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CHAPTER 1

MULTIPLE SCLEROSIS AND STEM CELL BASED THERAPY APPROACHES

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1. Multiple Sclerosis

Multiple sclerosis (MS) is a widespread, persistent, and inflammatory disease of the central nervous system (CNS) that is believed to result from a combination of genetic and environmental factors. The exact cause of the disease remains a mystery, but it is thought to involve immune-mediated mechanisms that are influenced by several factors. MS presents a range of symptoms, including vision problems, balance difficulties, muscle weakness, and cognitive impairment, which can affect different areas of the CNS. The hallmark of MS is the accumulation of demyelinating lesions in the brain, spinal cord, and brainstem. This condition primarily affects young adults between the ages of 20 and 40 and is a significant cause of non-traumatic disability in that age group (Bruscolini et al., 2018; Huda et al., 2019; Shahmohammadi et al., 2019; Ungureanu et al., 2018; Wildner et al., 2020; Wynford et al., 2019).

1.1. Etiology

MS is a chronic disease which is characterised by inflammation, demyelination and axonal and neuronal damage in the CNS. Although the important role of immune mechanisms and inflammation in the development of MS is well-known, it is still a matter of debate whether inflammation is the first event initiating the physiopathological chain of events or whether it is reactive to as yet undetermined infectious and neurodegenerative processes (Wootla et al., 2012). Immuno-genetic properties and environmental factors are among the etiology of MS. Environmental factors such as vitamin D deficiency, ultraviolet exposure, Epstein-Barr virus and smoking can be influential, while genetically, the presence of HLA DRB1*15 and HLADRB1*03 genes are major factors in the etiology of MS (Kantarcı, 2013).

1.2. Clinical Features and Prognosis of the Disease

Qualitative and quantitative analysis of the natural progression of MS phenotypes is possible. However, the direct and precise application of these analyses at the individual level still poses a number of challenges. The natural progression of MS is structured by the presence and interaction of two clinical phenomena corresponding to different physiopathological processes within the CNS: relapse and progression. Attacks (relapses) are very significant in the clinical prognosis of MS. Attacks are the clinical manifestation of acute, self-limiting focal inflammatory events that occur episodically in MS patients. Progression refers to a steady deterioration in neurological or other function over a period of at least 6 months. An MS attack is also defined as a period of manifestation of neurological disorders in which demyelinating and inflammatory lesions are demonstrated. Neurological symptoms persist for at least 24 hours. Although MS usually progresses with recurrent relapses, there

are also progressive forms that progress without attacks (Carlson and Fox, 2024). MS has been clinically classified in four different spectrums; Relapsing Remitting MS (RRMS), Primary Progressive MS, Secondary Progressive MS, Progressive Relapsing MS. Approximately 85% of patients have relapsing remitting progression. Relapses are temporary periods of neurological dysfunction and are the defining and diagnostic feature of RRMS. They are also referred to as "attacks" or "exacerbations". In general, relapses occur early and their onset is unpredictable (Kalincik, 2015; Repovic, 2019). Primary Progressive MS corresponds to the phenotype of continuous worsening of the disease from onset, with transient and brief minor remissions and plateau periods. Minor clinical fluctuations are recognised without significant attacks. Secondary Progressive MS corresponds to the phenotype representing a steady worsening of the disease with or without a progressive course of attacks, with attacks of decreasing frequency, often with or without transient and brief minor remissions and plateau periods. Progressive Relapsing MS corresponds to the phenotype in which there is a steady worsening of the disease from onset, with prominent attacks with complete or sequential recovery. The inter-attack duration is also characterised by continuous progression (Simone et al., 2022). As the existing phenotypes have a number of limitations, two new revised MS phenotypes were additionally defined. These revised MS phenotypes are Radiological Isolated Syndrome and Clinical Isolated Syndrome. Radiological Isolated Syndrome refers to the presence of incidental imaging findings suggestive of inflammatory demyelination based on lesion morphology and location in the absence of clinical signs and symptoms (Lebrun et al., 2020). Clinical Isolated Syndrome refers to the first clinical episode that is highly suggestive of the presence of inflammatory demyelisation in the CNS but not yet sufficient to establish the criteria for a clinically confirmed diagnosis of MS. The symptoms at admission are usually monofocal and develop acutely and subacutely over days or weeks. The localisation of symptoms is often to the optic nerve, spinal cord, brain stem or cerebellum, and more rarely to the cerebral hemispheres (Miller et al., 2012).

2. Diagnosis in MS

MS was first reported as "Sclerose en plaques" by Jean Martin Charcot in 1868 and then it was tried to be understood and redefined by various neuropathologists and clinicians. The definitions were generally based on clinical and autopsy studies due to the fact that it presented with different clinical features in each patient, the clinical progression varied in each patient, it could be confused with other diseases affecting the CNS and auxiliary examination methods were insufficient at that time. Concerning all chronic diseases, early and accurate diagnosis is the most significant step in the management of the conditions that may develop during the progress of

the disease as a patient and physician. The fact that clinical and radiological findings of MS could also be observed in other diseases and the absence of a disease-specific biomarker may complicate the diagnosis and sometimes lead to misdiagnosis of MS (Freedman et al., 2024). In the diagnostic with magnetic resonance imaging (MRI), cerebrospinal fluid evaluation and evoked potentials are utilised to examine the clinical status. Recognition of MS requires a challenging and long period of time. A number of diagnostic criteria have been established to differentiate MS from similar diseases. In 1965, the first diagnostic criteria were established (Schumacher et al. 1965). McDonald et al. also established the comprehensive assessment criteria bu utilization of MRI. The International Advisory Committee for Clinical Trials in MS met in 2017 and prepared a revision by updating the McDonald 2010 criteria. In the 2017 revision, in addition to the 2010 criteria; the clinicians could verdict in case of status of patients do not completely compatibel with the criteria, it is considered that the diagnosis is typically labeled as "probable MS". In addition, it is advisable to diagnose multiple sclerosis (MS) when magnetic resonance imaging (MRI) findings indicate conditions that facilitate the spread of the disease in both time and space (Thompson et al 2018).

3. Treatment Options of MS

Treatments performed in MS do not eliminate the disease, but pharmacological treatment and physiotherapy and rehabilitation can be applied to reduce the progression of existing symptoms and disability (Montalban et al., 2018).

3.1. Pharmacological Treatment

Pharmacological treatment in MS includes therapy of relapses, disease modifying interventions and symptomatic strategies. The majority of patients suffer from the RRMS type of MS. New or recurrent neurological impairment and other symptoms in MS are considered relapses (Brownlee et al., 2017). The use of high-dose glucocorticosteroids is a viable treatment option for relapses, however, steroid-resistant relapses may necessitate plasmapheresis. Both treatment options aid in the control of acute exacerbations and facilitate the recovery process by leveraging their anti-inflammatory effects (Ehler et al., 2015, Schweingruber et al., 2012). Therapies can be broadly categorized into four primary types, which include recombinant cytokines, complex peptide mixtures, monoclonal antibodies, and small molecules. It is important to note that these categories are inclusive of all therapeutic modalities currently in use (Winkelmann et al., 2016). In MS, drugs such as natalizumab, dimethyl fumarate, daclizumab, alemtuzumab, ocrelizumab are generally preferred in treatment (IFNβ). The most important improvement associated with the drug treatment is achieved in RRMS. The recommended doses and frequency of

usage of current medications vary according to the response of patients to the drug. Although studies suggest that MS attacks are triggered by environmental and exogenous factors such as viral infections in the genetic background, the factors that trigger the attack are not completely understood due to its very complex pathogenesis. Thus, the effectiveness of current immunosuppression-based treatments of MS is very limited.

3.2. Physiotherapy and Rehabilitation

The neurologist is at the primary responsibility in the diagnosis and drug treatment of MS. However, the fact that MS is a dynamic process that can initiate from a young age, and that the clinical pattern is diverse and not constant, requires that the process should be managed by a multidisciplinary approach. In addition to medical drug treatments at every stage of the disease, rehabilitation activities have significant positive contributions to the functional improvement of patients. Exercise has been demonstrated to have positive effects on general fitness, activity level, fatigue and quality of life in patients with MS (Mostert and Kesselring, 2002). The Expanded Disability Status Scale (EDSS) is a widely used test for assessing the effectiveness of clinical interventions in Multiple Sclerosis (MS) and for monitoring disease progression. It is commonly used in conjunction with a thorough neurological and musculoskeletal examination to evaluate disability levels. To ensure personalized care, individualized goals should be established for each patient based on their EDSS score and accompanying symptoms, and rehabilitation plans should be tailored accordingly (Nabizadeh et al., 2024).

MS Patients with EDSS Score <1 (Initial Period)

In this period, patients should be informed relate to their diseases and the necessity and importance of movements in this process from the early stages (Nabizadeh et al., 2024).

MS Patients with EDSS Score 1.5-5.5 (Early Stage)

Patients with this score, who can usually walk independently, have decreased walking distances and speeds. In the beginning of this period, it is crucial to increase the muscle strength of the distal muscles in the early stages and also the proximal muscles in the later stages. Spasticity can be managed with physiotherapy methods against mild spasticity that may occur in the early stages. Stretching exercises, cold and hot applications, orthosis and muscle relaxants may be used in the treatment of spasticity. Exercises against posture, gait and balance disorders should also be added to the treatment. Aerobic capacity of patients should be increased against endurance losses that may develop in this period and in the future (Nabizadeh et al., 2024).

MS Patients with EDSS Score Between 6-7

Patients with this score spend most of the day while seated. The patient who can walk with unilateral support at the beginning of this period needs bilateral support in the following periods and becomes wheelchair dependent at the end of the period. In this period, exercises should be planned to maintain the strength of the distal muscles and the function of the proximal muscles. Spasticity is also intense and widespread in a way to affect joint movements. Therefore, in addition to stretching exercises, oral antispastic drugs, local Botulinum toxin injections and anti-spasticity splints should be added to the treatment programme. Range of motion and stretching exercises should be performed to prevent contracture development. Swallowing and respiratory functions should be evaluated and exercises for these functions should be included in the rehabilitation programme (Nabizadeh et al., 2024).

MS Patients with EDSS Score 7.5-9.5 (Advanced Stage)

Patients with this score usually spend most of the day in a wheelchair or bedridden. They need support in their transfers. A preventive rehabilitation programme should be designed to maintain the existing muscle strength and to prevent complications. Patients in this period should be mobilised as much as possible and if the conditions are suitable, axial loading should be provided with standing upright positioning. Patients in wheelchairs are taught exercises and transfer methods that they may perform in the chair, they are advised to use air cushions against the development of pressure sores, to sit in the correct posture and not to sit for a long time. In terms of pulmonary complications that may develop in the future, patients should be positioned in bed with their head elevated, respiratory exercises should be started with a spirometer, and abundant fluid should be provided (Nabizadeh et al., 2024).

3.3. Stem Cell Treatment

Stem cells are undifferentiated cells with the capacity to divide indefinitely, exhibit high activity of enzymes related to cellular physiology and aging, such as telomerase and alkaline phosphatase, and possess the ability to self-renew. Stem cells can be categorized based on their differentiation potential, ranging from totipotent to oligopotent. Totipotent stem cells are capable of differentiation into embryonic and extra-embryonic structures, including the planenta and chorionic membrane. Pluripotent stem cells can differentiate into approximately 200 different types of somatic cells belonging to the 3 germ layers. Embryonic stem cells are a specific type of pluripotent stem cells. Multipotent stem cells can differentiate into a few cell types, while oligopotent stem cells can differentiate into fewer cell types. Additionally, stem cells can be categorized into 2 groups: embryonic stem cells and non-embryonic stem

cells, including adult stem cells, fetal stem cells, and induced pluripotent stem cells.

In stem cell and/or cell-based therapies, the cells whose properties are investigated can be somatic cells or stem cells. These cells can be used alone or in combination with a tissue graft to close the physiological deficit in the damaged area. When stem cells are considered, neural stem cells, mesenchymal stem cells, embryonic stem cells and induced pluripotent stem cells appear as stem cell types with potential. The transplantation methods of these cells used experimentally are direct lesional injection into the brain and systematic injection into the bloodstream. In addition, stem cells are also administered together with the graft by cobination of biomaterials consisting of stem cells and biodegradable scaffolds, or by genetic manipulation of stem cells by gene transfer/silencing to produce a cytokine that may be stimulatory for therapy and/or to differentiate into the relevant neuron type. In normal physiology, the lateral ventricle and hippocampus constitute the niche, which is called the necessary environment for stem cells to differentiate and function (LIronte at al., 2022). Stem cells have a number of characteristics to demonstrate their therapeutic effects in neurodegenerative diseases. These are stimulate the proliferation of reactive astrocytes stimulated by cytokines such as IL and TNF-α secreted from microglia due to damage or disease, stimulate angiogenesis of blood vessels, increase axonal remyelination, increases synaptic connections (synaptogenesis), suppresses macrophage infiltration and microglia activation, stimulates neuronal differentiation and proliferation (neurogenesis), secretes neurotrophic (BDNF, NGF, GDNF, neurotrophin-3 and neurotrophin-4 etc.) and angiogenic (FGF, PDGR, VEGF, angiopoietin-1 and angiopoietin-2 etc.) factors.

