Understanding Sjögren's syndrome as a systemic autoimmune disorder

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UNDERSTANDING SJÖGREN’S SYNDROME AS A SYSTEMIC AUTOIMMUNE DISORDER

By

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ABSTRACT

Sjögren’s syndrome is an autoimmune condition characterized by a dysfunction in the lachrymal and salivary glands which results in dry eyes and dry mouth. Since its first description in 1892, the disease is one of the most common autoimmune diseases after lupus erythematosus and rheumatoid arthritis in the United States. Despite its high prevalence in the general population, Sjögren’s syndrome remains hard to diagnose due to the wide range of symptoms associated with the disease that is also shared by other conditions. Furthermore, the mechanisms behind the pathogenesis are not properly understood even though multiple factors have been proposed to contribute to the disease. There is currently no approved cure or treatment for Sjögren’s syndrome, although the condition can be managed by treating the specific symptoms separately. This review will evaluate the genetic, epigenetic, hormonal, and environmental factors that are involved in the development of Sjögren’s syndrome. The different treatment options that are available or should be available to Sjögren’s syndrome patients will also be examined.
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Chapter 1: Sjögren’s Syndrome

Sjögren’s Syndrome and its Symptoms

Sjögren’s syndrome is a rare chronic autoimmune disease that occurs when the immune system attacks the gland that produces moisture in the eyes, mouth, and other areas of the body. The common symptoms are dry eyes and dry mouth. Dry eyes (keratoconjunctivitis sicca) can refer to a deficiency in the production of tears or their rapid evaporation. It can present itself with symptoms ranging from pain in the eyes to irritation and inflammation of the eye surface and lachrymal glands (Javadi and Feizi, 2011). Xerostomia or stomatitis sicca is a condition characterized by a reduced production of saliva and changes in the biochemical composition of saliva in patients (Altamimi, 2014). This condition can occur from time to time in healthy individuals, a chronic presentation of dry mouth is usually a sign of a serious medical condition. Sjögren’s syndrome is often accompanied by other immune disorders such as rheumatoid arthritis and systemic lupus erythematosus (NIH, 2021). Although the most common symptoms of the disease are dry eyes and mouth, other organs like the kidneys, gastrointestinal system, circulatory system, central nervous system, lungs, and liver can be affected (Columbia University Irving Medical Center, 2022). The disease is characterized by a triad of symptoms that include dryness of the mouth and eyes, fatigue, and joint pain. Focal mononuclear infiltration of the salivary and lachrymal glands leads to their gradual destruction and the process is often irreversible (Kuo et al., 2021).

Doctors usually divide Sjögren’s syndrome into two major categories based on their relationship with other autoimmune disorders (co-morbidities). Primary Sjögren’s syndrome is
usually not accompanied by other rheumatic diseases while secondary Sjögren’s syndrome is usually associated with another rheumatic disease such as rheumatoid arthritis, systemic lupus erythematosus, or polymyositis (Holdgate and St Clair, 2016).

**Morphology of the Salivary Glands**

Humans’ oral cavity contains three pairs of visible salivary glands: the parotid, submandibular and sublingual glands (Amano et al., 2012). The parotid gland is the largest of three macroscopic salivary glands and is formed of serous acinar tissue that secretes thin watery saliva through the Stensen’s duct. The parotid glands rely on sympathetic and parasympathetic innervation to stimulate and produce saliva (Chason and Downs, 2022). The submandibular gland constitutes the second largest of the three salivary glands and is formed of both serous and mucus acinar cells that secrete saliva with a thicker consistency through the Wharton duct. The mucus produced by the submandibular gland contains mucin, a substance that confers the saliva its antimicrobial properties while the serous substance produced by the acinar cells produces amylase that helps digest starch in the buccal cavity (Grewal et al., 2021). The submandibular gland is innervated by both the sympathetic and the parasympathetic nerve fibers that stimulate saliva production and inflammatory responses (Grewal et al., 2021). Although the sublingual salivary gland consists of both serous and mucous tissue, it is mainly composed of mucous acinar cells that produce thick mucus that further help in digestion and maintenance of the integrity of the oral cavity. The mucus produced by the sublingual gland is excreted through the Rivinus ducts and the glands are innervated by the parasympathetic nervous system which can increase saliva production (Grewal et al., 2022).

**Morphology of the Lachrymal Glands**
The lachrymal gland is an exocrine gland located in the eye orbit and consists of four distinct types of cells: acinar cell, duct cell, myoepithelial cell, and mesenchymal cell (He et al., 2022). The serous tissue of the lachrymal gland adds the serous component of the tear volume while the myoepithelial cells secrete IgA and IgG antibodies which confers the lachrymal gland with adaptive immune system properties (Machiele, et al., 2021). The myoepithelial cells surrounding the acinar cells and the ducts contract when neuronally stimulated to expulse the mucous and the serous component of the tears from the gland. The lachrymal gland is innervated by both the sympathetic and the parasympathetic nervous system (Machiele, et al., 2021).

**Incidence of Sjögren’s Syndrome**

The worldwide prevalence of primary Sjögren’s syndrome is roughly between 0.05 % and 4.8% and the female-to-male ratio is 9:1 (Kuo et al., 2021). A study looking at the incidence and the prevalence of adult primary Sjögren’s syndrome found that the incidence of primary Sjögren’s syndrome was highest among non-Latina Asian women and non-Latina white Women (Izmirly et al., 2019). However, in another study, it was demonstrated that primary Sjögren’s syndrome was diagnosed an average of seven years earlier in people identifying as Black or African American and the incidence was lower in this population (Brito-Zeron et al., 2016). Although primary Sjögren’s syndrome can develop in an individual at any age, most people are older than 40 at the time of diagnosis (Mayo Clinic, 2022).

**Diagnosis of Sjögren’s syndrome**

Primary and secondary Sjögren’s syndrome can be hard to diagnose in patients and the distinction between both types is important because it relates to the prognosis of the patient and
how they will be treated. The disease is diagnosed when symptoms are present with other systemic connective tissue diseases or autoimmune diseases (Sebastian et al., 2019). In the case of secondary Sjögren’s syndrome associated with rheumatoid arthritis, the healthy tissue of lachrymal glands, salivary glands, joints, and sometimes internal organs are attacked. Due to its rarity, Secondary Sjögren’s syndrome is often diagnosed based on the symptoms of dryness and connective tissue disease, which in turn can make the diagnosis subjective. Secondary Sjögren’s syndrome associated with rheumatoid arthritis is usually found in older, female patients who are seropositive for cyclic citrullinated peptide and the rheumatoid factor (Harrold et al., 2018). A positive result for cyclic citrullinated peptide antibodies (CCP) and the rheumatoid factor (RF+) are biological markers that indicate that a patient has rheumatoid arthritis (Atzeni et al., 2017).

Vasculitis refers to a group of disorders characterized by the destruction of the blood vessel walls due to inflammation. There are several types of vasculitis, and they can be classified based on the site and the type of blood vessels that are affected (Jatwani and Goyal, 2021). Vasculitis symptoms range from fever (between 38 and 39°C), weight loss, weakness, general malaise, arthralgia, and myalgia. These symptoms appear because of the inflammation of the blood vessels and the local tissue damage (skin rashes and lesions) (Snagolli and Lakshmi, 2019). Vasculitis seems to be a common symptom of Sjögren’s syndrome that can take many forms that do not include salivary and lachrymal gland inflammation (Scofield, 2011). In a study to analyze the several types of cutaneous vasculitis in 558 patients with primary Sjögren’s syndrome, it was found that 89 or 16% of them presented with cutaneous presentations. 52 of these 89 patients (58%) had cutaneous vasculitis (Ramos-Casals et al., 2004). The study also examined the clinical aspects of cutaneous vasculitis About half of the patients had a single episode of cutaneous vasculitis, with recurrent problems in the remaining half. The lower extremities were the most common site of the
rash associated with vasculitis. About 73% of the patients were treated with glucocorticoids. Some of them required higher doses of glucocorticoids or other immunosuppressant drugs. Nine of these patients received no treatment (Ramos-Casals et al., 2004). In conclusion, cutaneous vasculitis seems to be a common symptom in patients suffering from Sjögren’s syndrome.

