INTRODUCTION

Cystic fibrosis (CF) is the most common life-shortening pediatric respiratory disease among Caucasian populations, with a frequency of 1 in 2000 to 3000 live births ***(Rowe et al., 2006).***

In the United States, cystic fibrosis (CF) occurs in approximately 1:3000 Caucasians, 1:9200 Hispanics, 1:10,900 Native Americans, 1:15,000 African Americans, and 1:30,000 Asian Americans ***(Cystic Fibrosis Foundation, 2010).***

In Canada, there are approximately 4,000 people with cystic fibrosis. Approximately 1 in 25 people of European descent and one in 30 of Caucasian Americans is a carrier of a cystic fibrosis mutation. Although cystic fibrosis is less common in these groups, approximately 1 in 46 Hispanics, 1 in 65 Africans and 1 in 90 Asians carry at least one abnormal CFTR gene. Ireland has the world's highest incidence of cystic fibrosis at 1:1353 ***(Farrell et al., 2008).*** However, the incidence in populations of other ethnic backgrounds including Egyptians has not been yet established ***(Welsh et al., 2001).***

Cystic fibrosis (CF) is a multisystem disease affecting the digestive system, sweat glands, upper and lower respiratory tracts, and the reproductive tract, but progressive lung disease continues to be the major cause of morbidity and mortality ***(Kleven et al., 2008).***

Cystic fibrosis is caused by defects in the cystic fibrosis gene, which codes for a protein CF transmembrane conductance regulator (*CFTR*) that functions as a chloride channel and is regulated by cyclic adenosine monophosphate (cAMP). Mutations in the *CFTR* gene result in abnormalities of cAMP-regulated chloride transport across epithelial cells on mucosal surfaces ***(Flume et al., 2010).***

Defective CFTR results in decreased secretion of chloride and increased reabsorption of sodium and water across epithelial cells. The resultant reduced height of epithelial lining fluid and decreased hydration of mucus results in mucus that is stickier to bacteria, which promotes infection and inflammation. Secretions in the respiratory tract, pancreas, GI tract, sweat glands, and other exocrine tissues have increased viscosity, which makes them difficult to clear ***(Flume et al., 2010).***

As a genetic disease, CF begins at conception, though symptoms may not appear at first. Diagnosis is sometimes delayed for decades because of mildness of these symptoms or failure to recognize them. Typical symptoms include: salty-tasting skin (which parents often notice when they kiss their child), wheezing or shortness of breath, persistent cough and excessive mucus, frequent lung infections, such as pneumonia bronchitis, frequent sinus infections (sinusitis), nasal polyps, poor weight gain and growth, foul-smelling, greasy stools, swollen belly, accompanied by abdominal gas and discomfort, clubbing of the fingertips and toes ***(Sly et al., 2009).***

Early identification of CF through newborn screening programs has led to improved survival, better lung function and growth with less intensive therapy and reduced cost of therapy. The sweat test remains the standard diagnostic test for cystic fibrosis. It measures the amount of salt in a child's sweat, with a high salt level indicating that a person has cystic fibrosis. Genetic testing is available for cystic fibrosis, but it does not detect all of the mutations that can cause the disease ***(Kleven et al., 2008).***

The cornerstones of treatment for CF patients are antibiotics, airway clearance, and nutritional support. A standard treatment regimen includes airway clearance and exercise, mucolytic agents, bronchodilators, anti-inflammatory agents, supplemental oxygen, and nutritional support. CF patients should be cared for at a comprehensive cystic fibrosis care center by a multidisciplinary health care team that includes a physician, nurse, respiratory therapist, dietitian, and social worker ***(Borowitz et al., 2009).***

An early diagnosis of CF and a comprehensive treatment plan can improve both survival and quality of life. Follow-up and monitoring are very important. If possible, patients should be cared for at cystic fibrosis specialty clinics, which can be found in many communities. When children reach adulthood, they should be transferred to a cystic fibrosis specialty center for adults ***(Mogayzel et al., 2010).***

AIM OF THE WORK

The aim of this study is to screen for the presence of cystic fibrosis among Egyptian children with suggestive respiratory and gastrointestinal manifestations smiliar to cysic fibrosis and to identify causative genetic mutations in the (CFTR) gene which will help the efforts to design a specific mutational panel and new drugs that target specific mutations.