Treatment for MS depends on the severity and progression of the disease and may involve surgical interventions, pharmacological therapies, and stem cell treatments that have been experimentally investigated and executed. To understand the timeline of stem cell research, it is important to note the key developments in this field, including the demonstration of neurogenesis in the rat brain (Altman and Das GD, 1965), the discovery of neural stem cells in both young and old rat brains (Kaplan and Bell, 1984), the extraction of neural stem cells from embryonic rat brains (Temple, 1989), the demonstration of neurogenesis in the adult human brain (Eriksson et al, 1998), the derivation of neural stem cells from adult human brain tissue (Eriksson et al., 1998), the application of stem cells in the treatment of neurodegenerative diseases (Gould et al., 1998), the differentiation of functional dopaminergic neurons from human embryonic and induced pluripotent stem cells (Ma L et al, 2011), and the differentiation of functional serotonergic neurons from human embryonic and induced pluripotent stem cells and fibroblasts (Vadodaria et al., 2016). In recent years, there has been a significant increase in clinical trials

aimed at assessing the therapeutic potential of stem cells in the treatment of MS in humans. These studies primarily focus on neural stem cells and their products to ensure compatibility with the niche and are investigating treatment options for the different subtypes of MS. Recent research has also emphasized the importance of exosomes derived from stem cells as a potential mediator structure in demonstrating the healing and/or supportive function of these cells. Exosomes are particularly promising in the treatment of neuronal-based diseases, as they manipulate cells in the regions of neuronal damage at the molecular biological level through the genetic material and/or proteins they consist of. This enables them to mediate the differentiation of neuronal stem cells into neurons and the function of the differentiated neurons, as well as the suppression of neuroinflammation (Jafarinia et al., 2024). The role of mesenchymal and hematopoietic stem cells in immunoregulation is geared towards preventing cellular damage in MS by targeting neuronal stem cells. Employing autologous stem cells in clinical trials may emerge as one of the most effective treatment options, potentially resulting in a substantial reduction in morbidity rates (Sai Santhosha Mrudula et al., 2023). Stem cell therapies have gained considerable significance in the treatment of MS due to the incorporation of autologous and allogeneic stem cell modalities in research topics. As a result, the potential of stem cells and/or stem cell products in treating neurodegenerative diseases, including MS, which currently have no definitive cure, is receiving increased attention. It is anticipated that the use of stem cells in the treatment of such diseases will become more prevalent in the near future.

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CHAPTER 2

EFFECTS OF SHORT CHAIN FATTY ACIDS ON HUMAN METABOLISM AND RELATIONSHIP WITH DISEASES: A TRADITIONAL COMPILATION STUDY

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INTRODUCTION:

The gut microbiota affects our general health and nutritional condition in a number of ways. A growing body of research indicates that the physiology of the host is significantly influenced by microbial metabolites. Short-chain fatty acids (SCFAs) are volatile fatty acids that have less than six carbon atoms in both straight and branched chain topologies. Fermented food components that are not absorbed in the small intestine cause the gut microbiota in the large intestine to produce SCFAs.

The potential health benefits of SCFAs have been the focus of much research during the last few decades. Saturated fat acids (SCFAs) are the main byproducts of the fermentation of non-digestible carbohydrates (NDCs) that are accessible to the gut microbes. Short-chain fatty acids (SCFAs) are produced by fermentation of oligosaccharides, which are broken down from complex carbohydrates by the intestinal microbiota. These have been demonstrated to be beneficial to the host's health and are mostly made up of butyrate (C4), propionate (C3), and acetate (C2). In general, the mole ratios of C2, C3, and C4 are 60:20:20. In addition to providing energy, SCFAs are essential for maintaining intestinal integrity and metabolism once they are absorbed in the colon.

1) GUT, MICROBIOTA, FIBRE AND SCFA

1.1. Microbiota and İntestinal Barrier

The microbial barrier is made up of microorganisms that inhabit the human stomach, including bacteria, viruses, fungus, bacteriophages, and protists. Microbes are found in the core mucin layer, which is separated from the enterocyte surface in a healthy state. (3).

Trillions of microorganisms make up the complex ecosystem that is the human gut microbiota. The amount of bacteria per gram varies based on the area of the intestine: in the stomach, between 103 and 104 per gram; in the jejunum, between 105 and 106 per gram; in the terminal ileum, between 10 10 8 and 109 per gram; and in the colon, between 1012 and 1014 bacteria per gram of intestinal contents. The microbiota contains 150 times more microbial genes than the human genome and at least 1000 recognized kinds of bacteria. The microbial barrier is created by the many microorganisms that are present in the human gut, including bacteria, viruses, fungus, bacteriophages, and protists. Microbes thrive in the core mucin layer of the healthy gut, which is located away from the surface of the enterocytes. Trillions of microorganisms make up the human gut microbiota, which functions as a complex ecosystem. These microorganisms can be found in the stomach at concentrations of 103–104 per gram, in the terminal jejunum at 105–106, and in the ileum at

108–109. There are at least 1000 recognized bacterial species in the colon, where there are roughly 1012–1014 bacteria per gram of intestinal contents. Furthermore, compared to the human genome, the microbiota contains 150 times more microbial genes. (4)

The gut bacteria are a part of the dynamic, complex system known as the "intestinal barrier," which is the result of interactions between several functional levels. (5) (Table 1).

Table 1: *The intestinal barrier is made up of various anatomical and physical layers.*

• The gut microbiota (microbial barrier). • Mucus from the intestines that builds up at the point where the brush boundary of enterocytes and the intestinal lumen meet. • The interplay of stomach acid, biliary, and pancreatic secretions with gastrointestinal motility is known as the functional barrier. • Tight connections and the epithelium barrier (enterocytes). • The immunological barrier, which is made up of products from immune-competent cells. • Interface between the intestine and the blood vessel. • The hepatic filter, symbolized by the liver barrier.

The microbiota carries out essential tasks in the complex and linked gut environment, including the synthesis of micronutrients and the digestion and metabolism of nutrients like proteins, lipids, carbs, and vitamins. Its major biotransformation in the liver is also noteworthy. Note that acronyms for technical terms must be explained in detail the first time they are used. Furthermore, the microbiota contributes significantly to the synthesis of secondary intestinal bile acids, which are important hormone-like signaling molecules that facilitate the digestion of fat. The microbiota confers innate immunity and tolerance on the host, influencing the development and maturation of diverse cell types within the gut immune system's lymphoid tissues. (6)

Dietary modifications, probiotics, and prebiotics all modify the gut flora. The morphology and activity of gut bacteria are influenced by the substrates' bioavailability into the gut lumen. This in turn affects how certain bacterial models and the resulting metabolites are balanced. (7)

1.2 The Role of Dietary Fibre in SCFA Production

Short-chain fatty acids (SCFAs) cannot develop without dietary fiber, and there is a direct relationship between the gut microbiota and dietary nutrients. Monosaccharide polymers and oligomers combine to form molecules with varying diameters, which make up fibers. (8) Cereals, fruits, vegetables, and legumes are sources of dietary fibre. (9)

The enzyme profile of the human gut is inadequate for the complete metabolism of dietary fiber. Non-digestible oligosaccharides (NDOs), fermentable carbohydrates that are available to the microbiota, are represented by soluble fiber that the host is unable to digest. In addition to its potent ability to code genes, the microbiota plays a critical role in colonic and cecal fermentation, which facilitates full digestion of fiber. This ability to digest is dependent upon the high concentration of glycoside hydrolases in the vast microbial population (more than 17), whereas humans only have 260 distinct types of enzymes to break down carbohydrates. More than 100 trillion microorganisms are involved in this process. (Table 2). A MAC-rich diet in humans is linked to boosted colonic and faecal SCFA levels (10).

Table 2: *Dietary source, fibre substrates and SCFA-producing bacteria.*

Dietary Source	Fibre Substrates	SCFA Producing Bacteria
CASHEWS, GREEN BANANAS, WHITE BEANS, OATS AND POTATOES	Resistant starch	*Ruminococcus* , *Bacteroides*
SEAWEED AND CEREAL BRAN	Cellulose	*Bacteroides* , *Ruminococcus*
CEREAL BREAD	Haemi-celluloses (xylan and arabinoxylan)	*Bacteroides* , *Roseburia* , *Prevotella*
APPLES, APRICOTS, CHERRIES, ORANGES AND CARROTS	Pectin	*Eubacterium* , *Bacteroides, Fecalibacterium*
ASPARAGUS, LEEKS, ONIONS, BANANAS, WHEAT, GARLIC, CHICORY AND SPINACH	Fructans (inulin and fructooligosaccharides)	*Bacteroides* , *Fecalibacterium*
MOTHER'S MILK	Milk oligosaccharides	*bifidobacteria*
MILK, YOGHURT, BUTTERMILK AND CHEESE	Lactose (only in people with lactose intolerance)	*bifidobacteria*
OATS, BARLEY, WHEAT, RYE, MUSHROOMS AND SEAWEED	β-Glucan	*Eubacterium* , *Atopobium, Enterococcus*, *Lactobacillus, Prevotella*, *Clostridium* cluster XIVa
ACACIA TREE AND READY-TO-EAT FOOD ADDITIVE	Arabic gum	*Bifidobacterium* , *Lactobacillus* , *Ruminococcus*

1.3 Short-Chain Fatty Acids (SCFA);

This kind of organic fatty acid can have one to six atoms in its carbon chains. They are the primary anion produced in the colon by bacterial fermentation of polysaccharides, oligosaccharides, proteins, and glycopeptide precursors, as indicated in Table 3. Ultimately, fermentation yields various metabolic byproducts that are useful to the host in addition to metabolisable energy for microbial growth and maintenance. Numerous reactions and metabolic pathways are involved in the anaerobic microbial degradation of

organic material. The main byproducts of intestinal fermentation are heat, gases (CO2, CH4, and H2), and short-chain fatty acids (SCFAs). (11).

Our diet is the source of SCFAs, which are closely linked to it. Foods like butter and other dairy products, for instance, have high SCFA contents. The smallest and most structurally simple SCFA is acetate, which has just one carbon bonded to the carboxyl group. Propionate and butyrate, on the other hand, have two and three connected hydrocarbons, respectively(12).

Anaerobic fermentation by gut microorganisms produces most short-chain fatty acids (SCFAs), with the exception of a small portion that comes straight from meals. The specific amount varies based on a person's diet, the type and quantity of microbiomes in the body, and how long food takes to move through the digestive system. There is a 500–600 mmol daily production rate estimate, according to recent research(13).

Humans utilise SCFAs as a dietary energy source, accounting for around 10% of their caloric needs. These chemicals also affect intracellular functions, including regulation of hunger, mitochondrial activity in the liver, and brown adipose tissue (14). These chemicals also affect intracellular functions, including regulation of appetite, mitochondrial activity in the liver, and metabolism of brown adipose tissue(14). These chemicals also affect intracellular functions, including regulation of appetite, mitochondrial activity in the liver, and metabolism of brown adipose tissue(14).

2) Bacterial Fermentation and SCFA Production

In order to produce carbon and energy, a variety of gut microbiota species have the ability to ferment complex carbohydrates that the host is unable to assimilate. Short-chain fatty acids are produced as a result of this fermentation process in the colon (SCFAs). (15). Recent developments in metagenomics have allowed for the identification of the bacteria responsible for SCFA synthesis. The main precursor chemical for Short Chain Fatty Acids (SCFAs) is pyruvate, which is created following microbial hydrolysis through the glycolytic pathway for (deoxy-)hexoses and the pentose phosphate route for pentoses. (16)

All bacterial groups often include the mechanisms that lead to the formation of acetate, with the intestinal lumen being the place of maximal concentration. This is not the case for the more stable and substrate-dependent butyrate and propionate pathways. Two different processes can create propionate: the propanediol pathway, which is used by Lachnospiraceae, and the succinate pathway, which is used by Bacteroidetes and Negativicutes (Firmicutes phylum). Essential enzymes such as butyrate kinase and butyryl CoA:acetate CoA transferase aid in the production of butyrate. While certain

butyrogenic species, such Coprococcus, use the alternative pathway, most butyrate makers, such as Faecalibacterium prausnitzii, Eubacterium rectale, and Eubacterium hallii, employ the butyrate kinase pathway. (17)

3) SCFA ABSORPTION

Protonated SCFA diffusion and anion exchange are the two suggested absorption processes. 3) The colon and cecum effectively absorb short-chain fatty acids (SCFAs), with only 5–10% of them being eliminated in the feces. In the colon, SCFAs are quickly absorbed, improving the absorption of sodium and the excretion of bicarbonate.(10) Normally, SCFAs in human peripheral venous blood are low, with only significant amounts of acetate present.(18)

The three primary SCFAs—butyrate, propionate, and acetate—are absorbed at similar rates by various sections of the colon. Following absorption, SCFAs are metabolized at three primary sites in the body: (1) liver cells, which metabolize residual butyrate, use propionate for gluconeogenesis, and absorb 50–70% of acetate; (2) muscle cells, which derive energy from the oxidation of residual acetate; and (3) after being absorbed, ceco-colonic epithelial cells. (19)

4) EFFECTS OF SHORT-CHAIN FATTY ACIDS ON HUMAN METABOLISM

Short-chain fatty acids (SCFAs) have a beneficial effect on human energy metabolism, which includes the metabolism of glucose, lipids, and cholesterol in numerous organs (see Table 3) (20). Colonocytes transport SCFAs primarily using butyrate as their energy source after apical absorption. (21).

