Clinical features of chronic granulomatous disease: a series of 26 patients from a single center

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Chronic granulomatous disease is a genetically determined immunodeficiency disorder affecting phagocytic cells rendering them unable to kill certain bacteria and fungi. The present study is a single-center retrospective study that aimed to document the clinical course of 26 children, with a median age of 2.5 years, from 21 families diagnosed as chronic granulomatous disease from 1989-2008. A median delay of 39 months was observed between the onset of infections and age at diagnosis. Pneumonia was the most common initial manifestation of the disease followed by lymphadenitis, skin abscess and diarrhea. An AR inheritance was predominant in the study group. All patients received antibacterial and antifungal prophylaxis, resulting in a marked decrease in the incidence of infections. Overall mortality was 19.2%. These results showed that all features in our group (clinical, progression and outcome) were similar to the literature except for the predominance of autosomal recessive form.

Key words: chronic granulomatous disease, consanguinity, neutrophil.

Chronic granulomatous disease (CGD) is an inherited disorder characterized clinicopathologically by recurrent severe bacterial and fungal infections with granuloma formation[1]. It is caused by a genetic defect in one of the subcomponents of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase of the phagocytic cells[2]. The underlying defect is an inability of phagocytic cells to reduce molecular oxygen and create the reactive oxygen metabolites that are necessary for efficient intracellular microbicidal activity. This results in an increased susceptibility to a specific class of microorganisms, namely catalase-positive bacteria, mycobacteria, fungi, and other opportunistic agents[3].

The pattern of inheritance can be X-linked or autosomal recessive (AR). The most common form of the disease is X-linked, which is responsible for about 70% of cases[4]. It is due to a defect in $gp91^{phox}$, an integral membrane protein of NADPH oxidase encoded on the short arm of the X chromosome. Other forms of the disease are due to AR defects in other major components of the oxidase $p22^{phox}$, $p47^{phox}$ and $p67^{phox}$, each of which is encoded on a different autosomal chromosom[3]. In addition, CGD due to deficiency of cytosolic component Rac2 in two cases has been defined[4,5].

Here, we report the clinical features and follow-up of CGD patients from Hacettepe University Medical School.

Material and Methods

This is a retrospective study based on the patients' medical records. Patient and family history, physical examination and laboratory data were reviewed in 26 patients from 21 families diagnosed and followed at Hacettepe University Children's Hospital, Division of

Immunology, from 1989-2008. The patients who were lost to follow-up after diagnosis were excluded.

The diagnosis was made by the nitroblue tetrazolium test (NBT) and dihydrorhodamine 123 (DHR) assay in all patients, and the X-CGD patients were differentiated by bimodal histogram patterns in the DHR assay, which is specific for X-CGD carrier, in mothers of all patients; flow cytometric analysis using conjugated antibodies directed against the NADPH oxidase enzyme subunits could be done in 23 patients[6,7]. Mutations were studied and known in 21 patients and publication of the results are in progress (D Roos, CLB, Amsterdam, The Netherlands)[6,8].

All infections were defined based on the localization and etiologic agent, if demonstrated. An infection was accepted as severe when hospitalization or parenteral treatment was required.

Results

Demographics and Genetics

The study included 11 female (42%) and 15 male (56%) patients with a median diagnosis age of 2.5 years (range: 1 month to 19.5 years). The median time of follow-up was 4.5 years (range: 15 days to 13 years).

There was parental consanguinity in 71.4% (15 of 21 families) of the families. In only 1 patient, AR form was shown by mutation analysis despite absence of parenteral consanguinity. All of the studied patients had homozygous mutation. History of a known family member with CGD was present in 15.4% of the patients.

