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Table of Contents

Myxoid Angiomatoid Fibrous Histiocytoma: Report of An Uncommon Neoplasm with a Review of Literature

Marwa Abd ElAziz, Rania Gaber Aly and Samir S. Amr

22-25

Cystic Fibrosis Treatment; Overview of Small Molecule Modulators and Genetic Therapy

Salma Elmallah and Raafat Soliman

26-35

History of Dentistry in Ancient Egypt

Nadia Lotfy

36-41

Myxoid Angiomatoid Fibrous Histiocytoma: Report of An Uncommon Neoplasm with a Review of Literature

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Abstract:

Angiomatoid Fibrous Histiocytoma (AFH) is a rare soft tissue tumor usually seen in the extremities of children and adolescents. Classically AFH presents as a painless cystic mass that shows blood filled spaces on cut section and bland histiocyte-like cells on microscopic examination. Predominance of myxoid stroma is a rare finding in AFH, usually seen with other classical features. Herein, the researchers report a case of myxoid AFH with both unusual clinical presentation and uncommon histopathological features with a review of literature.

Key words: Myxoid, Angiomatoid fibrous histiocytoma.

Myxoid Angiomatoid Fibrous Histiocytoma: Report of an Uncommon Neoplasm with a Review of Literature

Case report

An 11-year-old male child presented by a painful thigh mass that had been noted for 7 months. MRI showed a deeply seated thigh mass measuring 7cm in maximum dimension not related to the underlying bone or the overlying skin. Surgical resection of the mass was done and showed a well-circumscribed solid firm mass having a whitish yellow cut section [Figure 1].



Fig. 1. A well circumscribed mass having a yellowish white tan cut surface.

Microscopically the mass was well circumscribed, lobulated composed predominantly of hypocellular areas and less frequently hypercellular zones surrounded by a rim of lymphoid tissue showing germinal centers [Figure 2]. The hypocellular areas showed myxoid basophilic stroma with oval to rounded cells having little pleomorphism and arranged in a reticular pattern. Some vacuolated cells were noted in these myxoid areas. Rare pseudovascular spaces filled with blood were observed. Scattered vessels were noted, most of which are slender and curvilinear. The hypercellular areas showed bland spindled cells arranged in fascicles with scant mitotic figures, but without abnormal forms. Collagen bundles were focally noted in some areas among loosely arranged spindled cells. Focal herringbone pattern was noted [Figure 3]. No necrosis was seen.

A panel of immunohistochemical antibodies (IHC) including desmin, smooth muscle actin (SMA), epithelial membrane antigen (EMA), S100 and MUC4 was done. Tumor cells showed diffuse strong cytoplasmic staining for desmin, focal staining for both smooth muscle actin (SMA) and epithelial membrane antigen (EMA), whereas they were negative for S100 and MUC4 [Figure 4]. So a diagnosis of myxoid variant of angiomatoid fibrous histiocytoma was made.

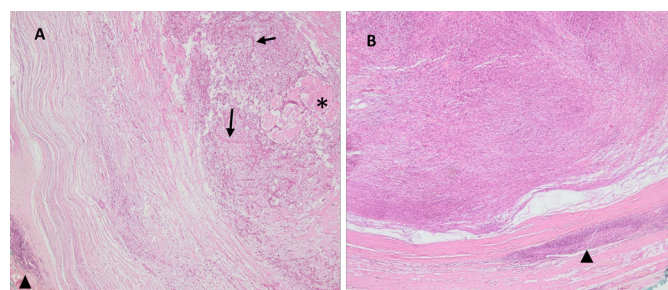


Fig. 2. A: A well circumscribed predominantly myxoid stoma-rich tumor composed of loose areas showing slender blood vessels (arrows) and cystic areas filled with myxoid material (asterisk). B: Hypercellular spindled areas were noted. The growth was surrounded by a rim of lymphoid tissue (arrowheads). H&E x10.

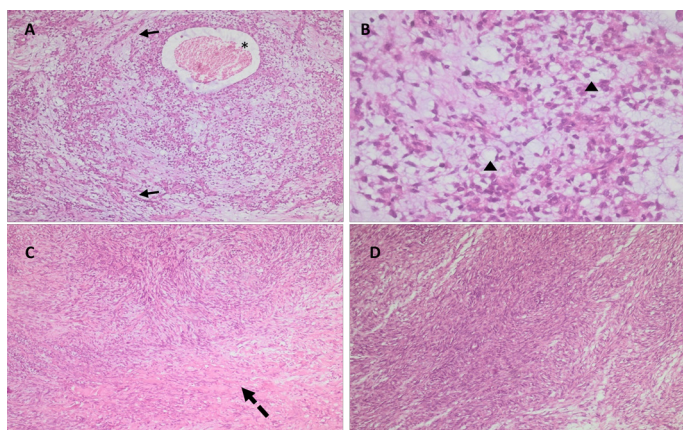


Fig. 3. A: The hypocellular areas showed loose myxoid areas with slender curvilinear blood vessels (arrows) and an occasional pseudovascular space (asterisk). H&E x10. B: The cells were small round to oval arranged in a reticulated pattern. Few vacuolated cells were noted (arrowheads). H&E x400. C: The hypercellular areas showed bland spindle cells with collagen bundles (dotted arrow). D: Herringbone pattern was noted. H&E x100.

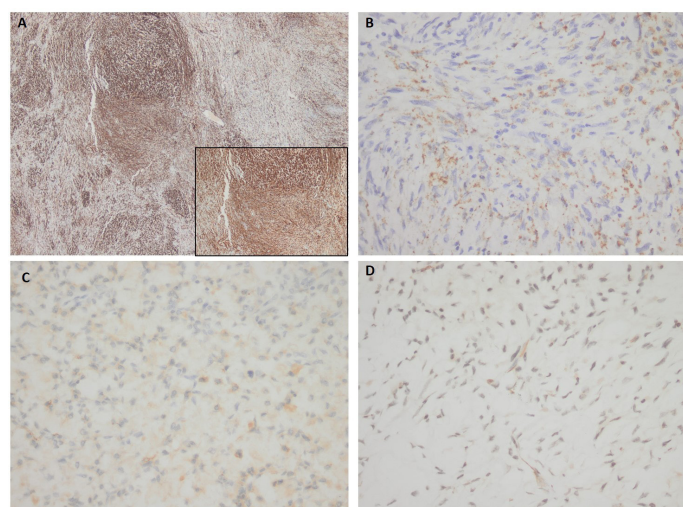


Fig. 4. A: Positive staining for desmin in hypocellular areas (x100) Inset: High power showing diffuse staining for desmin in hypercellular areas. B: Focal weak staining for CD68. C: Focal moderate staining for EMA. D: Focal moderate staining for SMA. (x400).

Discussion

Angiomatoid Fibrous Histiocytoma (AFH) is an uncommon soft tissue tumor representing 0.3% of all soft tissue neoplasms. It is a tumor of an uncertain type and an intermediate malignant potential [1]. It was previously referred to as 'angiomatoid malignant fibrous histiocytoma'; a term depicting its resemblance to vascular neoplasms and its potentially aggressive behavior [2].

AFH is seen mainly in children and adolescents. It usually arises on the extremities in the subcutaneous tissue as a nodular mass often with cystic areas. It shows a slow rate of growth [3]. Contrary to the present case, pain is not a common presenting symptom for this tumor; however, the secretion of cytokines – particularly IL-6 – can lead to systemic manifestations such as fever, anemia and weight loss. This has been attributed to binding of the IL-6 promotor region to the fusion protein EWSR1-CREB [4].

Classically, these tumors are well-demarcated masses of few centimeters in maximal dimension having a tan to brown cut section with grossly appreciable cystic spaces filled with blood. Microscopically, AFH shows central cystic hemorrhagic areas admixed with a uniform population of histiocyte-like cells surrounded by a dense peripheral rim of lymphoid tissue enclosed in a thick fibrous pseudocapsule. The lesional cells show a pale eosinophilic cytoplasm with bland round to oval nuclei showing no atypical features [5]. However, in rare instances severe atypia, multinucleation and hyperchromasia are encountered with no significant effect on the clinical outcome [6].

