ORIGINAL ARTICLE

Clinical and Immunological Characteristics of 63 Patients with Chronic Granulomatous Disease: Hacettepe Experience

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Abstract

Background Chronic granulomatous disease (CGD), one of the phagocytic system defects, is the primary immunodeficiency caused by dysfunction of the NADPH oxidase complex which generates reactive oxygen species (ROS), which are essential for killing pathogenic microorganisms, especially catalase-positive bacteria and fungi.

Objective The objective of our study was to assess the clinical and laboratory characteristics, treatment modalities, and prognosis of patients with CGD.

Methods We retrospectively reviewed 63 patients with CGD who have been diagnosed, treated, and/or followed-up between 1984 and 2018 in Hacettepe University, Ankara, in Turkey, as a developing country.

Results The number of female and male patients was 26/37. The median age at diagnosis was 3.8 (IQR: 1.0–9.6) years. The rate of consanguinity was 63.5%. The most common physical examination finding was lymphadenopathy (44/63), growth retardation (33/63), and hepatomegaly (27/63). One adult patient had squamous cell carcinoma of the lung. The most common infections were lung infection (53/63), skin abscess (43/63), and lymphadenitis (19/63). Of the 63 patients with CGD, 6 patients had inflammatory bowel disease (IBD). Twelve of the 63 patients died during follow-up. *CYBA*, *NCF1*, *CYBB*, and *NCF2* mutations were detected in 35%, 27.5%, 25%, and 12.5% of the patients, respectively.

Conclusion We identified 63 patients with CGD from a single center in Turkey. Unlike other cohort studies in Turkey, due to the high consanguineous marriage rate in our study group, AR form of CGD was more frequent, and gastrointestinal involvement were found at relatively lower rates. The rate of patients who treated with HSCT was lower in our research than in the literature. A majority of the patients in this study received conventional prophylactic therapies, which highlight on the outcome of individuals who have not undergone HSCT.

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Introduction

Chronic granulomatous disease (CGD) is a functional disorder of phagocytic leukocytes caused by mutations in the genes encoding the proteins of the leukocyte NADPH oxidase complex. This enzyme is located in neutrophilic and eosinophilic granulocytes and monocytes and generates reactive oxygen species (ROS), which are essential for killing pathogenic microorganisms [1]. Although the absence or impairment of reactive oxygen species (ROS) production in neutrophils and monocytes has been emphasized in the studies conducted so far, ROS production impairment was also observed in all myeloid cells [2]. The NADPH oxidase complex consists of three membrane-bound proteins, called gp91^{phox}, p22^{phox} and

EROS, and three cytosolic proteins called p40^{phox}, p47^{phox} and p67^{phox} [3]. During phagocytosis of pathogens, these proteins come together in one large complex that actively generates ROS. Inactivating variants of the genes *CYBA* (p22^{phox}), *NCF1* (p47^{phox}), *NCF2* (p67^{phox}), *NCF4* (p40^{phox}), and *CYBC1* (EROS) cause autosomal recessive chronic granulomatous disease (AR-CGD) [4, 5]. The X-linked form of the disease is caused by mutations in the *CYBB* gene that encodes gp91^{phox}.

The incidence of disease in the USA and Europe ranges from 1 in 200,000 to 250,000 births, while in the Israeli-Arab population, it is seen as 1 in 70,000 births [6, 7].

Severe infections are caused by bacteria, including *Staphylococcus aureus*, *Burkholderia cepacia*, *Serratia marcencens*, *Mycobacteria*, *Nocardia*, and *Salmonella*, and fungi such as *Candida* and *Aspergillus* species [8–10]. Among all primary immunodeficiencies, CGD has the highest prevalence of invasive fungal infections, which are seen in 20–40% of patients and with great impact on morbidity and mortality [11, 12]. The clinical spectrum of the disease comprises infections (lungs, lymph nodes, bones, liver, and skin); granuloma formation in the genitourinary and/or gastrointestinal tract; Crohn-like colitis; delayed wound healing due to excessive granulation; autoimmune findings; and growth retardation [13].

Hypergammaglobulinemia, increasing acute-phase reactants, chronic disease anemia, and hypoalbuminemia are frequently reported laboratory findings. The nitroblue tetrazolium (NBT) dye test, based on the determination of oxidase activity under light microscopy, can be used as a screening tool for the disease, while the dihydrorhodamine (DHR) test that measures the oxidation of dihydrorhodamine-1,2,3 to rhodamine-1,2,3 in neutrophils by flow cytometry can offer strongly guiding results for carrier states in heterozygous women for defects in CYBB or for defects with residual capacity of the enzyme, such as those observed in patients with hypomorphic variants in this gene [13–16]. Molecular assay approaches include serial single-gene testing, multiple-gene panel use, and more extensive genomic testing.

The objective of this study is to assess the clinical and laboratory characteristics, treatment modalities, and prognosis of patients with CGD who have been diagnosed and treated and/or followed up in a single immunology center in Turkey, as a developing country.

Methods

Patients and Study Design

We retrospectively reviewed patients with CGD from Hacettepe University Ihsan Dogramacı Children's Hospital Division of Pediatric Immunology, Ankara, Turkey, who have been diagnosed or followed-up between 1984 and 2018. The data of the patients with CGD were obtained from the hospital files and electronic records. The demographic characteristics of the patients (gender, age of diagnosis, consanguinity, etc.), clinical findings, laboratory findings, genetic mutations, treatment modalities, and survival were evaluated. The study design was in accordance with the Helsinki Declaration and was approved by the Hacettepe University Non-interventional Clinical Research Ethics Board. Informed consent was obtained from the patients and parents or legal guardians of the pediatric participants.

Methods

The patients were suspected as CGD patients based on clinical findings and diagnosed according to European Society of Immunodeficiencies (ESID) criteria. NBT was used to screen the patients' neutrophil NADPH oxidase capacity. The diagnosis was confirmed by DHR test in suspected patients.