A combination of blood tests, eye tests, imaging, and Biopsy can be used to successfully diagnose a patient with Sjögren’s syndrome. There is an established consensus on a set of criteria that can help medical professionals in their diagnosis. The patient must have at least one ocular symptom or sign, one oral symptom or sign, histopathology (abnormal lip biopsy), and autoantibodies. If a patient fits any 4 of the 6 listed criteria, they will get diagnosed with primary Sjögren’s syndrome. For a secondary Sjögren’s syndrome diagnosis, a patient must have been previously diagnosed with a major connective tissue disease and fit 2 of the 6 criteria (Vitali et al., 2002). The signs and symptoms vary between patients and the symptoms may be related to other conditions like age-related dry eyes and mouth and the side effects of certain medications like antidepressants (Kocer et al., 2015). Secondary Sjögren’s syndrome symptoms are closely related to those of rheumatoid arthritis and lupus (Dale and Popovitch, 2007). Aside from using the fact that secondary Sjögren’s syndrome is usually accompanied by another autoimmune disorder, Doctors started using positive results for the Schirmer’s test in addition to other anomalous findings on an eye exam. Schirmer’s test is used to determine whether the lachrymal glands are functioning properly. Strips of a non-filter paper are used and placed in both eyes for 5 minutes and are removed at the end of the test and the amount of wetting in the strips is measured. Deficient tears production will be indicated by a reading of less than 5mm (Johns Hopkins, 2022). To look at the functional integrity of the salivary glands, a sialogram may be performed. A contrast medium is injected into the salivary glands or ducts, the results are then viewed on an X-ray. Inflammation
or atrophy of the glands or ducts upon viewing the X-ray may reveal that the patient has an autoimmune disease or Sjögren’s syndrome (National Library of Medicine, 2021). Salivary Scintigraphy is another method that may be used to determine the functional activity of the salivary glands. A radioactive isotope is injected into the glands. The uptake of the substance by the salivary glands and its secretion with saliva is tracked over an hour. Rapid secretion of the substance can be used to determine whether the gland ducts function properly or not (Luk et al., 2017).

Antinuclear antibodies (ANA) are a class of antibodies that can be used to diagnose connective tissue immune disorders including Lupus and Sjögren’s syndrome (Nosal et al., 2022). Antibodies are produced by the body to fight invaders like bacteria and viruses. However, antinuclear antibodies attack the healthy cells of the affected individual (National Library of Medicine, 2020). A positive antinuclear antibody test may indicate that you have an autoimmune disease like Sjögren’s syndrome. However, the blood test alone is not sufficient to determine whether a patient has Sjögren’s syndrome and further analysis is often required. Labial Salivary gland biopsy is an important procedure in patients affected by Sjögren’s disease. An incision is made on the gland and the glandular tissue is collected and analyzed for the presence of inflammation and antinuclear antibodies (Giovelli et al., 2015). The sensitivity and accuracy of this diagnostic tool can rival the sialography and saliva flow rate measurement (Chisolm and Mason, 1968).

**Possible Causes of Sjögren’s Syndrome (Epigenetics, environment, Hormones, and Viruses)**

Sjögren’s syndrome is caused by an abnormal inflammatory response. However, identifying what exactly triggers this response might help find potential drug targets. There have been several genetic, environmental, hormonal, and viral factors that are thought to play a role in
the etiology of Sjögren’s syndrome (Reale et al., 2018). Because Sjögren’s syndrome is a multifactorial disease, there may be some biological, organic, and inorganic chemicals that might contribute to its pathogenesis (Bjork et al., 2020). It has been hypothesized that microbial infections, especially viruses might initiate inflammation in the salivary glands of Sjögren’s syndrome patients. This inflammation activates the production of IFN-a in glandular cells which in turn induces the adaptive immune response that we usually see in patients with Sjögren’s syndrome (Ambrosi and Wahren-Herlenius, 2015).

Viruses have been revealed as important drivers of evolution due to their ability to insert their genetic material into their hosts and their high mutation rates (Moelling and Broecker, 2019). Upon infection, viruses can elicit two different responses from the host: an innate response or an adaptive response. Due to their ability to trigger both subsystems of the immune system, viruses have long been thought to be involved in the development of autoimmune conditions (Fujinami, 2001). Many viruses like enteroviruses, rotavirus, Influenza A viruses, and viruses of the Herpesviridae family have been proposed as potential candidates for inducing inappropriate immune responses that can play a role in the development of autoimmune diseases (Smatti et al., 2019). The involvement of the Epstein Barr Virus has been studied in patients suffering from Sjögren’s syndrome. It was found that there is a high incidence of the reactivation of the virus in patients with Sjögren’s syndrome. A study looking at the role of the Epstein-Barr virus (EBV) in patients with Sjögren’s syndrome, found that seven out of twenty-one labial biopsies contained elevated levels of EBV Viruses. This suggests that the virus may be involved in the etiology of the disease (Yang et al, 1991). Viruses from the Picornaviridae family such as the hepatitis delta virus (HDV) also seem to play a role in the development of Sjögren’s syndrome. A study realized on patients with primary Sjögren’s syndrome has shown that 50% of the patients had elevated levels
of the hepatitis delta virus (Weller et al., 2016). The Human T-Lymphotropic Virus 1 or HTLV-1 is a virus that can cause multiple types of diseases such as Adult T cell Leukemia-Lymphoma, Tropical spastic Paraparesis, and HTLV-associated myelopathy (Ponce et al., 2019). This virus has also been studied as a possible factor in the etiology of Sjögren’s syndrome. A study looking into the involvement of HTLV 1 in patients suffering from Sjögren’s syndrome, found that the virus can directly infect epithelial cells of the salivary glands. It was also found that the infected epithelial cells can exacerbate the levels of inflammatory, migratory, and adhesive molecules (Nakamura et Kawakami, 2016). These findings demonstrate that viruses may play a role in the development of Sjögren’s syndrome.

Fluctuations in hormones can affect the body’s ability to properly regulate its metabolic functions. Hormones are thought to protect the body against diseases and variations of hormones between sexes have been the subject of multiple studies throughout the years. It is thought that the reason women are more likely to suffer from a heart attack is due to the protective effects that estrogen has against cardiovascular disease (Kent, 1979). This has been further demonstrated when it was discovered that women who have already gone through menopause are at a higher risk of suffering from cardiovascular disease than women who have not gone through it. These findings are particularly significant considering that estrogen levels are higher in premenopausal women than in menopausal women (Xiang et al., 2021). In the case of Sjögren’s syndrome, Scientists found that post-menopausal female patients suffering from Sjögren’s syndrome had elevated levels of prolactin in serum samples (Taiym et al., 2004). It is important to note that an increase in prolactin correlates with an increase in the immune response from mast cells, and macrophages due to the prolactin receptors expressed on the surface of these cells (Brandt et al., 2015). Another study found that some patients with Sjögren’s syndrome also suffer from thyroiditis and the gland
inflammation is also accompanied by a decrease in thyroid hormone production (Mavragani et al., 2012). This confirms that hormones play a key role in the modulation of the immune response seen in Sjögren’s syndrome.

A particular study realized with groups of Caucasian, Japanese, and Chinese patients found that there seems to be a genetic predisposition to Sjögren’s syndrome that can be attributed to the major histocompatibility complex (MHC) class II gene region, especially in HLA-DR and HLA-DQ alleles. The HLA- DQB1 gene belongs to a family of genes referred to as the human leukocyte antigen (HLA) complex ((MedlinePlus Genetics, 2020). These genes play a significant role in the immune system by helping it distinguish between self-proteins and peptides made by bacteria and viruses (MedlinePlus Genetics, 2020). HLA- DQB1 loci is associated with an increased risk of developing type 1 diabetes. While certain DQA1 and DQB1 alleles seem to reduce the incidence of the disease. A genetic study on Sjögren’s syndrome across various populations revealed the following: White patients had an increased frequency of the HLA-DRB1*0301-DRB3*0101-DQA1*0501-DQB1*0201 allele. Japanese patients had an increased frequency in HLA-DRB1*0405-DRB4*0101-DQA1*0301-DQB1*0401 and Chinese patients exhibited the risk haplotype of DRB1*0803-DQA1*0103-DQB1*0601. There also seems to be a shared amino acid motif among patients in the first domain of the DQB1 allele in each disease-associated haplotype (Kang et al., 1993). In conclusion, there seems to be a correlation between MHC class 2 antigen and Sjögren’s syndrome susceptibility.