The basolateral membrane carries the portion of SCFAs that are not used by the intestinal mucosa to the portal circulation. It is still mostly unclear how SCFAs are transferred from blood to different types of tissue. (20, 22)

SCFAs cause the mucus glands to secrete more secretions and accelerate blood flow. Above all, they produce acetyl-CoA, which is necessary for cell membrane formation and fat biosynthesis, protecting mucous membrane integrity. Certain indications suggest that SCFAs are essential for facilitating the advantageous impacts of gut microbiota. SCFAs have both direct and indirect impacts on markers of cardiovascular disease (CVD) risk. They also directly alter host health through a variety of tissue-specific pathways associated to intestinal barrier function, glucose homeostasis, immunomodulation, appetite regulation, and obesity. (23)

The majority of formic acid's current role in the intestines remains unclear. Research indicates that this acid is linked to the production of methane and is present during inflammation(24).

The cell's metabolism of fats and carbohydrates depends on acetic acid, which has the greatest concentration of all the short-chain fatty acids. Furthermore, the liver absorbs acetic acid, which aids in the creation of cholesterol there(25).

Additionally, it actively helps to prevent infections in the human gut by possessing antibacterial properties. (26) The inflammatory process of the intestinal mucosa, a typical feature in many medical circumstances, is primarily caused by a lack of energy. The main energy source for intestinal epithelial cells is butyric acid. The immunoregulatory effects of butyric acid help these cells as well as other mucosal populations. The way it alters gene expression affects both promoters and inhibitors of gene expression. (27)

Table 3*: Examples of trials on the effect of SCFAs on human health.*

SCFA TYPE	EFFECT ON HUMAN HEALTH
Acetate	• Defense against infection with E. Coli O157:H7.
	• Plays a part in the generation of cholesterol.
butyrate	• Intestinal epithelial cells get 70% of their energy from it.
	• Elevations in mucin synthesis and MUC2 gene expression.
	• Triggers the process of apoptosis and prevents the growth of tumor cells.
	• Prevents hydrogen peroxide and nitrosamides from having genotoxic effects.
	• Has an impact on immunomodulation.
	• It contributes to the prevention and management of cancer, Crohn's disease, and distal ulcerative colitis.
	• Lessens ulcerative colitis (UC) symptoms
Butyrate/acetate/ propionate	• Enhances the histology and macroscopic indicators of inflammation.
Propionate	• Enhances lipid metabolism and decreases the liver's production of cholesterol.

5) SCFA ASSOCIATION WITH DISEASES IN HUMAN METABOLISM

Plenty of evidence supports the crucial function of SCFA in maintaining health and causing various illnesses such as Irritable Bowel Syndrome, obesity, colorectal cancer, diabetes, non-alcoholic fatty liver disease, and cardiovascular diseases.

Plenty of evidence supports the crucial function of SCFA in maintaining health and causing various illnesses such as Irritable Bowel Syndrome, obesity, colorectal cancer, diabetes, non-alcoholic fatty liver disease, and cardiovascular diseases. SCFA is known to have many protective effects against human methanolysis.

The proximal colon has the highest quantities of SCFA, which are either absorbed by enterocytes or transferred into the circulation via the intestinal epithelium. There are two main signaling pathways associated with SCFA that have been identified: GPCR activation and histone deacetylase (HDAC) inhibition. Since HDACs regulate the expression of genes, inhibiting them has a wide range of secondary effects. We are still learning about SCFA-induced HDAC inhibition. (28) GPCRs, specifically GPR43, GPR41, and GPR109A, have been recognised as SCFA receptors. Research has demonstrated that these GPCRs have a crucial function in the modulation of metabolism, inflammation, and ailments.

Short chain fatty acids (SCFAs) have exhibited the ability to modify chemotaxis and phagocytosis, induce reactive oxygen species (ROS), alter cell proliferation and function, and possess anti-inflammatory, antitumour and antimicrobial effects while affecting gut integrity. Therefore, these discoveries highlight the critical part that SCFAs play in maintaining intestinal and immune homeostasis. (28)

5.1. Relationship between Colorectal Cancer (CRC) and SCFA

Colorectal cancer (CRC) is the third most common cause of cancer-related deaths worldwide, with the highest frequency occurring in developed countries. By 2035, 24.4 million more cases of colorectal cancer are expected to be detected worldwide. There is a substantial correlation between microbiome dysbiosis and low levels of short-chain fatty acids (SCFAs) and colorectal cancer (CRC).(29) Regulating SCFA-producing bacteria by fermentable fiber dietary intervention is now being explored as a potential colorectal cancer (CRC) treatment. (30)

Although it's unclear exactly how dietary fiber prevents colon cancer, it may reduce the risk of the disease. There are several theories that suggest fiber improves intestinal transit, reducing exposure to carcinogens in food, or that fiber absorbs carcinogens and alters the makeup of intestinal bacteria, altering bile salt and carcinogen metabolism. Moreover, fiber lowers colonic pH and alters the excretion of bile salts in the feces. Lastly, short-chain fatty acids (SCFAs), which have a variety of impacts on colonic epithelial cells at different phases of growth, development, transformation, and cell death, may be how dietary fiber exerts its protective benefits. This might potentially result in a decreased risk of cancer. A critical balance between the acquisition of new cells through mitosis and the loss of existing cells through differentiation and programmed cell death (apoptosis) is required to maintain tissue homeostasis. Changes in this equilibrium might be essential for the emergence of carcinogens. In colonic cells, SCFAs influence apoptosis; however, their effects

on normal and malignant lines differ. Different adenoma and carcinoma cell lines (RG/C2 and AA/C1) undergo apoptosis in response to SCFAs (32).

5.2. Inflammatory Bowel Diseases and SCFA Relationship

Dysbiosis, or changed microbial makeup, is a prevalent characteristic of inflammatory bowel diseases (IBD). In individuals with Crohn's disease (CD) and ulcerative colitis (UC), dysbiosis is linked to lower levels of SCFA-producing bacteria. Within the phylum Firmicutes, the majority of the fewer bacteria are members of the Ruminococcaceae and Lachnospiraceae families. (33)

The autoimmune disease known as inflammatory bowel disease, or IBD, has a severe negative effect on the quality of life of its sufferers. Regretfully, this illness currently has no known treatment. It is currently understood that nutrition, gut flora, and genetic factors all have a role in the etiology of IBD. TNF and JAK blockers are examples of targeted inflammatory cytokine inhibitors that have been shown to be beneficial in reducing IBD symptoms. It should be mentioned, nonetheless, that using them increases the risk of infectious illness in patients.(34)

A number of cytokines, including as IL10 and IL17, are important in the development of IBD; however, there are currently no IL10/17-targeted medications available for clinical use. Supplemental SCFAs can be added to one's diet, as they are beneficial to the host. This method controls the cytokine network and improves the detection of innate immune sensors in the gut. Thus, the intended result of stopping the overreaction of the immune system and impeding the advancement of IBD is accomplished. (34)

5.3. The Relationship between Obesity and SCFAs

Human body weight has been shown to decrease and satiety induced by fermentable dietary fiber has been found; it is also inversely correlated with obesity and weight growth. (35)

Short-chain fatty acids (SCFAs) are produced by the saccharolytic fermentation of non-digestible carbohydrates (NDCs) by bacteria in the colon. In the gut, acetate, propionate, and butyrate are the primary short-chain fatty acids (SCFAs). The amount of dietary fiber that is available and the variety of the gut flora affect their production. The colon's fermentation and absorption of SCFAs cause the release of gut hormones that promote fullness. These hormones subsequently communicate with the brain's appetite centers to reduce hunger. (36)

More study is required to completely understand the roles of acetate, in particular, although it may play a crucial role in the control of hunger. In

the hypothalamus, anorectic hormones like GLP-1 and PYY are produced in greater amounts when acetate is administered, as evidenced by certain studies. This leads to a decrease in food consumption. In rodent models, the glutamate-glutamine transcellular loop in the colon produces acetate, which triggers anorectic signaling in the ARC.(37) Perry et al. (2016), however, found the reverse, suggesting that acetate causes obesity via increasing the release of ghrelin and insulin.(38) It will need more investigation into the possible processes by which acetate affects appetite to ascertain whether it is an appetite-stimulating or appetite-inhibiting component of appetite regulation.(37)

Propionate is a short-chain fatty acid (SCFA) that decreases appetite by inducing fullness through gut hormones. Propionate acts through FFAR2/3, which is expressed in intestinal L-cells and increases the release of GLP-1 and PYY peptides. Furthermore, propionate has shown a propensity to limit the liver's ability to accumulate fat by suppressing the gene expression patterns linked to the production of fatty acids. Propionate administered directly to the proximal colon decreases food intake and weight gain in overweight individuals while also increasing PYY and GLP-1 levels following meal consumption. (39)

Butyrate works by interacting with the GPR109A receptor, which is required to maintain the integrity of the intestinal barrier and is associated with the colonic inflammatory response. Furthermore, it has been demonstrated that butyrate affects how much weight is regulated by increasing energy intake by interacting directly with skeletal muscle and initiating lipolysis in adipose tissue.(40)

Short-chain fatty acids (SCFAs) are a group of byproducts of microbial fermentation in the colon that activate anorectic gut hormones including PYY and GLP-1 to regulate hunger. In order to reduce obesity-related adiposity and weight gain in those who already have it, raising SCFA levels is a viable goal. (41)

Nonetheless, the data supporting the usefulness of SCFAs in managing weight is currently preliminary and mostly derived from research on animals. It is important to do research on the effects of SCFA administration in people, mainly by dietary fiber or oral or colonic infusions of specific SCFAs. One drawback of depending only on dietary fiber is that each person's gut flora has a different structure, which affects how the fiber breaks down and what SCFAs are produced. Another important thing to keep in mind is that SCFAs are an energy source in addition to perhaps stimulating hormones that control hunger. Therefore, more investigation is needed to fully determine the efficacy and any side effects of employing SCFAs as a therapy for obesity, especially in human subjects and the body as a whole. (36)

5.4. The Imbalance in the Relationship between Non-alcoholic Fatty Liver Disease

Systemic disorders eventually result from the imbalance in the connection between Non-alcoholic Fatty Liver Disease (NAFLD) and SCFA, which alters the composition and quantity of metabolites in the intestinal micro-ecosystem. This alteration affects the function and homeostasis of distal organs. Fat droplets occur in organs as a result of the buildup of extra dietary energy in vivo. Previous studies have shown that a diet high in fat and sugar increases the gut's production of lipopolysaccharide (LPS), which changes the gut's microbial composition and mucosal integrity. It also causes excessive hepatic steatosis, inflammation, and eventually the development of non-alcoholic fatty liver disease (NAFLD) in conjunction with obesity. A diet high in sugar and fat has been shown to increase the secretion of lipopolysaccharide (LPS) in the gut, which can change the microbial composition and integrity of the mucosa in the gut. It can also cause excessive hepatic steatosis, inflammation, and eventually lead to the development of non-alcoholic fatty liver disease (NAFLD) in conjunction with obesity. Research has shown a correlation between an increased frequency and severity of obesity and the amplification of NAFLD. The main focus of NAFLD linked with obesity prevention and therapy has been on caloric restriction or adopting a high-probiotic-fiber diet. (42)

Short-chain fatty acids (SCFAs) are produced by fermentation of dietary fibers, which intestinal bacteria are unable to digest. These metabolites are among the most common microbes in the digestive system. The colon is the principal site of microbial fermentation, and it is here that SCFAs govern the interplay between intestinal and host physiological processes. As signaling molecules, short-chain fatty acids (SCFAs) transmit information about gut microbiota and diet to the human metabolism. They have the ability to suppress inflammatory reactions brought on by LPS, which are connected to metabolic diseases. Furthermore, via blocking the synthesis of fatty acids and affecting gluconeogenesis, SCFAs impact the energy supply and metabolic equilibrium of the organism. Several studies indicate that short-chain fatty acids (SCFAs) produced from dietary fiber may mitigate metabolic diseases of the liver through the gut-liver axis or other pathways (42).

Through the regulation of lipid and glucose metabolism, inflammatory response, and liver tissue uptake after entering the liver through the portal vein, SCFAs have a beneficial effect on non-alcoholic fatty liver disease (NAFLD). The possible influence of SCFAs on NAFLD mediated by the intestinal barrier is equally significant as their direct effects. Through its ability to protect the intestinal barrier and alter the gut micro-ecology, SCFAs may be able to postpone the onset of diseases associated to non-alcoholic fatty liver disease.

