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Chronic granulomatous disease: A single-center experience in Central Anatolia

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ABSTRACT

Background: Chronic granulomatous disease (CGD), one of the phagocytic cell defects, is the primary immunodeficiency caused by dysfunction of the NADPH oxidase complex in neutrophils.
Methods: The clinical, demographic and laboratory findings of 17 CGD patients who were followed-up between 2002 and 2021 were obtained retrospectively from the records of the patients.
Results: The number of male and female patients was 10/7. The median age at diagnosis was 5.3 months (range 4–120) for 3 patients with X-CGD, and 42.4 months (range 8–350) for 14 patients with AR-CGD. We have investigated rare *CYBA* exon 3–6 deletion in 7 patients and hotspot mutation with delGT at the beginning of exon 2 of *NCF1* in 5 patients. The most common clinical findings were pneumonia and lymphadenitis with recurrent fever, respectively (41.2%, 35.3%). A total of 154 microbial infections requiring hospital admission (27 in 3 XL and 127 in 14 AR patients) were detected in the follow-up of the patients and median infection number for a patient was 9 in both groups. Eight of 17 patients had stem cell transplantation and the survival rate was 87.5%.
Conclusions: X-CGD patients are more rapidly recognized by family history and severe infections than those with AR-CGD and early prophylaxis may decrease infectious episodes. We have investigated the large deletion suggesting a possible founder effect for *CYBA* exon 3–6 deletion in Central Anatolia. Additionally, HSCT transplantation leads to a high survival rate for the patients with CGD.

1. Introduction

Chronic granulomatous disease (CGD) is a heterogeneous, inherited, primary immunodeficiency characterized by life-threatening recurrent bacterial and fungal infections, and granuloma formation [1]. It was first defined in 1957 [2]. It is estimated that the disease occurs in approximately one in 200,000 individuals and the incidence varies between regional populations [3].

CGD develops due to defects in the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase system, which is required for the respiratory burst of phagocytic cells and superoxide production. These genetic defects cause phagocytes (neutrophils, monocytes, and macrophages) to be unable to destroy certain microbes. The phagocyte NADPH oxidase is a complex of five proteins; membrane-bound components formed by the fusion of $gp91^{phox}$ and $p22^{phox}$ and cytosolic oxidase components $p47^{phox}$, $p67^{phox}$ and $p40^{phox}$. Any defect in *CYBB*, *CYBA*, *NCF1*, *NCF2* or *NCF4* genes destroys NADPH oxidase enzyme activity and leads chronic granulomatous disease. *CYBB*, the gene encoding the membrane-dependent $gp91^{phox}$ protein, is inherited as X-linked, while the others are autosomal recessive (AR) [4]. The X-linked (XL) form is more severe and occurs earlier, and it has a higher mortality [5–8].

The diagnosis is made with neutrophil function test for superoxide production (nitroblue tetrazolium reduction (NBT) or dihydrorhodamine [DHR] 123 assay). Today, the DHR-123 assay is preferred in many laboratories because it is rapid and sensitive even to a very small number of cells and it helps in the differentiation of X-linked and autosomal CGD form [9]. The molecular defect leading CGD phenotypes is found by genetic analysis [4,9]. Infections in patients with CGD are usually caused by catalase-positive microorganisms. *Staphylococci*, *Serratia*, *Burkholderia* and *Aspergillus* are frequent pathogens detected in

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patients with CGD. The most common infections observed in CGD patients are pneumonia, lymphadenitis and deep abscess. *Staph Aureus* and *Aspergillus* are among the most common infectious organisms reported in these infections [5,6].

Antimicrobial prophylaxis generally consists of long-term low-dose drugs such as Trimethoprim and Sulfamethoxazole (TMP-SMX) and itraconazole [4]. The major curative option for CGD is allogeneic stem cell transplantation [10]. The purpose of this study is to review our single-center experience that reveals clinical, laboratory, and demographic data of 17 patients with CGD in Konya province in Central Anatolia.

2. Materials and methods

The clinical and laboratory findings of 17 patients who were diagnosed with CGD between 2002 and 2021 were obtained retrospectively from the records of the patients. A data file was prepared to contain demographic data of the patients, complaints at the time of admission, clinical findings, accompanying disease, how it was diagnosed, the treatment received, and whether there was bone marrow transplantation (BMT). The study was approved by the Ethics Committee of Meram Medical School (Date: 21.01.2022/No: 2022/3609), Necmettin Erbakan University.

The DHR assay was performed as described by Köker et al. [9] In this test, clinical diagnosis of patients with CGD was confirmed from peripheral blood sample. The result is given by DHR stimulation index (SI), showing how many fold increase in neutrophil function after phorbol 12-myristate 13-acetate (PMA) stimulation. DHR assay also helps in the differentiation of XL and AR forms of disease by using carrier mother pattern (two different neutrophils populations after PMA stimulation) specifically observed in X-CGD form. Expression of flavocytochrome b_{558}, the membrane unit of NADPH oxidase consisting of a heterodimer of gp91^{phox} and p22^{phox}, was checked in all patient samples by gp91^{phox}/p22^{phox} antibody clone 44.1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA), which is negative if either p22^{phox} or gp91^{phox} is absent [9,11]. Molecular diagnosis of patients was confirmed by genescan and NGS analysis [4,11]. Laboratory and genetic testing of CGD patients was carried out in the Immune Deficiency Research Laboratory at the University of Erciyes, Kayseri, Turkey by Köker et al. The infections were specified according to the infective agent and infection site in a patient and causative microorganisms were isolated with cultures. The requirement for hospitalization with parenteral antibiotics or antifungals was accepted as severe infection.

