, Ochs H, Quie P, et al. 2000. Chronic granulomatous disease. Report on a national registry of 368 patients. Medicine. 79(3):155–69.

Wolach B, Gavrieli R, de Boer M, van Leeuwen K, Berger-Achituv S, Stauber T, Ben Ari J, Rottem M, Schlesinger Y, Grisaru-Soen G, et al. 2017. Chronic granulomatous disease: clinical, functional, molecular, and genetic studies. The Israeli experience with 84 patients. Am J Hematol. 92(1):28–36.

Wu J, Wang WF, Zhang YD, Chen TX. 2017. 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. J Immunol Res. 2017:8745254.

Yu G, Hong DK, Dionis KY, Rae J, Heyworth PG, Curnutte JT, Lewis DB. 2008. Focus on FOCIS: the continuing diagnostic challenge of autosomal recessive chronic granulomatous disease. Clin Immunol. 128(2):117–26.