Epigenetic modifications are inheritable alterations, usually reversible, that do not involve a change in the DNA sequence. These modifications can be methylations at specific DNA sites, Nucleosome alterations, and microRNA expression (Bordron et al., 2020). There is growing evidence that epigenetics might play a role in many autoimmune diseases including Sjögren’s
syndrome (Le Dantec et al., 2012). To test whether DNA methylation plays a role in Sjögren’s syndrome, Scientists tested global methylation in salivary glands epithelial cells of patients. They found that DNA methylation was greatly reduced and was accompanied by a decrease in the enzyme DNA methyl transferase (DNMT) and that there was an increase in the expression of Gadd45-alpha (a gene that promotes DNA demethylation) (Thabet et al., 2013). Another study established a link between hypermethylation of the promoter of the gene that encodes for BP230 (dystonin), and Sjögren’s syndrome (Gonzalez et al., 2011). A genome-wide association study performed on the minor salivary glands of patients suffering from primary Sjögren’s syndrome reported that 7820 methylate sites were differentially methylated. Of those 7820 sites, 5699 sites were hypomethylated and 2121 sites were hypermethylated (Cole et al., 2016). This demonstrates that there is a direct correlation between the proper methylation of genes and its involvement in the etiology of Sjögren’s syndrome.

Histone modifications are also thought to play a role in autoimmune diseases. It was demonstrated that there was a significant increase of Histone H3 acetylation and dimethylated H3 lysine 4 (H3K4me2) in patients with systemic lupus erythematosus which in turn raised CD70 mRNA levels in CD4⁺T cells (Zhou et al., 2011). In patients suffering from rheumatoid arthritis, it was found that the levels of the histone methyltransferase enhancer of zeste homolog 2 (EZH2) were elevated in synovial fibroblasts (Trenkmann et al., 2011). Synovial fibroblasts are responsible for the production of the extracellular matrix of the synovial fluid that lubricates the joints and reduces friction during movement (Ospelt, 2017). This proves that the chronic joint inflammation observed in rheumatoid arthritis can be associated with a change in the epigenetic profile of the cells responsible for the maintenance of the extracellular matrix. Knowing that secondary Sjögren’s syndrome can be associated with systemic lupus erythematosus (SLE) and rheumatoid
arthritis (RA), we can predict that the same results can be found in patients with secondary Sjögren’s syndrome.

MicroRNAs (miRNAs) are small RNA molecules produced in the nucleus that do not code for proteins but are involved in many important post-transcriptional modifications. Altered control of miRNAs can be associated with multiple inflammatory diseases including Sjögren’s syndrome (Konsta et al., 2014). Using polymerase chain reaction, scientists were able to show that microRNAs hsa-miR-768-3p and hsa-miR-574 have a protective role on salivary glandular cells in Sjögren’s syndrome patients with a reduced salivary flow. There was a clear upregulation in the expression of the microRNAs in these patients (Alevizos et al., 2011). In another study where miRNAs from the eye cells of patients suffering from primary Sjögren’s syndrome were analyzed, scientists identified a miRNA called miR-744-5p that was overly expressed. This miRNA decreased the activity of PELI3, a gene involved in the making of an anti-inflammatory protein called Pellino 3. The scientists were able to successfully increase PELI3 expression and reduce the formation of pro-inflammatory cytokines by using a miR-744-5p antagomir (Pilson et al., 2020). These results establish microRNAs as particular biochemical targets when it comes to treating Sjögren’s syndrome.
Figure 1. The proposed immunological mechanism behind the pathogenesis of Sjögren’s syndrome. A viral infection triggers the release of type I interferon (IFN1) and activates the innate immune system. The release of IFN leads to cell damage and the release of autoantigens. The autoantigens activate the B and T cells of the adaptive immune system that release antibodies into the targeted tissues. The autoreactive CD8⁺ T cells inflict further tissue damage to the salivary epithelium by releasing their cytotoxic granules. This augments the production of IFN1 by plasmacytoid dendritic cells (pDCs).

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The immune system and Sjögren’s Syndrome
The innate immune system is an evolutionary biological system that constitutes the first line of defense of the body against invaders and is composed of elements like TLRs (Toll-Like Receptors), Complement proteins that tag intruders for destruction, and finally phagocytic cells (Yattim and Lakkis, 2015). The presence of innate immune system cells such as macrophages and neutrophils are crucial to the mediation of inflammation and the ability of these cells to digest and present antigens to other immune system cells is key to the activation of the adaptive immune system (Kumar et al., 2018). Systemic Lupus Erythematosus (SLE) which is an autoimmune disease that affects tissues of the joints, skin, brain, lungs, blood vessels, and kidneys is shown to be caused by changes in the function and phenotype of neutrophils and macrophages (Mohan and Orme, 2012). Analysis of lesions in the minor salivary glands of patients with Sjögren’s syndrome had an increase in infiltration of macrophages and dendritic cells. There was an increased expression of IL-18 in macrophages in primary Sjögren’s syndrome patients (Manoussakis et al., 2007). Since secondary Sjögren’s syndrome is usually accompanied by a primary autoimmune disease like SLE, inflammation caused by innate immune system cells like macrophages and neutrophils could be associated with both diseases.

The innate response is modulated by a family of differentiation proteins called Toll-Like Receptors (TLR) (Neurath, 2008). Depending on the virulence of the pathogen, the lymphocytes of the adaptive immune system (B cells and T cells) may be required to fight specific pathogens (Alberts et al., 2002). The importance of the innate immune system in disease management and progression is further proven when it comes to activating the adaptive immune system. Macrophages and neutrophils while being participants in the first line of defense against pathogens, cannot always clear infection by themselves. However, they can initiate and direct primary adaptive immune system responses as well as clear out pathogens or infected cells targeted.
by members of the adaptive immune system (Janeway et al., 2001). The adaptive immune system is capable of targeting invaders directly by using white blood cells called lymphocytes (B cells and T cells) (Alberts et al., 2002). While taking slower to respond than the innate immune system, the adaptive immune system can retain “memory” that will be useful to fight the pathogen upon re-infection (IQWiG, 2006). The adaptive immune system relies on its capability to be able to differentiate between self-antigens and other pathogen-related antigens. Failure to differentiate those two different components can result in specific adaptive immune responses to the host self-antigens, causing autoimmune diseases (Alberts et al., 2002). Multiple studies have shown how the cells of the adaptive immune system can contribute to the progression of Sjögren’s syndrome. Analysis of tissues of patients affected by primary Sjögren’s syndrome shows that B cells are constantly activated (Riviere et al., 2020). Furthermore, CD4⁺ T cells are found to be present in great quantity in salivary glands during early disease progression (Fasano et al., 2020). A study of patients with secondary Sjögren’s syndrome associated with rheumatoid arthritis showed an increase of CD3⁺ and CD4⁺ T cells in salivary gland biopsies. IgG and IgA-producing B cells were also found to be present in the samples (Celengilil et al., 1990). Although most of the infiltrating lymphocytes are CD4⁺ T cells, CD8⁺ cytotoxic T cells also play a vital role in the pathology of Sjögren’s syndrome, especially in salivary hypofunction. It was found that there was an increase in cytotoxic T cells around the labial salivary gland of patients suffering from Sjögren’s syndrome. The presence of these CD8⁺ cells also correlated with apoptotic acinar and ductal cells (Kaneko et al., 2022). This shows that T cells are crucial to the autoimmunity of both primary and secondary Sjögren’s syndrome.
CHAPTER 2: FIBROSIS AND SJÖGREN’S SYNDROME

Mechanism of Fibrosis

Tissue damage can be caused by various events like inflammation, infections, toxins, and trauma. There are two crucial stages in tissue repair: regeneration and replacement. During regeneration, new cells replace part of the damaged tissue while in replacement the non-fixable parts of the damaged tissue are replaced by connective tissue which eventually leads to scarring (Krafts, 2010). Fibrosis is defined as the scarring and thickening of tissues because of excessive extracellular matrix production components like collagen (Wiley, 2008). During an injury, the damaged endothelial and epithelial cells release cytokines that attract immune system cells like macrophages, dendritic cells, and even mast cells to the site of infection (Shao et al., 2020). These immune system cells will release pro-fibrotic mediators such as TGF beta, platelet-derived growth factor (PDGF), and Interleukin-10 (IL10). These growth factors allow fibroblasts to proliferate and differentiate into myofibroblasts. These myofibroblasts in return will cause an excess in extracellular matrix production (Figure 2.)