Molecular Analysis

Genetic analyses were carried out by different centers in 40 of the 63 patients. Patients' DNA analyses were initially conducted in various centers by Sanger sequencing. Since 2017, our center has been working with the next-generation sequencing system, which has been extensively used recently. Targeted next-generation sequencing (TNGS) was performed after 2017 to reveal the molecular pathology of primary immune deficiency patients. TNGS work flow was based on an Ion AmpliseqTM Primary Immune Deficiency Research Panel designed for sequencing of 264 PID genes on an Ion S5 TM Sequencer. TNGS panel in our center includes *CYBB*, *CYBA*, *NCF*, *NCF2*, and *NCF4* genes. In 7 patients, this panel testing showed causative mutations for CGD.

An in silico analysis was performed to analyze the functional consequences of single-nucleotide variants found in CGD genes. Three different bioinformatic tools were used: PolyPhen (http://genetics.bwh.harvard.edu/pph/), Mutation Taster (http://www.mutationtaster.org), and SIFT (Sorting Intolerant From Tolerant) (http://sift.jcvi.org/www/SIFT_enst_submit.html).

Statistical Analysis

Data were analyzed with IBM SPSS Statistics 22.0 (IBM Corporation, Armonk, NY, USA). Factors associated with chronic granulomatous disease, clinical parameters, treatment approaches to patients, and clinical outcomes of patients were evaluated with descriptive statistics. In addition to descriptive statistics in the evaluation of groups, nonparametric Mann

Whitney U test and chi-square tests were used. Differences with $p < 0.05$ were considered statistically significant.

Results

Patient Characteristics

A total of 63 patients with a median age of 19 (min: 13–max:27) years were included in the study. The number of female and male patients was 26/37. The median age at diagnosis was 3.8 (IQR: 1.0–9.6) years. The rate of consanguinity was 63.5%. Family history of CGD was positive in 11 patients. In patients with a positive family history for CGD, 1 had X-linked and 7, had AR CGD. The genetic defect is unknown in the remaining 3 patients.

Clinical Manifestations

In 47 out of 63 patients, clinical findings on admission could be detected from the medical records. Forty percent of the patients were hospitalized for lung infections, 19% had lymphadenitis (number of patients with cervical, axillary, inguinal, and submandibular regions were 9, 5, 3, and 1, respectively), 19% had fever, 15% had skin abscess, and 8% had anal-perianal abscesses. Other symptoms are shown in

Table 1. The clinical findings in the follow-up period are shown in Fig. 1. The most common physical examination findings were lymphadenopathy (70%), growth retardation (52%), hepatomegaly (43%), splenomegaly (32%), prolonged/persistent fever (25%), chronic diarrhea (24%), and developmental delay (22%) (Table 2).

The most commonly seen infections were lung infections (53/63), skin abscess (43/63), and lymphadenitis (19/63), which were localized in the cervical region in 9 patients, in the axillary region in 5 patients, in the inguinal region in 3 patients, and in the submandibular region in 1 patient. Other commonly seen infectious presentations are liver abscess (17%), osteomyelitis (17%), otitis media (16%), and anal-perianal abscess (13%) (shown in Fig. 2). One adult patient who had history of smoking had squamous cell carcinoma in the lungs.

In follow-up, 28 patients received anti-tuberculosis treatment/prophylaxis. Six patients were followed up with a diagnosis of BCGitis/BCGosis, 2 patients with a diagnosis of pulmonary tuberculosis, 1 patient with Pott's disease, and 1 patient with tuberculosis dactylitis. *Mycobacterium tuberculosis* could be isolated in pus culture in only one patient.

Figure 3 displays the microorganisms found in different samples of CGD patients. In 35 of the patients, at least one microorganism was isolated. Twenty bacteria and 7

Table 1 Clinical and demographic characteristics of the study group		
Number of patients	63	
Sex (male)	58.7% (n=37)	
Age, median (IQR)	19 (13–27)years	
Age at diagnosis, median (IQR)	3.85 (1.05–9.64) years	
Consanguinity (positive)	63.5%	
Family history of CGD	17.5% (n=11)	
Mutations (AR/X linked) (n=40)	31/9	
Clinical findings on admission (n=47)	Lung infection 42.6% (n=20)	
	Lymphadenopathy 19.2% (n=9)	
	Fever 19.2% (n=9)	
	Skin abscess 14.9% (n=7)	
	Anal-perianal abscess 8.5% (n=4)	
	Diarrhea 8.5% (n=4)	
	Osteomyelitis 6.4% (3)	
	Moniliasis 2.1% (n=1)	
	Otitis media 2.1% (n=1)	
	Liver abscess 2.1% (n=1)	
Deaths	19.1% (n=12)	
Age of death(years), median(IQR)	5.50 (2.5–12.5)	
Reasons of death	Sepsis 6/12	
	Disseminated intravascular coagulation 3/12	
	Pulmonary infection 2/12	
	Myocarditis 1/12	

Table 2 Distribution of patients according to mutations

	Genes	Patients (n)	Sex (n) (M/F)	Age of onset (years)*	Age at diagnosis (years)*	Number of patients died
X-CGD	CYBB	10	10	1.8 (0.9–3.4)	11.6 (7.7–19.0)	2
AR-CGD	NCF 1	11	6/5	10.7 (3.0–16.2)	21.4 (18.9–23.8)	2
	NCF 2	5	4/1	3.9 (3.3–6.8)	3.9 (3.3–6.8)	1
	CYBA	14	6/8	2.3 (1.4–4.0)	2.3 (1.4–4.0)	1

*Median (inter-quartile range)

fungi, in total 27 different pathogens, were detected in sputum (19), pus (17), urine (12), blood (10), tissue (6), bronchoalveolar lavage fluid (BALF) (5), and central venous catheter (5) cultures. The most frequently detected microorganism in tissue cultures was *Aspergillus fumigatus* (lung, bone, paravertebral tissue, cerebellum).