Chapter 1

CYSTIC FIBROSIS

Definition and epidemiology:

Cystic fibrosis (CF) is a multisystem disease affecting the digestive system, sweat glands, upper and lower respiratory tracts, and the reproductive tract, but progressive lung disease continues to be the major cause of morbidity and mortality ***(Allen et al., 2010).***

Cystic fibrosis (CF) is the commonest lethal inherited autosomal recessive disease of white races, but it should be noted that no ethnic group is exempt from the disease, although prevalence varies across the continent ***(Hodson et al., 2013).***

Risk Factors and demographics:

1. age

The median age of diagnosis of cystic fibrosis is 14.5 months (interquartile range 4.2 to 65 months; two thirds of patients are diagnosed by 1 year of age ***(Flume et al., 2012a).***

2. Gender

CF occurs equally often in male and female patients. In general, female patients with CF are significantly worse than male patients. Female patients become infected with Pseudomonas aeruginosa earlier, diagnosed at a later age by at least 4 months

and have worse pulmonary function with increasing age, worse nutritional status, and earlier mortality ***(Sweezey et al., 2014).***

This "gender gap" is hypothesized to be due to the pro-inflammatory effects of estrogens, although it has been suggested that the increase in hormone secretion with puberty in females may interfere with the defense mechanisms of the immune system, thereby promoting progressive pulmonary involvement ***(Sweezey et al., 2014).***

3. Family history:

A positive history of CF in the family should raise the level of suspicion of CF in the patient ***(Elborn, 2013).***

4. Known carrier status of both parents:

If both parents are known carriers of mutant CFTR alleles, then each child conceived by those parents will have a 1 in 4 chance of having CF ***(Cystic Fibrosis Foundation, 2010).***

5. Ethnicity and place of distribution:

CF mainly affects individuals of Caucasian descent and white population with a rate of one in 2500 live births in all non Hispanics white population and considerably less in other ethnic groups ***(Rogan et al., 2011).***

Cystic fibrosis in the United States:

In USA, Approximately 30,000 Americans have CF, and there are an estimated 1,000 new cases diagnosed each year. The

overall birth prevalence of CF in the United States is 1 in 3,700 (Figure 1) ***(Cystic Fibrosis Foundation, 2010).***

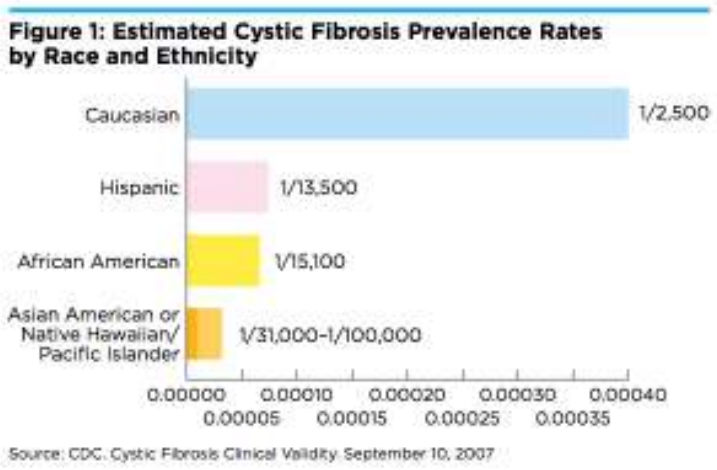

Figure (1): Demonsterates the prevelance rates of CF among Americans by their ethnicity ***(CDC, 2007).***

Survival: In the 1950s, few people with CF lived to go to elementary school. In 1985, the median survival age was about 25 years. In 2007, the predicted survival age was 37.4 years ***(Cystic Fibrosis Foundation, 2011).***

Deaths: Between 1999 and 2006, 3,708 people in the U.S died from cystic fibrosis.Most of these deaths were among Caucasians,178 among Hispanics/Latinos,26 among American Indians/Alaska Natives, lastly 16 among Asian Americans and Native Hawaiians/Pacific Islanders ***(Centers for Disease Control and Prevention, 2010).***