The extracellular matrix is a three-dimensional tissue support network composed of several macromolecules such as collagen, fibronectin, elastin, proteoglycans, glycosaminoglycans, and other glycoproteins (Theocharis, 2016). Each of these components provides the extracellular matrix with specific properties that help it carry its functions across tissues. Collagens are proteins present throughout the animal kingdom and play a significant role in maintaining the structural strength and malleability of the extracellular matrix across various tissues (Hynes and Naba, 2012). Fibronectin itself plays a critical role in embryonic development and participates in cell migration,
differentiation, growth, and adhesion (Pankov and Yamada, 2002) while elastin provides elasticity to some connective tissues such as the blood vessels (Wang et al., 2015). Proteoglycans are proteins that are associated with multiple carbohydrates. These complexes are responsible for the maintenance of pressure, osmosis, and viscoelastic qualities of the extracellular matrix. Proteoglycans have also been found to play a significant role in tissue remodeling during cancer, diabetes, inflammation, and atherosclerosis (Lozzo and Schaefer, 2016). Glycosaminoglycans and glycoproteins provide elasticity to specific connective tissues such as the synovial fluid that acts as a lubricant for the joints (Mann et al., 2022).

Due to the role that each of the components of the extracellular matrix plays, disruptions in their biochemical properties have been associated with the development of many disorders ranging from minor to severe (Frantz et al., 2010). Fibrosis is a process that results from a prolonged injury state and an increase in inflammation that characterizes the healing process (Lee and Kalluri, 2010). Fibrosis is caused by an accumulation of Type 1 collagen or fibrillar collagen in the extracellular matrix (Wight and Potter-Perigo, 2011). Collagen 1 is a critical component of the extracellular matrix as it promotes the differentiation and migration of myoblasts by releasing cytokine IL-6 (Liu et al., 2020). Therefore, anything that affects the production of collagen 1 can compromise the integrity of the ECM including modifications. Lysyl oxidase-like 2 (LOXL2), an enzyme that engages in the crosslinking of collagens and elastin, has been found to increase the stability of collagen assembly during fibrosis (NCBI, 2022), (Karsdal et al., 2017). Hyaluronan which is a type of glycosaminoglycan is an important player in wound healing when it is degraded. During degradation, hyaluronan fragments induce the formation of pro-inflammatory cytokines that promote keratinocyte and fibroblast migration and production. Therefore, it has been found
that levels of hyaluronan and its degraded products are elevated in scleroderma fibrosis and lung fibrosis respectively (Ghatak et al., 2015).

**Figure 2. Schematic of the mechanism of Fibrosis.** Damage to epithelial and endothelial cells triggers an immune response and the recruitment of white blood cells such as neutrophils and monocytes. These cells release pro-inflammatory cytokines. Presence of pro-fibrotic factors such as TGF-Beta and IL-13 and oxidative stress initiate fibroblast proliferation and differentiation into myofibroblasts. Chronic inflammation and repair cause the myofibroblasts to produce collagen and fibronectin in excess. The gradual deposition of these extracellular matrix components eventually leads to scarring.

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**Diagnosis of Fibrosis**

Fibrotic tissue can be detected and diagnosed in patients using multiple methods. The methods used will vary depending on the progression of the disease and the level of detail that the provider wants to achieve. Some invasive methods consist of using needle base biopsies, trichrome staining, and immunohistochemistry to identify specific types of extracellular matrix. This is especially useful when it comes to the accurate detection and selection of the right therapeutic approach (Baues et al., 2017). Trichrome staining is a technique that allows the observation of the extracellular matrix and can therefore help detect various stages of fibrosis (Bedossa et al., 2018). Immunohistochemistry or real-time PCR can be used to detect specific ECM molecules including immune system cell infiltrates and pro-inflammatory cytokines (Meng et al., 2019). Non-invasive techniques involve the use of anatomical Magnetic Resonance Imaging (MRI) instead of Computed Tomography (CT) and radiography since the latter methods may only detect changes in the tissue at later stages of the disease (Baues et al., 2017).

**Fibrosis in different organs**

Fibrosis can occur in multiple tissues within the body which cause it to be involved in many systemic organ diseases. Idiopathic pulmonary fibrosis is one of the most common types of lung fibrosis that can be observed and diagnosed in patients. This type of fibrosis usually does not have any known causes and results in dysfunctional epithelium formation and its inability to regenerate after repeated injuries (Barratt et al., 2018). Most patients diagnosed with idiopathic pulmonary fibrosis have a survival time of 2-3 years from the time of diagnosis. Although, recent data gathered
from clinical trials have shown that some patients can live longer than the allotted 2-3 years (Raghu, 2011). There is no cure for idiopathic pulmonary fibrosis. However, the right treatment can relieve the symptoms and halt the progression of the disease (NHS, 2019). Currently, two FDA-approved drugs can treat idiopathic pulmonary fibrosis: Nintedanib and Pirfenidone (American Lung Association, 2020). Nintedanib has antifibrotic properties and inhibits fibroblast proliferation by binding to fibroblast growth factor receptor (FGFR) (PubChem, 2019). Pirfenidone, on the other hand, can treat idiopathic pulmonary fibrosis by inhibiting collagen fiber formation (PubChem, 2005).

During Chronic kidney disease, for example, the final stage usually results in fibrosis and currently, there are no approved therapies to manage this disease besides dialysis and a kidney transplant (Cho et al., 2010). The fibrotic response observed during chronic kidney disease is usually the result of traumatic injuries, infection, metabolic disorders like diabetes, autoimmunity, and inflammation (Panizo et al., 2021). Although there are no specific medications that can treat chronic kidney disease (CKD), some medicine may be required to treat the symptoms or conditions that may arise from battling CKD. High blood pressure, for example, can constrict and damage the blood vessels of the kidney which can prevent it from filtering waste and fluids from the body. The extra fluid in the blood can put more pressure on the blood vessels of the kidney and worsen CKD (NIDDK, 2020). To halt the progression of kidney disease, lifestyle changes and combinations of high blood pressure medications such as Angiotensin-converting enzyme (ACE) inhibitors and angiotensin II receptor blockers (ARBs) that can keep the blood vessels from contracting, Calcium blockers that will allow the blood vessels to relax, diuretics to remove extra fluid from the blood, and finally beta blockers that will allow the blood to be pumped with less force in the blood vessels (NLM, 2021). Diabetes is the most common cause of CKD in the United
States and therefore treatments focusing on controlling glucose levels in patients suffering from diabetes can help manage and slow down the progression of CKD (Pyram et al., 2011). Dapagliflozin also known under the common name Farxiga, can help manage diabetes type 2 in adults and treat CKD by preventing glycosuria caused by glucose resorption in the nephron (PubChem, 2022).

Liver fibrosis is a chronic disease caused by many factors such as hepatitis viruses B and C, alcohol consumption, or non-fatty liver disease. If not managed, this can cause liver failure, liver cancer, or even death (Aydin and Akcali, 2018). Fibrosis of the liver can occur in stages and one of the ways of assessing the progression of the disease is to assign a score after taking a biopsy of the liver (Suk and Kim, 2015). One of these scoring systems is the METAVIR (Meta-analysis of Histological Data in Viral Hepatitis) which assigns fibrotic stages with scores ranging from 0 to 4. with zero corresponding to no observable fibrosis and 4 corresponding to definite or probable fibrosis (Krishna, 2021). Some drugs can treat hepatitis B and C and can reverse fibrosis caused by viral hepatitis. However, there is no specific drug regimen that can specifically target liver fibrosis (Berumen et al., 2021). New antifibrotic therapies that specifically target the deposition of fibrotic tissue and the accumulation of extracellular matrix proteins are currently being developed (Bataller and Brenner, 2005).

