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CYSTIC FIBROSIS: A PROBLEM APPROACH

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Abstract: Cystic Fibrosis (CF) – it's an autosomal recessive disorder, commonly seen in Americans and people of European descent. It generally affects more than 70,000 people every year worldwide. We have performed a systematic review which summarizes the various studies done in order to understand the disorder and all the various diagnostic methods being used in the hospitals. A person with cystic fibrosis will have increased levels of Cl- and Na+ in sweat. It is diagnosed by newborn screening (NBS), sweat chloride level tests, etc. Affected people generally have a problem with the movement of chloride across membranes of their cells, leading to a higher concentration of chloride in sweat. This sweat test which has been in use for more than 50 years has proved to be effective for the detection of abnormally high sweat chloride levels, hence it is considered as the diagnostic test for CF. But this testing also has its limitations, which necessitate the development of a better, faster, and more efficient approach as an alternative.

Keywords - Cystic fibrosis diagnosis, Sweat Chloride testing, New-born screening, Chloride-level quantification

I. INTRODUCTION

We have made significant progress over the last 2 decades in understanding Cystic fibrosis and its management. CF is a disease mostly concerned with lung and digestive system in young children, but over the years it has evolved into a complex disease affecting multiple systems and progressing to adulthood so that more adults suffer from cystic fibrosis than children. The average survival rate for this disorder in the 21st century has increased to more than 50 years, which is a substantial progress. The main reason for this was an increase in the rate of testing of new-borns.

CF is a genetic disorder that act on the lungs, and sometimes other organs of human body such as the pancreas, kidneys, intestine and liver. Symptoms of the disease are poor growth, sinus infections, fatty stools. It can also lead to clubbing of fingers and toes and male infertility. Long term effects of this disorder are difficulty in breathing, coughing mucus and patient may also experience frequent lung infections. [1]

CF is a genetic disorder, inherited in autosomal recessive manner. Genetic mutations in both the replicas of the protein Cystic Fibrosis Transmembrane Conductance (CFTR) are major cause of this disease. The people who have one mutated gene in CFTR protein are the carriers of this disease and are mostly healthy. CFTR's main functions include production of mucus, sweat and digestive fluids. CFTR, when not functional results in thickening of these secretions. Diagnosis of CF is made by a sweat tests and genetic testing. In countries like UK and USA infants are screened immediately within 48 hours of birth.

The protein CFTR essentially acts as the channel protein, responsible for the flow of H_2O and Cl^- ions through the lung cells. In a healthy person where CFTR protein is not defective, the ions are able to flow in and out of the cell freely. Although, whenever there is any sort of malfunction, these ions are not able to pass through these cells due to a blockage in the channel. This is what happens when a person has cystic fibrosis, which can be characterized by thick mucus buildup in the lungs of a patient.

There are different types of mutations occurring in a CFTR gene and resulting in defective CFTR protein, all of these defects end up causing cystic fibrosis though the severity of the disease may vary from different type of mutation. The CFTR protein mainly allows the passing of chloride ions from the mucus producing cells, immediately after which water is secreted resulting in thinning of mucus. In case of defective CFTR, there is generally thick and sticky mucus which obstructs the pathway, these blockages lead to different kinds of lung infections. Because of neutrophil infiltration, there is massive release of elastase, which ends up in overpowering the lung proteases, all of which directly contributing towards tissue destruction. Also, neutrophils which are degranulating have a tendency of releasing enormous quantities of nucleic acids whereas the protein cytosol matrix, mostly contributes in hyper-viscosity of mucus. In gastro-intestinal tract, the mucous plugs prevent the enzymes and the bile flow into the duodenum because of their obstruction in canaliculi of the pancreas as well as the gall bladder, all of which has a result in triggering malabsorption and other abnormalities in digestion.[2]