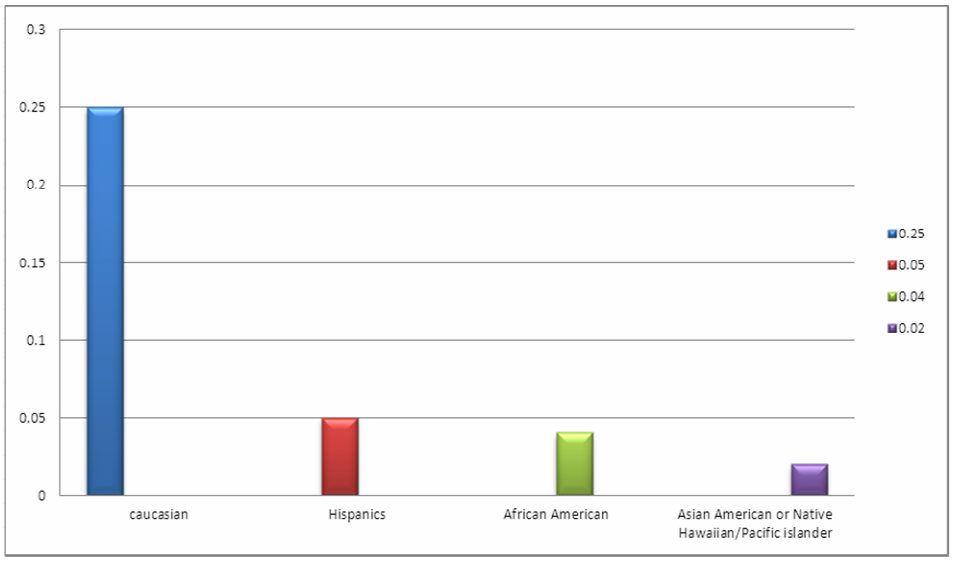

Figure (2): Cystic Fibrosis Death Rates Per 100,000 of the cystic fibrosis patients by race and ethnicity, 1999-2006 Source: NCHS 2005.

Cystic fibrosis in Europe:

Prevalence and incidence:

In Europe, CF affects nearly 30000 patients with annual incidence of 1: 2-3000 ***(Mccormick et al., 2010),*** 25,126 from European Union (EU) countries and 3,809 from non-EU countries. Also it affects 9000 patients in UK ***(Olesen et al., 2010).***

Annual mortality:

Mortality rate varies with age and is likely to be about 1–2% per year overall ***(Olesen et al., 2010).***

Cystic fibrosis in Arab population:

Epidemiological data about birth prevalence and carrier frequencies vary in the Arab population. For instance, the predicted

CF prevalence is 1:2500 in Jordanian neonates ***(Hamamy et al., 2007),*** 1: 4,243 in Saudi Arabia ***(Nazer et al., 1989)***, 1:1,680 to 1:4,150 in Morocco, 1:15,876 in the United Arab Emirates (UAE), In Iran, CF prevalence was 7.98 in 100 thousand during the 5-year period (2004-2008) ***(Dastgiri et al., 2011)***.In Oman, the predicted carrier frequency is 1:25 and the birth prevalence is similar to the Caucasian population ***(Ratbi et al., 2008).***

Cystic fibrosis in Egyptian population:

The disease was thought to be a rarity among Egyptians. However many studies have been done in Egypt to detect prevalence of cystic fibrosis, for example, ***Abdel-Salam (1986),*** reported a CF prevalence of 1:2664 in Egyptian newborns using the meconium albumin test and the sweat chloride test.