Although fibrosis of both the salivary glands and the lachrymal glands are observed in Sjögren’s syndrome, reports are scarce in the literature and more information is needed on the subject. Salivary gland fibrosis is one of the main highlights of Sjögren’s syndrome and is associated with progressive loss of gland function (Yin et al., 2021). Fibrosis of the salivary glands can occur at any age; however, it has been reported that labial salivary gland fibrosis is prevalent in Sjögren’s syndrome regardless of the age of the subjects (Leehan et al., 2018). The fibrotic
response may be accompanied by chronic inflammation and necrosis around the salivary glands (NTP, 2014). In patients suffering from primary Sjögren’s syndrome, fibrosis seems to be prevalent in the minor salivary glands (Leehan et al., 2017). Although dry eyes can be caused by many conditions such as Graft versus Host disease after bone marrow transplants or aging, it is also a common symptom of Sjögren’s syndrome (Zoukhri, 2006) (Akpek et al., 2011). Fibrosis of the lachrymal glands is also correlated with aging as analysis of lachrymal glands from patients over the age of fifty showed fibrosis around the ducts and other ductal abnormalities (Conrady et al., 2016). It has also been suggested that proinflammatory cytokines are responsible for the decrease in lachrymal gland function which in turn may result in low production of tears (Hayashi, 2011). This show that fibrosis is a process that can cause major damage to the organs and can even lead to death in certain cases.
CHAPTER 3: THE NON-OBESE DIABETIC MOUSE: AN ANIMAL MODEL FOR THE STUDY OF SJÖGREN’S SYNDROME

Mice as Model Organisms

Animals have evolved alongside humans for thousands of years and they share many commonalities such as their ability to think, communicate, eat, perform metabolic functions, and develop diseases. Understanding the basis behind most of these activities is key to curing diseases and countering biological challenges that affect humans. Therefore, animals have been used and are continued to be used in the field of biomedical research. Animals have been used since antiquity by physicians and scholars such as the Greeks Aristotle circa 384 – 322 BC, Erasistratus circa 304 – 258 BC, and Galen circa 129 – 199 / 217 or even the 12th-century Arab physician, Ibn Zuhr who practiced surgical procedures on animals before replicating them on humans (Hajar, 2011). Currently, the most used animals in biomedical research are purpose-bred rats and transgenic mice making them critical to the understanding and advancements made toward human health (NABR, 2020). Mice make great model organisms for human diseases because both mice and humans are genetically alike. Indeed, it has been reported that mice share more than 90% of their genome with humans (Mouse Genome Sequencing Consortium, 2002). Mice are easy to maintain, they do not take up a lot of space, have a short generation period, reproduce quickly, and can give birth to many offspring in the same litter (The Jackson Laboratory, 2022). This makes them one of the most useful model organisms in the lab.

Transgenic mice are mice whose DNA has been modified by inserting a chosen DNA from a foreign source into the nucleus of an egg from a female mouse (NRC, 1994). Transgenic mice
are specifically important because they allow scientists to study the mechanisms behind gene functions and their involvement in genetic diseases. The ability to insert or remove genetic material from these mice allow scientist to understand the phenotypic importance of gene dosage in multiple human diseases (Hickman Davis and Davis, 2006). It exists multiple transgenic mice that mimic the phenotypic and genotypic expression of various diseases. In the case of cancer by example, oncomice (mice expressing dominant oncogenes) have been used to study the involvement of oncogenes in the development of human cancers. Researchers used mice with mutations in the Myc and Ras oncogenes to evaluate the role of these oncogenes in developing breast cancer. They also studied the role of the Fos as an oncogene in transgenic mice and its involvement in bone cancer (Hanahan et al., 2007). A study that looked at the presence of the HLA-B27 gene, a gene that is associated with multiple inflammatory conditions such as spondylitis, inflammatory bowel disease (IBD), and psoriatic arthritis, found that this gene can change the microbiota found in the caeca of transgenic rats even when inflammation was not present (Lin et al., 2014). Considering that a change in the gut microbiota has been observed in patients suffering from IBD, this study can help get a better understanding of this gene and its implication in IBD. In conclusion, we can appreciate the importance of transgenic mice in studying, diagnosing, and eventually treating human illnesses.

The Non-Obese Mouse and Sjögren’s syndrome

The non-obese diabetic (NOD) mouse was first described in 1980 when the offspring of a female mouse with polyuria and glucosuria we selectively bred to give rise to a diabetic mouse strain (Makino et al., 1980). Type 1 diabetes also called type 1 diabetes mellitus (T1DM) is a chronic autoimmune disease that arises when T cells attack the insulin-producing Beta cells of the
pancreas (Katsarou, 2017). The NOD mouse has been used as a model to study type 1 Diabetes and has helped identify genes, immunological infiltrates involved in the development of the disease, and possible therapeutics that can help treat the disease (Pearson et al., 2017). Aside from diabetes, the NOD mouse does not have obesity but it is open to a wide range of autoimmune conditions. This can help researchers experimentally induce certain autoimmune issues by controlling certain genes. These genetic mutations have created NOD mouse models that developed disorders such as autoimmune thyroiditis, systemic lupus erythematosus (SLE) like disease, autoimmune cardiomyopathy, colitis wasting disease, and encephalomyelitis (Aoki et al., 2005).

The NOD mouse has also led to some incredible discoveries in the study of Sjögren’s syndrome. While examining the function of exocrine glands in the NOD.B10.H2b mouse strain, researchers found immune system cell infiltrates that are characteristic of Sjögren’s syndrome in the NOD mouse. However, this mouse strain did not develop insulitis and diabetes and became a new model for the study of Sjögren’s syndrome (Robinson, 1998). It has also been shown that interleukin 4 (IL-4) (a cytokine that promotes T cells differentiation and IgE isotype switching in B cells) participates in the development of Sjögren’s syndrome in the NOD mouse model (Gao et al., 2006). Another mouse that has been used for the study of Sjögren’s syndrome is the C57BL/6.NOD-Aec1Aec2 mouse. This mouse was made by breeding the C57BL/6.NOD-c3 mice carrying the insulin-dependent diabetes locus Idd3 (Aec1, autoimmune exocrinopathy 1) with the C57BL/6.NODc1t mouse carrying the Idd5 (Aec2) locus (Lee et al., 2012). Nineteen insulin-dependent diabetes (Idd) loci are thought to be implicated in the development of diabetes in the NOD mouse. However, the Idd3 and Idd5 loci are necessary for the apparition of sialadenitis, and the loss of secretory function seen in the disease (Lee et al., 2009). The
C57BL/6.NOD-Aec1Aec2 mouse exhibits the same Sjögren’s syndrome symptoms as the NOD mouse with minor differences. Nonetheless, the female C57BL/6.NOD-Aec1Aec2 did not show any apparent autoimmune anomalies in the lachrymal glands (Nguyen et al., 2006).

The Ductal Ligation and Resection Surgery Models of Salivary Glands in Mice

Injury models have proven themselves to be useful when it comes to studying the mechanisms that underlie many diseases. Disease models have been recreated in animals using surgical and technical methods. From these models, therapeutics can eventually be evaluated and tailored to specific diseases. Acute sialadenitis is the inflammation of the salivary glands due to bacterial infection (Wilson et al., 2013). If not treated, the inflammation can lead to complications such as the emergence of stones called sialoliths in the glandular ducts (Kao et al., 2019) and gland atrophy (Scott et al., 1999). To understand the etiology and the mechanisms behind the formation of sialoliths in patients suffering from obstructive sialadenitis, scientists designed a ligated model in rats. The parotid duct of the rat was ligated, and a micro clamp was used to obstruct it. This resulted in a significant decrease in the composition of acinar cells and a decrease in the total gland weight. However, after the removal of the clamp, there was a definite increase in the acinar cells which eventually lead to the complete regeneration of the gland (Cummins et al., 1994). Another duct ligation study realized on mice showed that obstructing the salivary flow from submandibular glands can induce autophagy which is important for maintaining glandular tissue survival. It was also found that after a long ligation period, there was an observation of acinar cell death (Lin et al., 2014). In conclusion, both studies were able to mimic the type of injury and inflammation
processes seen in human patients suffering from acute sialadenitis and open the door to possible treatments.

Resection is the surgical removal of a body part or organ as a treatment for many diseases. It has been used as the best choice for improving the quality of life of patients suffering from benign and malignant ailments alike (Marsh and Buicko, 2022). Patients suffering from recurrent salivary gland obstruction due to sialadenitis sometimes must get a total excision of the gland as a more permanent solution to the problem (Saunders et al., 1986). Total or partial resection has also been used as a treatment for benign submandibular gland tumors. It has also been proven that patients who went under a partial resection of their submandibular gland had more resting saliva flow than patients who underwent total resection a year after the surgical operation (Ge et al., 2016). To better understand the mechanism behind submandibular gland regeneration, scientists investigated the behavior of the female mice's submandibular gland after the ablation of 40% of the distal tip of the gland. It was found that there was a large proliferation of epithelial and stromal cells in localized regions of the gland. Furthermore, the secretory cells of the salivary gland maintained their integrity after the partial gland resection (O’keefe et al., 2020). Taken together, these results indicate that partial salivary gland resection is a more effective treatment for some salivary gland disorders as it sustains the functional properties of the gland.
CHAPTER 4: THE MOUTH MICROBIOME AND
SJÖGREN’S SYNDROME

The mouth contains the second largest and most diverse microbiota after the gut and houses multiple species of microorganisms such as bacteria, fungi, viruses, and protozoa (Deo and Deshmukh, 2019). Analysis from five clinically healthy individuals showed that the most common bacteria belonged to the genera of *Gemella*, *Granulicatella*, *Streptococcus*, and *Veillonella* (Aas et al., 2005). The streptococcus genus remains the most abundant genus among the characterized bacteria species from the oral flora (Burton et al., 2011). Various parts of the mouth such as the teeth, gingival sulcus, attached gingiva, tongue, cheek, lip, and the hard and soft palate harbor specific species of bacteria (Kilian et al., 2016). These species of bacteria can form diverse and complex communities called biofilms that can grow on surfaces (Nadell et al., 2008). Through this complex organization, bacteria have been found to communicate with each other and regulate physiological processes using a mechanism called quorum sensing (Li and Tian, 2012).