Cystic hemorrhagic areas are a diagnostic feature of AFH and have granted these tumors their name. They often lead to an erroneous diagnosis of a vascular tumor, however – unlike true vascular spaces – these pseudovascular spaces lack an endothelial lining. Small vessels can be seen in the vicinity of tumor nodules, though are often not a prominent feature. Hemosiderin deposition and fibrous tissue can be very prominent especially in longstanding tumors [5]. However, in a review of the spectrum of histological features in a series of 27 cases of AFH, the presence of pseudovascular spaces was observed in only 11 cases (43%) [7]. In another series of 13 cases from Taiwan, four cases had solid growth pattern, and did not show pseudovascular spaces [8]. The current case showed only a few occasional pseudovascular spaces.

Lymphoid tissue – a characteristic feature of AFH – can be admixed with plasma cells and eosinophils [7] but is more often in the form of lymphoid aggregates sometimes showing germinal centers – as seen in the present case. The presence of lymphoid follicles with germinal centers can lead to the initial consideration of lymph node metastasis. However, the lack of sinuses and the haphazard distribution of germinal centers not limited to subcapsular region stand against a nodal lesion [5].

Uncommon features of AFH include small cell change, alveolar pattern, predominantly myxoid areas and spindle cell pattern [5, 9, 10]. The predominance of these rare features can often be very misleading and direct the pathologist's attention to other lesions as round cell sarcomas particularly Ewing sarcoma, alveolar rhabdomyosarcoma, myxoid-rich tumors and spindle cell sarcomas [5].

Since AFH is a tumor of uncertain differentiation, the IHC features of these lesions can be very confusing and inconsistent. Despite the fact that histiocyte-like cells

predominate in these tumors, only half of the cases express CD68, a percentage surprisingly similar to the expression of CD99, desmin and EMA in AFH [11, 12]. Other less commonly expressed markers include ALK and other muscle markers as calponin and SMA [12, 13].

Multiple translocations with resultant fusion proteins were reported in AFH the most common of which is EWSR1–CREB1, followed by EWSR1–ATF1 and FUS–ATF1 being the least common [14, 15, 16]. None of these fusions appear to be associated with specific clinical or pathological features [5]. Of note, EWSR1–CREB1 and EWSR1–ATF1 are not unique to AFH and can be encountered in other tumors particularly clear cell sarcoma of the soft parts [17].

Myxoid AFH represents less than 5% of all cases of AFH. Myxoid stroma is expected to represent at least 60% of the tumor to be included in the myxoid AFH category [9]. The largest series of these tumors was reported in 2014 by Schaefer et al. [9] followed by few reports of similar findings [18, 19]. It has recently been reported in uncommon sites including the intracranial location [20]. Apart from the predominance of myxoid stroma, the histological, immunohistochemical and cytogenetic features of this variant is similar to those of the classic AFH. However, not all tested cases for EWSR1 and FUS genes by Schaefer et al. showed rearrangement in myxoid AFH challenging their diagnostic utility [9]. Intracranial tumors show a unique fusion between EWSR1–CREM – a gene belonging to the CREB family [20].

The present case showed a constellation of rare histological features. The predominance of curvilinear slender vessels with only a very few pseudovascular spaces were seen. No hemosiderin pigment was observed. The complete absence of the classic histiocyte-like cells with the stark predominance of myxoid areas occupying more than 80% of the tumor along with the herringbone like spindled areas; all posed a diagnostic challenge. Only the fibrous pseudocapsule and the peripheral lymphoid follicles gave the initial clue to the possibility of myxoid AFH, however, other myxoid-rich lesions were initially considered; particularly low grade fibromyxoid sarcoma, neurogenic benign tumors, extraskeletal myxoid chondrosarcoma and myxoid liposarcoma.

In accordance with the literature, the present case showed strong desmin expression but weaker EMA and SMA. Only scattered cells were positive for CD99. The absence of histiocyte-like cells characteristic of this lesion came in agreement with the weak and focal CD68 expression. Positivity for desmin and EMA excluded the prospects of extraskeletal myxoid chondrosarcoma and myxoid liposarcoma. MUC4 and S100 negativity excluded the diagnosis of low grade fibromyxoid sarcoma and neurogenic tumors, respectively. Negativity for S100 also excluded myoepithelial tumors that can show some degree of similarity to myxoid AFH.

As previously mentioned, AFH was initially thought to be an aggressive tumor [2]. However, follow-up of large series showed that local recurrence does not exceed 12 % of cases and is mostly related to incomplete excision and

that distant metastasis is seen around 5% of cases [6, 12]. Therefore, in the recent classifications of soft tissue tumors the “malignant” designation was removed from the name of these lesions [1]. Only surgical resection with negative resection margins is curative for these lesions [5].

Conclusion

AFH can present as a painful solid mass. Some of the classic features of these lesions may be very focal – as the blood-filled-cystic spaces – while others can be completely absent – as the histiocyte-like cells. In these cases, identification of even few cystic spaces filled with blood, along with other classical features on histopathological examination can be a clue to the diagnosis. The integration of clinical data with gross and microscopic features is mandatory in such challenging cases to reach an accurate diagnosis.

Conflicts of interest

The authors have no conflicts of interest to declare.

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Cystic Fibrosis Treatment; Overview of Small Molecule Modulators and Genetic Therapy

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Abstract:

Cystic fibrosis, CF, is a life-shortening genetic disease affecting Caucasian population. It is a recessive genetic disease that is mainly caused by different types of mutations affecting the gene encoding for the cystic fibrosis transmembrane conductance regulator, CFTR, protein. A malfunctioning CFTR protein would lead to the accumulation of a thick viscous mucous layer blocking pancreatic ducts, intestines and airways which is the primary reason of death. Treatment of cystic fibrosis was mainly addressing the symptoms to overcome the complications of the disease. Since the early 2010s, the development of an actual therapy has reached great milestones including small molecule modulators and genetic therapy. Small molecule therapy depends on the development of small pharmacological agents that can, through different mechanisms, restore the function of the mutated CFTR protein. On the other hand, gene-editing techniques are evolving showing very promising results. Gene therapy entails the relocation of a proper copy of the CFTR gene in the aim of expressing a functional CFTR protein. Interesting advances in the development of small molecule and genetic therapies are discussed in this review article with a highlight on their benefits and limitations.

Key words: cystic fibrosis, treatment, disease, drug, pulmonary, small molecule modulators, gene therapy

INTRODUCTION

1. Background

Cystic fibrosis (CF) is one of the most common life-threatening genetic diseases among people of Caucasian origin. It affects over 90,000 individuals who are heterogeneously distributed worldwide where the United States of America contains the highest number of registered patients, (Figure 1).^[1] It is an autosomal recessive disorder characterized by a widespread dysfunction of exocrine glands particularly in lungs and

the gastrointestinal tract leading to severe complications and often early death.^[2] CF was first recognized in 1938 by the pathologist Dorothy Andersen and since then the interest in understanding the pathophysiology and the underlying mechanism of the disease has been in continuous growth.^[2] In the 1950's, it was noted by Kessler and Andersen that cystic fibrosis patients show an abnormal electrolyte composition of the sweat (excess sodium chloride, NaCl).^[3] Further investigations suggested that the disturbance in fluids and electrolyte transport is associated with an abnormally low chloride ion permeability through epithelial membranes that leads to poor NaCl reabsorption in sweat ducts and consequently high concentration of NaCl in the sweat of cystic fibrosis patients.^[4]

The underlying cellular defect of this abnormality remained unknown until the identification of the gene mutation responsible for this defect in 1989.^[2] Molecular cloning experiments have allowed the isolation of DNA segments containing the cystic fibrosis gene locus. The CF gene is located on the long arm of chromosome 7, embracing 180000 base pairs.^[5] In 1989, John Riordan et al. were able to fully characterize the product of the CF gene and it was given the name of CFTR (cystic fibrosis transmembrane conductance regulator) protein.^[5]