As of now, there is no cure for this disorder, the lung infections are treated like any other infections with antibiotics. Inhalation of hypertonic saline and salbutamol has also been found useful. If the lung function continues to worsen, the patient can consider lung transplantation whereas if the pancreatic function worsens, the patient must be supplemented with fat soluble vitamin. Chest physiotherapy such as airway clearance is also helpful but has only short-term benefits. The average life expectancy of patients suffering from cystic fibrosis is around 50 years. Lung problems cause most of the problems which result in death of patients. This disorder is most common in people from western and European countries where it affects one out of 3000 new-borns every year. It is least common in African and Asian people almost non-existential. The name cystic fibrosis is given because of the cysts that are formed within the pancreas. [3]

II. STATISTICS

Among people of European ancestry, CF is the most usually occurring autosomal recessive disorder. About 30,000 people in the US are diagnosed with cystic fibrosis at age of 6 months. About 4000 people from Canada, one in 30 white Americans, and about 1 in 25 people from Europe are carriers of CF. One in 90 Asians, 1 in 65 Africans, 1 in 46 Hispanics carry at least one abnormal CFTR gene even though it is uncommon in these groups. CF which is a genetic disease that shortens the lives is marked as one of the most widespread disease. In nations within the western world CF is more common. In Finland only one person in 80 manages to carry CF mutation creating an exception. Ireland had world's highest generality of CF that is 1 in 1353. "In the European Union, one in 2000 to 3000 children are diagnosed with CF" states the WHO. One in 3500 babies is born with CF in the US. One in 3300 babies in the US was born with CF in 1997. But for Asian Americans, the rate was reduced to 1 in 32000 and only one in 15000 African American children was diagnosed with CF. [4]

In the UK around 7000 people suffer from CF. the prevalence of this genetically inherited disease is more in white people. Even after this, CF is keenly recognized in non-white people. Specialists have 1 in 2 patients with CF and management takes place usually in specialist centers, in case of small number of patients' pediatricians will be involved. [5]

In past 20 years the actual grip of the disease and the effect of the management has been expeditious.CF was merely a digestive disease and also lung disease in kids but the complexity has been increased. [6] With present condition CF will be more common in adults when compared to children. The babies which were born in 21st century was predicted with increased median survival that is around 50 years. Hence, treatment standards are increased, newborn screening implementation started increasing, and therapeutic strategies are focused. [7]

III. PATHOPHYSIOLOGY

The abnormalities in CFTR causes CF. In the sick Kids database >2000 mutations of this protein were reported knowing all of these won't cause diseases. "How does CFTR abnormalities lead to chronic respiratory infections, inflammation, and bronchiectasis?"

Transport of Chlorides and bicarbonates directly to the surface and bringing water along with these ions is actively done by CFTR protein. Other ion channels are also affected by CFTR; most of them block the incursion of Na inside the cell via epithelial Ns channel. The acidification and decreased water and ion transit are the result of abnormality in CFTR. This led to 2 CF research teams with two different perspectives on the production of airway disease by CFTR. This controversy is referred to as the salt wars in the CF community. The University of North Carolina at Chapel Hill reported that dehydration, a decrease in osmotic pressure to the two airway lumen leads to a deterioration of the periciliary fluid layer in cystic fibrosis and a gradual reduction in mucociliary flow causing CF pathogenesis.[8] Restoration of periciliary fluid layer using osmotic agents, dry powder mannitol and hypertonic saline.

Airway acidification was identified by the CF research group in Iowa. This group explains that acidification of airway can lead to various abnormalities mainly in the defence mechanism of host in airway in the cystic fibrosis mice and pH balance restoration can be restored by the host defence.[9] These studies have led to the investigation of alkaline solution evaluation as a therapy for this disease.[11]

IV. DISEASE CONDITION

A discovery was made based on the fact that cystic fibrosis sweat contains elevated concentrations of Cl^- and Na^+, that sweat glands are relatively impermeable to Cl^-. this inability to absorb chloride which resulted in salty sweat became a diagnostic characteristic for cystic fibrosis. Based on the evidence provided by certain researchers' defective chloride transport was found in CF airway epithelia which resulted in enhanced Na+ permeability. This cystic fibrosis epithelial defect was commonly restricted to the apical membrane. In usual airway of epithelia, increased cAMP opens apical Cl- channels which a pathway is created for Cl^- ions to flow through the membrane. When there is a defect in cAMP Cl- secretion there is defective salt transport across pancreatic duct epithelia. This led to a hypothesis that CF causes abnormal epithelial salt transport.