Dental Plaque

Dental plaque is a layer of microorganisms that forms on tooth surfaces. It is comprised of microbes and secreted macromolecules from bacteria and saliva alike (Marsh and Bradshaw, 1995). Roughly 80% to 90% of the plaque is made of water while 70% of its dry weight is bacteria. The remainder contains a matrix made of polysaccharides, saliva proteins, and glycoproteins (Marsh and Bradshaw, 1995). The microbiome is important for the maintenance of the integrity of the mouth. If that integrity is compromised, it can lead to inflammatory diseases like gingivitis and
periodontitis. These diseases are usually caused by the bacteria that have accumulated in the gingival sulcus (space between the surrounding gums and the teeth) (Avila et al., 2009). The microbiome that usually lines the gingival sulcus is gram-positive and facultative anaerobes bacteria like *Streptococcus anginosus* and *Actinomyces naeslundii*. However, if there is a lack of oral hygiene, the dental plaque will contain more species of the gram-negative kind such as spirochetes, *Fusobacterium nucleatum*, and *Bacteroides* species. The latter species are present during gingivitis while periodontitis in its more severe stages is linked to species such as *Porphyromonas gingivalis*, *Bacteroides forsythus*, and *Treponema denticola* (Ruby and Barbeau, 2002). This proves that specific species of bacteria are associated with different areas of the mouth and that changes associated with bacterial species can lead to inflammatory disease in the oral cavity.

**Dry mouth and its effects on the oral cavity**

Saliva is a liquid produced by salivary glands that is important for the health of the oral cavity. It is composed of 99.5% of water, 0.2% of inorganic salts and enzymes, and 0.3% in proteins (Roblegg et al., 2019). One of the most important enzymes present in saliva, alpha-amylase, plays a role in the digestion of polysaccharides and may be involved in the formation of cavities by contributing to the production of glucose used by bacteria inhabiting teeth surfaces (Scannapiecco et al., 1993). Kallikrein, an enzyme that is found in small quantities in saliva can impact leucocyte migration. It also can break down kininogens into bradykinin-like peptides which can play a significant role in regulating blood pressure, inflammation, and vasodilation (Modeer, 1977) (Kashuba et al., 2013). Lingual lipase is an enzyme produced by the von Ebner glands of the tongue and engages in the digestion of triglycerides in the stomach (Hamosh, 1990).
Lysozymes are enzymes present in tears, mucus, sweat, breastmilk, and saliva that have antimicrobial, antiviral, and antifungal properties (Ferraboschi et al., 2021). As an antibacterial agent, it can destroy bacterial the peptidoglycan that makes up the bacteria’s cell walls. It can also work with other polypeptides with similar antimicrobial properties to get rid of bacteria (Ganz, 2006). However, certain diseases can cause the saliva’s composition to vary. In the case of Sjögren’s syndrome, it was revealed that the protein composition of the patient’s saliva was different from healthy people. Many of the patients suffering from the disease showed elevated amounts of certain proteins like lactoferrin, beta (2)-microglobulin, sodium, lysozyme C, and cystatin C. While these same patients had reduced production of amylase and carbonic anhydrase (Mathews et al., 2008). All this shows that any disruption in the chemical and physical properties of the saliva can have detrimental effects on the health of the oral cavity.

One of these effects is dry mouth or xerostomia. Dry mouth is particularly common among people of old age. One of the possible explanations is that older people must take more medications on average than any other age group. Some of these medications (antidepressants, antihistamines, and diuretics) can reduce the saliva flow rate and therefore cause dry mouth as a side effect (Thomson, 2015). Xerostomia can also be caused by diseases. A study found that 60.8% of patients suffering from Parkinson’s also had a dry mouth as a symptom. However, amongst these patients, only 12% of them reported this symptom to their physician. This shows that this symptom may be a neglected symptom that may be an initial sign of Parkinson’s disease (Cersosimo, 2011). Sjögren’s syndrome is another disease that has been associated with dry mouth as a symptom. Dry mouth results from the progressive inflammation and fibrosis of the salivary glands by immune system cell infiltrates (Fox, 2005). Viruses like the cytomegalovirus, HIV, and mumps can also cause dry mouth (Fathi et al., 2021). Radiation therapy for head and neck cancer can cause damage
to the salivary glands and lead to salivary gland hypofunction. It was found that the inflicted
damage to the gland can be due to the type of treatment used or the amount of radiation received
by the glands (Pinna et al., 2015). This proves that dry mouth can be a result of multiple factors
such as side effects of medication, neurodegenerative diseases like Parkinson, autoimmune
diseases like Sjögren’s syndrome, and viral infections.

Xerostomia can cause terrible damage to the oral cavity including cavities, mouth
infections and pain, difficulty eating and swallowing, and an overall change in the oral microflora
(Arany et al., 2021). A study that looked at the composition of the microflora of patients who had
saliva hypofunction found that a lot of them had elevated levels of lactobacillus and candida
species. A study that looked at patients suffering from Sjögren’s syndrome found that there was
an increased level of Veillonella bacterial species in the disease group when compared to the
healthy controls. It was also discovered that Sjögren’s syndrome patients lacked bacteria belonging
to the genus Neisseria and Porphyromonas (Rusthen et al., 2019). This study is particularly
important since it has been proposed that Veillonella may allow opportunistic bacterial species,
part of the dental plaque, the ability to thrive and induce cavities and periodontal inflammation
(Zhou et al., 2021). We can use this information to confirm that any change to the oral microbiome
can cause detrimental issues to the mouth, especially in the absence of proper salivation.
CHAPTER 5: DRUG THERAPIES FOR SJÖGREN’S SYNDROME

Sjögren’s syndrome is a long-term condition that does not have a targeted treatment, but it can be managed with the use of immunosuppressants, eye drops, medication, and even surgery. Whether it is primary or secondary Sjögren’s syndrome, treatment varies from individual to individual and is usually provided by an interdisciplinary team consisting of a primary care doctor, rheumatologists, ophthalmologists, ETN specialists, and dentists (Stefanski et al., 2017). Keratoconjunctivitis sicca (KCS) is one of the main symptoms of both primary and secondary Sjögren’s syndrome and is characterized by a reduced production of the aqueous layer of the tear film (Roberts, 1991). Artificial tears or lubricating eyedrops are prescribed as a standard treatment for dry eyes since they can improve the quality of life of the patient (Messmer, 2015). However, long-term use of artificial tears only provides temporary relief and does not treat the underlying problem, especially if the condition is chronic.

Approved Treatments for Dry Eyes

Cyclosporine A which is an immunosuppressant drug that has been approved by the FDA for two decades can be used to suppress the immune system of patients after an organ transplant but has been shown to increase tears production in patients presenting with dry eyes symptoms (PubChem, 2022). Cyclosporin also decreased inflammation in dry eyes by protecting the conjunctival epithelial cells from apoptosis and by promoting the apoptosis of infiltrating T-cells in return (Gao et al., 2013). Despite the benefits provided as an immunosuppressant, cyclosporine
A can cause some negative side effects on organs like nephrotoxicity, hepatotoxicity, gingival hypertrophy, tremors, and an increase in blood pressure (Rezzani, 2004). It has also been revealed that the use of Cyclosporine A can emulsion can cause ocular burning in certain patients (Lei et al, 2011).

Corticosteroids often just called steroids are a class of natural hormones produced by the adrenal glands and include various synthetic analogs. These drugs can be used as a treatment for many conditions, especially to treat inflammation and suppress the immune system (Annane et al., 2019). It has been shown that a combined application of artificial tears and corticosteroid drops in patients with moderate to severe dry eyes, significantly reduced the symptoms and inflammation in conjunctival epithelial cells. However, these drugs had no remarkable effect on the Schirmer’s test of said patients (Avunduk et al., 2003). Ophthalmic Corticosteroids can be exceptionally potent when it comes to treating eye inflammation. However, it is not without its risks as it can cause ptosis, limitation of ocular movements, orbital fat atrophy, and epithelial toxicity. (Mc Ghee et al., 2002).