3. Statistical analysis

SSPSS program (version 25.0, IBM Corp., Armonk, NY, USA) was used for statistical analysis. Continuous variables were expressed as means ± standard deviations or medians. For comparisons of continuous variables, the Mann-Whitney *U* test was used. $P < 0.05$ was considered statistically significant and 95% confidence intervals were reported as appropriate.

4. Results

Of the 17 patients from 15 families included in the study, 10 were male (58.8%) and 7 were female (41.2%) and median age was 36.7 (4–350) months. Firstly, neutrophil function was measured by DHR-123 assay and the results confirmed CGD in all the patients; DHR stimulation index (SI) was between (SI:1–11) (normal range 70–100) [4]. Additionally, among the family members, 3 of 15 mothers (F2, F4 and F13) have mosaic pattern (i.e., those with functionally two different neutrophil or monocytes populations) in DHR assay and these mothers were X-CGD carriers (Fig. 1A) (Table 1). Carrier mothers had normal mosaic pattern with approximately half of the neutrophils and monocytes showing functional oxidase activity (51%, 50%, and 46.5%, respectively) and they had no clinical findings. Therefore, an extra test for skewing of lyonization (the X-chromosome inactivation) was not

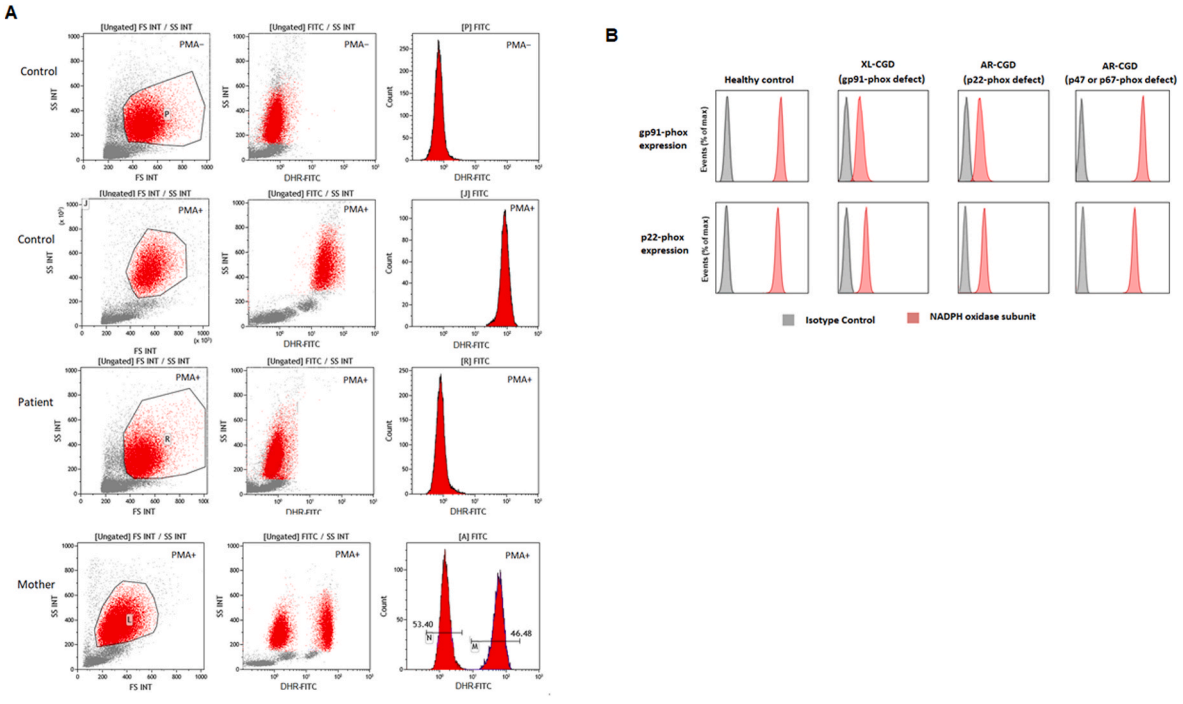


Fig. 1. (A) X-CGD patient (P13) and carrier mother represented by DHR 123 assay. (Upper line control without PMA stimulation, line 2. control with PMA, line 3. Patient with PMA and line 4. Mother with PMA.) Mother has two neutrophil population including diseased and normal neutrophils, specific for X-CGD. **(B)** Expression of flavocytochrome b_{558}, the membrane unit of NADPH oxidase consisting of a heterodimer of gp91^{phox} and p22^{phox}, was checked in all patient samples by gp91^{phox}/p22^{phox} antibody, which is negative if either p22^{phox} or gp91^{phox} was absent in patients 2,4 and 13 with XL-CGD and also absent in patients 1,3–4,6-9 and 14 with AR-CGD.

Table 1
Demographic and genetic data of patients and CGD subtypes.