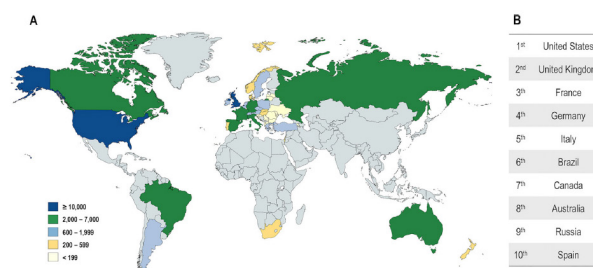


Figure 1: Demography of cystic fibrosis patients in different countries. (A) Distribution according to the total number of patients registered. (B) Top 10 countries with the highest number of patients registered. ^[1]

2. Structure of CFTR Protein

The CFTR glycoprotein is comprised of 1480 amino acids and is a member of the Adenosine TriPhosphate (ATP) Binding Cassette (ABC) superfamily; it is a family of active transporters that use the energy of ATP hydrolysis to translocate ions across epithelial membranes based on concentration gradient.^[6, 7] Being a multi-domain membrane, CFTR protein consists of two membrane spanning domains (MSD1 and MSD2), two intracellular nucleotide binding domains (NBD1 and NBD2) and an intracellular regulatory domain (R) that connects both halves of the protein and regulates the gating of the CFTR channel, (Figure 2).^[8, 9]

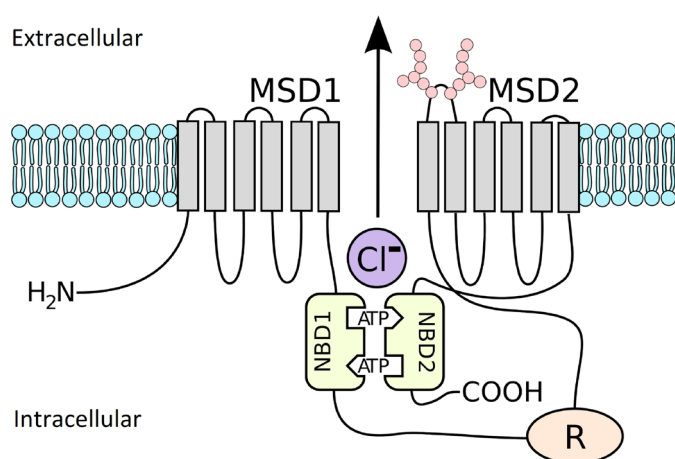


Figure 2: Predicted topology of CFTR protein.^[7]

Each MSD subunit consists of six hydrophobic transmembrane domains that together form the channel pore of the protein to allow ion conductance.^[8] Activation of the CFTR protein requires 2 main factors; it starts with the phosphorylation of the R domain, which is a highly charged sequence containing several phosphorylation sites, by various protein kinases such as PKA, PKC and AMP-activated protein kinase.^[10] The second factor is the binding and hydrolysis of ATP molecules at the NBDs which stimulates the opening and closure of the chloride channel respectively.^[11] CFTR is a multifunctional protein where besides being a chloride and bicarbonate channel, it can also regulate the transport of other ions by having either a positive or a negative effect on other channels and transporters. A working CFTR protein can help in the efflux of Cl^- , $(\text{HCO}_3)^-$ and K^+ ions through different apical channels.^[12] Furthermore, it down regulates the reabsorption of Na^+ ions from epithelial Na^+ channels (ENaC).^[13] Decreased secretion of Cl^- ions and increased reabsorption of Na^+ ions is the main biomarker of CF as patients would have salty sweat due to increased sodium and chloride levels.

2.1. Normal CFTR Protein Synthesis:

Since the characterization of the CFTR gene, over 1600 different gene mutations have been identified.^[14] These mutations interfere with the normal pathway of CFTR from the nucleus to the plasma membrane leading to either no Cl^- channel or a malfunctioning one. The journey of the CFTR protein includes many

cellular proteins within multiple compartments.^[15] It first starts with the transcription of the DNA code, within the nucleus, into mRNA. The mRNA leaves the nucleus to be translated, mainly in the endoplasmic reticulum (ER), into a nascent polypeptide. Within the ER lipid bilayer, the nascent protein undergoes further protein maturation and a cotranslational folding process.^[7] Any formed misfolded CFTR protein will be bound to a specific chaperone protein to carry it through an ER-associated degradation process, ubiquitination, followed by degradation in the ubiquitin proteasome system. The cytoplasmic transfer of the properly folded CFTR protein to Golgi apparatus is done through a variety of chaperone proteins, where it will undergo posttranslational modification, notably glycosylation. The CFTR processing involves its conversion from mannose-enriched to a mature oligosaccharide side chain attached to the asparagine residues located in the fourth loop of MSD2.^[7] The mature CFTR will be shuttled, by clathrin-coated vesicles, from the Golgi apparatus and stationed in the plasma membrane. Once inserted into the plasma membrane, CFTR turns over at a rate of 10% per minute and has a half-life of ~12–24 hrs. Finally, the internalization of the mature protein proceeds through clathrin-coated endosomes.^[7]

2.2. CFTR Mutations:

There are more than 2000 identified mutations that can affect the CFTR gene leading to a wide range of phenotypes since the discovery of the CFTR gene in 1989. They can be classified into six main classes according to their resulting defect in the CFTR production, (Figure 3).^[16]

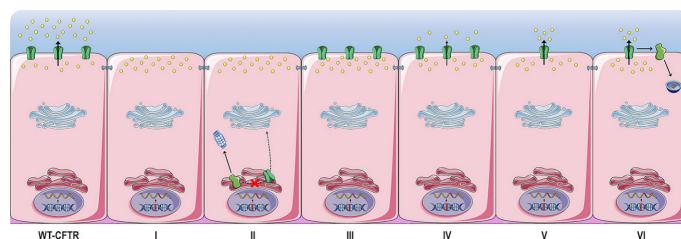


Figure 3: Schematic representation of the different classes of CFTR gene mutations.^[1]

2.2.1. Class I Mutations, Defective Synthesis:

This class of mutations results in truncated or unstable mRNA leading to defective expression of CFTR gene and consequently defective synthesis of CFTR protein.^[17] This will lead to complete absence of the protein at the cell surface and it affects ~7% of CF patients worldwide.^[17] Class I mutation most common examples are; W1282X and G542X.^[17]

2.2.2. Class II Mutations, Defective Processing:

This class of mutation results in defective protein processing. The formed misfolded CFTR protein will be retained at the endoplasmic reticulum leading to premature degradation and preventing the trafficking

of the protein to the plasma membrane.^[17] The most common examples of this class are; F508del and N1303K.^[17] This class includes F508del, also termed as Δ F508, mutation which is the most frequent CF mutation affecting ~90% of CF patients in at least one allele.^[19] This mutation involves the deletion of phenylalanine amino acid located at position 508 at the NBD1 of the CFTR protein.^[19]

2.2.3. Class III Mutations, Defective Gating:

In this type of mutation, the CFTR protein successfully inserted at the epithelial membrane will suffer from defective gating. This will lead to a closed channel or reduced open probability of the CFTR channel and consequently decreased chloride ion efflux.^[18] The most common examples of this class are; G551D and S549R.^[17] G551D is the third most common type of mutation affecting ~5% of CF patients worldwide.^[20] It involves the replacement of the glycine amino acid located at position 551 of the NBD1 of the CFTR protein with aspartic acid and it leads to a reduced open probability of tenfold less than the normal CFTR channel.^[21]

2.2.4. Class IV Mutations, Defective Conductance:

This class of mutation involves defects in the channel pore leading to decreased open probability to be around one third that of the normal protein.^[7] This will consequently lead to lower permeation and conductance of chloride ions through the CFTR channel. It affects mainly the MSD of the CFTR protein.^[22] The most frequent examples of this class are; R117H and R334W.

