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Cystic Fibrosis in Gaza Strip: Mutation Analysis and Major Disease Manifestations

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Abstract: Cystic fibrosis (CF) is a devastating autosomal recessive disorder caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene the product of which is responsible for the transport of chloride ions across the membranes of epithelial cells. The disease is characterized by abnormally high sweat electrolytes, exocrine pancreatic insufficiency and chronic pulmonary obstruction, causing progressive disability and ultimately death. The aim of the present work was to determine for the first time in Gaza strip the common CF mutations and the major disease manifestations in Palestinian CF patients. The study population consisted of one hundred CF cases, 71% of them were males. The average age of the subjects was 70.6±44 months. Ninety three percent of the cases had white colored skin. Most of the CF cases (85%) were initially diagnosed by clinical manifestations and sweat test. The most frequent respiratory manifestations among CF cases were recurrent chest infections and chronic cough with viscid sputum whereas, the most frequent gastrointestinal manifestations were recurrent gastroenteritis, abdominal colic and flatulence, and malabsorption. Regarding the anthropometric measurements most CF cases had short stature (86%), 94% were underweight, and 65% had wasting. Also, 82% of CF cases were anemic. Allele-specific PCR was employed for screening DNA samples available from 64 patients for nine mutations: ΔF508, 3120+1G>A, N1303K, G85E, 1717+1G>A, G542X, W1282X, and 1209G>A, whereas, mutation 3120+1kbdel8.6kb was assayed by multiplex PCR. The results of mutation testing revealed that 61% of mutation-identified CF cases have at least a single ΔF508 allele. Moreover, 12.2% were homozygous for the 3120+1G>A *CFTR* mutation. Homozygous N1303K, G85E and 3120+1kbdel8.6kb mutations occurred in equal proportions and collectively represented 14.7%. The recorded *CFTR* homozygous mutations could be classified as: 51.2% class II, 19.5% class V, 4.9% class I and 2.4% class IV. The study shows that CF is not uncommon in Gaza strip and the findings will be useful for planning future screening and proper genetic counseling programs for Palestinian CF patients.

Key Words: Cystic fibrosis, mutations, manifestations, Gaza strip-Palestine.

Introduction

Cystic fibrosis (CF) or mucoviscidosis is an autosomal recessive genetic disorder of the exocrine glands, primarily affecting the gastro-intestinal and respiratory systems, and it is usually characterized by chronic obstructive pulmonary disorder, exocrine pancreatic insufficiency, and abnormally high sweat electrolytes, causing progressive disability and often early death [1].

CF is caused by mutations in the cystic fibrosis transmembrane conductance regulator (*CFTR*) gene. This gene helps in releasing sweat, digestive juices, and mucus. CF develops when both copies of the *CFTR* gene become mutated [2]. The defective gene and its abnormal protein result in a defective flow of Cl^- and Na^+ ions leading to high concentrations of these ions in sweat and cause the body to produce unusually thick, sticky mucus that; clogs the lungs and leads to life-threatening lung infections, obstructs the pancreas, and stops natural enzymes from helping the body digest and absorb food [3].

The *CFTR* gene is mapped on chromosome 7q31.2. The normal allelic variant for this gene is about 250,000 base pairs (bp) long and contains 27 exons that is transcribed into 6129 bp mRNA which in turn codes for the 1480 amino acids CFTR protein [4].

CFTR is a type of protein classified as an ABC (ATP-binding cassette) transporter. CFTR transports Cl^- ions across the membranes of cells of the lungs, liver, pancreas, digestive tract, reproductive tract, and skin. CFTR is made up of five domains: two membrane-spanning domains (MSD1 and MSD2) that form the chloride ion channel, two nucleotide-binding domains (NBD1 and NBD2) that bind and hydrolyze ATP and a regulatory (R) domain [5].

The *CFTR* gene is characterized by a high number of mutations where more than 1980 different mutations have been described so far. Delta F508 (ΔF508) however, represents the most commonly encountered mutation in white people [6].