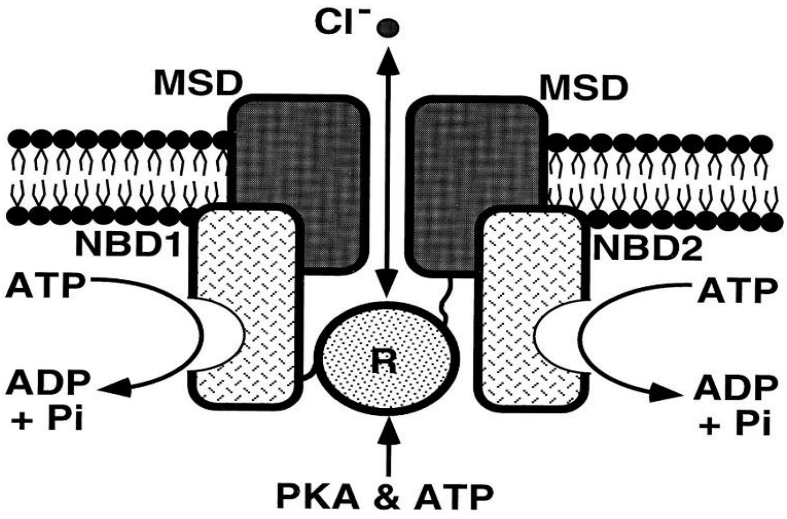

Figure 1 The figure shows domain structure of CFTR. Where, MSD - membrane-spanning domain; NBD - nucleotide-binding domain; R - regulatory domain; and PKA is cAMP-dependent protein kinase.[12]

Primary sequence was identified by the first insights about CFTR. From this sequence a potential structure was proposed where 1480 amino acids of CFTR potentially formed 5 domains:

- 2 membrane spanning domains (made of 6 transmembrane segments)
- R domain (containing phosphorylation sequences)
- 2 nucleotide binding domains (interacting with ATP)

Certain features which place CFTR in the ABC transporter family are sequence similarity, its overall topology and prediction of two MSD's ABC transporter family is mostly associated in energetic transport of substrate through cell membranes along with NBD powered ATP hydrolysis. [12]

CFTR is a synchronized Cl– channel-$^{-}$ located in the epithelial apical membrane: $^{Cl-}$ channels are generated when CFTR is expressed in different group of cell types. Properties of channels of Cl- is altered by site- directed mutagenesis. Integration of CFTR with planar lipid bilayers forms Cl- channels. Even though CFTR is a Cl- channel it can still have additional functions such as regulation of Na+ K+ and can affect ATP efflux from cells and also influences recycling of endosomal membrane.
CFTR Cl- channel regulation is a complex mechanism. The activity of Cl- channel requires both R domain phosphorylation and binding of ATP and NBDs reaction with water. The R domain is phosphorylated by the cAMP dependent protein kinase (PKA) at several sites. Domain R mostly plays an inhibitory role to keep the channel closed, although after phosphorylation it enhances the interaction of ATP with the NBDs by acting as a stimulatory agent. The NBDs being the novel regulatory feature of CFTR bind and hydrolyse ATP to control the open and shut of the channel. It is evident that ATP hydrolysis is not coupled to Cl- flow in a stoichiometric way, but a set of conformational changes is produced by the energy released through this type of ATP hydrolysis which generates a gating cycle where the channel is able to move through open and closed states.
After it was found that CFTR epithelial Cl- channel was indeed regulated by phosphorylation, it leads to the observation that epithelia is in fact the site of the disease.