Eighteen patients were tested for *Aspergillus*, and 8 found to be positive. *Aspergillus* antigen test was performed once in 5 three times in 6, minimum four times in 7 patients.

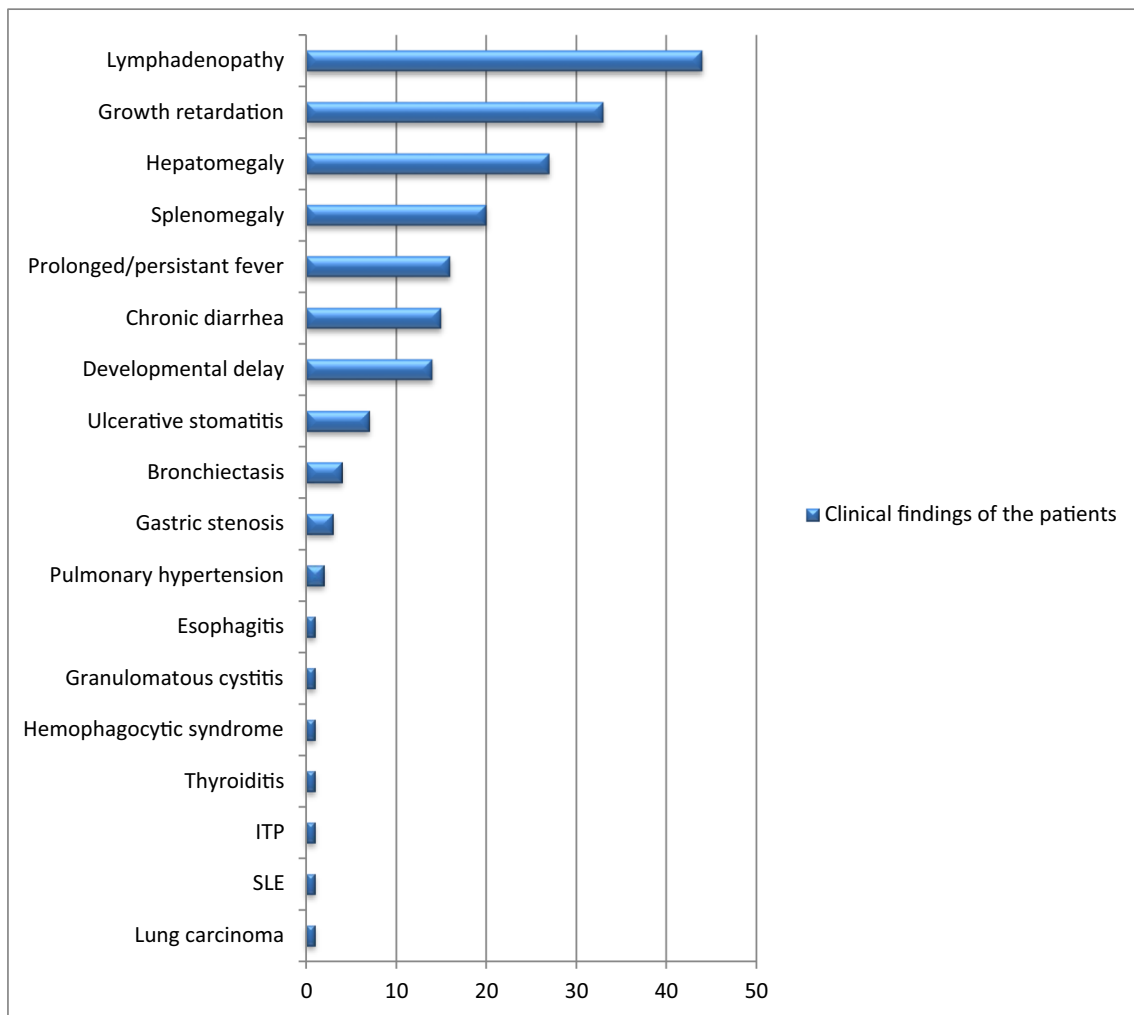Of the 63 patients with CGD, 6 patients had inflammatory bowel disease (IBD). The diagnosis of IBD was made by