Mutations in the *CFTR* gene have been classified into five different groups according to the mechanism by which they disrupt CFTR function; however these classes are not mutually exclusive. Therefore, it appears that the variability in disease severity in patients with CF is a result of different gene mutations, together with additional factors,

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genetic and/or environmental [7]. This study was conducted in order to determine for the first time in Gaza strip (Palestine) the common CF mutations and correlate them with the major disease manifestations of the CF patients.

Materials and Methods

The study population consisted of 100 patients diagnosed as having CF and were recruited from Gaza strip hospitals. Patients' data regarding their personal, socio-demographic, environmental and history of chronic diseases were collected by face-to-face interview with the patients and/or their families in addition to reviewing their medical records. Hemoglobin examination and anthropometric measurements to assess height to age, weight to age, and weight to height z scores were recorded for all participants. Additionally, records of health care facilities for the past (2000 to 2010) ten years were reviewed retrospectively to determine the number of children who were diagnosed and confirmed as CF and their outcomes and to assess the magnitude, trend and outcome of CF disease.

Genomic DNA for downstream mutation analysis was extracted from the available 64 EDTA-blood samples using Wizard Genomic DNA purification Kit (Promega, USA) following the manufacturer's instructions. Allele-specific PCR was employed for screening the DNA samples for eight mutations: ΔF508, 3120+1G>A, N1303K, G85E, 1717+1G>A, G542X, W1282X and 1209G>A as described previously [8,9] whereas, mutation 3120+1kbdel8.6kb was assayed by multiplex PCR [9].

Results

The average incidence rate of CF for the years (2000-2010) in Gaza strip is 1.26 case: 5000 live births and a prevalence of 3.72 cases: 100,000 persons for the same time period. Data analysis also revealed that there is variation in the case fatality rate of CF through the ten years where, the highest case fatality rate was 26.67% in 2000, while the lowest rate was 1.18% in 2008.

Regarding disease manifestation, the results revealed that only 3% of CF cases complained of chronic diseases other than CF. The respiratory system was affected in 43% of CF cases, both gastrointestinal and respiratory systems were affected in 52% of cases, while multisystem were affected in 5% of the cases.

The respiratory system manifestations included recurrent chest infection, chronic cough, chronic dyspnea, viscid sputum, cyanosis and recurrent sinusitis (100%, 94%, 83%, 92%, 57%, and 45%, respectively), Figure 1. As for gastrointestinal manifestations, 61% of the cases were complaining of recurrent gastroenteritis, 41% of chronic diarrhea, 38% of steatorrhea, 17% of chronic constipation and 54% of recurrent attacks of abdominal colic and flatulence and 50% of malabsorption, Figure 2. Also the results revealed that all cases were recurrently admitted to hospital. The average admission time was 3.51±1.63 times/year among CF cases.

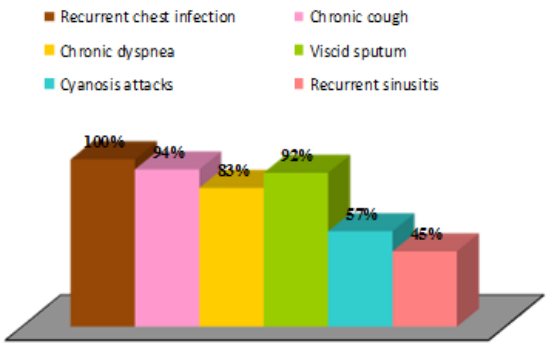

Figure 1: Distribution of cystic fibrosis cases by respiratory manifestations (Gaza, 2010)

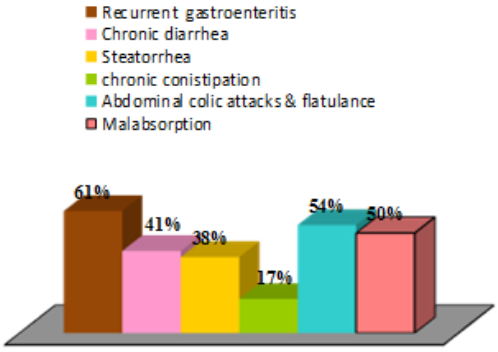

Figure 2: Distribution of cystic fibrosis cases by gastrointestinal manifestations (Gaza, 2010)