2.2.5. Class V Mutations, Defective mRNA Stability:

This class of mutation results in splicing errors which will lead to defective mRNA stability. The defective stability will lead to the formation of a mixture of both correct and aberrant protein and thus reduced synthesis of the normal CFTR channel.^[7] As a result, a reduced total amount of CFTR protein is found at the plasma membrane leading to decreased chloride ion secretion. The most frequent examples of this class are; A455E and c.1680–886A>G.^[17]

2.2.6. Class VI Mutations, Defective CFTR Stability:

This class of mutation will cause instability of the CFTR channel already localized at the plasma membrane leading to an accelerated turnover rate from the cell surface. The most frequent examples of this class are; 4326delTC and 4279insA.^[17]

Broadly speaking, mutations of classes I, II and III are the most severe types as they are associated with few or no CFTR protein at the plasma membrane. While classes IV, V and VI have milder disease conditions as they involve only partial loss of the CFTR function activity.^[17]

3. Symptoms of Cystic Fibrosis:

The lack of functioning CFTR at the proper location

in the epithelial membrane, due to different types of mutations, leads to malfunctioning CFTR-dependent ion channels. This results in the disruption of ion transport homeostasis through epithelial membranes in different organs.^[2] In the lungs, CFTR and ENaC are important for the regulation of the water volume and height of the airway surface liquid (ASL).^[23] ASL is the layer covering the lung surface. It is composed of mucus and periciliary liquid and it is necessary for mucociliary clearance.^[2] In CF, overexpression of ENaC leads to increased absorption of salt and water from airway surfaces and ASL depletion.^[23] Dehydration of airway surface liquid leads to defective mucociliary clearance and reduced bacterial clearance.

Mucus accumulation provides a good environment for bacterial colonization and subsequently chronic airway diseases, chronic inflammation and respiratory failure. Pulmonary disease is the primary cause of morbidity in cystic fibrosis (>90%).^[2, 24] Thick mucus can also accumulate in the pancreas and intestines, leading to the obstruction of pancreatic ducts and preventing the passage of digestive enzymes to small intestines. This leads to pancreatic insufficiency, maldigestion, poor growth and weight loss. Other symptoms of cystic fibrosis are liver cirrhosis, rectal prolapse, male infertility, diabetes, and a salty sweat.

4. Towards The Treatment of CF:

Previously, CF therapy was limited to symptomatic treatment. The main goal was to alleviate the symptoms; improving the quality of life of CF patients by treating downstream disease processes that are secondary to CFTR malfunction, without curing the disease itself.^[25] Symptomatic treatment includes antibiotics and anti-inflammatory agents that help to eradicate chronic bacterial infections such as *Pseudomonas Aeruginosa*. Antibiotics used are mainly in the inhaled form such as; azithromycin, tobramycin, aztreonam and levofloxacin. Other antibiotics are also recommended such as; ciprofloxacin, cephalexin, amoxicillin and doxycycline according to sensitivity patterns.^[27, 28] Secondary airway inflammation is controlled using NSAIDs, inhaled and systemic steroids and cromolyn.^[29]

Mucolytic agents to break down mucus and clear up airways and pancreatic ducts are recommended along with the combination of inhaled β -agonists with humidified oxygen; a 3–6% hypertonic saline solution and dornase alfa to decrease viscoelasticity of mucus and dilating the airways.^[30, 31] CF patients are also given pancreatic enzymes to help digestion; combinations of proteases, lipases and amylases, and supplementary vitamins to ensure good nutrition.^[32] More agents for symptomatic improvements are in the pipeline.

Isolation of CFTR protein and understanding of molecular mechanisms behind the clinical manifestations of the disease has helped in the development of new treatments tackling the main cause of cystic fibrosis. At present, different drug approaches are being developed to overcome the underlying genetic defect causing the disease and help to halt or reduce the progression of the disease. Agents focusing on correcting structural and

functional abnormalities of CFTR protein can be divided mainly into; small molecule modulators and gene therapy. Figure 4 summarizes the main abnormalities and treatment approaches in CF patients.^[33]

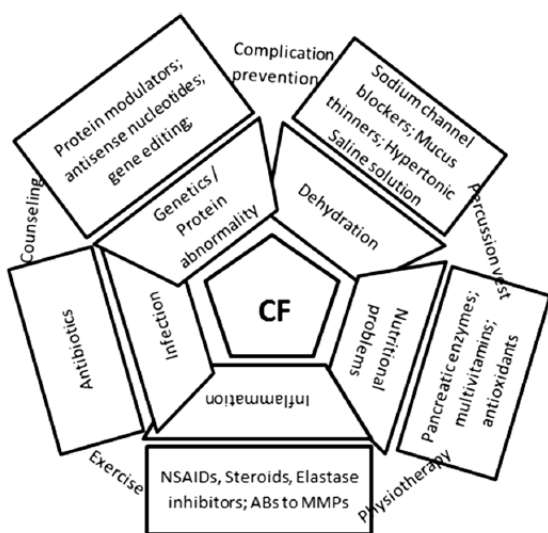


Figure 4: The main physiological dysfunctions and treatment approaches for CF patients. Inner trapezoid boxes depict the physiological abnormalities and outer rectangular boxes depict the main treatments. The texts connecting the outer boxes show nonpharmacological management. [33]

4.1. Small Molecule Modulators:

The discovery of small molecules, drug candidates, which can interact with the malfunctioning CFTR protein, has evolved over the years. This therapy has the advantage over gene therapy, as it avoids the potential treatment of wrong cells and/or losing the physiological CFTR regulation.^[19] Small molecule therapy mainly involves the development of new compounds that can overcome the underlying CFTR defects and help in the restoration of its function. These defects can be mainly summarized into 2 points:

1. Aberrant CFTR folding and premature degradation,
2. Defective channel gating.

Development of such pharmacological agents is usually done through high throughput screening. High throughput screening involves testing a large collection of small molecules, either natural or synthetic drug-like compounds, using an automated assay designed to recognize active compounds with high efficiency and reliability.^[34] The active candidates in the initial primary screen are further evaluated and the lead compounds are then optimized and intensively screened as potential new drugs.^[34] Small molecule modulators can be classified according to their mechanism of activity into four main classes; correctors, potentiators, amplifiers and stabilizers.^[1]

4.1.1. Potentiators:

Potentiators are small molecules that can repair the gating defect of CFTR ion channel by interacting with mutant CFTR protein, localized at the cell surface. They potentiate the channel activity of mutant CFTR and increase Cl^- transport across the membrane.^[35] VX-770 is the first potentiator that was able to pass in vitro and clinical trials to be the first CF drug approved by US FDA on January 31, 2012 after an unusually fast 3 months review, Figure 5.^[36] It was developed from quinolinone-3-carboxamide moiety and was given the generic name of Ivacaftor, then was released in the market under the name of KalydecoTM.^[37] Ivacaftor was discovered via high throughput on Fisher rat thyroid (FRT) cells.^[17] Using fluorescence based assays, changes in the membrane potential after applying Kalydeco due to CFTR-mediated chloride efflux were measured.^[37]

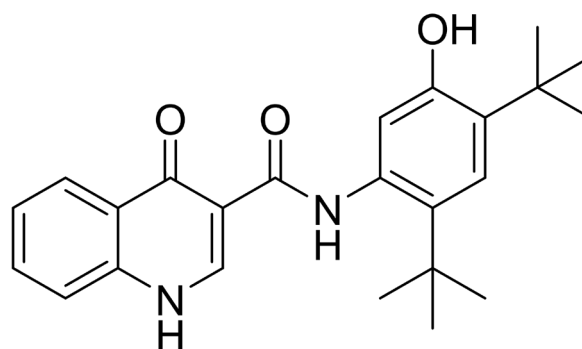


Figure 5: Chemical structure of VX-770, ivacaftor.