This scenario emphasizes how crucial it is to investigate the possible health advantages of SCFAs further.(43)

5.5. The Relationship between Blood Pressure (Hypertension) and SCFA

Having high blood pressure is a major risk factor for heart disease. Although genetics only accounts for a tiny percentage (<5%) of the illness, genome-wide association studies have shown that genetics play a significant role in the occurrence of hypertension. However, some lifestyle variables, such as body mass index (BMI) and salt consumption, can alter blood pressure by 5 mmHg, indicating that lifestyle plays a substantial role in blood pressure regulation. Several dietary regimens, like the DASH (Dietary Approaches to Stop Hypertension) and Mediterranean diets, have demonstrated a link between lower blood pressure and higher intake of fruits, vegetables, and fiber. Crucial metabolites generated by the gut microbiota, SCFAs, have been shown to rise with a Mediterranean diet.(44)

Research indicates that eating a diet high in fiber might alter the composition of the gut microbiota and increase the number of bacteria that produce acetate, which will ultimately help to mitigate gut dysbiosis and lower blood pressure. Acetate produced from gut microbiota also lowers blood pressure when taken directly, suggesting that it has a protective effect on blood pressure regulation. Furthermore, moderate amounts of propionate generated by gut bacteria significantly decreased angiotensin II-induced hypertension in mice via preserving regulatory T cell-dependent immunological homeostasis. It has been demonstrated that butyrate suppresses the renal (pro)renin receptor-mediated intrarenal renin-angiotensin system, hence preventing angiotensin II-treated hypertension. Furthermore, studies have shown that metabolites of short-chain fatty acids (SCFA) that are generated from gut microbiota can reduce blood pressure in animals. (45)

5.6 Diabetes and SCFA Relationship

Diabetes mellitus (TD) is partially influenced by genetic predisposition, but environmental variables also play a significant role in the aetiology of TD and other autoimmune and allergic illnesses. (46) Non-obese diabetic (NOD) mice were fed special diets containing acetylated or butylated resistant starches, which release high quantities of acetate or butyrate in the colon following bacterial fermentation. This prevented the animals from developing diabetes. The results suggest that cooperation between microbiota and high-fiber diets may be able to reduce the risk of TD in susceptible people. It is remarkable that complete protection may be obtained by delivering a diet that includes both butyrate and acetate, suggesting that both SCFAs function through different processes. (47)

Although the diet treated with acetate alone changed the makeup of B cell subsets in the spleen, both diets lowered the frequency of T cells that are autoreactive in lymphoid tissues and the expression of CD86 in mature B cells that produce IL-12 in the marginal zone. The butyrate-treated diet increased Treg number and effectiveness, consistent with previous findings (described before with reference to the periphery). Proteomic and enteroendocrine beta cells, which are crucial for glucose tolerance, may have metabolite-sensing GPCRs, such as GPCR43, activated in the prevention of diabetes by SCFAs(48).

5.7 Blood-Brain Barrier and Neuroimmunoendocrine Functions and SCFA Relationship

The role of bacteria in the interrelated gut-brain axis is gaining more and more attention. SCFAs are already linked to the control of neuroendocrine and neuroimmune processes. (49). A network of neurons that are innervated into the intestinal mucosa controls digestion processes in response to immunological effector molecules produced into the extracellular milieu. Much closer to the intestinal lumen, the internal submucosal plexus innervates the mucosa and muscularis mucosa, performing a sensory role associated with controlling intestinal blood flow and epithelial activities. Neurons, particularly vagal afferent nerves, which are essential for mood, stress response, and satiety, may be directly impacted by SCFAs carried throughout the stomach. (50). Mice protected against the aftereffects of chronic psychosocial stress were given a week's supply of oral acetate, propionate, and butyrate supplemented drinking water. Oral supplementation of acetate, propionate and butyrate in drinking water for one week offered protection against the subsequent effects of chronic psychosocial stress in mice Oral supplementation of acetate, propionate and butyrate in drinking water for one week offered protection against the subsequent effects of chronic psychosocial stress in mice (51).

Furthermore, SCFAs that enter blood arteries have the ability to pass across the blood-brain barrier (BBB) and into the CSF and brain. Transportable neurotrophic factors control the development and differentiation of neurons and synapses in the brain. Short-chain fatty acids (SCFAs) have been associated with many brain diseases that influence learning and memory and enhance appetite suppression through neuropeptides, while the exact processes behind these effects are yet unknown. (52)

Furthermore, SCFAs contribute to the blood-brain barrier's permeability. Compared to typical mice, the blood-brain barrier (BBB) of germ-free (GF) mice is more permeable to smaller molecules. However, recolonization with a diverse microbiota or bacteria that produce short-chain fatty acids (SCFAs) can restore the integrity of the blood-brain barrier in GF animals. (53)

5.8. Anti-İnflammatory Effects Of SCFA

Intestinal epithelial cells (IECs) and immune cells have G protein-coupled receptors (GPCRs), which are activated by short-chain fatty acids (SCFAs) and which inhibit histone deacetylases (HDACs) to reduce inflammation in the intestinal mucosa. Using GPR109A in vitro and ex vivo colonic cell lines in mouse colon models, butyrate inhibits the activation of NF-κB produced by lipopolysaccharide (LPS). Furthermore, HT-29 and NMC460 colon cells are stimulated by the acetate/GPR43 pathway to undergo hyperpolarization and potassium influx, which activates NLRP3. This is in line with reports that IL-18 is activated in colonic epithelial cells from animals who were fed a high-fibre diet after developing dextran sulfate sodium (DSS)-colitis. Hence, these results demonstrate that SCFAs activate GPR109A and GPR43, which regulate inflammation and promote colonic epithelial healing. GPR109A is necessary for the interesting impact that butyrate has on MCT1 surface expression on the colonic cell line C2BBe1, suggesting that these proteins work together to mediate the effects of butyrate. (54).

When it comes to innate immune functions, SCFAs reduce inflammatory reactions in human monocytes by triggering the production of prostaglandin E2 and PTX-sensitive GPCRs that promote the expression of the anti-inflammatory cytokine IL-10. In addition to their anti-inflammatory effects via microbial breakdown of the fibers against SCFAs, dietary fibers can also have a direct impact on the gut immune system.(55)

Furthermore, SCFAs, particularly butyrate, influence the regulation of genes related to cell division, proliferation, and inflammatory response by blocking HDACs' ability to promote histone acetylation. This reduces the risk of cancer and preserves intestinal balance. Through TLR- and IFN-induced gene expression, HDACs regulate the formation of myeloid cells, innate immunity pathways, and the inflammatory response. Moreover, colonic proinflammatory cytokines (TNF-α, IFN-γ, and IL-6) are inhibited by HDAC inhibitors such valproic acid, which also lowers the severity of the illness in experimental mouse colitis. These results validate the importance of butyrate as an HDAC inhibitor and are encouraging for the search for substitutes in the treatment of inflammatory bowel disease. (56)

CONCLUSION:

Short-chain fatty acids (SCFAs) may be important for colon health as well as for the treatment and prevention of certain diseases, according to a growing body of research. The investigation of SCFAs has taken on additional

significance due to the possible advantages of prebiotics and probiotics. Dietary carbohydrates were previously investigated in isolation, ignoring the fact that the amount and type of fermentable substrates consumed in human diets varies greatly, impacting the amount and pattern of SCFA synthesis. The synergy between functional foods made from different types of carbohydrates and their effects on the generation of SCFA and general health need more study. To identify the fundamental mechanisms of action, basic research—including in vitro research—must be conducted.

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CHAPTER 3

EVALUATING CHAOS THEORY IN HEALTH MANAGEMENT

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1. INTRODUCTION

It is well known that, until recently, the mechanical management approach prevailed in organisational structures. As a result, the actions that could be taken under different evolving conditions were predictable in advance. Despite attempts to generate alternative approaches to the problems encountered in mechanical management until the last quarter of the twentieth century, these approaches are also perceived as structures similar to mechanical systems. However, due to the criticism brought by the emergence of the new concept of science, it is observed that there is a transition from the mechanical approach to the chaotic management approach (Kamacı, 2010).

This change and transformation brings unexpected results and closely affects organisations, which are indispensable elements of social life. It is observed that even the most reputable sectors can suddenly lose all their advantages, while other sectors can unexpectedly become more valuable. This situation shows that it is no longer possible for business organisations to sustain their existence and achieve competitive advantage in a simple and stable environment in the new business world (Öge, 2005).

Although the first scientist to use the term chaos was Henri Poincare in the 1900s, the studies of Edward Lorenz, a meteorologist, in 1960 were influential in the emergence of chaos theory. Working on a weather model, Lorenz entered the data into a computer environment and represented it with graphs. By chance, Lorenz predicts that if he makes insignificant changes to the system (for example, moving the results of the model to three decimal places and rounding the number 0.506127 to 0.506), there will be no change in the graphs. However, he unexpectedly encounters a completely different graph. Observing that the data form a butterfly pattern through long-term repetition, Lorenz concludes that the results of non-linear systems cannot be predicted (Öge, 2005).

Chaos theory, which focuses on nonlinear, chaotic and dynamical systems, has influenced almost every field of science in the last 20 years. Due to the chaotic structures of biological systems, the life sciences are one of the best application areas for chaos theory (Orhan, 2013). Chaos theory, which attempts to explain and interpret natural events through physical, chemical and mathematical experiments, first emerged in the natural sciences (Samur & İntepeler, 2016).

Healthy systems are thought to be in a chaotic state throughout their lives. The loss of chaotic structure leads to disease. After chaos theory, many classical ideas about health and disease became controversial (Orhan, 2013). The chaotic nature of healthcare systems in terms of patients, healthcare workers and

hospital environments, as well as the need for advanced technology, provides an area where chaos theory can be applied. In particular, chaos theory is used to explain the behaviour of chaotic systems and to understand the sources of change (Samur & İntepeler, 2016).

Learning chaos theory is important because this theory can open new horizons in understanding the underlying mechanisms of health behaviour and diseases and provide more appropriate treatments for patients (Orhan, 2013). The aim of this study is to evaluate health management from the perspective of chaos theory by explaining chaos theory. In line with this aim, chaos theory is first explained. The aspects of health care institutions that have characteristics of chaos science are evaluated. Examples of national and international studies conducted in the health sector using chaos theory are examined.

The significance of this study lies in highlighting the potential use of chaos theory in health management and how it can contribute to more effective management of health systems. Healthcare is a complex domain with many interacting variables, and is therefore fraught with uncertainty. Chaos theory provides an important framework for understanding and managing the behaviour of such complex systems. It can be effectively applied in various areas such as health policy development, quality improvement in health care, hospital management and patient satisfaction. In addition, this study provides health managers and researchers with a guide to understanding the potential of chaos theory in health management practice and how it can contribute to better management of health systems. As a result, it can improve the efficiency of health services, ensure more effective use of resources and better serve public health.

Research into the potential of chaos theory in health management can contribute to a better understanding and management of health systems. This, in turn, can lead to significant outcomes such as improved quality of care, more effective use of resources, and improved public health. By bringing a fresh perspective to the health management literature, this study can contribute to the development of the field and serve as a foundation for future research efforts.

2. Chaos Theory

Chaos theory, considered to be the foundation of postmodern social sciences, emphasises that relationships in organisations, which are chaotic systems, are non-linear and constitute a mechanism that can produce unexpected outcomes and unpredictable alternatives (Töremen, 2000). Unlike other systems, nonlinear chaotic systems involve the presence of multiple

interactions that are both ordered and chaotic. Random perturbations within these internal chaotic systems can lead to turbulence, resulting in unpredictable events and relationships that generate new patterns of change. Despite all unpredictability, consistent order always emerges and persists from random developments and chaos (Morgan, 1998).

Chaos theory is concerned with non-linear dynamics that show the probability of occurrence in very high-dimensional systems, exhibit predictable behaviour in the short term, never repeat themselves, and produce significant qualitative effects from small changes within the process (Tekel, 2006).

When chaos is mentioned, words such as anarchy and freedom may come to mind. Scientifically, however, chaos theory has nothing to do with these. Chaos theory is more concerned with questioning the order within disorder (Tosun, 2006). The term 'chaos' was first used by the scientist Henry Poincaré at the beginning of the 20th century. Poincaré conducted studies to prove whether the solar system was stable. As a result of his work, Poincaré concluded that the solution of the system of equations describing the motion of the solar system was highly dependent on initial conditions, but that these initial conditions could not be precisely determined. This finding proved that it was not possible to determine whether the solar system was stable. Poincaré used the term "chaos" to describe this unpredictability in orbit prediction (Erturk, 2012). According to Henry Poincaré, when a system consists of several strongly interacting parts, unexpected behaviour can occur. This is the starting point of chaos theory (Fettahoğlu, 2009).