Anthropometric measurements (Table 1) of the CF cases showed that the average height is 99.64±20.10 cm and that the average of height to age Z score (HAZ) is -2.42±1.80. The average weight of the cases is 14.70±5.84 kg, the average of weight to age Z score (WAZ) is -2.48±1.05 and the average of weight to height Z score (WHZ) is -1.67±1.82. The anthropometric measurements reflected that short stature, underweight, and wasting are common among the CF patients (Table 2) where, the percentage of short stature among CF cases was 86%. Also the percentage of underweight among cases was 94% and the percentage of wasting among cases was 65%. The average of hemoglobin among CF cases was 10.44±0.54 g/dl. Also the percentage of anemia among them was 82% as illustrated in Tables 1 and 2.

Table 1: Averages of anthropometric measurements and Hb level of CF cases (Gaza, 2010)

Variable	CF Cases		
	No	Average	S.D
Height (cm)	100	99.64	20.10
HAZ	100	-2.42	1.80
Weight (kg)	100	14.70	5.84
WAZ	100	-2.48	1.05
WHZ	100	-1.67	1.82
Hb (g/dl)	**100**	10.44	0.54

Table 2: Distribution of cystic fibrosis cases by short stature, underweight, wasting and anemia (Gaza, 2010)

Variable	CF Cases	
	No	%
Short stature		
Yes	86	86
No	14	14
Underweight		
Yes	94	94
No	6	6
Wasting		
Yes	65	65
No	35	35
Anemia		
Yes	82	82
No	18	18
Total	100	100

Regarding consanguinity, 81% of the cases' parents were first cousins (3rd degree relatives) and 8% of the cases' parents were more distant relatives. In this study, we could obtain DNA samples from 64 of the cases which were screened for nine CFTR mutations (ΔF508, 3120+1G>A, N1303K, G85E, 1717+1G>A, G542X, W1282X, 3120+1kbdel8.6kb and 1209G>A). The results of genetic mutation testing identified CFTR mutation(s) in 41 CF cases from the 64 cases (64.1%).

Thirty nine percent of the mutation-identified cases were homozygous for ΔF508 mutation. Moreover, 61% of the mutation-identified cases have at least one ΔF508 allele i.e., about 22% of mutations were heterozygous for ΔF508, two-third of them were of unknown mutation on the other allele, while one third of them had G542X mutation or W1282X mutation on the other allele. Additionally, 12.2% of mutation-identified cases were homozygous for 3120+1G>A, 14.7% were homozygous in equal proportions for the three N1303K, G85E and 3120+1kbdel8.6kb mutations. Homozygous 1717 + 1G > A, and 1209G > A were encountered in 2.4% each. Results of identified mutations are summarized in Table 3.

Table 3: Number and frequency of detected *CFTR* mutations.

CFTR mutations	CF Cases	
	No.	%
Homo ΔF508	16	39
Homo 3120+1G>A	5	12.2
Homo N1303K	2	4.9
Homo G85E	2	4.9
Homo 3120+1kbdel8.6kb	2	4.9
Homo 1717+1G>A	1	2.4
Homo 1209G>A Heter	1	2.4
Hetero ΔF508/G542X	2	4.9
Hetero ΔF508/W1282X	1	2.4
Hetero ΔF508/Unknown	6	14.7
Hetero G542X/Unknown	1	2.4
Hetero W1282X/Unknown	2	4.9
Total	41	100

According to the detected *CFTR* mutations, 51.2% of them belonged to class II, 19.5% to class V, 4.9% to class I, 2.4% to class IV, and 22% could not be assigned to any class (Table 4).