Patient (family) Number	Sex	Protein Expression (Cyto b_{558})	Subtype	Gene	Mutation	Nucleotide change	Aa change	Cons
P1 (F1)	F	Absent	AR-CGD, $p22^0$	CYBA	Deletion	Exon 3-6del	Large deletion	Yes
P2 (F2)	M	Absent	XL-CGD, $gp91^0$	CYBB	Deletion	c.80_83delTCTG	p.[Val27Glyfs*33]	No
P3 (F3)	M	Absent	AR-CGD, $p22^0$	CYBA	Deletion	Exon 3-6del	Large deletion	Yes
P4 (F4)	M	Absent	XL-CGD, $gp91^0$	CYBB	Deletion	c.80_83delTCTG	p.[Val27Glyfs*33]	No
P5 (F5)	M	+	AR-CGD, $p47^0$	NCF1	Deletion	c.75_76delGT	p.[Tyr26Hisfs*26]	Yes
P6 (F6)	M	Absent	AR-CGD, $p22^0$	CYBA	Deletion	Exon 3-6del	Large deletion	Yes
P7 (F7)	M	Absent	AR-CGD, $p22^0$	CYBA	Deletion	Exon 3-6del	Large deletion	Yes
P8 (F7)	F	Absent	AR-CGD, $p22^0$	CYBA	Deletion	Exon 3-6del	Large deletion	Yes
P9 (F8)	M	Absent	AR-CGD, $p22^0$	CYBA	Deletion	Exon 3-6del	Large deletion	Yes
P10 (F9)	F	+	AR-CGD, $p47^0$	NCF1	Deletion	c.75_76delGT	p.[Tyr26Hisfs*26]	Yes
P11 (F9)	F	+	AR-CGD, $p47^0$	NCF1	Deletion	c.75_76delGT	p.[Tyr26Hisfs*26]	Yes
P12 (F10)	F	+	AR-CGD, $P47^0$	NCF1	Deletion	c.75_76delGT	p.[Tyr26Hisfs*26]	Yes
P13 (F11)	M	Absent	XL-CGD, $gp91^0$	CYBB	Deletion	Exon 1-13del	CYBB del	No
P14 (F12)	M	Absent	AR-CGD, $p22^0$	CYBA	Deletion	Exon3-6 del	Large deletion	Yes
P15 (F13)	F	+	AR-CGD, $p67^0$	NCF2	Duplication	1034dupA	p.[Leu346Alafs*36]	Yes
P16 (F14)	F	+	AR-CGD, $p47^0$	NCF1	Deletion	c.75_76delGT	p.[Tyr26Hisfs*26]	Yes
P17 (F15)	M	+	AR-CGD, $p47^0$	NCF1	Deletion	c.75_76delGT	p.[Tyr26Hisfs*26]	No

M: Male, F: Female, Cons: Consanguinity, ^{0}: No Expression.

needed. Mothers who did not have a mosaic neutrophil pattern in DHR assay were suspected to be AR-CGD. We also checked candidate protein expression of flavocytochrome b_{558}, the membrane unit of NADPH oxidase, in all patient samples by flow cytometry with $gp91^{phox}$/$p22^{phox}$-specific antibody. The results showed that both $p22^{phox}$ and $gp91^{phox}$ expressions were absent in three X-CGD patients and seven AR-CGD patients (Fig. 1B) (Table 1).

Genetic analysis with NGS in both groups revealed large deletion encompassing exon 3–6 at *CYBA* (p22) in six families, c.80_83 delTCTG at *CYBB* (gp91) in two families and *CYBB* del in a family, and 1034 dupA at *NCF2* (p67) in a family (Table 1). NGS results showed exon 3–6 deletion at *CYBA* in seven AR-CGD patients in parallel with the protein expression study (Table 1). Due to the pseudogene conflict in NGS, a hotspot mutation c.75_76 delGT at exon 2 of *NCF1* in five families was investigated by genescan analysis. One out (F15) of all parents in AR group was consanguineous. The rate of consanguinity was 91.7% in AR group and all the biallelic mutations had homozygote characteristic in AR group and as listed in Table 1.

The demographic data of the patients are listed in Table 3 and the mean age at diagnosis was 43.1 ± 66.6 months, median age 5.3 months (4–120) in the X-linked group, and 102 ± 102.1 months and 42.4 months (8–350) in the AR-CGD group, respectively (Fig. 2).

The most common hospital admission symptom was pneumonia in both group. Patients were usually admitted to the hospital with more than one clinical finding, among which the leading three were lung infection, lymphadenitis and recurrent fever (Fig. 3) (see Table 1).

4.1. Non-infectious complications

The radiological finding in computed tomography was defined as pulmonary granulomatosis and this was observed in 4 patients (P1, P6, P7, P9) with AR-CGD. Granulomatous skin reaction in the perineal area was found in one case with $P47^{phox}$ defect and pyloric outlet obstruction because granuloma was observed in one case with $p22^{phox}$ defect (Table 2). However, inflammatory bowel disease (IBD) and perianal fistula were not observed in any of the patients. Hepatomegaly and splenomegaly were observed in 8 and 6 patients, respectively; and no hepato-splenomegaly was seen in 7 patients (Table 2). Bladder granuloma and urinary outflow obstruction were observed in one male patient with $p22^{phox}$ defect and in a female case with $p22^{phox}$ defect who had a multicystic dysplastic kidney (Table 2). Chorioretinitis and nystagmus were observed in a X-CGD case (P4). Growth retardation was found in 7 (41.2%) patients (Table 2). Autoimmune findings were observed in 2 patients with p47phox defect; one case (P12) had symptoms of autoimmune disease (Behçet's disease, FMF) sporadically accompanied by JIA (Table 2). Another patient had sarcoidosis (Table 2).