Chaos theorists have been interested in events that occur when a system is dragged from a state of equilibrium within the system to a "chaos threshold", expressing how systems can transform themselves. In such scenarios there are 'bifurcation points' where systems diverge into very different futures. At these points, the energy within the system can self-organise through unpredictable leaps towards different system states. If the former dominant attractor dissipates energy and instability, potential changes can dissipate and the system can revert to a variation of its former state. Conversely, if a new attractor takes precedence, it can draw energies towards a new configuration (Morgan, 1998).

According to chaos theory, when a system begins to move beyond its current equilibrium, it is pulled in one direction by "strong attractors" (Toremen, 2000). Such a pull towards one can render the other insignificant. For example, if a student is intensely focused on studying a book in his or her room, external sounds may be reduced to an inaudible level. However, if the student pays attention to external sounds, he or she may be influenced by

these sounds and perhaps abandon his or her studies. Chaos theorists have emphasised that chaotic systems can be subject to different types of attractors.

While some of these attractors pull systems towards equilibrium or near-equilibrium states, others can push the system towards complete change (Morgan, 1998). The most famous example of the latter group is the "Lorenz attractor" (Woods & Grant, 2004).

In summary, chaos theory is a scientific approach to understanding the patterns and behaviours behind order in nature and complex systems. This theory was developed to model the behaviour of complex systems from simple mathematical equations. Chaos theory emphasises that systems are often not regular and predictable, and that even a small change can have significant consequences.

3.CHARACTERISTICS OF CHAOTIC SYSTEMS

The general characteristics of chaotic systems can be listed as follows:

3.1. Non-linearity and Uncertainty

According to Newton, the universe operates in a rational, deterministic and clockwork mechanism. Small events cause small things, while large events cause large things (Stacey et al., 2000). This idea contradicts chaotic thinking because chaos theory does not accept linearity, causality and predictability; it argues that predicting the future is very difficult due to the dynamics of chaotic systems. Therefore, uncertainty prevails in chaotic systems (Morrison, 2008).

3.2. The Butterfly Effect

One of the greatest contributions to Chaos Theory is credited to Edward Lorenz, who introduced and popularised the concept of the "butterfly effect". While inputting data into the computer to produce weather forecasts at his university, Lorenz used a different application and rounded numbers with very small differences to each other. This small difference, which amounted to a difference of one thousandth when rounded, led to different results (Ucar, 2010). Lorenz's work gained prominence after three years when it was published in a journal (Tekel, 2006). Lorenz's study showed that it would be impossible to produce accurate long-term weather forecasts due to even small changes in conditions over long-term processes (Özoran, 2017). According to the concept of the butterfly effect and the dependence on the starting point, which is one of the two fundamental points explained by chaos theory, "the flapping of a butterfly's wings in Beijing can cause a storm in New York a

month later". In other words, even an event with a very small impact, when combined with other events in a chain reaction, can affect the entire system, emphasising the crisis point that can affect various outcomes, not just negative or positive (Lorenz, 1993). Another example of the butterfly effect was demonstrated by Bishop (2017). According to him, a person walking past a building in the morning may be hit by a tile falling from the roof, resulting in death. However, leaving the house earlier or later than usual could change the time of passing and thus the consequences. This in turn would affect many subsequent events, creating another butterfly effect.

3.3. Interconnectedness and Mutual Interaction

The presence of many independent parts alone is not sufficient for the existence of a chaotic system. The parts within a chaotic system must be interconnected (Morrison, 2008). The close interconnectedness of the parts within a chaotic system indicates that a change in one part will directly and indirectly affect other parts and subject them to change (Mitleton-Kelly, 2003).

3.4. Self-Organisation

Self-organisation is the state of a system that spontaneously acquires a new order without external intervention regarding what, when and how individuals within the same system will act (Saygan, 2014). Due to the continuous interaction with the environment, open systems need to be compatible with the environment in order to maintain their existence. Through feedback, the system adapts various inputs from the environment and adjusts them to make the system compatible with the environment. As the system progresses through input-process-output, it reorganises itself by processing different information received from the environment, thus achieving harmony with the external environment. Feedback consists of two different loops: compensatory and reinforcing. Self-organisation is only possible with reinforcing feedback. In balancing feedback, chaos is an undesirable state. Its goal is to eliminate inputs that could create chaos in order to maintain the current state of the system and prevent disturbance of the equilibrium. Reinforcing feedback, on the other hand, has an amplifying effect on the deviation. In positive or negative reinforcing feedback, unexpected changes are rapidly reinforced from the moment they begin. Reinforcing feedback is dynamic and ensures that the system continuously moves to another point and reorganises itself, maintaining its existence in a new state of normality (Öge, 2005).

3.5. Impossibility of Planning, Design and Prediction

Although chaotic systems may experience turbulent fluctuations, they eventually establish a consistent order (Morgan, 1998). Therefore, in their final

states (after the chaos threshold), chaotic systems possess properties that arise spontaneously rather than those provided by traditional control mechanisms such as planning, design and hierarchy (Morrison, 2008).

3.6. Emergence

"Emergence refers to the totality of interactions between individual and small components. Therefore, in chaotic systems, it is not considered appropriate to study the components that make up the whole separately. The system cannot be reduced to its constituent parts (Morrison, 2008). This is because the whole represents a value that is different, excessive and unpredictable from the sum of its parts. What matters is the whole formed by the parts. "Emergence is related to the whole. The whole made up of interacting parts is important (Mitleton-Kelly, 2003). In conclusion, chaotic systems have a holistic character. The holistic structure results from the synergy effect. Emergence shows why the whole is more than the sum of its parts (McMillan, 2004).

3.7. Coevolution

The concept of coevolution refers to the response of variables in one system to changes in variables in another system. The reciprocal changes and evolution of the environment and the organisation express the logic of the theory (Porter, 2006). Coevolution assumes that the environment and the organisation cause changes in each other by influencing each other. In other words, instead of a one-way interaction, there is a two-way interaction. Coevolution is therefore essentially a feedback approach. As one coevolving entity influences the other, the affected entity also influences the influencer. From the perspective of the feedback approach, it is possible to say that mutual interactions form a loop (Baum & Singh, 1994).

3.8. Deviation From Equilibrium

Chaos theory includes the open system approach (Cilliers, 1998). An open system is defined as a system that exchanges energy, matter and information with its environment. The exchange of energy, material and information with the environment moves the system out of equilibrium. Chaotic systems that exchange with the environment, by adopting the logic of open systems, have the property of deviating from equilibrium (Mitleton-Kelly, 2003).

3.9. Diversity of Probability Spaces

Chaotic systems contain many interactions that are both regular and chaotic in nature. Due to the inherent chaos, small variables can lead to fluctuations. Fluctuations can lead to unpredictable outcomes (Morgan, 1998).

Therefore, systems with chaotic characteristics can diversify structurally in different ways, indicating the high probability of potential outcomes resulting from fluctuations (Mitleton-Kelly, 2003).

3.10. Threshold of Chaos

The threshold of chaos refers to the divergence point at which a system moves from disorder to order, where its order is disrupted and it loses its regularity. It is used to explain the direction in which the system changes as it moves from a state of equilibrium to a disordered state. This divergence point, which can lead the system to different states after leaving the state of equilibrium, appears at bifurcation points. A new order emerges from the complex system at the threshold of chaos (Sayğan, 2014).

The threshold of chaos lies between order and chaos, where the determined movements in the ordered structure are replaced by irregular, unpredictable movements, and the system reaches the threshold of chaos. After this point, the process of change begins, putting the system at risk. If the system has the necessary dynamic structure to maintain its continuity, it can transition to a new order; otherwise, it may be adversely affected. In a system that has become chaotic and has reached the threshold of chaos, completely uncertain, irregular and unpredictable situations occur (Berber, 2003).

3.11. Positive Feedback

Feedback can be either negative or positive. Negative feedback aims to balance, regulate and reduce the existing difference in unbalanced states (Wheatley, 2006). Positive feedback, on the other hand, aims to promote change, strengthen and increase impact (Morrison, 2008). Unlike balancing, positive feedback strengthens by increasing the difference between the components. The creation of substantial effects from small inputs indicates the presence of positive feedback in chaotic systems (Mitleton-Kelly, 2003).

3.12. Path Dependence

In chaotic chemical systems, two stable states can emerge simultaneously under the same limited conditions, a phenomenon referred to as "bistability" (Prigogine, 1987). This situation, applicable to organizations as well, implies that a change in one unit of the chaotic system results in changing another unit as well. For instance, the emergence of one technological development leading to another technological advancement can be cited as an example of "path dependence" (Mitleton-Kelly, 2003). The indirect and direct interactions of two or more evolving units causing evolutionary effects on each other are defined as path dependence (Nitecki, 1983).

3.13. Emergence of New Order

Chaotic systems spontaneously organize and form a new "order" through self-organization and the resulting self-generated cycles without any intervention (Mitleton-Kelly, 2003). In chaotic systems, tiny variables can lead to significant fluctuations (Prigogine, 1987). Despite the emergence of unpredictable outcomes due to fluctuations, the most crucial aspect is the consistent emergence of order from random and chaotic developments (Morgan, 1998).

4.CHAOS THEORY IN HEALTHCARE MANAGEMENT

The social sciences are in constant interaction with the natural sciences, with human behaviour at the heart of the social sciences. Human beings have free will, and their attitudes and behaviours will differ under the same conditions. Therefore, human beings are chaotic beings. People are multidimensional and complex beings (Mendenhall, 1999). They perceive based on their experiences, so interpretations vary. It is difficult to objectively evaluate tasks performed according to human perceptions. Since both providers and recipients of health care services are human, the likelihood that any service will result in chaotic outcomes is very high. The efficiency metrics of healthcare services are multidimensional, as are the dynamics of errors. Therefore, adopting a chaotic perspective in healthcare management can be a guide to prevent chaotic outcomes (Çıraklı et al., 2017).

Chaos theory plays an important role in the field of management. Within the framework of this theory, some guiding points for management are as follows (Morgan, 1998):

Rethinking organisations: In chaotic systems, when the threshold of chaos is reached, new orders emerge. However, these orders cannot be planned or predetermined. This situation can be unsettling for managers accustomed to traditional control mechanisms. In chaotic systems, hierarchies based on the needs of clusters and aimed at solving unexpected problems replace the top-down hierarchy. Moreover, these hierarchies can be established and directed from any point in the system.

Changing management and context: The primary role of managers is to create contexts in which self-organisation can take place. Changing the old context is necessary for innovative change to take place. New contexts can be created by developing new understandings and initiating new actions.

Creating big effects from small changes: Small but critical changes can trigger significant change. The example of the 'butterfly effect' shows that small changes can have significant effects.

Adapting to emergent situations: In chaotic systems, no one can control or shape system activities. Organisations therefore need to review their structure and adapt to change. Accepting continuous transformation as a natural process and managing change can offer significant opportunities.

These points are important in understanding the role of chaos theory in management. Hospitals, as organisations that include numerous professional groups, with a wide variety of products, under the influence of a large number of environmental factors in highly chaotic relationships, are constantly under legal obligations because of the potential for errors to result in the termination of human life. In addition, they struggle to achieve efficiency and productivity due to the intensity of technology and labour, which makes their existence precarious, especially for organisations structured in a matrix format. In particular, the abundance of actors in the environment in which hospitals operate and their power over the organisation make the health sector the most valid sector in which chaos theory applies. In addition, all these characteristics facilitate the transition of hospitals into a chaotic state. Therefore, it is necessary for hospital managers to have a holistic view of the organisation, constantly analysing environmental changes and dynamics, considering the possible impact on the hospital, positioning the organisation in the market accordingly, developing the necessary strategies and choosing to be proactive rather than reactive. Internally, they should have a structure that understands the expectations and desires of the employees and keeps their motivation high so that the organisation can easily adapt to these changes in a flexible and lean manner (Ataman, 2001).

The information asymmetry between patients and doctors creates an unusual interdependence. In general, there is a high degree of technological and professional heterogeneity in any healthcare institution, which makes it difficult to understand the organisation as a whole. In addition, the mystical nature of health services further complicates the understanding of health service management (McDaniel & Driebe, 2001).

Individuals working as part of a clinical team have unique patterns of behaviour that emerge over time as a group, with a set of rules that guide the behaviour of each individual in the group. This situation results in health services having a chaotic operational structure (Litaker et al., 2006). The current management and policy issues in health care are products of the cultural environment within health care, which makes the chaos approach useful in health care institutions (McDaniel & Driebe, 2001).

Health services are intertwined in the fields of practice, theory and education, with no sharp boundaries. In recent years, Turkey has accelerated

the transition from a "classical" structure to an "integrated" education system in health education. In terms of explaining health and disease by relating the parts to each other and emphasising the effects of the parts on each other, rather than dividing health into parts, it seems to be compatible with chaos theory (Demirsoy et al., 2001). Similarly, the chaos perspective is consistent with the 'holistic' approach of caring theories. According to this perspective, health care for an individual should not be limited to meeting only physical needs, but should also aim to meet all of the individual's needs (emotional, psychosocial, cultural, religious, etc.) holistically (Dossey & Keegan, 2009).