Mutation analysis, which could be performed in 22 patients, revealed 5 patients (P18-22) from 3 families having X-CGD with mutation in the CYBB gene (22.7%). The underlying

Table I. Sex, Age and Genotype List of 22 CGD Patients

P No	Sex	Age of onset	Carrier mother*	Subtype	DNA Analysis
P1F1	F	1.5 y	-	A67°	+
P2F2	F	1.5 y	-	A67°	+
P3F3	F	5 y	-	A67°	+
P4F4	F	2 mo	-	A47°	+◊
P5F4	F	3 mo	-	A47°	+◊
P6F5	M	7 y	-	A47°	+◊
P7F6	M	4 y	-	A47°	+◊
P8F7	M	5 mo	-	A47°	+◊
P9F8	M	6 y	-	A47°	+
P10F8	M	7 y	-	A47°	+
P11F9	M	2 mo	-	A22°	+□
P12F10	F	1.5 mo	-	A22°	+□
P13F11	M	1.5 mo	-	A22°	+□
P14F11	F	1 mo	-	A22°	+□
P15F12	F	7 y	-	A22°	+□
P16F13	F	1.5 mo	-	A22 °	+□
P17F14	M	20 days	-	A22°	+□
P18F15	M	1.5 mo	+	X91°	+□
P19F15	M	5 mo	+	X91°	+□
P20F15	M	1 mo	+	X91°	+□
P21F16	M	1 mo	+	X91°	+
P22F17	M	1.5 mo	+	X91°	+
P23F18	F	1 mo	-	AR-CGD	ND
P24F19	M	8 mo	-	AR-CGD	ND
P25F20	M	10 y	-	AR-CGD	ND
P26F21	F	5 y 7 mo	-	AR-CGD	ND

CGD: Chronic granulomatous disease. y: Year. mo: Month. ND: Not done.
*: In dihydro rhodamine 123 (DHR) assay two neutrophil population
◊: Described in Roos et al., 2006
□: Described in Köker et al., 2009

genetic defects were *NCF2* gene mutation in 3 (13.6%) (P1-3), *NCF1* in 7 (31.8%) (P4-10) and *CYBA* gene in 7 of 17 (31.8%) (P11-17) patients who had AR CGD (Table I). Mutation analysis was not available in 4 patients (2 female, 2 male). In flow cytometric analysis of these patients using monoclonal antibodies targeting the subunits of NADPH oxidase, the presence of consanguinity and absence of two neutrophil populations in flow cytometric burst pattern of the phagocytic cells of the mothers were suggestive for the AR form.

Age of Onset

The age of onset was before the age of 2 years in 18 patients (range: 1-18 months) (69.2%), whereas 8 patients (30.8%) presented after 2 years of age (range: 4-10 years). Among patients with late onset (> 2y), 7 (87.5%) had mutations causing AR form of the disease. Although AR form was considered in the remaining 1 patient, molecular diagnosis could not be done. In 2 patients with X-linked disease and 2 patients with AR form, the age of onset was the first month of life. Overall, 3 patients (11.5%) were diagnosed in the very early postnatal life (<2 months of age), and 2 of them were diagnosed before any infection developed owing to the sibling history of CGD. These 3 patients (2 AR form and 1 X-linked) did well under prophylaxis without severe infections during follow-up. A comparison of the clinical features of patients with the X-linked and AR forms showed that infections tended to begin at earlier ages in the X-linked form.

Clinical Presentation and Course After Diagnosis

Pneumonia was the most common presentation and the main responsible agents were fungi. Pneumonia was followed by lymphadenitis, skin abscess and diarrhea (Table II). There were 168 infectious episodes in total, and the annual incidences of overall and serious infections were 0.71 and 0.25, respectively.

At first admission, 10 patients presented with severe infections: 2 intracranial abscess due to Aspergillus spp., 3 pneumonia (2 Aspergillus spp., 1 *Mycobacterium tuberculosis*), 1 liver abscess and 1 splenic abscess of unknown origin, 1 osteomyelitis (*Staphylococcus aureus*), 1 endocarditis accompanying pulmonary aspergillosis, and 1 lymphadenitis due to *S. aureus*. Five of these patients could receive additional interferon (IFN)γ treatment and all responded well to the treatment.

Six patients developed new deep organ infections: chest wall mass due to *Aspergillus fumigatus* in 2 patients, a vertebral osteomyelitis caused by *Staphylococcus haemolyticus* and hepatic abscess in 1 patient each, and intracranial aspergillosis in 2 patients while on prophylaxis.