Kalydeco was able to increase the transepithelial current (I_{sc}) four to six folds by increasing both the frequency and the duration of the channel openings.^[17] It showed excellent activity and pharmacokinetic properties which qualified it to be the first human therapeutic agent that actually targets the main cause of the disease. Ivacaftor is a potentiator where it binds to the NBD1 of the CFTR protein already inserted into the plasma membrane to solve the gating problem by increasing the channel open probability (P_o), Figure 6.^[38]

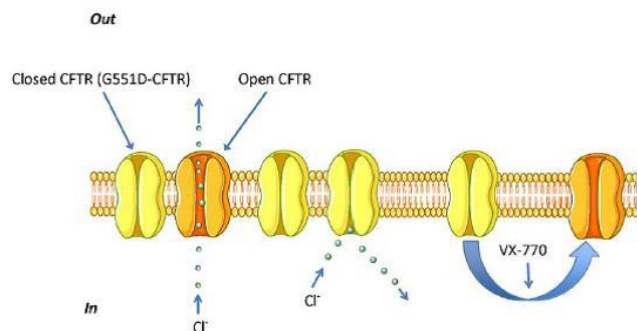


Figure 6: Effect of ivacaftor, VX-770, on G551D mutated CFTR protein. VX-770 allows CFTR channels to remain open, thereby, restoring chloride transport function. [38]

Children aged 6 years and older carrying class III mutation were able to benefit from ivacaftor by taking one tablet, 150 mg, every 12 hrs. Patients reported significant improvement in lung function, weight gain, markedly reduced sweat salt concentrations and overall improved lifestyle quality.^[39]

Being in the market for over 7 years, patients treated with ivacaftor showed good resistance to secondary infection with *Pseudomonas Aeruginosa* and other pathogens, improved body mass index and quality of life.^[40] With the aim of extending the target mutations of ivacaftor, a series of experiments and clinical trials were done. Ivacaftor is now an approved drug for 38 other gating mutations increasing the number of patients who may benefit from the drug.^[1]

Being a successful example, ivacaftor analogs are being developed with the aim of acquiring improved pharmacokinetic properties and a better metabolic profile with low toxic byproducts. VX-561, formerly known as CTP-656, is a deuterated ivacaftor analog that is now in phase II clinical trials showing enhanced stability in vitro and healthy volunteers compared to ivacaftor, figure 7.^[41] Having improved clinical profile, VX-561 is planned to be taken once daily rather than twice in the case of ivacaftor offering better patient compliance. CF therapy based on multiple potentiators has also been tested.

A combination of ivacaftor with natural food components like genistein and curcumin was tested on CF patients carrying gating mutations. The double or triple combination was found to successfully synergize restoring CFTR-dependent fluid secretion.^[43] The inspiring story of ivacaftor being the first CF treatment has opened the way for the development of more small molecule modulators acting either as potentiators or having different mechanisms of action.

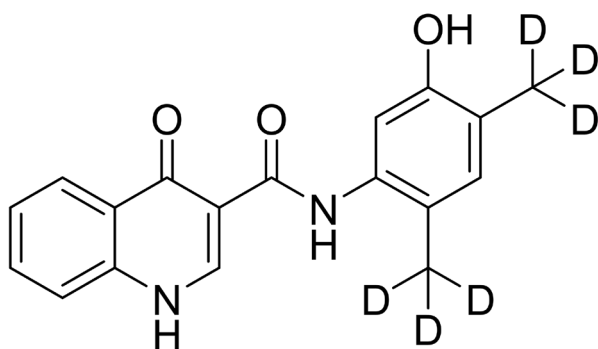


Figure 7: Chemical structure of VX-561, Deutivacaftor.

4.1.2. Correctors:

Correctors are pharmacological agents that overcome the defective processing of mutant CFTR. They can interact with either chaperone proteins to block CFTR premature degradation, or with the CFTR protein itself

to help its proper folding and trafficking to the cell membrane, thus rescuing the cell surface expression of the mutant CFTR.^[43] They have been classified according to their molecular targets into 3 main classes; class 1 correctors which can stabilize NBD1-TMD1 and/or NBD1-TMD2 interfaces, class 2 correctors having the ability to stabilize NBD2 and its interfaces with other CFTR domains and class 3 correctors which directly stabilize NBD1.^[44] This classification can be useful to evaluate corrector combinations from different classes to have a complementary effect.

The great need to discover an effective therapy that can treat CF patients carrying the most common mutation, F508del, has fueled the innovation of a plethora of correctors. Lumacaftor, VX-809, is the fruit of research advances, Figure 8. It is developed by Vertex pharmaceuticals through high throughput screening.

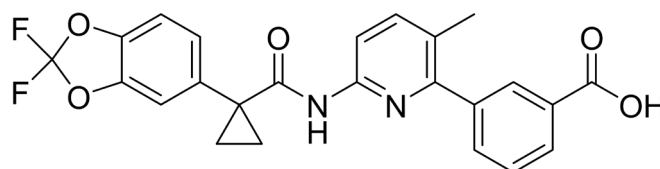


Figure 8: Chemical structure of VX-809, lumacaftor.

Lumacaftor is a corrector that can bind to the F508del mutated CFTR promoting proper folding of the protein, its trafficking to the plasma membrane escaping the endoplasmic reticulum premature degradation and maintaining its stability at the cell surface.^[45] It has been proposed that the binding site of lumacaftor in the pocket created by the deletion of phenylalanine at position 508 found at the interface between NBD1 and the fourth transmembrane domain of MSD2.^[46] It was found that lumacaftor can rescue 30% of CFTR protein from degradation.^[47] Lumacaftor has no effect on the open probability of the CFTR chloride channel, gating, and the efflux of ions. Therefore, a combination of a lumacaftor and ivacaftor is needed in order to solve both trafficking and gating problems associated with F508del mutation.

Orkambi® is the first combination medicine of “lumacaftor and ivacaftor” to target CF patients carrying the most frequent mutation, F508del. Orkambi represents a breakthrough in CF therapy and it was approved by the US FDA in July, 2015.^[47] It exerts its effect through a synergistic mechanism between a corrector and a potentiator.

It combines a chemical corrector, lumacaftor, that helps proper folding and trafficking of the protein and therefore increasing the total amount of channels at the cell membrane and a potentiator, ivacaftor, to increase the frequency and duration of the channel opening which in turn will increase the total movement of chloride across the membrane, Figure 9.^[48]

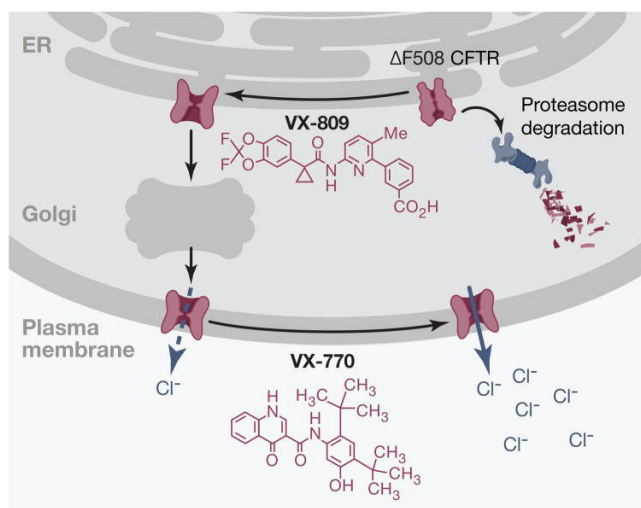


Figure 9: Orkambi's dual effect as corrector and potentiator. Lumacaftor rescues CFTR mutant from premature degradation while ivacaftor increases the channel open probability. [48]

Clinical trials proved that Orkambi has a significant but modest effect on lung function in patients carrying F508del mutation with a 2.6 to 4% increase in FEV₁.^[49] FEV₁ or forced expiratory volume in one second is a standard test for lung function that measures the amount of air that can be exhaled in one second.^[49] Orkambi is administered as two orally bioavailable combination tablets consisting of 200 mg lumacaftor and 125 mg ivacaftor to be taken every 12 hrs with a good safety profile in the short and long term.^[46] Due to the lipophilicity of ivacaftor and lumacaftor, taking Orkambi with high fat-containing food highly increased their absorption. It was found that co-administration with high fat meal improved the absorption of lumacaftor with 2 fold and that of ivacaftor was 3 fold higher.^[16]

Albeit patients treated with Orkambi have experienced an improvement in their quality of life, the drug-induced improvement in lung function was modest. This limited effect is maybe due to an inhibitory action exerted by ivacaftor on lumacaftor.^[50] Further in vitro studies were conducted in order to identify the main cause behind Orkambi's reduced efficacy. These studies revealed that the reduced efficacy was related to the negative impact of ivacaftor, VX770, on the stability of the lumacaftor rescued F508del CFTR protein.^[51] It determined that

ivacaftor has a non-specific destabilizing effect on the lipid bilayer of the plasma membrane as it increased its fluidity or lipid packing and reorganized the membrane.^[51] This membrane disruption causes a decreased abundance of the corrected protein leading to an inhibitory effect on the lumacaftor mediated correction of the CFTR protein. The negative effect of ivacaftor was correlated to its predicted lipophilicity as less lipophilic ivacaftor derivatives had milder inhibitory effect.^[51]