4.2. Infection complications

A total of 154 infections requiring hospital admission were detected in the follow-up of the patients. Of these, there were 27 infections in the 3 XL patients and 127 infections in the AR group. The average number of infections per patient was 1.96 per year during the follow up. The number of infections requiring hospitalization and intravenous antibiotic treatment was defined as serious infection 81 cases. Of these, there were 12 infections in the XL group and 69 infections in the AR group. The average annual incidence of serious infections per patient was 0.86 (XL group, 1.16; AR group, 0.79).

In this study, it was observed that the first affected organs by infection or granulomatous reaction was the lungs, followed by cutaneous/subcutaneous lymph node and gastro-intestinal system. During the follow-up of the patients, a common infectious manifestation was pneumonia, with 48 episodes in 13 of 17 patients (76.4%). Skin infection was observed (21 episodes in 10/17 patients) (XL group: n = 3, AR group: n = 8), as well as skin abscess (5 episodes in 4 patients) and lymph node abscess (3 episodes in 2 patients) (Table 2). Additionally lymphadenitis was observed (22 episodes in 10 patients) (Table 2). Among the gastrointestinal findings, enteric infection was observed in 29.4% (9 episodes in 5 patients), and perianal abscess was observed in one patient. In addition, dental/peritonsillar abscess was seen in 11.7% (2 episodes in 2 patients). Liver abscess was observed at a rate of 17.6% with 3 episodes in 3 cases. Renal or urinary tract involvement was observed in 23.5% of the patients (7 episodes in 4 patients) (Table 2). Otitis media was observed with 11 episodes in the 5 patients, and mastoid abscess was observed in only one case. Conjunctivitis and blepharitis were observed in one patient (Table 2).

When all infectious attacks are considered together, the most common microorganisms isolated in cultures were *S. aureus*, *Aspergillus* spp. (23%, n = 6) (Table 2), *Pseudomonas aeruginosa* (19.2%, n = 5), and agents listed in Table 4. The most frequently isolated microorganisms in sputum cultures were *Aspergillus* spp (n = 3), *Pseudomonas aeruginosa* (n = 2), and *Klebsiella* (n = 2); and *C.albicans* was observed in one case. *S. aureus* was the most frequently isolated bacterium in cultures for skin infections, as expected. In most lymphadenitis, the causative pathogen was isolated because the cultures were negative or because fine needle aspiration or biopsy was not performed. One patient with the *Salmonella* group in blood culture also had enteritis. *S. aureus* was isolated in one case culture from peritonsillar abscess. The most frequently isolated pathogens in liver abscess were *aspergillus/mucormycosis* in one case and *S. aureus* in the other. *Aspergillus* was isolated from a brain abscess

Table 2
Infectious, non-infectious findings and follow up treatment options of patients with CGD.

Patients (Subtype)	Infections//N infections/N serious infection		Microorganisms	Non-infectious Manifestations	Treatment	Outcome
P1 $p22^{phox}$	Septicaemia	1//1	*Burkholderia cepacia*	Lung granuloma	Antimicrobial treatment	Died
			CNS	HLH		
				Hepatomegaly		
				Splenomegaly/nodule		
				Aplastic anemia		
P2	Pneumonia	2//0	*Staphylococcus aureus*	CHD	Antimicrobial treatment	Died
$gp91^{phox}$	Skin abscess	1//1	*Serratia marcescens*	Hepatomegaly	IFN-γ	
	lymph node abscess	2//2	*Enterococcus faecium*			
	Cutaneous/subcutaneous infections	1/0	*Aspergillus* spp			
	Lymphadenitis	1//0				
	Septicaemia	1//1				
	Enteric infections	1//0				
	Dental/peritonsillar abscess	1//0				
	Liver abscess	1//1				
	Urinary tract infections	2//0				
P3 $p22^{phox}$	Pneumonia	2//2		Pyloric outlet obstruction	Antimicrobial treatment	Died
	Septicaemia	1//1				
P4 $gp91^{phox}$	Pneumonia	2//2	*Staphylococcus aureus*	Hepatomegaly	Antimicrobial treatment,	Alive
	Cutaneous/subcutaneous infections	2//0	*CNS*	Chorioretinal disease/ nystagmus		
	Lymphadenitis	3//1			HSCT, from matched related donor	
	Otitis media	1/0				
P5 $p47^{phox}$	Pneumonia	2//2	*Pseudomonas aeruginosa,*	Hepatomegaly	Anti TB and Antimicrobial treatment, IFN-γ	Alive
	Miliary tbc	1//1	*Klebsiella pneumonia*			
	Lymphadenitis	2//2	*C.albicans*			
	Otitis media	2//1	*enterobacter spp*			
	Conjunctivitis/blepharitis	1//0	*Mycobacterium tuberculosis*			
P6 $p22^{phox}$	Pneumonia	10//8	*Aspergillus fumigatus*	Lung granuloma	Antimicrobials and Anti TB Treatment,	Died
	Cutaneous/subcutaneous infections	3//0	*Serratia marcescens*	Hepatomegaly		
	Tbc pneumonia	1//1	*Klebsiella pneumonia*	Splenomegaly		
	lymph node abscess	1//1	*Staphylococcus aureus*	Bladder granuloma	HSCT, from matched related donor	
	Lymphadenitis	4//1	*Mycobacterium tuberculosis*	Urinary outflow obstruction		
	Osteomyelitis	1//1	*Staphylococcus aureus*			
	Enteric infections	3//1	*CNS*			
	Otitis media	4//0				
	Urinary tract infections	2//0				
	Dental/peritonsillar abscess	1//1				
P7 $p22^{phox}$	Pneumonia	4//4	*Pseudomonas aeruginosa*	Lung granuloma	Anti TB treatment	Alive
	Lymphadenitis	1//0	*Aspergillus* spp	Hepatomegaly	HSCT, unrelated	
	BCGitis	1//1		Splenomegaly		
	Liver abscess	1//1				
P8 $p22^{phox}$	Pneumonia	3//3	*Serratia marcescens*	MDK	Antimicrobials and Anti TB treatment,	Alive
	Tbc pneumonia	1//1	*Mycobacterium tuberculosis*	Hepatomegaly		
	Mastoid abscess	1//1	*CNS*	Splenomegaly		
	Lymphadenitis	1//0			HSCT, unrelated	
	Septicaemia	1//1				
	GVHD (cutaneous)	1//1				
	Urinary tract infections	1//0				
	Otitis media	1//0				
P9 $p22^{phox}$	Pneumonia	3//3	*Staphylococcus aureus*	Lung granuloma		Alive
	Skin abscess	1//1	*Acinetobacter*	Hepatomegaly	HSCT, from matched related donor	
	Lymphadenitis	3//0	*Pseudomonas luteola*	Splenomegaly		
	Cutaneous/subcutaneous infections	1//1	*Aspergillus* spp/ *mucormycosis*			
	Liver abscess	1//1	Achromobacter denitrificans			
	Brain abscess	1//1				
	Septicaemia	1//1				
	Osteomyelitis	1//1				
	Otitis media	3//0				
P10 $p47^{phox}$	Pneumonia	1//1	*Mycobacterium tuberculosis*		Anti TB treatment, IFN-γ	Alive
	Tbc pneumonia	1//1				
P11 $p47^{phox}$	Cutaneous/subcutaneous infections	2//0	*Mycobacterium tuberculosis*		Anti TB treatment, IFN-γ	Alive
	Tbc pneumonia	1//1				