In a study done on 1000 Egyptian children aged 3-14 years considered as high-risk patients for cystic fibrosis. They were screened by sweat chloride test. The prevalence of this group was (1:22) ***(Abdel-Salam et al., 1993).***

Another study was done in Egypt to screen for cystic fibrosis in 80 patients with their ages ranged between 11 months and 15 years,). Fecal fat test was positive in 90% of patients. 20 patients (25%) were proved to carry Δ F508 mutation. Of them, only one was homozygous and 19 were heterozygous for that mutation ***(Shawky et al., 2003).***

While in another Egyptian study, Sixty-one patients from the Chest Unit, Cairo University Children's Hospital, were

included. Patients were screened using the sweat test system.Out of the 61 patients, 12 (20%) had positive sweat chloride screening of whom eight CFTR sequence changes were identified in seven affected probands and two were confirmed in one sibling by direct DNA sequencing ***(Naguib et al., 2007).***

In another recent study done in Egypt, A total of 100 patients clinically suspected of having CF were recruited from the CF clinic, Children's Hospital, Cairo University. Sweat chloride testing was done for all patients. Patients positive for sweat chloride were tested for the DF508 mutation. Thirty-six patients (36%) had a positive sweat chloride test. Positive DF508 mutation was detected in 22 (68.8%) patients, 8 (25%) were homozygous, 14 (43.8%) were heterozygous ***(El-Falaki et al., 2014).***

Genetics of Cystic Fibrosis

Introduction and historical background:

CF has been described in the medical literature as long ago as 1606 with the description of "a child who tastes salty being be witched and will soon die" ***(Quinton, 1999).*** It was more formally described in the 1940s ***(Snelling, 1942).*** In 1985, The cystic fibrosis transmembrane conductance regulator gene (CFTR gene) that caused this disease was localised to the long arm of chromosome 7 ***(Rommens et al., 1989),*** with the sequence being fully identified in 1989 ***(Wainwright et al., 1985).***

CFTR gene:

1. CFTR structure

The (CFTR)) is a single large gene located on chromosome 7 at position q31.2 spanning 250 kilobases that encodes the instructions for the body to make the (CFTR) protein ***(Rogan et al., 2011).***

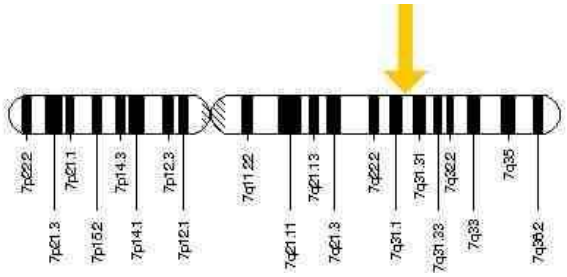

Figure (3): Showing that the *CFTR* gene is located on the long (q) arm of chromosome 7 at position 31.2 ***(CFTR 2, 2012).***

More precisely, the *CFTR* gene is located from base pair 117,470,771 to base pair 117,668,664 on chromosome 7 and is composed initially of 27 exons numbered 1-24 with subdivisions a and b for exons 6,14 and 17.The mature transcript is 6,129 nucleotides long including an open reading frame of 4,440 coding bases.

2. Inheritance

The inheritance of CF is described by geneticists as autosomal recessive'; the patient must possess two defective *CFTR* genes by inheriting a mutant copy of one *CFTR* gene from each parent. Each time two carriers of the defective gene conceive, there is a 25 percent chance that the child will have CF. There is a 50 percent chance that the child will be a carrier of the gene, and 25 percent chance that the child will not have the gene at all ***(Cystic Fibrosis Foundation, 2010).***

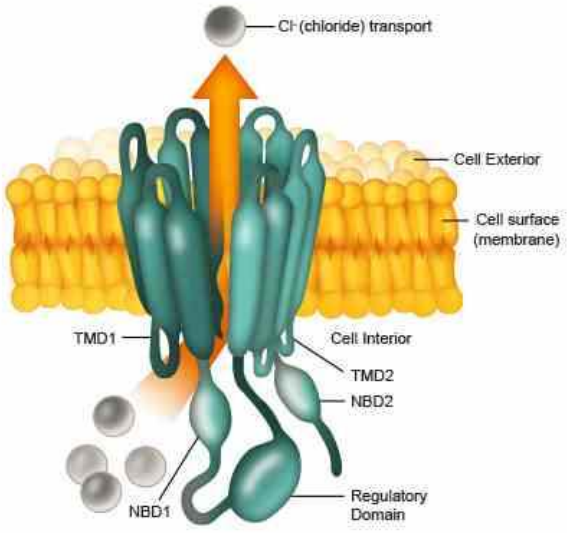

Figure (4): Showing CFTR protein structure from ***(CFTR 2, 2012).***

The CFTR protein

1. CFTR protein structure:

The CFTR protein is found in the top layer (epithelial layer) of specific cells (respiratory epithelium, sweat glands, pancreas, reproductive system). It is a member of the ABC **(A**TP **B**inding **C**assette) transporter super family of proteins that use the energy from nucleotide (ATP) hydrolysis to transport molecules across the membrane ***(CFTR 2, 2012).***