According to Kernick (2006), there are three key characteristics of the chaos approach to health care. First, it emphasises the avoidance of a reductionist approach to understanding the chaotic nature of health services. Second, it emphasises the importance of understanding patterns of order and how they develop and organise themselves. Third, it emphasises the importance of focusing not on the components themselves or the outcomes they produce, but on the interaction between these components.

"Sensitivity to initial conditions is a key feature of chaos theory. Our small decisions today can have significant and unexpected consequences tomorrow. If life itself and our health behaviours are chaotic, then society's health policies should, at least in part, follow a "chaotic pattern". Health policies that do not follow a chaotic pattern become disconnected from social reality over time and become meaningless. Chaos plays an important role in human motivation and behaviour (Resnicow & Vaughan, 2006).

In the context of health behaviour change, the initial conditions can be said to consist of the following: level of knowledge; current trends and mood; frequency, duration and intensity of the target behaviour; social support; social norms; genetic make-up; and numerous other psychological and environmental conditions and characteristics. A small change in these initial conditions can lead to significant changes in a person's health behaviour (either positive or negative). For example, according to the principle of sensitivity to initial conditions, a single word uttered by a health care provider or a disapproving look when prescribing medication to a patient in a clinic can cause the patient to lose trust in the health care provider, the hospital and even the medication, resulting in non-adherence to the prescription and potentially causing significant harm or even loss of life to the individual. Chaotic systems do not behave randomly. However, they can be affected by random events according to the principle of sensitivity to initial conditions. For example, suddenly giving up a bad habit after years of unsuccessful attempts or taking up a previously abandoned bad habit after years can be examples of this (Orhan, 2013).

Another characteristic of chaotic systems is the existence of "attractors". Analysing and detailing the attractors in a chaotic system makes it possible to analyse the system's behaviour and thus partially predict the future. For example, among first year nursing students attending classes taught by 10 different teachers, if two teachers stand out significantly in terms of student interest, mutual communication, modelling and other variables, these two teachers become the "attractors" of the system. The analysis of these attractors (which shows which characteristics of these two teachers are more liked by the students, or which qualities the students value in their teachers) can lead other teachers to adjust their behaviour accordingly, and the school management to organise the educational system accordingly. In this way, the quality of education and the level of satisfaction with education can be increased (Orhan, 2013).

In health care institutions, chaos can be perceived as a new opportunity, a source of motivation and professional success for some, while it can cause discord and depression for others. For example, a nurse temporarily reassigned to another clinic may see this situation as an opportunity to improve her knowledge, skills and relationships, while another nurse experiencing the same situation may become frustrated and complain. In such a scenario, the nursing manager should recognise and evaluate the probabilities and discrepancies (Çıraklı et al., 2017).

Overall, looking at the literature, as research on chaos theory continues, fractal 'signatures' of preventive health behaviours and health promotion behaviours can be identified, which may lead to a shift towards 'healthier' behaviours for individuals and society in the future (Orhan, 2013).

Self-organising structures enable organisations to act autonomously through the rules they set in relevant situations. Using a complex systems perspective and modern technologies, this autonomous structure can now be more easily modelled. As a result of this modelling, risks are reduced and responses are prepared in advance. In this way, solutions are autonomously activated when relevant problems recur. A recent example of this is the Autonomy Oriented Computing system used by all regional hospitals in Ontario, Canada, which calculates patient arrival times and waiting times for heart surgery, eliminating time problems in the healthcare system and producing evidence-based mathematical solutions. Therefore, many similar problems can be solved through such mathematical models (Tao & Liu, 2015).

The benefits of chaos theory for managers have been demonstrated by the results of studies. Understanding this theory allows managers to find more effective solutions to the problems they face. Therefore, it is important for those working in management to be familiar with chaos theory. Chaos

also plays an important role in education because the rapid pace of change in education renders traditional methods inadequate. The concept of chaos is therefore becoming increasingly important in education (Altun, 2001).

A similar situation exists in health care management. Complexity and chaos in health care systems help us to understand the structure of the system and facilitate the creation of rules for dealing with problems. The solutions proposed by healthcare professionals enable the implementation of new rules, such as emergency departments refusing non-urgent patients or charging extra fees in case of excessive congestion (Söyük & Kurtuluş, 2016).

Chaos theory provides a framework for understanding complexity and uncertainty in natural and social systems. Healthcare management stands out as an ever-changing and complex field where many variables interact. Therefore, chaos theory plays an important role in healthcare management as it can help to address the challenges of understanding and managing healthcare systems.

Chaos theory offers a different perspective for addressing the complexity and uncertainty in healthcare systems. The principles and methods of chaos theory can be used to increase the efficiency of healthcare services and manage them more effectively. For instance, studying the behavior of chaotic systems can assist in understanding the variability and uncertainty in healthcare, thus aiding in the formulation and implementation of health policies.

In general terms, the application of chaos theory in healthcare management can be summarized as follows:

Extreme Sensitivity to Initial Conditions: Chaos theory emphasizes that even the smallest changes in systems can lead to significant effects. From the perspective of healthcare management, this principle enables us to understand the complexity in healthcare systems. For example, it can help us understand how a small change in a healthcare policy or practice may result in unexpected consequences on the quality or accessibility of healthcare services. Healthcare managers, by understanding extreme sensitivity to initial conditions and evaluating risks accordingly, can make better decisions.

Fractal Structure: The fractal structures of chaotic systems represent the complexity and self-similarity of the system. From the perspective of healthcare management, fractal structures enable us to understand the diversity and uniqueness of healthcare services. For example, understanding how healthcare services exhibit self-similarity at different times and in different areas can help in more effectively allocating resources and improving services.

Strange Attractors: In chaotic systems, attractors represent specific focal points. In healthcare management, these attractors may represent significant goals, such as patient satisfaction or health outcomes, for a healthcare institution. Focusing on these attractors can help healthcare managers develop more effective strategies and direct the system towards these goals.

Chaos Patterns: Chaos theory suggests that disorder precedes order, and the universe contains numerous chaos patterns. In healthcare management, this principle helps us comprehend the complexity and diversity of healthcare systems. Each aspect of healthcare services has its own order and chaos patterns. This understanding enables healthcare managers to manage variations and uncertainties more effectively and plan systems more efficiently.

5.LITERATURE REVIEW

Upon a general review of the literature, it is observed that studies related to Chaos theory in healthcare tend to be theoretical rather than empirical, focusing on explaining the aspects of healthcare institutions that exhibit characteristics of Chaos science.

Begun and Luke (2001) examined new organizational arrangements that emerged in local healthcare service markets in 1995. The study identified market characteristics such as geographic region, population size, age distribution, minority ratio, income, education level, hospital and specialist physician ratio, and ratio of major employers as initial conditions. Begun and Luke (2001) found that changes in initial conditions influenced the formation of new organizational structures. The path of change is determined by initial conditions and feedback from newly formed organizational structures. Therefore, some experts suggest that adopting a chaos science perspective could be advantageous (Begun & Luke, 2001).

Plsek and Wilson (2001) stress the significance of relationships between parts rather than the parts themselves, and propose that minimal specifications foster more creativity than detailed plans. Treating organizations as Complex Adaptive Systems enables the emergence of a new and more productive management style in healthcare services.

Plsek and Greenhalgh (2001) argue that linear models are inadequate for coping with the increasing chaos in healthcare services. They suggest that unpredictability should be acknowledged, autonomy and creativity should be respected, and these characteristics should be utilised. Furthermore, they recommend providing flexible responses to emerging patterns and opportunities. The science of chaotic adaptive systems provides valuable concepts and tools to tackle the current challenges in healthcare services.

Clinical practice, organization, knowledge management, research, education, and professional development are interconnected and built around multiple self-adjusting and interacting systems. Due to the structures of chaotic systems, some events may remain inexplicable.

According to Haigh (2002), even a small change in the implementation of nursing services can lead to significant outcomes. Therefore, Haigh emphasizes the importance of adopting a chaotic perspective in nursing education and professional life, due to the chaotic nature of nursing services related to human-focused issues. It is predicted that adopting a chaotic perspective in nursing management can provide significant benefits.

The human body has a complex structure and generally maintains its physiological systems. However, with the aging process, the adaptability of each subsystem decreases (Peng et al., 2002).

In her study "Using chaos theory: the implications for nursing," Haigh (2002) argues that a small change in the implementation of nursing services can lead to significant outcomes. Therefore, she suggests that nursing services, which involve human subjects, are chaotic, and nurses should be equipped with a chaotic perspective in nursing education and professional life.

Resnicow and Vaughan (2006) express that fractal structures are an important feature of chaos theory and can be used to explain health behaviors. They note that although behaviors vary depending on factors such as knowledge, trend, norm, intention, there is still a "recurrence" (similarity-fractal) within an individual's behaviors and even among individuals. When this "recurrence" can be revealed, the health behaviors of individuals become predictable.

Physiological structures of the respiratory system indicate a decrease in fractal structures and adaptability due to aging. Furthermore, it is stated that many physiological data such as blood pressure, heart rate, muscle electrical activity, blood sugar level exhibit chaotic properties from a mathematical perspective and have a fractal structure (Varela et al., 2010).

Sturmberg et al. (2010) propose that redesigning the Australian healthcare system with a chaotic perspective, which places individuals and their health at the center of the system, will offer significant opportunities. The authors emphasize the importance of evaluating the healthcare system with chaotic thinking to achieve more successful outcomes.

Baghbanian and Torkfar (2012) suggest that conducting economic evaluations in healthcare services from a chaotic perspective can lead to more

accurate and meaningful results. This perspective provides a broader view of the organization and enables the development of innovative solutions.

Erturk (2012) argues that chaos does not exist within the systems themselves but rather due to limitations in human perception. In summary, systems are sensitive to both the initial variables and subsequent factors that affect them, and they continue to operate in this manner. However, humans cannot develop a comprehensive analysis method that encompasses all of these variables, so they cannot accurately predict the behaviour of systems. This is where chaos arises.

Orhan (2013) recognises that healthy systems maintain their complex and chaotic nature throughout their lifetimes. The author emphasises the importance of healthcare managers learning Chaos theory because it can open up new horizons for understanding the underlying mechanisms of health behaviour and diseases, and providing more suitable treatments to patients. Chaos theory can challenge existing assumptions about health-disease concepts and treatments, leading to more accurate solutions.

Chaos and uncertainty are fundamental characteristics of primary healthcare services. Variables in living systems have non-linear distributions, meaning that small changes can lead to significantly different behaviors. Implementing system tools can aid in comprehending chaotic problems and guide the development of solutions (Sturmberg, 2015).

Samur and İntepeler (2016) highlight the importance of nurses understanding the nature of chaotic systems and taking this into account during their assessments, given their involvement in such systems. It is suggested that nurse managers should not fear chaos, but instead work towards empowering nurses to solve their own problems within the healthcare system and promote team harmony and creativity. To achieve this, strategies such as identifying attractive elements and leveraging their effects, using feedback mechanisms, and investigating the origins of chaotic behavior should be developed. Therefore, nurse managers can also apply Chaos theory and its principles to managerial issues.

Çıraklı et al. (2017) suggest that healthcare institutions are suitable for Chaos science due to certain characteristics. These include information asymmetry between service providers and recipients in healthcare services, significant technological and professional heterogeneity in healthcare institutions, and current managerial and political issues in healthcare services being a result of the past of healthcare services within the cultural environment.

Perceptions vary based on personal experiences, therefore interpretations should be marked as subjective. Healthcare service efficiency criteria are multidimensional, as are error dynamics. It is important to be aware of nonlinear situations in healthcare management, as this can guide the prevention of chaotic outcomes (Çıraklı et al., 2017).

Khan et al. (2018) highlight the significance of viewing healthcare service structural challenges as opportunities for adaptation. This approach advocates for promoting innovative solutions to achieve positive adaptation, supporting the social system in generating and disseminating ideas that will emerge and spread within the system, and, most importantly, accepting these adaptable actions as part of the system's behaviour. Embracing uncertainty and adapting innovatively, chaos thinking can enable actors to participate meaningfully and comfortably in healthcare system transformation.

Yıldız (2020) concludes from their study that the butterfly effect, strange attractors, chaos, fractal structures, bifurcation, and self-organization play roles in the occurrence of earthquakes. It is observed that the butterfly effect, bifurcation, and chaos concepts are explanatory in the process of turning into a disaster. In the transition to a new order, the roles of strange attractors and self-organization are important. Researchers are recommended to conduct quantitative studies on the relationship between chaos theory and disasters, and it is emphasized that chaos theory approach should be included in disaster management planning.

Kuşçu and Şimşek (2020) indicate in their study that the healthcare system contains a dynamic element like humans and is actually nourished by dynamic systems despite deriving strength from linear systems. It is emphasized that human behavior, being a social entity, is generally shaped according to new paradigms. Factors such as personality, environment, ideals, ambition, hormones influence behavior, and it is stated that the deterministic approach is insufficient. In this context, it is emphasized that the human element is present at every step from planning healthcare services in healthcare institutions to putting them into operation, and therefore, the system has a "non-linear" structure.