(*continued on next page*)

Table 2 (*continued*)

Patients (Subtype)	Infections//N infections/N serious infection		Microorganisms	Non-infectious Manifestations	Treatment	Outcome
P12 p47^{phox}	Pneumonia	6//3	*Pseudomonas aeruginosa*	Perineal granuloma	Antimicrobial treatment	Alive
	Cutaneous/subcutaneous infections	3//0	*Aspergillus* spp	Hepatomegaly		
	Skin abscess	2//1	*Klebsiella pneumonia*	Splenomegaly		
	Lymphadenitis	3//0	*E.coli,*	JIA/MAS/Behçet/FMF	HSCT, unrelated	
	Septicaemia	2//2	*S. Epidermidis*			
	Enteric infections	2//0	*Stafhaemolyticus*			
	Urinary tract infections	2//1	*Staphylococcus aureus*			
P13 gp91^{phox}	Pneumonia	1//1	*Pseudomonas aeruginosa*		Antimicrobials and Anti TB treatment, IFN-γ	Alive
	Cutaneous/subcutaneous infections	2//1	*E.coli (ESBL+)*			
	Lymphadenitis	1//0				
	Enteric infections	1//1				
	BCGitis	1//1				
P14 P22^{phox}	Pneumonia	4//3	*Aspergillus* spp.		Antimicrobials and Anti TB treatment,	Alive
	Cutaneous/subcutaneous infections	5//0				
	Lymphadenitis	3//0	*Staphylococcus aureus*			
	Enteric infections	2//1	*Klebsiella pneumonia*		HSCT, related	
	BCGitis	1//1				
	Septicaemia	1//1				
	Perianal abscess	1//1				
P15 p67^{phox}	Skin abscess	1//0			HSCT, unrelated	Alive
P16 p47^{phox}	Pneumonia	8//6		Sarcoidosis		Alive
	Cutaneous/subcutaneous infections	1//0				
P17 p47^{phox}	Cutaneous/subcutaneous infections	1//0				Alive

HLH: Hemophagocytic lymphohistiocytosis, MDK: Multicystic dysplastic kidney, CHD: Congenital heart disease, JIA: Juvenile idiopathic arthritis, MAS: macrophage activation syndrome, FMF: familial mediterranean fever, HSCT: Hematopoietic stem cell transplantation, CNS: Coagulase-negative Staphylococcus, Anti TB treatment: (isoniazid, rifampicin, ethambutol, pyrazinamide), Antimicrobials: specific for the pathogens.

Table 3
Age characteristics of the patients with CGD.