The CFTR protein is composed of 1,480 amino acids arranged into five functional domains, which join to form the single chain protein. Each domain plays a different role in trying to send the chloride through the cell membrane. The severity of CF symptoms will depend upon which domain is affected ***(Patrick et al., 2012; CFTR 2, 2012).*** The five domains are***:***

1-TMD or transmembrane domains:

Around 19% of total CFTR protein is composed of two groups of six membrane-spanning regions (TMD1 and TMD2), which form the channel pore allowing transport of chloride ions across the membrane.

2-NBD or nucleotide-binding domains:

These two intracellular nucleotide-binding domains (NBDs) bind the nucleotide molecule ATP (a vehicle of chemical

energy). Opening and closing of the channel (or 'gating') requires ATP to bind to this domain.

3-Regulatory domain or R:

The highly charged "R domain" containing multiple phosphorylation sites, regulates the channel activity and can be considered to be the 'trigger' governing whether the channel opens or closes, to activate the channel.

Opening of the CFTR channel requires two main changes to occur in the structure of the protein:

1. Phosphate molecules bind to the regulatory domain (R) in a process called phosphorylation by protein kinase A.
2. Recruitment of nucleotides (ATP, ADP) which bind to the nucleotide binding domains (NBD1 and NBD2). Under normal condition, these domains dimerise to open the channel pore allowing chloride efflux and subsequent water efflux. Upon subsequent ATP hydrolysis, the NBDs disassociate to close the channel and prevent chloride efflux. The entire process of channel opening and closing is aptly termed channel ''gating ***(Kirk and Wang, 2011).***

These events bring about conformational changes in the structure of CFTR that allow the channel to open.

As this is reversible, CFTR can open and close at varying frequencies – also known as the '**open probability.**

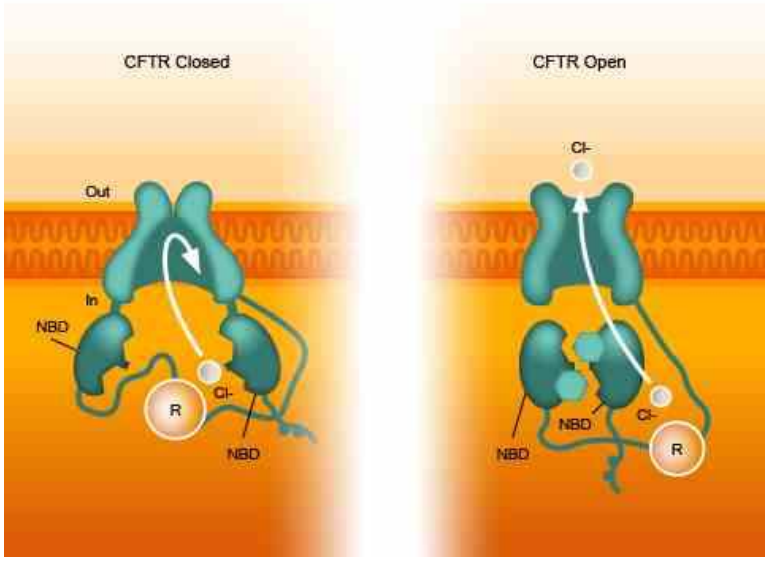

Figure (5): Showing opening of CFTR channel ***(CFTR, 2012).***

2. CFTR journey:

The entire process of CFTR protein synthesis can be broken down into 5 main steps ***(Rogan et al, 2011; Kirk and Wang, 2011).***

1. ***Transcription***
2. ***Translation and protein folding***
3. ***Post-translational modification***
4. ***Protein trafficking***
5. ***Surface expression***