Köse's (2023) descriptive evaluation examines the concept of the "new normal" in the uncertain and changing environment caused globally by the COVID-19 pandemic. The pandemic has created great uncertainty and change in every aspect of life, reshaping people's habits and norms. The article discusses efforts to establish a new order and create new normals within the irregularities of the pandemic process from the perspective of Chaos Theory.

6.CONCLUSION

This study highlights the significance and potential of implementing chaos theory in healthcare management. Chaos theory can aid in comprehending and managing the intricacies and uncertainties of healthcare systems. Various strategies can be developed within this framework to enhance the quality of healthcare services, optimize resource utilization, and improve public health.

This study offers a guide on how chaos theory can be applied in healthcare management practice. Healthcare managers can make more effective decisions by understanding the principles of chaos theory and evaluating its application to healthcare systems.

In conclusion, this study provides a new perspective to the healthcare management literature, demonstrating that chaos theory could be an important tool for better understanding and managing healthcare systems. Therefore, healthcare managers and researchers should consider exploring and implementing the potential of chaos theory in healthcare management more closely.

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CHAPTER 4

INTERVENTIONS TO PREVENT SUBSTANCE USE IN ADOLESCENTS

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1. Introduction

Adolescence is defined as the period of life between childhood and adulthood, encompassing individuals between the ages of 10 and 19 (WHO, 2022). Adolescence is a critical developmental stage often associated with unhealthy behaviors such as risk-taking tendencies and substance use (Gray & Squeglia, 2018).

Substance use is relatively common among adolescents, including the use of cigarettes, alcohol, tobacco, cannabis, and illicit drugs (Trucco, 2020). Substance use is recognized as a leading public health problem worldwide due to its serious health consequences, social consequences, and prevalence, especially among young people (Sunday et al., 2021). Risky behaviors such as tobacco use, antisocial behaviors, hazardous alcohol consumption, physical inactivity, and unprotected sexual intercourse usually begin during adolescence (Patton et al., 2016). It has been shown that these risky behaviors emerge with adolescence and that engaging in one behavior increases the likelihood of engaging in others. The co-occurrence of risky behaviors during adolescence may lead to substance use disorders (Tinner et al., 2022), substance-related diseases, and increased mortality rates (Alves et al., 2017).

Risky behaviors among adolescents are the leading cause of preventable deaths. It is estimated that approximately 1600 young people aged between 12 and 17 smoke their first cigarette every day, 8 million people die annually due to tobacco and tobacco use worldwide, and approximately 5.6 million adolescents alive today will die prematurely from smoking-related diseases (US Preventive Services Task Force, 2020). Substance use can also expose adolescents to short-term problems such as poor school performance (Liu et al., 2023) and truancy (Sunday et al., 2020), as well as long-term problems such as impaired neurological development by affecting brain function, cognition, attention and mood (US Preventive Services Task Force, 2020). In addition, engaging in risky behaviors involving substance use can lead to negative social consequences (Tinner et al., 2022).

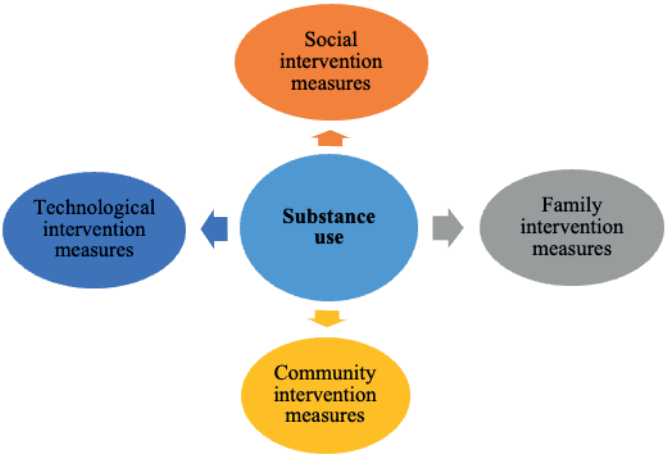
Understanding the underlying factors associated with substance use, reducing its prevalence through intervention programs, and addressing substance use-related problems among youth is critical (Sunday et al., 2023). Considering that all adolescents are at risk of substance use initiation, interventions to prevent substance use initiation should be provided regardless of the presence or absence of other risk factors (US Preventive Services Task Force, 2020).

2. Substance Use Prevention Programs

The existing literature suggests various intervention measures based on the severity of adolescents' substance use problems (Liu et al., 2023). The aim of programs to prevent substance use in adolescents is to increase protective factors, and reduce or eliminate risk (Bulut & Yesilkayali, 2020). In the literature, it is emphasized that substance use problems in adolescents may occur when various risk factors such as individual, family, school, group, and social factors come together (Liu et al., 2023). Factors that increase the risk in adolescents can be listed as being male, white race, repeating a grade (Bahar & Soyler, 2021), being from rural areas, low parental education level, family problems (Kantarci Bingol, 2022), substance use in the immediate environment (Bahar & Soyler, 2021; Liu et al., 2023), curiosity (Kantarci Bingol, 2022), childhood friends who smoke, extremely stressful life and perceiving tobacco use as low risk. Exposure to these risk factors not only causes physical health consequences (Stockings et al., 2016) but can also lead to psychological health problems such as anxiety and depression in adolescents (Cao et al., 2023). Therefore, it is important to identify substance use problems in the adolescent population as early as possible and take timely intervention measures (Liu et al., 2023). Early intervention in risk factors distracts individuals from negative behaviors and reduces substance use (Yoldas & Demircioglu, 2020).

Prevention programs are the primary step that should be implemented to evaluate leisure time activities in a specific population, mostly children and adolescents at school. In a recent study, interventions for substance use in adolescents were grouped according to their implementation topics; based on social intervention measures, based on family intervention measures, based on community intervention measures, and based on technological intervention measures (Liu et al., 2023) (Figure 1). Having intervention plans based on different topics can be an effective strategy for solving the substance use problems of adolescents.

Figure 1. Interventions for substance use in adolescents



Reference: Liu et al., 2023

2.1. Prevention programs based on social intervention measures

Broader prevention policies to change the environment in which adolescents live can be used as part of a comprehensive approach to reducing substance use (Jones et al., 2020). In this direction, it is important to introduce laws that control the sale of substances as part of social intervention measures (Haegerich et al., 2019). It is necessary to clarify the scope of legal substance use at the universal and national level, introduce minimum age limits, control substance use through taxes, reduce smoking areas, limit cigarette advertising, and adopt appropriate preventive policies. In addition, community leaders and decision-makers should encourage policies against substance use and the steps to be taken in this context can reduce substance use behavior in adolescents (Korkmaz & Simsek, 2017).

2.2. Prevention programs based on family intervention measures

Many factors such as family relationships, different parenting styles, family functioning, and limited parental supervision (Jones et al., 2020) affect substance use in adolescents. The literature reports that family members play a major role in negative substance use (Bahar & Soyler, 2021; Mahdi & Ali, 2021). The main goal of this prevention program is to reduce the risk of substance use by creating strong family relationships. In this direction, training programs or courses should be organized for parents and adolescents (Vega-López et al., 2020). Guidelines should be developed for families to help them evaluate the characteristics, behaviors, and attitudes of adolescents, and individual and family therapies should be organized (Kantarci Bingol, 2022).

2.3. Prevention programs based on social intervention measures

Community-based interventions can be effective in dealing with adolescents' negative substance use behaviors (Korkmaz & Simsek, 2017) and reduce recidivism rates (Atac, 2023). Community-based intervention plans usually require community leaders, community workers, professionals, and family and school-level stakeholders to work together (Liu et al., 2023). Promoting and highlighting non-smoking role models can increase the likelihood that young people will not choose to smoke. In particular, the attitudes of athletes, artists, and other celebrities towards non-smoking can set a positive example for young people (Kantarci Bingol, 2022). Awareness can be raised by organizing awareness-raising campaigns involving substance use for the society (Korkmaz & Simsek, 2017). Support can be provided to families and adolescents by organizing community-based smoking cessation programs that include methods such as individual counseling, group therapy, and smoking cessation medications. In addition, May 31 has been accepted as World No Tobacco Day to raise awareness in society. Therefore, as always, activities and information on the harms of tobacco use should be organized on this special day to raise public awareness.

It is also important for schools and community organizations, including primary health care providers, to collaborate on joint projects to combat substance use, develop adolescent and parent support programs, and provide more comprehensive assistance to young people and their families. To prevent tobacco use in school-age children and adolescents, primary care behavioral interventions, including education or brief counseling, were found to have a moderate net benefit (US Preventive Services Task Force, 2020). Information dissemination and awareness-raising activities on substance use can be organized at school events, community festivals, or health fairs. Training programs and seminars on substance use and coping techniques can be organized in schools to raise awareness of adolescents about substance use (Korkmaz & Simsek, 2017). In these programs, printed materials and posters can be used to prevent substance use. Adolescents can be offered services such as creating substance use cessation plans, providing emotional support, and referring to other resources if necessary. In addition, smoking cessation support groups can be established in schools. These groups can be a support and sharing platform for students who want to gain determination to quit smoking. The groups can enable students to share their experiences, cheer each other up, and cope with the difficulties they face. Schools can reduce young people's smoking habits by providing an environment where smoking is banned (Ucuncu & Dikici, 2022). Areas where smoking is prohibited can be designated and penal sanctions can be imposed on students who smoke. Schools can raise awareness among young people about smoking addiction

and encourage them to quit smoking by organizing smoking cessation weeks and activities (Korkmaz & Simsek, 2017).

In recent years, studies on substance use have emphasized interventions aimed at behavior change. In this regard, it is stated that multifaceted training can be provided in schools using curriculum interventions, exercise interventions, and peer-family-school interventions that focus on improving cognitive behavioral skills (Oesterle et al., 2018). Gomez et al. (2021) reported that the primavera prevention program was effective in reducing alcohol consumption in a randomized controlled cluster study in which they tried to improve cognitive-behavioral skills in school students aged 10-12. Brick et al. (2017) reported a significant decrease in the prevalence of smoking and drinking in an intervention study conducted using a multi-attribute benefit measurement approach in students, aiming to prevent substance use and ensure a healthy energy balance by reducing physical activity, fruit, and vegetable consumption, and sedentary behavior. Similarly, Butzer et al. (2017) in a randomized controlled study conducted on students, it was concluded that doing yoga in schools helps reduce the smoking tendency of both men and women.

2.4. Prevention programs based on technological intervention measures

Just-in-time interventions provided through mobile devices or telephone hotlines aim to help people make healthy decisions in the moment and can therefore influence health behaviors (Gunter et al., 2020). Therefore, mobile applications can be used to monitor young people's substance use habits, identify cessation goals, provide motivation, and identify triggering factors, and can be effective for substance use abstinence. The study by Haug et al. (2020) on the evaluation of the effectiveness of a just-in-time planning intervention provided via cell phone to reduce alcohol use in adolescents: a randomized controlled crossover trial showed that just-in-time interventions can be tested and implemented in the field of addiction and that digitally provided alcohol planning interventions can reduce alcohol use in adolescents who report excessive alcohol consumption. However, for these communication tools to be effective, they should focus on the right target audience and appropriate messages, and attract the attention of adolescents with constantly renewed and diversified content.

In addition, internet-based online support groups and forums enable young people to communicate with each other and share their experiences in the process of quitting smoking which can help increase their motivation (Tulucu, 2022). In addition, educational videos and interactive online resources

on substance use can provide adolescents with goals and strategies. Similarly, social media platforms can be an effective tool to provide information and raise awareness about substance use.

Additionally, mass media interventions are also one of the effective methods to prevent substance use. Informative television and radio advertisements and organized campaigns and programs can encourage the prevention of substance use.

3. Conclusion

Scientific evidence for effective prevention of substance use indicates the importance of interventions targeting risk and protective factors at the individual, family, and community levels to maximize public health impacts. More research is needed based on theoretical perspectives supporting behavioral change in youth alcohol use, with adequate intervention and follow-up periods, and using a sufficiently strong sample size in a variety of settings. Involving young people in study design and implementation can also help sustain results and translate them into effective public health policy and practice.

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CHAPTER 5

DECELLULARIZATION TECHNIQUES IN TISSUE ENGINEERING AND MEDICINE

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1. Tissue Engineering

The human body can suffer traumas, which can lead to various injuries and dysfunctions in the affected tissues and organs. Fortunately, stem cells and other vital components, such as somatic cells and extracellular matrix (ECM) elements, possess significant potential for repairing these damages. However, in cases where the trauma is severe enough to threaten the body's vitality, this potential may not be sufficient to resolve the issue. In such situations, stem cell and tissue engineering research has developed regenerative and reparative treatment approaches that provide the necessary potential to repair serious damage. These approaches combine tissue engineering techniques with stem cell-based therapies to trigger or enhance the body's regenerative power, maintain normal physiological function, and repair damaged tissues or organs.