Table 4: Distribution of CF classes in the genotyped patients

CF disease classes	CF Cases	
	No.	%
Class I	2	4.9
Class II	21	51.2
Class III	0	0
Class IV	1	2.4
Class V	8	19.5
Compound	9	22
Total	41	100

Discussion

The CF cases investigated in this study are of the severe form of the disease as their clinical features were similar to those described for classic CF which include acute/persistent respiratory symptoms, malnutrition and steatorrhea due to pancreatic insufficiency (10-16).

The anthropometric measurements and hemoglobin level of CF cases enrolled in this study reflects that short stature, underweight, wasting and anemia are very common, and indeed about 65- 94% of the cases were found to be in different malnutrition stages for their weight and height to age and weight to height. Moreover, the rates of malnutrition indicators can be considered very high in comparison to those reported for CF patients in other Arab and western countries. Also, the Z scores of HA, WA, and WH were lower among CF cases in comparison to controls or other Arab and western countries (13, 17-19).

Poor growth, weight loss and anemia are results of three major reasons for the energy imbalance among CF patients. First, the high energy requirements, where CF cases work hard to breathe, and that work requires energy. It is estimated that the work of

breathing alone increases the energy requirement to 9% more than that of a healthy person with the same body weight (20). Second, the poor absorption because of pancreatic insufficiency, where the body of CF patients is not able to absorb much energy from the food that is eaten. Even if a person with CF eats large amounts of food, most of the potential energy is excreted unused. Third, the poor intake. In spite that most of CF patients have good appetite, there are other issues going on that make it difficult for CF patients to eat, such as acid reflux, breathing problems, or struggles with depression (20). The aforementioned reasons justify the high rates of malnutrition indicators such as short stature, underweight, wasting and anemia or the low scores of HAZ, WAZ, WHZ and hemoglobin level observed in this study. Most of CF children in the GS were of severe form and most of them were complaining of recurrent severe chest manifestations such as dyspnea and cyanosis. Also more than half of CF patients were suffering from chronic gastroenteritis and malabsorption.

According to the current study results, about 61% of mutation-identified CF cases have at least a single allele of ΔF508, 12.2% were homozygous for 3120+1G>A mutation. Also homo N1303K, homo G85E and homo 3120+1kbdel8.6kb *CFTR* mutations were found among 14.7% of the mutation-identified CF cases. These findings are consistent with those reported in the Middle East and many other regions in the world in terms of ΔF508, N1303, W1282X and 3120+1G>A mutations (21,22). The most frequent mutation, present in about 67 percent of cystic fibrosis chromosomes worldwide, results in the deletion of a phenylalanine residue at codon 508 (ΔF508). The clinical manifestations in patients homozygous for this mutation have been extensively studied. They generally have pancreatic insufficiency of early onset with markedly elevated sweat chloride concentrations, but the pulmonary manifestations are widely variable (23-29). The same conclusion could be extracted from the current study. About 29-40% of CF children were complaining of severe short stature, wasting, underweight and moderate anemia. These proportions are almost similar to the proportion of CF cases of homo ΔF508 (39%). This could indicate that the CF children of homo ΔF508 could be presented with severe form of CF, where they had failure to thrive. Also this proportion is almost coinciding with that of CF cases who were complaining of malabsorption (50%) which is a sign of pancreatic insufficiency. The frequency of pulmonary manifestations among CF children in the GS was very high (45-100%). This is compatible with the hypothesis which assumes that pancreatic phenotype can sometimes be predicted by genotype, and pancreatic insufficiency is almost invariably associated with two severe mutations. The correlation between genotype and phenotype for pulmonary phenotype is not reliably predictive and the course of lung disease in CF is especially vulnerable to environmental and modifier gene (30).

Conclusion

Cystic fibrosis disease is not rare in the GS, where the incidence and prevalence rates of CF are high and around the rates of Caucasian Western Europe populations. Respiratory system was the most system affected by CF disease. The anthropometric measurements and hemoglobin level of CF cases in the GS reflected that short stature, underweight, wasting and anemia were very common. About two thirds of mutation-identified CF cases have at least a single allele of ΔF508, which is considered of severe type of CFTR mutations. On other hand more than half cases belong to class II of CF disease (severe form of disease).

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