	XL	AR			Total
	gp91^{phox}; 3f, n:3	p22^{phox},6f, n:7	p47^{phox}; 5f, n:6	p67^{phox}, n:1	15 families 17 patient
	Mean ± SD Median (min-max)	Mean ± SD Median (min-max)	Mean ± SD Median (min-max)	Mean ± SD Median (min-max)	Mean ± SD Median (min-max)
Age of onset of complaints (month)	4.5 ± 3.97 3 (2–9)	9.3 ± 12.0 6 (1–36)	97.2 ± 85.4 90 (3–216)	2	39.0 ± 65.6 6 (1–216)
Age at diagnosis (month)	43.1 ± 66.7 5.3 (4–120)	38.9 ± 47.6 21.6 (8–144)	184.7 ± 99.9 180.7 (35–350)	48	91.6 ± 97.7 36.7 (4–350)
Current age	104.3 ± 45.7 110 (56–146)	150.2 ± 78.3 171 (12–263)	273.0 ± 133.0 280 (45–457)	70	180.7 ± 116.4 171 (12–457)
Delay in diagnosis	38.7 ± 62.7 4 (1–111)	30.0 ± 36.3 20.6 (3–108)	87.5 ± 71.7 90.7 (3–188)	46	52.8 ± 57.5 27.6 (1–188)
Mean follow-up time (months)	4461.3 ± 39.8 52 (27–105)	111.0 ± 76.0 111 (3–241)	88.2 ± 45.2 104 (10–142)	22	88.9 ± 60.5 104 (3–241)
Deceased	1/3	3/14			4/17

culture in a case. Tibial osteomyelitis (11.7%) was observed in 2 male patients with p22-phox defect; the isolated microorganisms were *Aspergillus fumigatus, Serratia marcescens, S. Aureus,* etc. (Table 4).

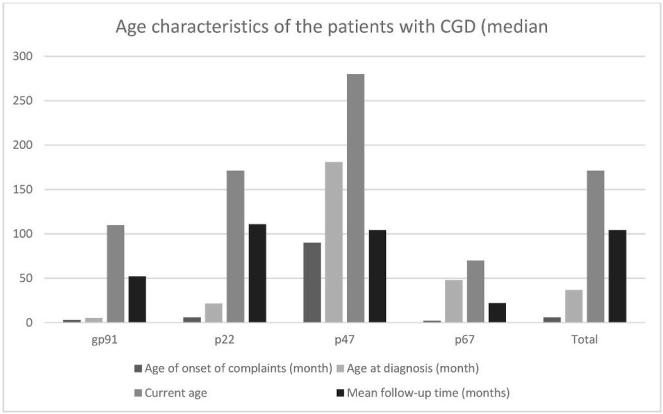


Fig. 2. CGD subtypes and median age characteristics of in the patients.

Septicaemia occurred in 41.2% of patients (8 episodes in 7 patients) and 3 patients died of sepsis. Coagulase-negative Staphylococcus was most frequently isolated from blood cultures in patients with sepsis. *Burkholderia cepacia*, which often causes fulminant pneumonia in CGD, was isolated from only one patient's blood and CSF culture. This case was accompanied by hemophagocytic lymphohistiocytosis (HLH) and aplastic anemia, and the patient (P1) died as a result. *Serratia marcescens* was isolated from the blood culture of another patient who died and valvular aortic stenosis was also present. In the other two patients who died, no microorganisms were detected.

According to the routine vaccination schedule in Turkey, 16 of 17 patients were vaccinated with (Bacilli Calmette-Guérin) BCG during infancy before CGD diagnosis. Tuberculosis infection was observed in 47.1% (8/17) of the patients. A widespread reaction was observed in 5 of 17 patients (29.4%), and a local or regional reaction (BCG-itis) was

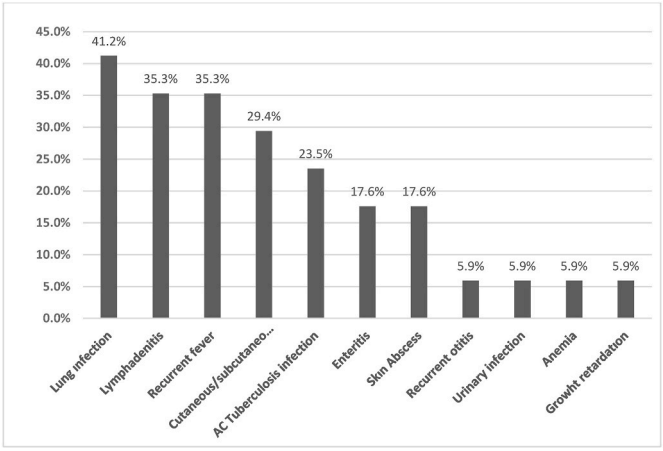


Fig. 3. Clinical findings during hospital admission symptoms of the patients with CGD.

Table 4
The infectious pathogens identified in cultures of the patients with CGD.

Infection	Pathogen	N patient of isolates
Blood culture (sepsis, pneumonia, enteritis)	*Coagulase-negative Staphylococcus, Burkholderia cepacia*	1
	Staf aureus	1
	Aspergillus spp.	1
	Pseudomonas aeruginosa	1
	Serratia marcescens, Streptococcus mitis/ oralis, Salmonella group	1
	Achromobacter denitrificans + *Coagulase-negative Staphylococcus*	1
	Stafhaemolyticus	1
	Enterococcus faecium	1
Sputum culture (pneumonia)	*Aspergillus* spp.	2
	Aspergillus spp, *Klebsiella pneumonia, P. aeruginosa*	1
	C.albicans, Klebsiella pneumonia, Pseudomonas aeruginosa, enterobacter spp	1
Throat culture	*Klebsiella pneumonia*	1
Peritonsillar abscess	*Staf aureus*	1
Skin abscess	*Staf aureus*	1
	Staf aureus/serratia marcescens/ enterococcus faecium	1
	Pseudomonas aeruginosa, E.coli (ESBL+)	1
Cutaneous/subcutaneous infections	*S. Epidermidis, Klebsiella pneumonia*	1
Liver abscess	*Staf aureus*	1
	Aspergillus spp/*mucormycosis*	1
Brain abscess apse	*Aspergillus* spp/*mucormycosis*	1
Wound culture (osteomyelitis)	*Aspergillus fumigatus Serratia marcescens*	1
	S.aureus, Pseudomonas luteola, Acinetobacter, Coagulase-negative Staphylococcus	1
Meningitis	*Burkholderia cepacia (P1)*	1
Urine culture	*Coagulase-negative Staphylococcus*	1
	E.coli, Pseudomonas aeruginosa, Klebsiella pneumonia	1