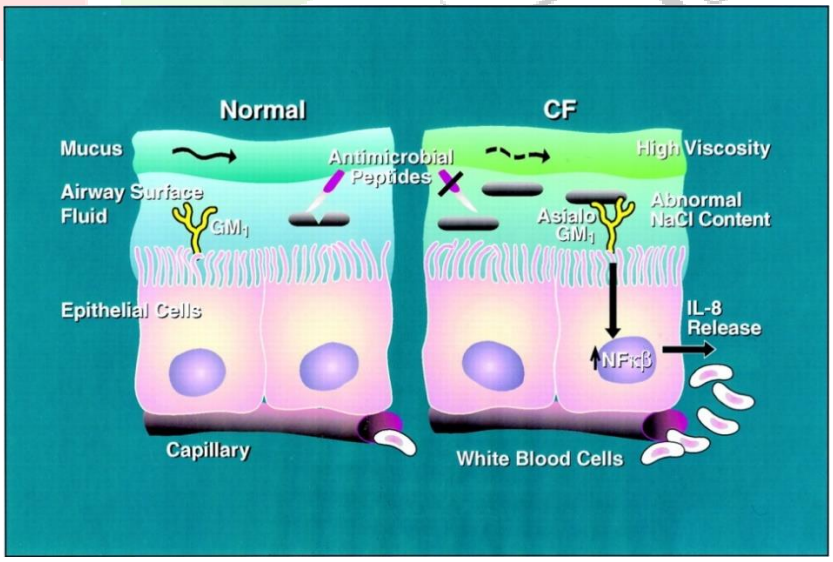

Figure 2 Pathogenesis of CF airway disease [12]

There are generally four different mechanisms which lead to CFTR function disruption, they are listed as follows:

- Class I mutations – premature termination signals lead to defective production of proteins.
- Class II mutations – defect in refining of protein.
- Class III mutations – channel opening control is disrupted.
- Class IV mutations – channels have defects in their ion conduction mechanism.

The most usual mutation which leads to CF is deletion of phenylalanine at remnant 508, which is responsible for almost 70% cases of CF in the North America. [13]

V. CYSTIC FIBROSIS - DIAGNOSIS

Earlier CF diagnosis was dependent on physical similarities by clinical recognition of its respective signs and symptoms. But due to the widespread screening of new-borns (NBS) at the very low 64% of Cystic Fibrosis cases in the US are successfully done for patients who are asymptomatic or those who have minimum symptoms who have been tested positive for screening test. The infants who have been tested positive are again tested by a confirmatory test which shows high chloride concentrations.
The best method to diagnostically test for cystic fibrosis is to measure electrolyte levels in sweat. Patients have elevated concentrations of sodium and that of chloride (>60 mmol/l). However, we have also seen in cystic fibrosis cases with normal sweat chloride levels as well. New age methods have reduced the amount of sweat that is required. The test should be performed by a trained person and the procedure must be followed strictly. Hence the diagnosis is usually made in secondary centres and tertiary centres, even though the investigation is done by primary care centres. In cases where a region of doubt exists, other tests are available.

Figure 3 Macro duct system attached to an arm during sweat test [15]

The following figure shows an arm of a child with macro duct system during sweat test. First to stimulate sweat pilocarpine iontophoresis is induced, the capillary which is closed is then put on to the forearm skin. Sweat will easily be detected as it enters the tubing (blue); electrolyte analysis has made it possible to analyse sweat as little as 50 µl of sweat with reliability.

In UK the screening programme for all new-borns include using a Guthrie blood spot test. An initial screening is done to detect the elevated levels of immunoreactive trypsinogen. Samples which are positive are tested for mutations in the CFTR gene which are second screened for immunoreactive trypsinogen if at all necessary.

Programmes for screening are commonly done in many places around the world, but the results cannot be absolute in certain countries with a low ubiquity of mutations in CFTR genes. Early diagnosis and detections can be a boon for many as it can give access to nutritional care; specialised care; diagnostic uncertainty is fairly reduced; and parental counselling for testing. Screening programs have a lot of ambiguity attached to it as well. Heterozygous patients are often misunderstood as carriers this can lead to a lot of mental stresses for the family to face until there is a thorough conclusion. However, in some cases actual cystic fibrosis patients' diagnosis can also turn out to be faulty.