Hydroxychloroquine is a derivative of the antimalarial and anti-inflammatory drug chloroquine (PubChem, 2022). Hydroxychloroquine reduces inflammation by blocking key inflammatory pathways and Toll-Like Receptor activation of the innate immune system (Danza et al., 2016). This drug has been used to treat multiple autoimmune diseases, including systemic lupus erythematosus (SLE). Administration of the drug to patients suffering from SLE showed a reduction in the risks of flare, a reduction in the organ damage associated with the disease, and even a reduction in the dosage of steroids required to control the disease (Ponticelli and Moroni, 2017). Hydroxychloroquine is sometimes prescribed to patients with Sjögren’s syndrome to improve their symptoms. However, the efficacy of such treatment is sometimes disputed. One
study has shown an increase in the secretion of the salivary and lachrymal glands, a reduction in pain, and an improvement in systemic inflammatory markers such as SST and C-reactive protein (Danza et al., 2016). Another study showed that Hydroxychloroquine showed no significant difference from the placebo administered to patients with primary Sjögren’s syndrome presenting with dry eyes and dry mouth (Wang et al., 2017). However, it was found that long-term use of hydroxychloroquine may decrease the risk of coronary artery disease in patients with Sjögren’s syndrome (Yang et al., 2020). More studies are needed to examine the effects of hydroxychloroquine on the lachrymal and salivary glands, especially in patients with secondary Sjögren syndrome associated with SLE.

**Approved Treatments for Dry Mouth**

Cevimeline is an FDA-approved derivative of acetylcholine that is used to treat dry mouth in patients with Sjögren’s syndrome or patients dealing with post-radiation treatment associated with head and neck cancer. It is a muscarinic agonist given in patients who do not respond to artificial saliva or mechanical stimulation of the salivary glands (Pakala et al., 2021). The recommended administered dose is usually 30 mg three times a day and long-term use of the drug has been shown to increase saliva production and improve dry eye symptoms (Weber and Keating, 2008). The medication is available under the brand name Exovac and is associated with mild side effects ranging from rhinitis, sweating, and nausea to headaches, dizziness, diarrhea, and fatigue. However, no liver injury was recorded among users during the drug trials (NIDDK, 2012).

Pilocarpine is a drug that can be used to treat dry mouth in patients suffering from Sjögren’s syndrome (PubChem, 2022). This drug induces flow from exocrine glands and may improve salivary flow from the salivary glands. Patients who were administered pilocarpine tablets of 20
mg or more per day showed amelioration of their dry mouth symptoms (Vivino, 2001). Another study that was trying to elucidate and compare the benefits of pilocarpine hydrochloride and artificial saliva as a treatment for dry eyes and dry mouth, found that pilocarpine was more successful at treating dry mouth than artificial saliva. Pilocarpine also improved dry eye symptoms by increasing lacrimal flow (Cifuentes et al., 2018). Pilocarpine is associated with many side effects such as hypersalivation, vomiting, slow heart rate, bronchospasm, urination, diarrhea, and sweating (Panarese and Moshirfar, 2021).

**Treatment for Pain in Sjögren’s syndrome**

Chronic pain is one of the main symptoms of primary Sjögren’s syndrome (Segal et al., 2014). Non-steroidal anti-inflammatory drugs (NSAIDs) such as ibuprofen or naproxen may be taken to manage pain caused by the disease (NYU Langone Health, 2022). It has also been demonstrated that NSAIDs in form of eye drops can treat sicca symptoms in patients with Sjögren’s syndrome. Diclofenac, an NSAID has been found to improve corneal sensitivity caused by dry eyes (Aragona et al., 2004). Despite their reputation as notable painkillers, NSAIDS can cause multiple side effects for instance: gastrointestinal issues (stomach irritation, bleeding, ulcers), cardiovascular accidents (stroke, heart attack), high blood pressure, liver injuries, and renal problems (Bindu et al., 2020) (Bjarnason et al., 1993).

Since Sjögren’s syndrome is a systemic disease, many of the symptoms must be managed separately and some of them require a specific drug regimen. If not treated Sjögren’s syndrome can lead to serious complications in the future and even death. Therefore, more research is needed on the subject.
CHAPTER 6: FUTURE TREATMENTS AND THERAPIES FOR SJÖGREN’S SYNDROME

Most of the current drugs and treatments available to patients suffering from Sjögren’s syndrome treat separate symptoms and not the disease. Here in this chapter, we want to evaluate treatment routes that could be explored, current drugs that are being investigated in clinical trials, and other treatment options that are successful in animal models.

Biomarkers and their potential role in the diagnosis of Sjögren’s syndrome

Biomarkers are biological or chemical substances that can be objectively measured or quantified in a cell or organism (Califf, 2018). Biomarkers play a key role in the diagnostic process and treatment of diseases. Specific biomarkers can help identify various stages of a particular disease. In autoimmune diseases such as rheumatoid arthritis, several biomarkers such as the erythrocyte sedimentation rate (ESR) and the levels of C-reactive protein (CRP) can indicate the degree of disease activity (Atzeni et al., 2017). In the case of Sjögren’s syndrome, interferon type 1 (IFN-1) is thought to be involved with the pathophysiology of Sjögren’s syndrome since higher levels of IFN-1 correlate with the presence of anti-Ro/SSA or anti-La/SSB autoantibodies (Chen et al., 2015). However, since multiple factors are thought to contribute to the pathogenesis of Sjögren’s syndrome, several biomarkers should be considered since there is a clear alteration in their levels in patients suffering from the disease. Such biomarkers can be long non-coding RNAs (lncRNAs), microRNAs (miRNAs), and proteins present in certain biological fluids. A study found
that two species of lncRNAs, LOC100652951 and LOC100506036 were significantly upregulated in the T cells of patients suffering from rheumatoid arthritis (Lu et al., 2016). Micro RNAs could also be used as potential biomarkers for Sjögren’s syndrome. A study looked at the MicroRNA expression in healthy controls, patients with systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and Sjögren’s syndrome and found that patients suffering from Sjögren’s syndrome had the highest levels of miRNA upregulation in all four groups. One of these micro RNAs, miR-34b-3p, was significantly upregulated in Sjögren’s syndrome when compared to the control or RA group (Williams et al., 2016). This shows that these RNA species could be used as biomarkers in the diagnosis and treatment of autoimmune diseases like Sjögren’s syndrome. 

Proteomics is the study of the structure and activity of proteins in organisms or cells (McArdle and Menikou, 2021). In the study of proteomics, the most used tool is Mass spectrometry with Liquid Chromatography with Tandem Mass Spectroscopy (LC-MS-MS) and Matrix-associated laser desorption/ionization-time of flight (MALDI-TOF/TOF) (Aslam et al., 2017). Considering that protein structure, composition and expression can be altered in certain diseases, we can use protein analysis to diagnose or even treat diseases. A study investigating the salivary biomarker profiles of patients suffering from primary Sjögren’s syndrome, rheumatoid arthritis, and healthy controls found that 61 and 55 proteins were significantly changed in patients with Sjögren’s syndrome when compared to patients without the disease (Delaleu, 2015). Raman spectroscopy is a powerful tool that can be used to perform proteomics analyses due to its ability to provide information on the biomolecular structures in cells and tissues (Zhang et al., 2011). A Raman spectrum contains multiple peaks that represent the vibrational modes that form during the collision of a molecule and a photon (O’Brien et al., 2014). It has been proposed that Raman spectroscopy can be used to diagnose oral diseases because it can detect metabolic changes in the
bacteria that comprised the biofilm (Zhang et al., 2022). Using Raman spectroscopy, scientists looked at the minor salivary gland of patients with primary Sjögren’s syndrome. They found that there were biomolecular differences between controls and disease samples. There were increased levels of proteins, nucleic acids, and keratin in the disease samples while the lipid levels were decreased (Xue et al., 2014). However, Raman spectroscopy has several limitations when it comes to protein analysis such as long measurement times, low sensitivity, slow imaging by point scanning, and advanced data analysis is sometimes necessary (Eberhardt et al., 2015).