observed in the other 3 (17.6%) patients. Four of the disseminated BCG disease cases involved lymph nodes and lung tissue (BCG pneumonia) (P6, P8, P10, P11), while one patient had miliary tuberculosis (P5) and *Mycobacterium tuberculosis* (TBC) was isolated by gastric lavage smears. All patients with BCG-itis (P7, P13, P14) showed local or regional involvement at the vaccine injection site and developed ipsilateral regional lymph node enlargement after BCG vaccination. The diagnosis of BCG-itis was made entirely based on laboratory, histological and clinical criteria, and mycobacteria could not be isolated in cultures. No non-tuberculous mycobacterial infection was observed. Antituberculosis treatment was given for a year in all eight patients and until all patients were under control.

Prophylaxis with TMP-SMX and antifungal was used for all patients except one lost to follow up and another quitting at first admission to the hospital. In severe cases interferon-γ (IFN-γ) was added to the treatment protocol. Curative treatment for CGD is stem cell transplantation. Hematopoietic stem cell transplantation was performed in 8 patients (47.1%), 4 of them from relatives and 4 from an unrelated fully matched allogenic donor (Table 2). Cutaneous graft versus host reaction (GVHD) developed in a patient (P8). The survival rate after HSCT transplantation was 87.5%, 3 (17.6%) died due to sepsis and one due to pneumonia, and the mean age of death was 152.6 ± 105.7 months with a median of 167 months (12–263). Of those who died, 3 were AR (p22phox defect) and one was an X-linked case.

5. Discussion

This is a single-center study which revealed the features of 17 CGD patients from Central Anatolia, and the most common CGD genotype was (81.25%) AR-CGD. The prevalence was higher than in the western literature review, for which the high rate of consanguinity (n = 13) (86.5%) was the underlying cause in our region [4]. Our city population is around 2 million and since not all CGD cases may be diagnosed before death, and a few patients followed by other hospitals were not included in our list, the results suggested that the estimated prevalence of CGD was around one in 100,000 people, which is higher than expected in the literature.

The mean age at diagnosis was also significantly different between the XL and AR-CGD subtypes. The mean age at diagnosis in was 7.6 years, with a median of 3 years. The mean delay in diagnosis was found to be 4.4 years. Compared to individuals with AR-CGD, patients with X-CGD had a lower mean age upon diagnosis (3.6 vs. 8.5 years). Köker *et al* found in a series of 89 patients that the mean age at diagnosis was 5.2 in AR-CGD and 2.7 in X-CGD [4]. Patients with the AR form have milder clinical symptoms than those with the XL form and this may due to residual oxidase activity [4,5,11]. Milder clinical progress and late-onset symptoms may cause later age of diagnosis [11]. Growth retardation was detected in 41.2% (7/17) of our study group. Their height and weight were below the 3rd percentile for their age. Growth retardation was a finding observed in immunodeficiencies, and growth and development were also affected in CGD patients. As a result, it is evident that early diagnosis and treatment would contribute positively to growth.

Our study showed that the most common targeted organ due to infectious findings was lung, followed by skin, lymphadenitis and gastrointestinal tract. At least one lung infection was identified in 76.4 % of the patients. Skin/subcutaneous infections and lymphadenitis were observed with a frequency of 58.8%. These results are consistent with publications on the clinical presentation of CGD [5,6,8,12]. One of the gastrointestinal findings in our study was an enteric infection, which was observed with a frequency of 29.4 %. Perianal abscesses were observed in 5.9%. A study conducted in the USA reported that GI involvement was present in 32.8% of patients with CGD, which was similar to our findings. Studies reported that 75% of patients with CGD who had perianal abscesses had X-linked CGD [13]. However, our patient with a perianal abscess had an AR subtype. In their research, Zhou *et al* found a 7% prevalence of perianal infection [14]. Compared to the literature, our patients had a higher rate of sepsis (41.2%), which resulted in the mortality of three patients (17.6%). Sepsis was found in 5% of patients in an Italian cohort [8] and 20% in a European one [6].