After confirmation of diagnosis, members belonging to the same family are also screened. The disease is most often pre-symptomatic or unrecognised which makes it even more necessary to screen all the siblings for the disease. Asymptomatic adult relatives are offered to screen for carrier status if they wish to and to also to educate them about prenatal screening for informed decisions in the future. Screening and counselling is readily provided by primary care but on the other hand it is all the more important for genetic laboratories to coordinate and ensure rapid, cost effective testing facility.[15]

CF diagnosis requires clinical presentation and CFTR gene dysfunction. Disease confirmation of sweat chloride is first considered, followed by a genetic analysis of CFTR, and then a physiologic CFTR test. Rarely when chloride level in sweat is <30 mmol/L it can be considered that the individual has the disease, if the other confirmatory tests support CF (genetic, physiologic testing). IN some cases, on analysis identification of only one CFTR variant is done, which makes it necessary to further extend the scope of CFTR testing. Cystic Fibrosis occurs if both alleles have Cystic Fibrosis-causing, MVCC mutations. In extreme cases where CF is not resolved even after all methodical tests, CRMS/CFSPID is considered.[16]

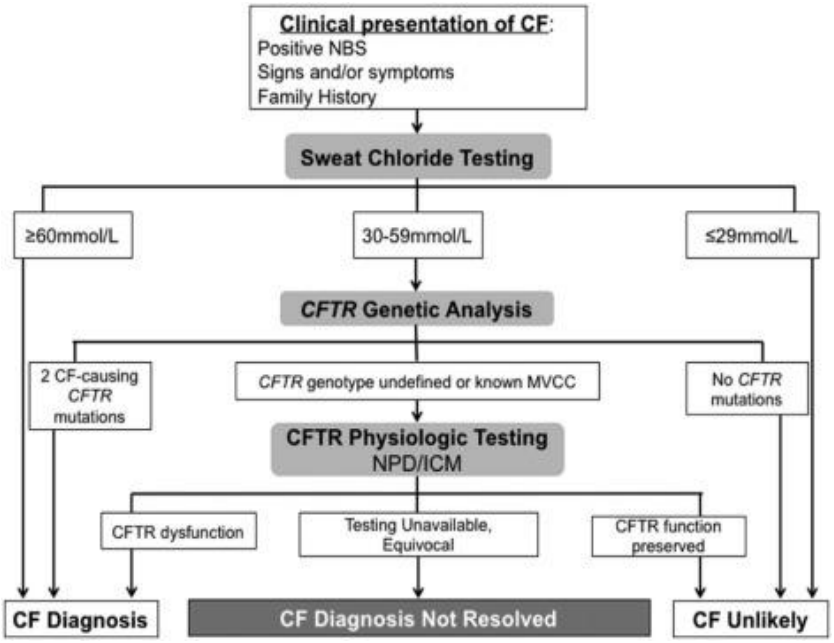

Figure 4: hierarchical protocol for the diagnosis of CF. [14]

VI. DRAWBACKS OF CURRENT METHOD OF DETECTION

Sweat chloride levels are detected by sweat test for children within 48 hours of birth of the child. This disease is very common in the United States that almost all the children are screened for it immediately after birth. [17]

Generally, in a sweat test the amount of chloride (salt) is measured. This is done by collecting the sweat from upper part of leg of a baby or through their arm. People with CF face problem in movement of chloride through cell membranes, which will further result in elevated concentrations of sweat chloride. This test for sweat has proved to be effective for detection of abnormally elevated levels of sweat chloride, hence it will be considered as the diagnostic test for CF. [18]

The following table illustrates the various types of methods used for sweat induction and testing and their respective limitations: [19]