Looking for other agents having better synergistic effect with ivacaftor, other correctors were developed. Tezacaftor, VX-661, was developed based on the structure of lumacaftor and it showed very good clinical results, Figure 10. The combination of tezacaftor and ivacaftor was approved by the FDA in 2018 under the name of Symdeko® but it failed to have any better results than orkambi.^[1]

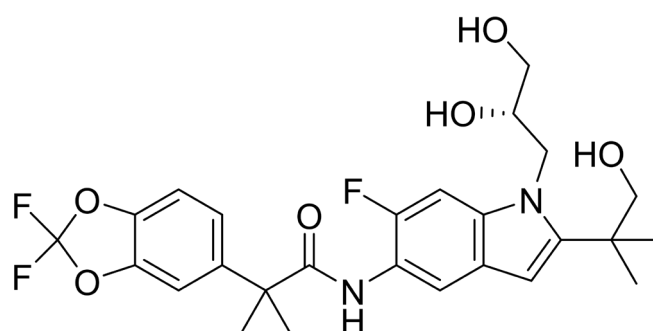


Figure 10: Chemical structure of VX-661, tezacaftor.

In the hope of having better synergistic results, a triple combination of lumacaftor-ivacaftor with new correctors; such as elexacaftor and bamocafactor, were developed, Figure 11. The combination of lumacaftor-ivacaftor-elexacaftor is recently approved by FDA under the name of Trikafta®.^[1] The long term effects of this combination are still ongoing. Recently, another corrector-potentiator triple therapy of elexacaftor-tezacaftor-ivacaftor, kaftrio, is being tested showing very promising results.^[67] The clinical trials of kaftrio showed improved lung function and increased body mass index with decreased sweat chloride levels.^[68] More and more correctors and combinations are being in the loop offering big hopes to CF patients carrying the most frequent mutation.

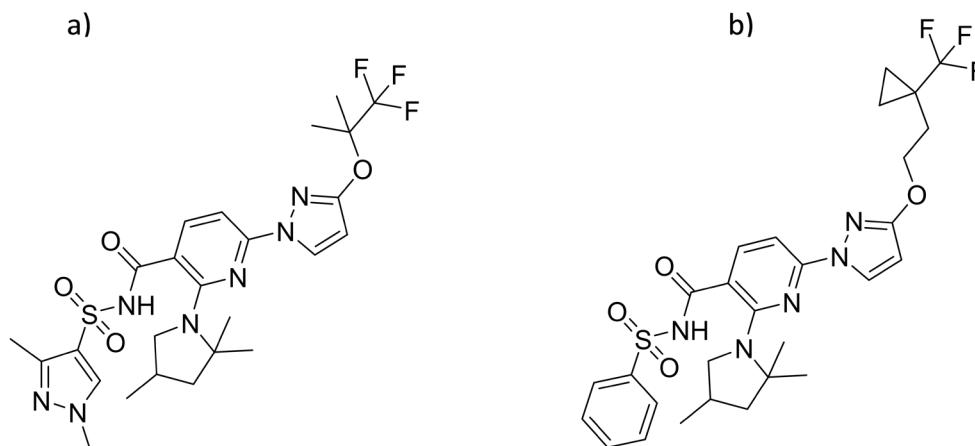


Figure 11: Chemical structure of; a) elexacaftor, b) bamocafactor.

4.1.3. Stabilizers:

Stabilizers are agents that anchor the CFTR protein at the cell surface. They are mainly useful in class VI mutation preventing CFTR removal and degradation by lysosomes.^[1] They can be used alone or in combination with other classes of modulators to rectify the intrinsic stability of the CFTR protein and increase its residence time at the cell surface.^[52] One of the most common stabilizers is the hepatocyte growth factor (HGF). It has the ability to enhance the CFTR stabilization at the plasma membrane by activating Rac1 GTPase signaling.^[53] HGF can be combined with correctors such as lumacaftor in the treatment of F508del mutation to further enhance CFTR maturation and anchoring at the cell surface.^[54] Prolonged HGF treatment showed a beneficial effect on ivacaftor/lumacaftor combination by preventing ivacaftor mediated destabilization of lumacaftor-rescued CFTR in F508del-expressing cells.^[55]

Other stabilizers have been developed such as vasoactive intestinal peptide (VIP) and Cavosonstat. VIP enhances the phosphorylation of the actin-binding complex ezrin/radixin/moesin (ERM) which interacts with the scaffolding protein Na⁺/H⁺ exchange factor1 (NHERF-1) and CFTR to prevent CFTR endocytosis and lysosomal degradation.^[56] Cavosonstat (N91115) is the first stabilizer to reach clinical trials. It can inhibit S-nitrosoglutathione (GSNO) reductase increasing cellular GSNO and stabilizing cell surface expression of F508del CFTR.^[57] Unfortunately, cavosonstat failed to show promising clinical results in terms of lung function and sweat chloride concentration even when combined with modulators such as ivacaftor or lumacaftor reaching a dead end in clinical development.^[58]

4.1.4. Amplifiers:

Amplifiers are pharmacological agents that amplify the expression of CFTR mRNA and, consequently, the biosynthesis of the CFTR protein.^[59] The strategy is to augment the abundance of the CFTR protein which can then be folded properly and have a corrected function with the help of correctors and potentiators. The first developed amplifier nesolicaftor (PTI-428) was able to pass phase II clinical trials. Albeit, clinical testing of nesolicaftor combined with lumacaftor-ivacaftor therapy showed promising results in terms of lung function and reduction of sweat chloride concentration levels, further clinical studies are now discontinued.^[57]

4.2. Gene Therapy:

Being a monogenic disease, treatment of CF with gene therapy sounds a very promising approach having the advantage of targeting all CF patients regardless of the CFTR mutation present.^[60] Gene therapy involves the relocation of proper copies of CFTR gene into the epithelial cell layer of the affected organs mainly the airways.^[61] The gene translocation is done through vectors either viral, e.g. adenovirus vector, or nonviral which will be cationic molecules able to form a complex with the negatively charged DNA.^[62] Gene therapy has been subjected to clinical trials but several challenges were faced such as the low delivery of functional genes

by most available vectors, short half-life of therapeutic gene expression leading to low efficacy, and undesirable inflammatory responses.^[63] To overcome the limited effect of gene therapy, the development of efficient vectors to deliver the cDNA to the airway cells is under progress.

4.2.1. Gene editing:

Gene editing is a genetic approach that implies the permanent correction of the mutated CFTR gene.^[62] CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 approach is a gene editing strategy built on the same idea as bacterial defense mechanisms against foreign DNA, Figure 12.^[61] It is currently the simplest, most versatile and precise method of genetic manipulation where the corrective DNA containing multiple small pieces is incorporated into a locus consisting of short palindromic repeats, called CRISPR. Cas9 is an enzyme acting as a pair of molecular scissors that can cut the two strands of DNA at a specific location in the genome so that introduced DNA can be added.^[64]

This technique has been in *in vitro* clinical trials since 2013 showing very promising results.^[65] Gene editing strategy has the advantage of allowing life-long expression and natural adjustment in the cell but no clinical data have been reported yet.^[62]

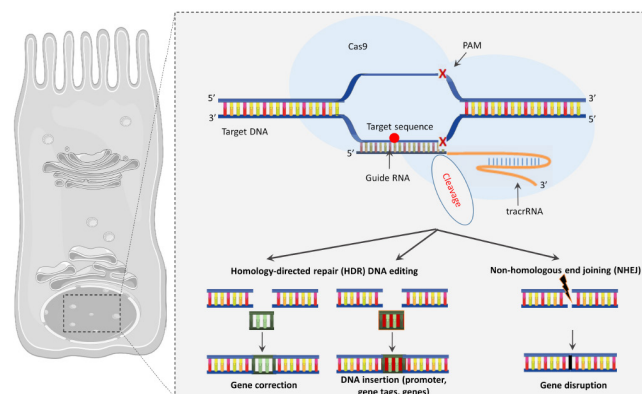


Figure 12: The CRISPR/Cas9 gene editing technique. Cas9 endonuclease binds to the target site using a guide RNA to precisely cut DNA allowing genome editing. CRISPR/Cas9 may drive to gene correction or DNA insertion. ^[61]

4.2.2. mRNA repair:

mRNA repair technique implies repairing the abnormal CFTR mRNA via the insertion of the missing bases. Eluforsen, formerly known as QR010, is an antisense RNA-based oligonucleotide sequence that is formulated as an aqueous solution to be administered by inhalation.^[66]

It is designed to mainly target the abnormal mRNA in patients with the F508del mutation. Eluforsen has the ability to bind to the mRNA region around the F508-encoding deletion and restore the CFTR protein function in the airway epithelium.^[66] The drug is still in clinical trials showing promising results in terms of safety and efficacy.