Fig. 1 Clinical findings during follow-up of the patients (*X*-axis: number of patients)
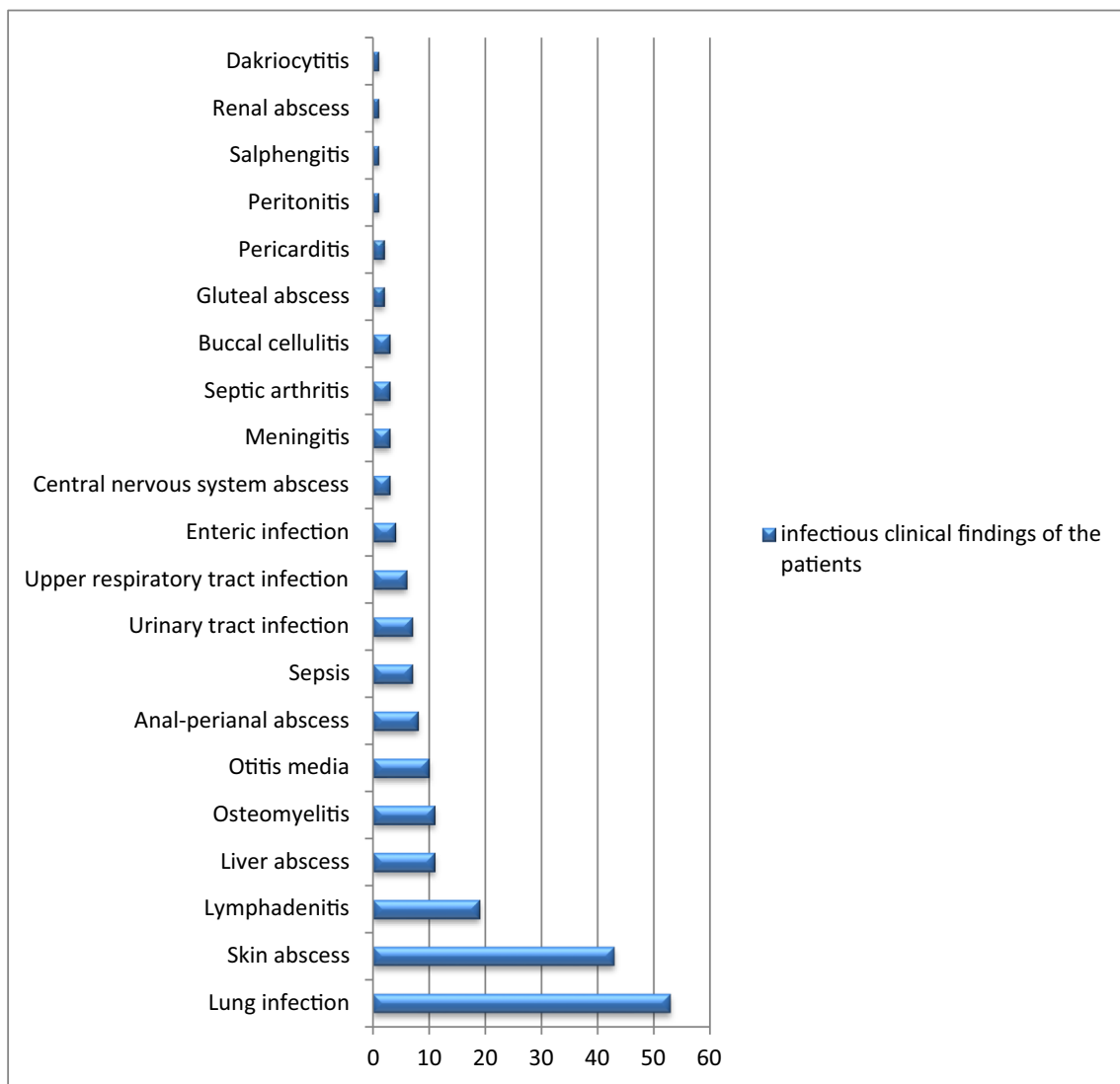
Fig. 2 Infectious diseases associating CGD in our cohort (*X*-axis: number of patients)

endoscopic imaging and pathological examination of the specimens. IBD was suspected in patients with clinical findings such as diarrhea, persistent fever, tenderness, GIS bleeding, and high acute phase reactant. These patients were evaluated with colonoscopy for the presence of superficial intestinal mucosal ulceration, involvement of terminal ileum, and cobblestone appearance. The presence of active colitis and/or ileitis with lymphocyte infiltration in the mucosa in pathological examinations supported the diagnosis of IBD. All patients had diarrhea, 3 of them had rectal bleeding, and 2 of them had abdominal pain. During follow-up, one patient was complicated with intestinal perforation.

Twelve of the 63 patients died during follow-up. The median age of death was 5.5 (2.5–12.5) years. Cause of death was sepsis in 6 patients, diffuse intravascular coagulation (DIC) in 3 patients, pulmonary infection in 2 patients, and myocarditis in one patient.

Laboratory Features

NBT test was performed in all patients in the study group. NBT result was 0 in all patients. DHR was found to be abnormal in 38 patients.

Hemoglobin levels were found to be below the reference values in 60% of the patients [17]. Leukocyte numbers were higher than the reference values in 29% of the patients. Leukopenia was found in 5% of patients during infection episodes associated with medication usage, especially antipyretics.