Diagnostic method[19]	Inference[19]
Sweat Stimulation by pilocarpine iontophoresis which was later Collected by filter paper Analysis is done by sodium – flame photometry	This test reported only the concentration of sweat sodium levels.
Sweat Stimulation by pilocarpine iontophoresis which was later collected on a filter paper for 30 minutes. Analysis is done by sodium – flame photometry	This test measured only the sweat sodium levels without reporting mean sodium concentrations.
Stimulation was done by pilocarpine iontophoresis. Storage and mixing: This was carried out by two different ways: 15-90 minutes in gauze squares (3x3inch) and 30 minutes in a paper filter (2.5cm) for chloride polarographic.	- This was not a healthy control group, so- then it is possible that these subjects had a condition that affected their sweat electrolytes or had a mild CF phenotype. - Although this study had been done before the innovation of the CFTR gene.
Stimulation was done by pilocarpine iontophoresis which was Collected on filter paper for 30 minutes. Analysis of chloride done by the coulometric method	- This test had a very small number of subjects in each group. - Brothers and sisters generally have a 2/3 risk of being a carrier.
Stimulation was done by pilocarpine iontophoresis method which was collected on a filer paper for 20 minutes or less than that. Analysis done by sodium – flame photometry or chloride – potentiometric method	- It was found that non-symptomatic subjects may have a condition that affects sweat electrolytes or have a mild CF phenotype. - There wasn't a single CFTR gene mutation analysis to exclude carriers or mild CF.

VII. PRESUMPTIVE DIAGNOSIS AND SWEAT CHLORIDE TESTING [20]

For other people	Sweat chloride tests are performed as per the approved principles following, international protocols of the Clinical and Laboratory Standards Guidelines. (2009)
For new-borns	Newborns who test positive for new-born CF screening, should perform the tests with adequate amount of sweat collected when the infant weighs around 2 kgs.
New-borns greater than 36 weeks	It is best advised to perform the sweat chloride testing within 10 days of birth, before the neonatal period ends.
In infants who have undergone NBS and identified with CF.	Finding the right amount of sweat and sweat chloride should not be delayed and other appropriate treatments should start as soon as possible.
For other people	The level of chloride in sweat should be analyzed within hours of sweat collection, the results should be delivered to the relevant authorities, parents or patients, on the same day. Prompt reporting is encouraged by helping patients and family help deal with stress better.

Results <30 mmol/L [20]

New born	When newborns are tested, sweat chloride level <30 mmol / L indicates that CF is less likely. Sweat chloride testing should also be performed if there is a history from a indicated family member, or if symptoms persist.
For other people	Sweat chloride tests level which is indicated below is 30 mmol/L shows CF is unlikely for all age groups.

Results 30-59 mmol/L [20]

For other people	Values of chloride amount in sweat in a range of 30-59 mmol/L may have CF. Extended gene analysis (CFTR) is to be performed. further studies are necessary to rule out or confirm CF diagnosis. Evidence can be supported by CFTR genotyping or by further CFTR physiological tests.

Results ≥60 mmol/L [20]

For other people	For people presenting a PNS (Positive New-born Screening), or a history, diagnosis can be performed in case the value is ≥60 mmol/L. The genetic analysis should also be included in many NBS programs, DNA misinterpretations should be avoided.
For newborns	If it is a positive sweat test, 2 mutation causing CF should be checked and confirmed.

VIII. CONCLUSION

The various testing methods as explained in this article show how Cystic Fibrosis is detected and how the current methods which are being used are incomprehensible and inefficient towards the process. It is therefore need of the hour to come up with alternatives in order to find a quick and easier way for the diagnosis of CF. One of the preferable ways to do this would be by the use of sensors. Sensors can be manipulated in such a way to detect concentration of chloride levels in the human sweat without employing the long and tedious procedure for collection of large amounts of sweat and also quicker detection. Also, a sensor would provide easy portability and would cut the time required in the lab for detection of chloride levels in the lab using colorimetric titrations.

There have been several studies for development of a sensor that can successfully detect and monitor various types of diseases, despite these improvements CF still remains left to be tested using the traditional diagnostic methods. Such sensors would also help in constant monitoring of glucose levels or can be incorporated to monitor pulse, not unlike a pulse oximeter. These studies are a step in the direction of developing various biomedical instruments for simplifying diagnosis of various diseases, Cystic Fibrosis being one of them.

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