**Differential Expression Analysis of long non-coding RNAs in Patients with Sjögren’s syndrome**

Long non-coding RNAs (lncRNA) are RNA molecules that are more than 200 nucleotides long and do not encode for any proteins (Chen et al., 2021). Since their discovery, long noncoding RNAs are implicated in the pathogenesis of several autoimmune disorders such as rheumatoid arthritis, systemic lupus erythematosus, multiple sclerosis, and type 1 diabetes (Lodde et al., 2020). One study wanted to investigate differentially expressed mRNAs and lncRNAs in the peripheral blood mononuclear cells in 4 patients with Sjögren’s syndrome and 4 healthy controls. The study revealed that there were 497 upregulated lncRNAs and 256 upregulated mRNAs while the downregulated genes included 683 lncRNAs and 384 mRNAs (Chen et al., 2021). Differential gene analysis showed that two lncRNA species: GABPB1-AS1 and PSMA3-AS1 were significantly upregulated. GABPB1-AS1 also corresponded with the percentage of B cells and the levels of IgG (Chen et al., 2021). We decided to analyze the data used in this study and retrieved the FASTQ from the Gene Expression Omnibus (GEO) database and used R studio and the DESQ2 program to perform a gene expression analysis of the data. From the data, we generated 2 plots a heatmap
and a normalized count plot where the most statistically significant gene-based was picked based on the adjusted p-value. The heatmap included the first 15 most differentially expressed genes and showed that many of the genes were either upregulated or downregulated in the disease samples when compared to the controls. However, our normalized counts plot revealed that there was a pseudogene that was significantly downregulated in patients with Sjögren’s syndrome when compared to controls. Using the ENSEMBL IDs that are assigned to both mRNA and IncRNA genes, we were able to determine that the RP4-765C7.2 pseudogene was the gene that was significantly downregulated in patients with Sjögren’s syndrome. This pseudogene encodes for a long noncoding RNA and had a log2 fold change of -8.02. Not much is known about this pseudogene other than the fact that it only has one splice variant, that it is situated on chromosome 1, and that it is associated with ribosomal protein S14 (The Human Gene Database, 2022).

However, our results were different than what was found during the study because we used different methods and programs for the analysis. We used R studio and DESEQ2 while the scientists used GraphPad prism for their analysis. While we used the same p-value as the one used in the paper (p-value < 0.05), we used a log2 fold change > than the absolute value of 1.5 instead of the fold change > to 2 that was used in the study. We also analyzed both mRNA and IncRNA transcriptomes together. The scientists also performed the two-tailed unpaired t-test or Mann-Whitney U test to evaluate the differential expression of genes between the groups while we did not.

From this differential gene analysis, we can conclude that there is great variability in the gene expression pattern of the mRNAs and IncRNAs of patients with Sjögren’s syndrome. We can suggest the use of the RP4-765C7.2 pseudogene as a potential biomarker.
Figure 3. Differential gene analysis of long noncoding RNAs and mRNAs in patients with Sjögren’s Syndrome. Heatmap with the ENSEMBL IDs of 15 of the most differentially expressed mRNAs and lncRNAs genes. Upregulation is represented in yellow, and downregulation is represented in blue. The ENSEMBL ID ENSG00000213058 corresponds to the RP4-765C7.2 pseudogene. This pseudogene is significantly downregulated in disease when compared to healthy controls.
Figure 4. ENSG00000213058 corresponds to the RP4-765C7.2 gene. The normalized count plot picked the most statistically significant gene based on the adjusted P-value. This gene is a pseudogene that has an adjusted p-value of $1.94 \times 10^{-27}$ and is associated with ribosomal protein S14 (RPS14).
Drug Therapies

Rituximab is an FDA-approved drug that can be used to treat multiple diseases such as rheumatoid arthritis, non-Hodgkin lymphoma, lymphocytic leukemia, and granulomatosis (PubChem, 2022). It has been found that rituximab can cause the death of malignant B cells in patients with cancers such as lymphomas. The way that rituximab can induce cell death in vivo is not fully understood but it is thought that the drug can do so either by directly activating complement proteins or by inducing cell lysis of a target cell through a process called antibody-dependent cell-mediated cytotoxicity (Weiner, 2010). Rituximab has also been used in multiple studies with patients suffering from primary Sjögren’s syndrome. However, there is variation in the reported effects on the salivary and lachrymal glands. Some studies reported an improvement in salivary gland function after treatment and this seems to be the result of the decrease of B cell infiltrates in the gland (Verstappen, 2017). A study wanting to investigate the effects of Rituximab on twelve female patients with Sjögren’s syndrome showed that there was no significant change in lachrymal and salivary gland function regardless of the decrease in B cells in the blood (St Clair et al., 2014).

Abatacept is a drug that has been approved for the treatment of rheumatoid arthritis, and psoriatic arthritis, and for the prevention of Acute Graft versus Host Disease. The drug prevents the activation of T cells (DailyMed, 2021). The effects of this drug vary between studies and the benefits reported differ vastly between patient groups. Indeed, a study looking into the effectiveness of the drug for the treatment of Sjögren’s syndrome associated with rheumatoid arthritis found that there was a definite increase in the tear volume and the saliva flow of the patients (Tsuboi et al., 2014). These results are significant since it helps confirm the role that T
cells play in the pathogenesis of Sjögren’s syndrome. A double-blind study that looked at the effects of Abatacept on patients suffering from primary Sjögren’s syndrome found that there was no significant difference between the group on placebo and the group on Abatacept. However, there were decreased levels of certain biomarkers such as IgG, IgA, and IgM-rheumatoid factor in the individuals receiving the drug (Baer et al., 2020). This proves that more research is needed on Abatacept and its interactions with immune system cells, especially B cells.

Methotrexate is an immunosuppressive drug that can be used to treat certain cancers such as leukemia, lymphomas, and autoimmune diseases like rheumatoid arthritis and psoriasis (PubChem, 2022). In autoimmune diseases, it has been found that methotrexate can repress phosphoribosylaminimidazolecarboxamide formyltransferase (AICAR transformylase) which may prevent guanine and adenosine from being metabolized. The buildup of adenosine combined with its anti-inflammatory effects prevents T cell activation and B cell over-reactivity (Hannoodee and Mittal, 2022). The effects of methotrexate have been studied in patients suffering from primary Sjögren’s syndrome. There was an improvement in the main symptoms (dry eyes and dry mouth) reported by the patients. Although, no significant amelioration in the objective parameters of the dry eyes and dry mouth symptoms was detected (Skopouli et al., 1996).

Current Gene Therapies that are being investigated for the treatment of Sjögren’s Syndrome

Gene therapy is a scientific technique that involves the manipulation of genes to treat human diseases (Tang and Xu, 2020). In recent years, advancements in gene therapies especially CRISPR-cas9-based therapies, have been especially useful in the design of possible therapeutics for many human diseases (Uddin et al., 2020). Gene therapies have proven themselves to be effective in the
management of symptoms of Sjögren’s syndrome. Aquaporin 1 (AQP1) is a small water channel protein that is found in a variety of tissues. However, it has been localized in the myoepithelial and endothelial cells of the salivary glands (Delporte et al., 2016). In the eye, AQP1 is expressed in the corneal epithelium (Verkman et al., 2014). Scientists found that the introduction of an aquaporin 1 adeno-associated virus (AAV) vector in the submandibular salivary of a mouse with primary Sjögren’s syndrome-like condition can restore fluid circulation in the salivary and lachrymal gland (Lai et al., 2016). Another study found that transferring the AQP1 cDNA using an adenovirus vector into the parotid gland of surviving patients of head and neck cancers could increase the saliva flow rate in some of the patients and improve dry mouth symptoms (Baum et al., 2012). In conclusion, we can say that these studies prove that AQP1 therapy can improve dryness symptoms in patients with Sjögren’s syndrome. Currently, a pharmaceutical company is investigating AAV-AQP1 as potential gene therapy for patients with Sjögren’s syndrome (MeiraGTx, 2018).

**Concluding Statement**

In this review, we evaluated the genetic, epigenetic, hormonal, and environmental factors that participate in the pathogenesis of Sjögren’s syndrome. We also assessed the different treatment options that are available or should be available to Sjögren’s syndrome patients. The biggest challenges addressed in this review are the need for better diagnostic biomarkers and treatment methods. Finding a cure for Sjögren’s syndrome is especially challenging considering that the immune system cells such as T cells and B cells play a significant role in the
inflammatory process seen in the disease. A solution would be to find an effective way to eliminate or increase immune tolerance in these immune system cells.


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