All patients were put on co-trimoxazole and itraconazole prophylaxis from the day of diagnosis. Despite continuous prophylaxis, 12 patients developed at least one episode of severe infections during the follow-up. IFNγ could be given for prophylaxis in addition to co-trimoxazole and itraconazole in only 5 patients, and in 4 of them, severe infections recurred without any benefit.

The etiologic agent could be identified in only 34 of 168 of the total infectious episodes. The responsible infectious agents isolated at each infectious episode are given in Table III. The most frequent responsible agent was Aspergillus spp. followed by Staphylococcus spp. The search for microorganisms was not performed in most episodes occurring in local hospitals, and despite proper procedure, negative cultures were obtained in the rest of the episodes that were evaluated in our hospital.

Except for one patient who had esophagitis leading to esophageal stricture, none of the patients had developed any complications reported previously in CGD patients such as gastric outlet obstruction, urinary tract obstruction or granulomatous colitis.

Five patients (P11,20,22,23,26) died during the follow-up period, with a mortality rate of 19.2%. One patient died at the age of 4.5 years and another at the age of 10 years due to unknown reasons at home, whereas 3 patients died of mixed infections. Two of the latter patients died of overwhelming sepsis (*S. aureus, Candida parapsilosis and C. krusei* in 1 and *A. fumigatus* and *Klebsiella pneumoniae* in the other) within 10 days after diagnosis at 22 months of age and at 12 months of age. The third died of respiratory insufficiency due to

Table II. Type of Infections at Presentation and During Follow-Up Period

Clinical presentation	Before diagnosis		Follow-up	
	N patients (%)	N episodes	N patients (%)	N episodes
Pneumonia	16 (61.5)*	35	8 (30.7)	18
Lymphadenitis	9 (34.6)	26	5 (19.2)	25
Skin abscess	4 (15.3)	15	1 (3.8)	5
Diarrhea	4 (15.3)	10	1 (3.8)	7
Intracranial abscess	2 (7.6)	2	2 (7.6)	2
Liver abscess	2 (7.6)	2	1 (3.8)	2
Perirectal abscess	2 (7.6)	2	2 (7.6)	4
Chest wall mass	-	-	2 (7.6)	4
Osteomyelitis	1 (3.8)	1	2 (7.6)	2
Splenic abscess	1 (3.8)	1	-	-
Septic arthritis	-	-	1	1
Other (vaginal candidiasis, ascites, endocarditis)	3 (11.5)	5	-	-

* numbers in parentheses indicate the percentage of patients

pulmonary infection (*A. fumigatus and C. krusei*) at 5.5 years of age.

Growth and Development

In 17 patients, regular weight percentile records could be obtained. Among these 17 patients, 10 (58.8%) were below the 10th centile for weight at diagnosis. Among these 10 patients, only 3 were still below the 10th centile at the last visit, whereas 7 patients had significant weight gain. There were regular height centile records in only 13 patients. In 5 of these patients (38.5%), the height was below the 10th centile at the time of diagnosis. Three of these patients had an increase in height centile during the follow-up.

Discussion

Chronic granulomatous disease (CGD) is a disease of phagocytes in which the molecular defect has been shown to be in one of the subunits of the superoxide generating phagocyte NADPH oxidase system, leading to an inability of bactericidal activity against organisms. Since the first description of CGD by Berendes et al.[9] in 1957, the prognosis of patients has improved dramatically due to the advent of antibiotics, antifungals and IFN-γ.

The overall mortality has changed over the last 40 years. In 1967, only 21% of children with CGD survived beyond five years of age[10]. There has been an increase in the mean age of the survivors from 8 years in 1985 to 16 years in 1998, although the overall mortality remained unchanged at 23% over the last 13 years of the study[11]. During the five years of a recent study, overall mortality was found to be 13%, which is slightly lower than the rate in the present study, which is 19.2%[12]. Overall, five patients died during the follow-up period (range: 15 days to 13 years). Two of them had X-linked form and one had AR form confirmed by mutation analysis. The remaining two female patients had flow cytometric results suggestive of AR pattern.