Acute-phase reactants were evaluated on admission in 54 of the 63 patients. The median ESR was 27.5 (IQR: 14–48) mm/h; the median CRP was 3.5 (IQR: 0.7–8.3) mg/dL. At the time of diagnosis, IgG values were found to be higher than the reference intervals in 27% of the patients, IgA in 35% of the patients, and IgM values in 16% of the patients [18]. The median IgE was 100 (IQR: 30–560) IU/mL.
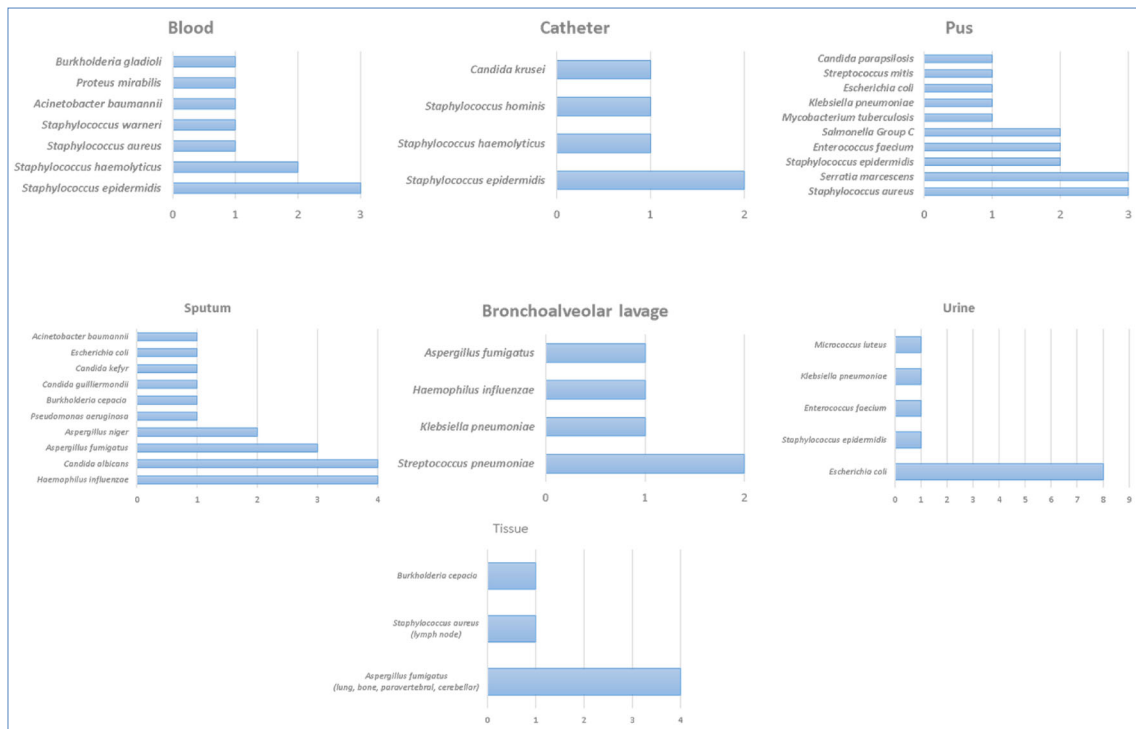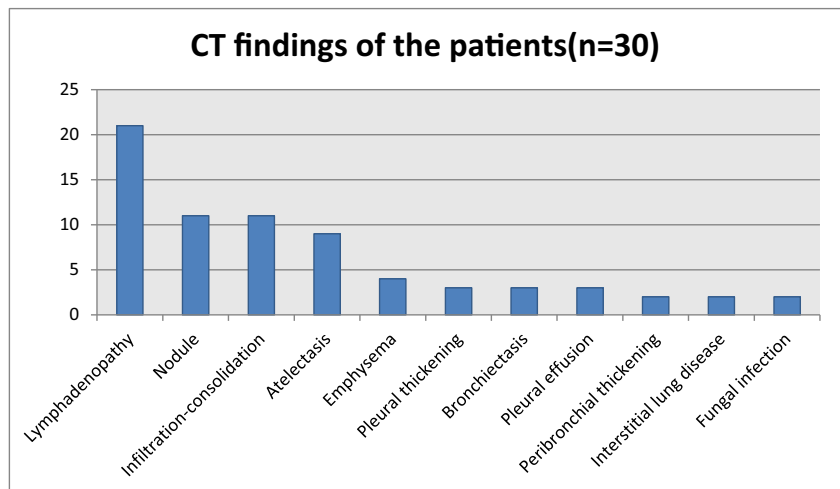
Fig. 3 Distribution of pathogenic agents in patients with CGD

All patients with IBD had elevated CRP (median: 6.7 (2–12) mg/dL and ESR (median: 59 (14–100) mm/h). Fecal calprotectin was evaluated in two of the 6 patients and found to be 214.2 and 42 μg/g stool (reference value: < 60 μg/g stool).

Thirty patients are evaluated with thorax computed tomography (CT) for the suspicion of pneumonia and/or pulmonary involvement, as shown in Fig. 4. Most common findings were lymphadenopathy (70%) (mostly mediastinal and hilar), nodules (37%), infiltration- consolidation (37%), and atelectasis (30%). Evidences of bronchiectasis are present 4% of study group, as shown in Fig. 1.

Treatment

Fifty-eight patients received antimicrobial prophylaxis with TMP-SMX. All patients were treated with antifungal prophylaxis [itraconazole (56), voriconazole (6), posaconazole (2), fluconazole (1)]. IFN-gamma prophylaxis was used in 34 patients with a dosage of 50 mcg/m^2 three times a week. Fever as a side effect was encountered in a few of our patients.

Twenty-eight patients have been treated with anti-tuberculosis drugs. Twenty-one patients used only isoniazide and rifampicin combination, the rest of the patients used other anti-tuberculosis drugs in addition to

Fig. 4 Thorax CT findings of the patients (Y-axis: number of patients)

isoniazide-rifampicin combination [pyrazinamide (3), streptomycin (3), ethambutol (4)].

During follow-up, 6 patients were transfused with granulocytes for the treatment of acute infections, including pneumonia (3), osteomyelitis (2), and cerebellar abscess (1). Granulocyte transfusions significantly contributed to the clinical improvement of the patients.

Five of the patients were treated with HSCT, from a matched related donor (3) or from a matched unrelated donor (2). Two of these patients developed graft-versus-host disease (GVHD): one suffered from BK virus nephropathy and fungemia after transplantation, and one of them died because of sepsis. One patient was transplanted for the second time because of graft loss. One patient is being followed up for chronic GVHD. The patients received cyclosporine for GVHD prophylaxis after transplantation. The clinical characteristics of the patients who underwent hematopoietic stem cell transplantation in our center are given in Table 3.

Molecular Diagnosis

Mutations causing CGD were evaluated in 40 of the 63 patients (Fig. 5). The mutations detected in our patients are shown in Fig. 4. *CYBA* (p22^{phox}), *NCF1* (p47^{phox}), *CYBB* (gp91^{phox}), and *NCF2* (p67^{phox}) mutations are detected in 35%, 27%, 25% and 12%, of the patients, respectively (Table 2). Known mutation details of the patients are given in Table 4.

Deceased patients had a mutation in *CYBB* (2), *NCF1* (2), *CYBA* (1), or *NCF2* (1).

Discussion

In the present study, we investigated clinical and immunological characteristics of 63 patients with CGD from a single center in Turkey. AR CGD was present in 75% of the patients.