CONCLUSION:

Cystic Fibrosis is a recessive genetic disease caused by a plethora of CFTR gene mutations. The treatment of CF remained symptomatic until the development of new therapies that tackle the main root of the disease. Since the structure of the CFTR protein is fully elucidated, small molecule modulators that have the ability to bind to the mutated CFTR protein and restore its function were developed. Small molecule modulators can be classified into; correctors, potentiators, stabilizers and amplifiers.

They are personalized to specific types of mutations and it is a therapy that should be taken for a lifetime. Several modulators have been successfully approved by the FDA and EMA and released in the market increasing the life expectancy of CF patients from less than 20 years to over 50. Small molecule modulators have the advantage of improving the life quality of CF patients but being an expensive lifetime therapy that should be taken once or twice daily fueled the urge to the development of a permanent treatment.

Gene therapy involves the relocation of proper copies of CFTR gene into the epithelial cell layer. It is a permanent therapy targeting all different classes of mutations. Different techniques for gene editing are being developed and some of them have reached clinical trials with promising results. Gene therapy has the advantage of being a permanent treatment with a broad spectrum regardless of the type of mutation although its development is challenging.

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History of Dentistry in Ancient Egypt

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Abstract:

Dental profession in ancient Egypt was highly advanced and practiced in very professional ways. Ancient Egyptians had the honor of having the earliest dentist in the world, he was Hesi-ra.

Medical papyri revealed that ancient Egyptians had some diseases such as tooth wear, dental abscess and dental caries. They had skilled dentists that managed those problems relying on actual treatment, they used natural substances extracted from vegetable, fruit and oils for making drugs.

They were pioneers in making tooth fillings, Dental restoration and performing dental surgeries. Furthermore, the idea of dental implants dated back to the ancient Egyptians.

Keywords: Ancient Egyptian diseases, Ancient Egyptian Dentistry

INTRODUCTION

The Egyptian nation was, without doubt, the one in which civilization first took its rise and had its earliest development from the time of Menes, first King of Egypt (3892 B.C). Therefore, the healing art was flourished earlier in Egypt than elsewhere.⁽¹⁾

Knowledge of medicine and dentistry as practiced among ancient Egyptians is now no longer limited; it is documented by many ways such as:

1. The study of ancient remains

Ancient remains (mandible, piece of bone, instrument) demonstrated the dental practice and dental diseases. Human remains are able to tell the story of all lifestyle⁽²⁾ (Figure 1).



Fig.1. Ancient remains

2. The study of ancient teeth

Enamel is the hardest material in the human body, and teeth are often preserved even when bones are not. Teeth are one of the most informative parts of the human body. Teeth provide information about numerous issues including health, diet, stress, occupation, and diseases in ancient humans⁽³⁾ (Figure 2).



Fig. 2. An open mouth of a mummy with complete dentition

3. Mummification

The unique mummification in ancient Egyptians resulted in survival of large numbers of well-preserved soft tissue body remains that are ideal research material.⁽⁴⁾

4. Modern medical technology

Modern medical technology such as X-Rays, CT Scans and electron microscopes helped to view the bones and organs of mummies and allowed the understanding of ancient dentistry.^(5,6)

5. Forensic techniques

Teeth are chemically stable tissues in the body. Analysis of teeth provides insight on health, disease, nutrition, and social organization within selected communities.^(7,8)

6. Translation of the Rosetta stone

This stone was written in hieroglyphic, demotic and Greek language. It was discovered in early 1800s, by Champillon, who translated it and that led to knowing of ancient Egyptian hieroglyphic inscriptions and translation of papyri⁽⁹⁾ (Figure3).



Fig. 3. The three languages of Rosetta stone

7. Ancient papyri

Several Egyptian medical papyri were discovered and translated. The most important are:

- **Ebers papyrus** ^(10,11)
The Ebers Papyrus was discovered by Georg Ebers in 1862 at Luxor during the exploration of a mummy's tomb. It is believed to be dated back to 3500 BC. Ebers papyrus is considered as the most important medical textbooks of ancient time. The papyrus contains over 700 medical remedies covering all aspects of medicine.

Dentistry as a profession is also included in all its aspects such as, diagnosing, prevention and treating dental diseases. There were eleven remedies contained in this papyrus that related directly to dental problems.

- **Edwin Smith Papyrus** ^(12,13)
It was written by Imhotep, the god of Healing. His statue stands today in the Hall of College of Surgeons in Chicago.

It is the world's oldest surgical document. It is viewed as a textbook on surgery. It discussed facial trauma and fractures, stitches and cauterization, treatment of abscesses and suturing of lip wound (Figure 4).



Fig. 4. Imhotep and Edwin Smith Papyrus

Dentists in Ancient Egypt

Ancient Egypt showed a high degree of medical specialization at early stage of the history. Medicine was highly specialized and there were specialists for treatment of the eyes, internal medicine, head, heart, etc. There was distinction between 'physicians' and 'dentists'. Dentists were called Toother.⁽¹⁴⁾

There were skilled dentists in ancient Egypt who practiced what today is considered to be highly skilled techniques.⁽¹⁵⁾

The hieroglyphic symbols of a dentist were: Eye of Horus which denoted to a physician and tusk (elephant canine) as a symbol for a tooth⁽¹⁶⁾ (Figure 5).



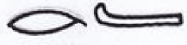
	Hieroglyphic eye of Horus
	Tusk denote tooth
	Eye and Tusk
	One who makes or handles – denoting treatment
	'toother', he who deals with teeth
	One who treats or makes teeth, 'Tooth maker'

Fig. 5. Dentists in hieroglyphic language

The Earliest Dentist in the World (Hesi-ra)⁽¹⁷⁾

Hesi-ra or Hesi-re (hsj-r') is the earliest recorded dentist not only in Egypt, but also he had the honor of being the first dentist in the world. His name means blessed by Ra (sun-god, Ra).⁽¹⁸⁾

Hesi-ra was a man of great distinction who carried many titles. He was called" Head of Dentists", "Chief of the toothers," and "Greatest of teeth". Hesi-ra was honored to be buried beside Djoser's Step Pyramid at Saqqara. Hesi-Ra is also well known for his richly decorated panels found in his tomb ^(18,19) (Figure 6).



Fig. 6. Hesi-ra was on his wooden panel

Achievements of Hesi-Ra.⁽¹⁶⁾

Although dentistry at that time was crude by today's standards; yet Hesi-Ra had many achievements in dentistry:

- He drilled holes in teeth to help drain the infection.
- He performed advanced dental techniques such as root canal therapy.
- He also recognized symptom of Diabetes, and described its signs (polyuria) or "frequent urination".