Non-infectious complications as well as inflammatory and rheumatic conditions have been commonly reported in patients with CGD [15,16]. Non-infectious inflammatory complications were observed in at least one episode in 58.8% of the participants in our study (66.6% in patients with X-linked CGD, 57.1% in patients with AR-CGD). 17.6% of our cohort was X-linked. A similar analysis was performed in a French

cohort; 69.4% of patients reported inflammatory symptoms, but 71.4% of the cohort was XL-CGD, and patients with X-linked CGD had twice the rate of inflammatory complications compared with patients with AR-CGD [17,18]. In our study, inflammatory pulmonary symptoms were observed in 23.5% (granulomatous lung disease: 17.6%, interstitial pulmonary fibrosis: 5.9%), which was similar to that in the literature. Non-infectious granulomatous lung diseases were treated with anti-inflammatory therapy. In comparison to the literature, inflammatory gastrointestinal involvement (chronic diarrhea: 17.6%, pyloric outlet obstruction: 5.9%) was detected at the low rate of 23.5 % in our study [6,17,18]. In addition to inflammatory GI disease, liver involvement was common and important. Patients with CGD may develop nodular hyperplasia, non-cirrhotic portal hypertension, hepatosplenomegaly, and splenic sequestration [17,19]. 47.1% of our study group had hepatomegaly, 35.3% had splenomegaly, and 41.2% had no organomegaly. Autoimmune findings were rare in this study; Behçet's, FMF, and sporadic JIA were observed in one patient, whereas sarcoidosis occurred in another. Macrophage activation syndrome has also been reported in CGD patients, a potentially life-threatening inflammatory complication [17,20]. Macrophage activation syndrome was observed in one of our patients (P1). This patient died during follow-up.

The incidence of infection has been accepted as a measure to evaluate clinical course of the disease and effectiveness of various prophylactic treatments. The annual incidence of infection in CGD patients has been reported to be between 0.64 and 0.79, depending on the treatment received [8]. The mean number of infections per patient during follow-up was 1.96 per year in our study group. In total, 81 cases of infection were hospitalized requiring intravenous antibiotics and antifungal treatment and these were defined as severe infections. The mean annual incidence of severe infection was 0.86 (XL group: 1.16, AR group: 0.79). That is slightly higher than in the literature because the follow-up time of a patient was very short (about three months).

Studies have shown that infection with *Burkholderia cepacia*, which is a usual pathogen in CGD, may be severe and fatal [6]. It was isolated in a patient (P1) accompanied by hemophagocytic lympho-histiocytosis (HLH) and aplastic anemia, and the patient eventually died from *B. cepacia* sepsis. The incidence of mycobacterial infection increased significantly in last decade, and mycobacterial infection was one of the most important clinical problems observed during follow-up of our patients. Mycobacterial infections are common in CGD patients in countries where tuberculosis is endemic, especially in areas where BCG vaccination is routinely given [21]. Similar to our study, probable or definite BCG disease occurred in 59.2% (77/130) of patients in a cohort from China, 93.5% of whom were vaccinated with the BCG strain [14].

Two patients received daily prophylaxis with TMP-SMX and antifungal azole derivatives. Our results were similar to reports from Turkish, European and US cohorts [4–6]. The low annual incidence of serious infections in this study can be attributed to prophylaxis, as well as intensive follow-up with laboratory support and specialist management. Thus, it seems that prophylaxis management plays an important role in controlling severe infections and quality of the life in CGD patients.

Hematopoietic stem cell transplantation (HSCT) is the only curative treatment for CGD [17]. From diagnosis to the last follow-up day, 8 patients received stem cell transplantation and one had Cutaneous GVHD. The survival rate after HSCT transplantation was 87.5% among our patients, and stem cell transplantation was performed in one of four cases who died, which was similar to the literature [22]. The mortality rate of 23.5% appears to be similar to those reported in a large series in developed countries since 2000 (7–16 years follow-up mortality 9%–23%) [7,8,23].

In conclusion, the clinical signs and symptoms of CGD in this study largely confirm previous assumptions about the causative microorganisms. X-CGD patients are more recognized by family history and severe infection than AR-CGD. Early prophylaxis with TMP-SMX and an azole derivative help to prevent severe infections and may decrease infectious episodes. We found rare large deletion at exon 3–6 on *CYBA* gene in six unrelated families (Table 1). Most families inhabited Konya city and its environs, suggesting a possible founder effect for *CYBA* exon 3–6 deletion in the central part of Türkiye which was similar to the literature [24]. We also found a mutation at the hotspot point, a GT deletion at the beginning of exon 2 of *NCF1,* in 5 families with AR-CGD [2]. This mutation may result in residual oxidase activity in AR-CGD with p47phox defect causing a delay in onset, diagnosis and late clinical presentation. The protective role of residual activity is limited while the infection is ongoing, however [11]. Awareness of late presentation of CGD is important for management of CGD to achieve diagnosis and early prophylaxis.

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Ethical approval

This research study was conducted retrospectively from data obtained for clinical purposes. The study was approved by Necmettin Erbakan University Meram Medical School Ethics Committee (Date: 21.01.2022/No: 2022/3609).

Consent to publish

Not applicable.

Consent to participate

Not applicable.

Author contributions

Dr. Yahya GUL analyzed and interpreted the data and wrote the paper. Dr. İsmail Reisli drafted the manuscript and as analyzed/interpreted the study data. Dr. Sevgi Keleş, Dr. Şükrü Nail Guner analyzed and interpreted the clinical data and provided statistical support. Dr. Mustafa Yavuz Köker analyzed and interpreted the clinical and laboratory data and responded to comments. Rabia Nayir PhD provided genetic and flow cytometric support. Sinan Kütük PhD provided DHR123 assay and expression study. Dr. Esra Hazar and Dr. Hasan Kapaklı were tasked with acquisition of clinical data. All authors read and approved the final manuscript.

Declaration of competing interest

The authors declare that there are no conflicts of interest

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