The CGD prevalance has been reported as 1/200.000 in Europe [19], but is not known in Turkey which is among the most populous countries of the world with high consanguinity rate (20–25%) and high fertility rate [20]. The rate of consanguinity was detected as 63.5% in our study which caused the high incidence of AR form of the disease in our study. Koker et al. evaluated 89 patients with CGD from 73 Turkish families in a multicenter study and concluded that most of the families (55%) have an AR genotype, and 38% have an X-linked genotype; patients from 5 families with a suspected AR genotype (7%) were not fully characterized [21]. In a separate study of fewer patients from a different region of our country, consanguinity was found to be 54% and AR-CGD to be 41.6% [22]. This research showed a higher rate of consanguinity rate and AR form of CGD than other studies from Turkey in literature.

The X-linked form of CGD is more common in America, Europe, and Japan [6, 19, 23], whereas AR-CGD is more prevalent in countries like Egypt, Iran, and Israel [17, 18, 24], where consanguineous marriage is more common. Recently, in a study published by Blancas-Galicia et al., including Mexican national CGD data, 64 of 89 patients with known genetics were shown to have X-linked inheritance [25].

The median age at diagnosis was 3.9 (1.1–9.6) years in our study whereas 8 months in a Chinese cohort [26], 4.4 years in an Italian cohort [6], and 4 years in the study from Israel [17]. In the study from Turkey including 89 patients, mean age at diagnosis of patients was found to be 5.2 years in AR-CGD and 2.7 years in X-CGD. Median diagnostic delay was approximately 3 years in our study, which was a much longer duration when compared with a French cohort, in which age at diagnosis was found to be 2.5 years and diagnostic delay was found to be 1.2 years [27]. In studies conducted in our country, ages of diagnosis were found to be similar, although different in AR and X-CGDs. Time of diagnosis is late compared to European countries which may be due to the earlier presentation of clinical findings of X- CGD and higher awareness.

Table 3 The clinical characteristics of the patients who underwent hematopoietic stem cell transplantation

Patient number	Sex	Age At diagnosis	Age At HSCT	Donor	Conditioning regimen	Complications	Current Status
	F	11 years	17 years	10/10 matched sibling	+	BK virus nephropathy, pulmonary hemorrhage, fungemia, chronic GVHD, hyperglycemia, encephalytis, invasive CNS aspergilosis	Exitus
	F	10 years	19 years	10/10 matched sibling	+	–	Live
	M	13 months	5 years	10/10 matched Unrelated	+	Neutropenia septicemia Prolonged intensive care hospitalization	Live
	M	3 years	8 years/19 years	10/10 matched sibling Unrealed	+	Graft rejection/none	Live
	F	13 months	5 years	10/10 matched father	+	aGVHD	Live

Table 4 Genetic mutations of the patients

	Sex	Consangunity	Gene	Exon/intron	Mutation (nucleotide change)	Protein change	
P1	M	–	CYBB	Exon 7	c.736C>T	p.Gln246Ter	Reported
P2	M	+	CYBB	Intron 3	c.253-1G>A	skip exon 4	Reported
P3	M	+	CYBB	Exon 5	c.469C>T	p.Arg157Ter	Reported
P4	M	–	CYBB	NA	NA	NA	NA
P5	M	–	CYBB	Exon 4	c.271C>T	p.Arg91Ter	Reported
P6	M	–	CYBB	Exon 5	c.450_451delAA	p.Ser151LeufsTer13	Reported
P7	M	–	CYBB	All exons deleted	c.0		Novel?
P8	M	–	CYBB	Exon 11	c.1415delG	p.Gly472AlafsTer30	Reported
P9	M	–	CYBB	Exon 9	c.1031C>T	p.Ser344Phe	Reported
P10	M	+	CYBA	Intron 5	c.369+1G>A homozygous	exon 5 deleted	Reported
P11	M	+	CYBA	Exon 6	c.408delC homozygous	p.Lys137SerfsTer54	Reported
P12	M	+	CYBA	Exon 3	c.166dupC homozygous	p.Arg56ProfsTer157	Reported
P13	F	+	CYBA	Exon 3	c.166dupC homozygous	p.Arg56ProfsTer157	Reported
P14	F	+	CYBA	Exon 2	c.70G>A homozygous	p.Gly24Arg	Reported
P15	F	+	CYBA	Intron 5	c.369+1G>A homozygous	exon 5 deleted	Reported
P16	F	+	CYBA	Intron 5	c.369+1G>A homozygous	exon 5 deleted	Reported
P17	F	+	CYBA	Intron 1	c.58+4_58+7del AGTG homozygous	p.Ile20SerfsTer77	Reported
P18	M	–	CYBA	Intron 1	c.58+4_58+7del AGTG homozygous	p.Ile20SerfsTer77	Reported
P19	F	+	CYBA	Exon 2	c.70G>A homozygous	p.Gly24Arg	Reported
P20	F	+	CYBA	Exon 6	c.339C>A homozygous	p.Cys113Ter	Reported
P21	M	+	CYBA	Exon 6	c.408delC homozygous	p.Lys137SerfsX54	Reported
P22	F	–	CYBA	Exon 2	c.70G>A homozygous	p.Gly24Trp	Reported
P23	M	–	CYBA	Exon 2	c.74G>A homozygous	p.Gly25Asp	Reported
P24	F	+	NCF1	Exon 6	c.574G>A homozygous	splice defect	Reported
P25	M	–	NCF1	Exon 2 Exon 7	c.75_76delGT c.579G>A	p.(Tyr26HisfsTer26) p.Trp193Ter	Reported
P26	M	+	NCF1	Exon 6	c.574G>A homozygous	splice defect	Reported
P27	M	+	NCF1	Exon 2	c.75_76delGT homozygous	p.(Tyr26HisfsTer26)	Reported
P28	F	+	NCF1	Exon 2	c.75_76delGT homozygous	p.(Tyr26HisfsTer26)	Reported
P29	F	+	NCF1	Exon 2	c.75_76delGT homozygous	p.(Tyr26HisfsTer26)	Reported
P30	F	+	NCF1	Exon 2	c.75_76delGT homozygous	p.(Tyr26HisfsTer26)	Reported
P31	M	+	NCF1	Exon 2	c.75_76delGT homozygous	p.(Tyr26HisfsTer26)	Reported
P32	M	–	NCF1	Exon 2	c.75_76delGT homozygous	p.(Tyr26HisfsTer26)	Reported
P33	M	–	NCF1	Exon 2	c.75_76delGT homozygous	p.(Tyr26HisfsTer26)	Reported
P34	Female	–	NCF1	Exon 2	c.75_76delGT homozygous	p.(Tyr26HisfsTer26)	Reported
P35	Female	–	NCF2	NA	NA	NA	NA
P36	Female	–	NCF2	Exon 4+Intron 4	c.[279C>G+366+1G>C] compound heterozygous	p.Asp93Glu	Reported
P37	Female	+	NCF2	Exon 4	c.304C>T homozygous	p.Arg102Ter	Reported
P38	Female	+	NCF2	Exon 6	c.605C>T homozygous	p.Ala202Val	Reported
P39	Male	–	NCF2	Exon 2	c.G38>A homozygous	p.Gly13Glu	Novel?
P40	Male	+	NCF2	Exon 8	c.767_768dupAA homozygous	p.Glu257LysfsTer14	Reported