Not only Hesi-ra who had this place of honor in ancient Egypt, dentists had great respect by the ancient Pharaohs. A tomb of three dentists discovered at Saqqara next to Djoser's Step Pyramid. Their tombs are made of limestone not of mud as other people.⁽²⁰⁾

Dental Diseases and Common Problems in Ancient Egyptians⁽²¹⁾

Because teeth are the best preserved parts of a body, dental diseases are evident. Surveys on mummies suggest that, far from having healthy dentitions, some of ancient Egyptians suffered from dental disease. Ebers Papyrus refers to diseases of the teeth and various toothache remedies.⁽¹¹⁾

Ancient Egyptians suffered from:

1. Tooth wear

This condition was found in most of the skulls. Teeth were extremely worn. There was loss of the crown resulting in pulp exposure and apical infection⁽²²⁾ (Figure 7). This condition was due to abrasion by inorganic particles of food.^(23, 24)

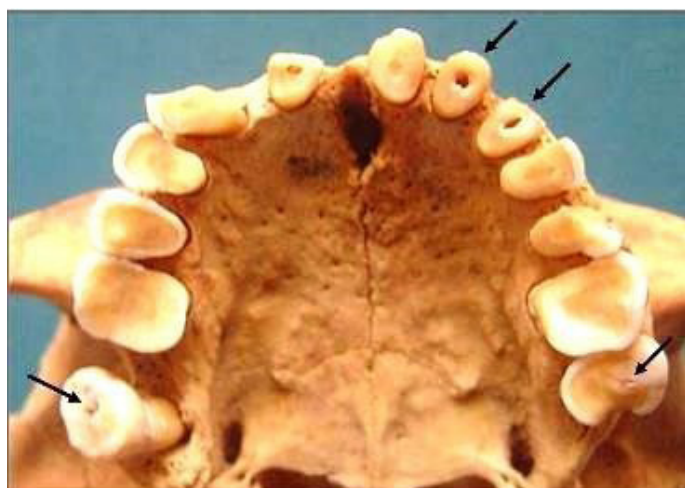


Fig. 7. Tooth wears in ancient skull

2. Dental abscesses⁽²¹⁾

Apical infection was observed in a number of skulls. This was due to extreme wear when the pulp cavity becomes exposed (Figure 8).



Fig. 8. Apical abscesses

3. Disease of the gum⁽²⁵⁾

This appears in the form of presence of calculus on the roots of the teeth and presence of pocketing (Figure 9).



Fig. 9. Calculus and pocketing on the roots of teeth in a mummy

4. Dental caries

Dental caries was not frequent due to lack of fermentable carbohydrates in diet and fibrous abrasive food, which inhibited retention of plaque on the tooth surface. "A worm that grows into the tooth" was thought to be the cause of dental caries.⁽²⁶⁾

Ancient Egyptians Managed their Diseases by Many Ways

Ancient Egyptians took great care to treat their diseases. They had skilled dentists and they were relying on actual treatment not in magic spells and prayers to the Gods. Ebers papyrus has recipes to treat many oral diseases.⁽¹¹⁾

In their treatment they did the following:

1. Utilization of drugs

Treatments consisted of natural substances that had therapeutic properties which were extracted from vegetable, fruit, mineral oils, seeds and onions in various combinations and applied to the aching tooth. Many of this ancient pharmacology are still in use, both in medicine and dentistry.⁽²⁷⁾

2. Mouth washes

Ancient Egyptians had various recipes for mouth washes; in different compositions. Some of the mouthwashes were for the specific purpose and for maintaining a healthy mouth and teeth.⁽¹¹⁾

3. Ancient Egyptian toothpaste

A formula included mint, salt, grains of pepper and the most active component was dried Iris flower. This was the world's oldest recipe for toothpaste. Effectiveness of Iris flower against gum disease had been recently discovered and used commercially now. This toothpaste was described until 1873, when Colgate released the first commercially prepared toothpaste.⁽²⁸⁾

4. Tooth whitening

Ancient Egyptians described a recipe "for white and perfect teeth". It was made of rocks and oyster shells, crushed as a tooth powder and used by rubbing directly on the teeth with a finger.⁽²⁸⁾

5. Tooth brushing

A form of a toothbrush was found within the pyramids of ancient Egyptians. It was crafted from a stick; its end was flayed so that the fibers of the wood were softer.⁽²⁹⁾

6. Management of carious teeth⁽³⁰⁾

This was performed by tooth fillings. Descriptions of how to make ancient fillings were recorded. Teeth in mummies were found with filling material which included:

- a) Filling material looks like a composite filling. It consisted of mixture of a powder (burnt ox hoof ash) added into other ingredient (Figure 10).
- b) Lenin was used for large painful cavity. Lenin was treated by substance to treat the pain.⁽³¹⁾



Fig. 10. Filling material in teeth of an ancient mummy

7. Surgical treatment

Ancient Egyptians practiced oral surgery from as early as 2,500 BC.⁽³²⁾

Evidences for practicing surgery:

a. Edwin Smith Surgical Papyrus which includes remedies for gums and swelling, treatment of loose teeth and dental abscess.

Holes drilled into the jaw bone in order to drain abscesses were found. The holes were the result of man-made drillings, in order to drain the pus from the apical abscess⁽¹⁶⁾ (Figure 11).

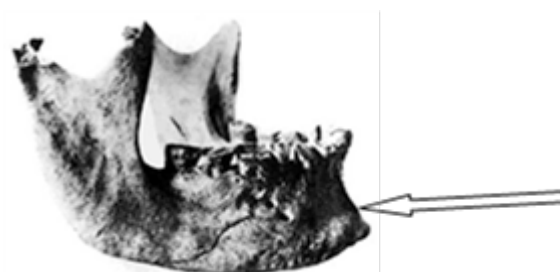


Fig.11. Holes drillings in a mandible to drain the pus

b. Dental instruments⁽³³⁾

A collection of nearly 40 surgical instruments found on the entrance of a temple of Kom Ombo beside the Nile. These surgical instruments include various medical and surgical instruments like bone saws, knives, scalpels, retractors and chisels (Figure12).

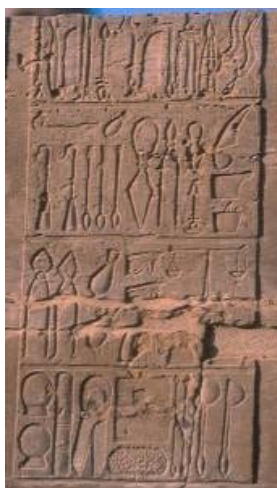


Fig. 12. Surgical instruments

8. Tooth extraction

Tooth extraction was rarely practiced, although some mummies showed sign of forced tooth removal. It is believed that dentists performed extraction for many problems of the mouth. This assumption is based on the fact that forceps are pictured numerous times in the wall carvings of the Ancient Egyptians. All of these procedures were performed without the use of modern day anesthetics.

Some drugs and hypnosis were used to cause the patient to sleep during a surgical procedure “anesthesia”. Opium was well known and was given against severe pain.⁽³⁴⁾

9. Dental restoration

Early tooth replacement took place in ancient Egypt. An attempt at replacing lost teeth was discovered by archeologists.

A dental bridge was found where lost teeth substituted with natural teeth which reattached by means of a gold wire to the surrounding teeth⁽³⁵⁾ (Figure 13).

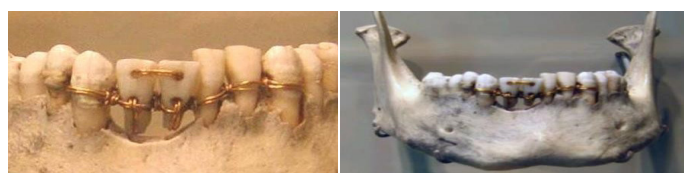


Fig. 13. A mandible of a mummy (2500 BC) with a gold wire bridge



Fig. 14. Maxillary incisors supported by golden band

In another bridge, replaced maxillary incisors was discovered and supported by golden band.⁽³⁶⁾ (Figure 14). “The maker of teeth” did this bridge. He was like dental technician in these days.⁽³⁷⁾

10. Implant⁽³⁸⁾

The idea of dental implants dated back to the ancient Egyptians. Ancient Egyptians hammered teeth-like pieces of shell, ivory, and animal bone directly into the gingiva to replace missing teeth (Figure 15).



Fig. 15. Early dental implants

Conclusion

- The dental skills of ancient Egyptians were far beyond their time with the writing of the first medical texts, performance of the first surgical techniques, use of the first splints and bandages, the first drug therapies, and the first medical dictionary.
- The ancient Egyptians made a path and framework for the advances in all fields of dentistry that exist in our world today.
- It is difficult for man to appreciate completely those people in terms of their arts, sciences, technology and their overall philosophy of life that enabled them to experience a culture that lasted for thousands of years.
- What one knows about them is just a little of what they had.
- More will be discovered through excavations, discoveries and use of technology.
- There should great appreciation to our ancestors, makers of civilization, hoping to follow their way.

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