All of the patients received prophylactic antifungal and co-trimoxazole, whereas additional IFN-γ treatment was given as therapeutic and prophylactic regimen in only five patients because of the presence of severe infections, and all recovered from infection. There are conflicting reports regarding the prophylactic effect of IFN-γ, which is an immunomodulatory cytokine shown to restore NADPH oxidase activity partially in the neutrophils and monocytes of patients with X-linked CGD. While ongoing studies in Europe and the United States have demonstrated the safety of IFN-γ prophylaxis, it is not used universally[13]. In the present study, the number of patients who received IFN-γ prophylaxis

Table III. Microorganisms Isolated from CGD Patients During 34 Infections

Etiologic agents	Number of infectious episodes
Aspergillus spp	9
Staphylococcus aureus	5
Staphylococcus haemolyticus	2
Candida spp	6
Klebsiella pneumoniae	2
Enterococcus faecalis	1
Salmonella spp	2
Mycobacterium tuberculosis	1
Atypical mycobacteria	4
Streptococcus pneumoniae	1
Pseudomonas aeruginosa	1

CGD: Chronic granulomatous disease.

is too small to compare with patients who received only the conventional prophylaxis.

The age at the onset of symptoms in patients with CGD is usually early in life. In one study, 50% of patients had the onset of infections before 1 year of age and 79% before 2 years of age[14]. In our series, the age of onset was before 2 years of age in 18 patients (69.2%) and overall, 8 patients including 6 with AR form had a late onset (4-10 years of age). The median delay between the onset of infections and the diagnosis was shown to be 39 months in our patient group, which is higher than the delay of 18 months reported by Finn et al.[14], of 13 months reported by Cale et al.[15] and of 2 years reported by Martire et al.[12].

Winkelstein et al.[3] analyzed the inheritance pattern of 368 CGD patients and found X-linked recessive form of the disease in 70% and AR form in 22%, whereas in 8% of the patients, the data was not sufficient to draw a conclusion. We demonstrated that 17 patients from 14 families (82%) were of AR form, whereas only five patients from three families had X-linked form of the disease[6] based on mutation analysis results. In the remaining five patients who lacked molecular analysis, three were predicted to have AR form based on the immunocytometric analysis. The predominance of AR form in our patient group may be explained by the high incidence of consanguineous marriages in the general population.

In the present study, pneumonia was the most common presenting manifestation, followed by lymphadenitis, skin abscess and diarrhea. The initial clinical manifestation varies among studies, with lymphadenitis being the most common followed by pneumonia in some studies[2,3,12]. During the follow-up, incidences of both pneumonia (61.5%, in 16 cases) and lymphadenitis (30.7%, in 8 cases) were reduced to 34.6% (9 cases) and to 19.2% (5 cases) respectively, in our patients. Although the reduction in the incidence was not significant, the infectious episodes were milder under co-trimoxazole and antifungal prophylaxis. The rates of overall infections per year were 0.79, 0.64 and 0.70 as reported by Liese et al.[2], Martire et al.[12] and Cale et al.[15], respectively.

The rates of severe infections per year were reported as 0.27 by Liese et al.[2] and 0.26 by Martire et al.[12]. These are comparable with the incidences found in the present study, where the annual incidence of overall infections was 0.73 and the rate of severe infections was 0.24.

Since the definition of CGD in 1957, the isolated infectious agents have markedly changed. The leading agent was staphylococci initially, replaced later by Aspergillus spp.[16,17]. In our patient group, the leading cause of infections was Aspergillus spp. followed by staphylococci spp., while Candida spp. were the third causative agent.

Although the number of the patients was too small to analyze the relation between the CGD subgroups and phenotype, the comparison of the clinical features of our patients with the X-linked and AR forms of the disease revealed that the initial clinical manifestation was lymphadenitis in the X-linked group (4 patients) and pneumonia followed by lymphadenitis in the AR group. The median age of onset was 18 months in the AR patients while in five X-linked CGD patients, it was 1.5 months. Although the number of patients was too small, it seems that the age of onset of infections is earlier in the X-linked group.

These results showed that the clinical features, progression and outcome in our group are

similar to those reported in the literature, but there is a predominance of AR form in contrast to the literature.

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