The most common clinical manifestation of CGD in our patients was recurrent lung infections, consistent with other studies in the literature [6, 17, 19, 27]. Other common findings were lymphadenitis, fever, and tissue abscess. In the follow-up period, lymphoproliferative findings, growth retardation, and inflammatory findings like fever and diarrhea were most commonly seen in our cohort. Most common infectious sites were the lungs, skin, and lymph nodes, like in other studies [17–19]. In our study, at least one lung problem was noted in 84.1% of patients. Marciano et al. also showed the lungs as the most common infection site with 87% [8]. The most commonly isolated pathogens were *Staphylococcus* and *Aspergillus*

Fig. 5 Genetic defects of the patients (total number of patients with a genetic diagnosis: 40 out of 63 patients)

CGD mutations(n=40)

NCF2(AR)
13% (n:5)

CYBA(AR)
35% (n:14)

CYBB(X-linked)
22% (n:9)

NCF1(AR)
30% (n:12)
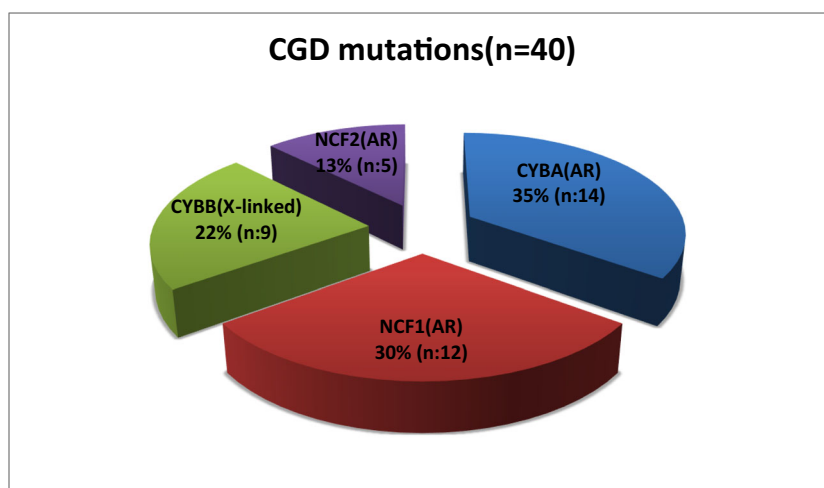
species. Staphylococci were detected in almost all sample types, whereas *Aspergillus* species were detected mostly in sputum and tissue samples.

One of the important problems in patients with CGD is internal organ abscesses. Liver abscess, which is difficult to cope with, is associated with portal hypertension, nodular regenerative hyperplasia, cirrhosis, splenomegaly and splenic sequestration, and mortality in patients [28]. In our study, 11 patients (17.5%) had liver abscess; 2 patients underwent surgery; 3 patients had percutaneous drainage; the remaining 6 patients were treated with antibiotics and antifungal drugs. In our study group, 4 out of 11 patients with liver abscesses died during follow-up. Another major problem is the susceptibility to mycobacterial infections. According to the routine vaccination schedule of our country's ministry of health, all infants are vaccinated with BCG since 1953 between birth and 2 months, so all patients received BCG vaccine. In follow-up patients, 44.4% of them received anti-tuberculosis treatment for the mycobacterial infections (6 patients for BCGitis/BCGosis, 1 patient for Pott's disease, 1 patient for tuberculosis dactylitis, and rest of them received anti-tuberculosis treatment for pulmonary tuberculosis.).

The frequency of mycobacterial infection was expected to be higher in our study because of routine vaccination in our country. However, since it is difficult to detect the bacilli especially in sputum and fasting gastric juice samples taken from children, we only had one pus sample positive for mycobacterial infection. Additionally, similar to our clinical practice, a study from China suggested to treat vaccinated CGD patients with anti-tuberculosis drugs even in the absence of symptoms and emphasized the need of a guideline for the treatment methods of these patients [29]. A previous study showed recombinant human IFN-gamma treatment together with anti-tuberculosis drugs could provide better control of BCGosis [30]. In the mentioned study among the 23 CGD patients, 19 received routine anti-tuberculosis treatment. Among the 19 patients received routine anti-tuberculosis

treatment, 7 (36.8%) received recombinant human IFN-gamma treatment (1 MIU/m2, twice a week) together with anti-tuberculosis treatment. It is reported that in all the 7 patients, BCGosis/BCGitis was cured after 1 year of treatment.

As seen in many other primary immunodeficiencies, it is not only the immune deficiency that is a clinical problem but also emerging immune dysregulation. In patients with CGD, noninfectious complications (gastrointestinal, pulmonary, or rheumatological) may occur as a result of chronic and uncontrolled inflammation as well as recurrent infections [31]. Noninfectious complications such as interstitial lung disease, pulmonary nodule, pleural effusion, and granuloma formation may occur secondary to chronic inflammation in CGD [32–35]. In a retrospective French study, 28% of 67 adult patients had noninfectious pulmonary events [33]. Radiological pulmonary findings were generally detected as nonspecific consolidation, ground glass appearance, tree-in-bud opacities, scattered nodules, and bronchiectasis [33, 36, 37]. In our study, the most common findings in 30 patients evaluated with lung CT were mediastinal and hiler LAP (70%), nodules (36.7%), and infiltration-consolidation (36.7%).

Gastrointestinal system (GIS) involvement is another problem that may occur secondary to inflammation. In our study, there were 15 patients (23.8%) with chronic diarrhea; 6 of the patients (9.5%) were diagnosed with inflammatory bowel disease by endoscopic and pathological findings. Metin et al. reported a 15-year old boy with gastric antral stricture due to CGD from our center in 1999 [38]. In a study of pediatric patients with CGD from the US National Institutes of Health (NIH), the rate of gastrointestinal involvement was found to be 32.8% [39]. According to the USIDNET registry, 38% of patients with CGD have gastrointestinal system findings. GIS involvement rate is lower in our study, which may be due to the fact that our patients are mostly AR-CGD and inflammatory complications were found to be twice more frequent in X-CGD [8, 19]. Steroids or anti-TNF treatments may be needed

in patients with chronic inflammation. These treatments should be used with caution in periods of infections, especially with *Aspergillus*.

A consequence of chronic inflammation and recurrent infections may cause malnutrition in patients with CGD. As previously reported [23, 40, 41], 33 patients (52.3%) had growth retardation in our cohort. Growth retardation was found in 37.5–58% of patients in studies conducted in our country.

Along with the increase in life span with the treatments applied in PIDs, malignancies have started to be seen more frequently. Chronic inflammation in CGD may also facilitate tumor development in these patients. Lymphoma, leukemia, retinoblastoma, melanoma, rhabdomyosarcoma of the liver, and glioblastoma multiform are the types of tumors detected in patients with CGD so far [42–45]. Lung cancer was detected in one of our adult patients with smoking history. He was a 57-year-old male patient who was diagnosed as CGD during the investigation for recurrent lung infections and skin abscess. Upon detecting a mass lesion in the radiological imaging of the lung, left upper lobectomy was performed, and its pathology was compatible with squamous cell carcinoma.

Antimicrobial drugs (TMP-SMX), antifungal drugs (mainly, itraconazole), and interferon gamma are recommended for effective prophylaxis in CGD [46–48] as we use in routine clinical practice. Treatment of acute infections should be started empirically according to the suspected microorganism. In some patients, we transfused granulocytes for the treatment of acute invasive infections and abcesses and found it beneficial since there is a functional disorder of the patients' neutrophils.

The rate of patients who treated with hematopoietic stem cell transplantation (HSCT) was lower in our research than in the literature. This was mainly due to factors such as the preference of patients and families for traditional follow-up, the failure to find a matched donor or the development of end organ damage. In the last 10 years in our clinic, the HSCT has been considered earlier in CGD patients because of the improvement in HSCT success. A majority of the patients in this study received conventional prophylactic therapies, which highlights on the outcome of individuals who have not undergone HSCT.

Hematopoietic stem cell transplantation (HSCT) is the only cure for CGD and eliminates infectious and inflammatory complications [1]. However, patients are prone to graft failure and post-transplant complication. Organ dysfunction prior to transplantation may exacerbate transplantion-related complications [49]. HSCT was found to be the most successful, especially when applied before the 8 years of age and in the presence of a fully compatible donor. A recent literature about conventional treatment vs HSCT offers more intense conditioning to avoid graft rejection. Risks and benefits should be carefully considered when making a decision for HSCT in adolescents and adults [50]. In the report published by the

US Immunodeficiency Network, it was reported that 50 of 507 CGD patients underwent allogeneic stem cell transplantation between 1953 and 2016. Allogeneic transplantation did not change the risk of mortality but was associated with a reduced incidence of infection and increased function. Although HSCT is the definitive treatment of CGD, the survival rates with and without HSCT are roughly comparable, especially in patients with AR form of the disease [51]. Our patient group covers the years between 1986 and 2018. HSCT has recently been discussed as an effective treatment option and is used in clinical practice. The rate of patients who treated with hematopoietic stem cell transplantation (HSCT) was lower in our research than in the literature. This was mainly due to factors such as the preference of patients and families for traditional follow-up, the failure to find a matched donor, or the development of end organ damage. In the last 10 years in our clinic, the HSCT has been considered earlier in CGD patients because of the improvement in HSCT success. A majority of the patients in this study received conventional prophylactic therapies, which highlights on the outcome of individuals who have not undergone HSCT.

In the current clinical practice, gene therapy as a promising treatment modality remains limited to patients without alternative treatment options [52].

Conclusion

We identified 63 patients with CGD from a single center in Turkey. Unlike other cohort studies in Turkey, due to the high consanguineous marriage rate in our study group, AR form of CGD was more frequent, and GI involvement were found at lower rates. The rate of patients who treated with HSCT was lower in our research than in the literature. A majority of the patients in this study received conventional prophylactic therapies, which highlight the outcome of individuals who have not undergone HSCT. Early diagnosis, appropriate treatments and genetic counseling prevent life-threatening infections, noninfectious complications in patients with CGD.

Declarations

Conflict of Interest   The authors delare no competing interests.

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