Tissue engineering is a multidisciplinary field that brings together various disciplines, including medicine, mathematics, physics, materials science, and others, to create functional tissues using cells, biologically active signaling molecules, and scaffolds. The goal of this constantly evolving science is to develop biological structures necessary for regenerative and reparative medicine applications, as well as to replicate structures as closely as possible to those found in nature.

Stem cells are a key component of the body's self-renewal and repair mechanisms. Most of the information on their regenerative and reparative potential comes from studies conducted under classical culture conditions, which involve two-dimensional (2-D) cell culture on plastic dishes. However, these conditions are insufficient for fully mimicking the functions of stem cells in the body, as the cells grow and proliferate in a flat layer or layers and cannot fully replicate the three-dimensional (3-D) architecture of organs in terms of morphology, physiology, and histology. To overcome these limitations, tissue engineering and stem cell-based 3-D culture studies have been developed, which can better mimic many cellular functions, such as proliferation, differentiation, morphology, physiology, responses to stimuli, gene and protein expression profiles, migration capacities, and drug metabolism. This mimetic feature offered by tissue engineering science enables more potential tissue or organ design.

The three fundamental elements of tissue engineering-cells, scaffolds, and biologically active molecules-interact with each other. In tissue engineering studies, artificial scaffolds made of materials like polylactic acid (PLA), polylactic coglycolic acid (PLGA), polyethylene glycol (PEG), and polycaprolactone (PCL) are frequently employed in conjunction with

decellularized natural scaffolds. Cells such as mesenchymal stem cells, embryonic stem cells, or induced pluripotent stem cells, which are grown or differentiated on these scaffolds, can develop into cells with the correct morphology and function due to the signals they receive from biologically active molecules. These signals ultimately result in the formation of the main functional cell types of the tissue and organ to be produced. For instance, molecules like vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), and angiopoietin promote the formation of the vascular network in the scaffold (Chiu and Radisic 2010, Freeman and Cohen 2009).

The advancement of technology has facilitated the production of various biological structures for tissue engineering. These laboratory-produced functional tissues and organs have opened up new possibilities for repairing damaged tissues and organs. However, significant challenges still persist in the field of tissue engineering, particularly with regard to artificial scaffolds that do not possess the necessary vascular network and are not biocompatible. To address these issues, decellularized natural scaffolds are increasingly being utilized in research. These scaffolds are not only biocompatible but also possess an ECM and vascular network that are nearly perfect. Unlike artificially produced scaffolds, natural scaffolds also provide an architecture with an appropriate size and number of pores that facilitate cell settlement, movement, interaction, and exchange of substances. Despite these advancements, there is still a need for more information about the properties and functions of cells, scaffolds, and other biologically active molecules in tissue engineering. The production of tissues and organs that are functionally complete and intricately structured remains a distant objective.

2. Decellularization Agents

Tissues and organs primarily comprise two fundamental structures: cells, which perform physiological functions within tissues and organs, and ECM components that regulate and support cellular functions. Decellularization is a technique used in tissue engineering studies to obtain natural scaffolds. The aim of decellularization is to preserve the original architecture of the tissue by removing cells and their interactions with ECM components using various physical and/or chemical agents. This process involves not only the elimination of cells and nuclear materials but also the minimization of damage to the 3-D tissue architecture caused by the agent used. A combination of chemical and physical applications is typically more effective in preserving the integrity of the matrix and its functionality during decellularization. However, the biochemical content of the tissue plays a crucial role in determining the success of the technique.

One of the primary challenges in both xenogeneic and allogeneic transplants is the recognition of cellular antigens as foreign by the recipient's immune system, leading to an immune response within the body. ECM components, however, are conserved across species and are increasingly being studied for their potential in regenerative and reparative medicine (Exposito et al., 1992, Mazza et al., 2015). In fact, extracellular matrices from a variety of organs and tissues, such as heart valves (Schenke-Layland et al., 2003), blood vessels (Dahl et al., 2003), skin (Chen et al., 2004), skeletal muscle (Borschel et al., 2004), urinary bladder (Freytes et al., 2004), and liver (Lin et al., 2004), have been studied for tissue engineering and regenerative medicine purposes. Advancements in decellularization and tissue engineering have made whole organ decellularization a reality (Shupe et al., 2010, Guyette et al., 2014). However, challenges such as donor limitations, tissue incompatibility after transplantation, the need for lifelong immunosuppressive drug use, and graft versus host diseases make organ failure a more serious issue. Solutions to these issues include natural scaffolds obtained through decellularization and the subsequent re-equipping of the individual's own cells (recellularization). The efficacy of decellularization is contingent upon the extent to which residual biological substances persist on the scaffold. In light of decellularization's pivotal role in achieving recellularization, it is crucial to refine the technique before incorporating it into clinical practice (Brown et al., 2010). ECM has been found to possess a significant influence on cell mitogenicity (Antebi et al., 2015), chemotaxis (You et al., 2023), and cell differentiation (Xu et al., 2023). The effects of the ECM are likely attributable to its surface topography, 3-D structure, and the content of matrix proteins.

In the field of transplantation, the recognition of xenogenic and allogenic cellular antigens as foreign by the host organism can lead to either an inflammatory response or tissue rejection through the immune system. This poses significant challenges, particularly in finding suitable donors and eliciting immune responses against the transplanted biological material. However, ECM components are generally conserved across species and can be tolerated in xenogeneic transplantations, thereby avoiding immune rejection or significant inflammation (Kasravi et al., 2023). In the context of tissue and organ transplantation, it is crucial to evaluate the efficacy of decellularization methods when using tissue materials. This is a critical factor for the clinical success of such procedures. Recent advances in the decellularization process have made it possible to recellularize these materials, potentially rendering them invaluable for clinicians in the near future.

2.1. Chemical Decellularization Agents

Chemical agents such as acids, bases, hypotonic and hypertonic solutions, detergents, and alcohols are commonly used in the process of decellularizing organs and tissues. These agents are often employed in combination with

methods such as perfusion, exposure to a pressure gradient, and soaking or shaking in liquid. Decellularization by perfusion involves utilizing a peristaltic system connected to the vascular network of the tissue or organ. This method, in conjunction with the appropriate chemical enzymatic agent, has been demonstrated to effectively decellularize major organs such as the heart (Guyette et al., 2014), liver (Öztürk 2015), lung (Hoffmand et al., 2023), and kidney (Liu et al., 2015). The pressure gradient method is an auxiliary technique that can increase the success of decellularization when performed with enzymatic agents. In the soaking or shaking in liquid method, the agent used should diffuse into the tissue by diffusion and exhibit its effects. This method has been used to effectively decellularize tissues or organs such as the cornea (Wilson et al., 2016), testis (Baert et al., 2015), bladder (Yang et al., 2010), cartilage and vessels (Duisit et al., 2018), and tendons (Deeken et al., 2011) that allow for effective diffusion. It is important to note that the specific agents and methods used can vary depending on the tissue or organ being decellularized and the desired outcome. The factors that must be considered when selecting an agent and method for desellarisation include the protein, lipid, and carbohydrate composition of the tissue, whether it is layered or not, its volume, and the density of cells in the tissue. Furthermore, the histological characteristics of the tissue determine the duration of the desellarisation process. It is essential to evaluate these variables to ensure the most effective and efficient treatment.

Acids and bases are agents that initiate hydrolytic degradation in the cytoplasmic components of cells as well as in nuclear materials such as DNA and RNA. Chemical agents such as acetic acid, hydrochloric acid, sulphuric acid and sodium hydroxide, ammonium hydroxide are highly influential for the disintegration of cell membrane and organelles (Falke et al., 2003). Peracetic acid is structurally effective for the decellularization of thick and non-dense tissues and is a widely used agent for the sterilisation of the decellularized matrix even at low concentrations such as 0.10-0.15% (Hodde and Hiles 2002). Whilst the use of bases for decellularization gives effective results for tissues such as the dermis, it is also very limited due to the damage it causes to the 3-D architecture of the tissue. In other words, bases such as sodium hydroxide and ammonium hydroxide are not preferred for decellularization due to the damage they cause to collagen fibrils and glycosaminoglycan (GAG) molecules and the removal of growth factors in the matrix along with the cells (Reing et al., 2010). Ionic detergents such as Triton X-100, sodium dodecyl sulphate (SDS), sodium deoxycholate and anionic detergents such as Triton X-100 are widely used for decellularization. Detergents are highly effective agents in the dissociation of cellular and nuclear structures from each other and from the ECM and in the denaturing of proteins. Decellularization with detergent carries a certain risk in terms of loss of GAG and collagen constituents in

the extracellular matrix. In this respect, the type, amount, application time, tissue volume and molecular content of the detergent to be used are the most important factors affecting the success of decellularization and the integrity of the ECM (Rieder et al., 2004, Hudson et al., 2004, Cebotari et al., 2010). Unlike Triton X-100, SDS is a more potent chemical agent for decellularization of tissues such as kidney, liver and heart and removal of cellular debris from the matrix (Uygun et al., 2010, Cebotari et al., 2010). Although it ensures an effective decellularization, it is also acknowledged that SDS damages the natural structure of the tissue during the decellularization process and leads to a decrease in GAG concentration and loss of collagen integrity. Therefore, it is intended to reduce these detrimental effects to a minimally significant degree by decellularization achieved with low concentrations of SDS (Gilbert et al., 2006).

In addition, hypertonic and hypotonic solutions are also used in the process of decellularization of tissues or organs because they disrupt DNA-protein interaction and cause osmotic shock in cells. Hypertonic and hypotonic solutions, which can be used as an auxiliary agent in the removal of lysed cells from the extracellular matrix, can show an effective result when used sequentially and in combination with other decellularization agents. Alcohols function as auxiliary agents in the process of removing water from tissues and breaking down lipid-based cellular components (Prasertung et al., 2008; Flynn et al., 2010). Although hypotonic and hypertonic solutions, as well as alcohols, can cause harm to the ECM to some degree, like other chemical agents, it is crucial to optimize their use in terms of quantity and duration for successful decellularization.

2.2. Physical Decellularization Agents

Physical agents, such as those used in freeze-thaw, pressure application, sonication, and agitation, are commonly utilized in conjunction with other agents to facilitate desellarisation. Applications of physical agents for desellarisation include freeze-thaw, pressure application, sonication, and agitation. During the freeze-thaw process, the formation of intracellular ice crystals leads to the breakdown of membrane structures and cell lysis. It is important to control temperature changes during this process, as the formation of ice crystals can also pose a risk to the extracellular matrix's microarchitecture. Additionally, the removal of cellular debris from the environment is required when using the freeze-thaw technique for desellarisation. This technique has been used to desellarise structures such as tendons, ligaments, and nervous tissue. The pressure application method is another auxiliary technique used for cell disruption, but it is not suitable for desellarisation due to its potential to cause high damage to the ECM and is

therefore not recommended for organs or tissues with tight connective tissue. Physical agents such as mechanical agitation and sonication are often used in combination with chemical agents to remove cells from tissues.

2.3. Enzymatic Decellularization Agents

Enzymatic decellularization, a highly effective method for removing cellular and nuclear components from the matrix, employs enzymes such as lipase, endonuclease, exonuclease, and trypsin (Kasimir et al., 2003). While this method can be effective, it may not always yield optimal results on its own. Therefore, it is necessary to combine it with other agents to achieve optimal outcomes. The use of enzymatic agents can impact cellular and nuclear structures, as well as protein, lipid, and carbohydrate-derived components in the extracellular matrix. Therefore, the duration of enzyme treatment of the tissue is crucial in determining the success of decellularization. Inadequate enzyme treatment may not provide sufficient cellular and nuclear purification, while excessive treatment can cause undesirable disintegration of ECM components such as laminin, fibronectin, elastin, and GAG. Additionally, the optimal temperature required for the enzymes to function is a critical factor in achieving successful decellularization with enzymatic agents. Moreover, the utilization of protease inhibitors such as phenylmethylsulfonylfluoride (PMSF), aprotinin, and leupeptin, in conjunction with enzymatic agents, is essential for protecting the protein components of the ECM from being degraded by proteases released from lysed cells.

2.4. Evaluation of Decellularization Quality

The methods and agents used to remove cells from the tissue during the decellularization process cause certain structural alterations in the 3-D architecture of the extracellular matrix. Effective and successful decellularization can be accomplished by the combination of physical, enzymatic and chemical agents. In general description of decellularization protocols, the process involves lysis of cells using ionic solutions or physical treatments followed by removal of cellular and nuclear components from the tissue using enzymatic agents and detergents. Chemical and biological agents used in the process of decellularization of organs and cellular residues in the matrix after the process cause the development of immunological responses and decrease in biocompatibility. Consequently, it is crucial to ensure that the ECM obtained after decellularization is biologically acceptable. For this purpose, the cellular elements such as DNA, protein and phospholipids in the matrix can be examined to obtain information about the success of decellularization. In addition, the techniques used for decellularization are not completely effective in removing these cellular elements from the

extracellular matrix. Nonetheless, there are also several criteria indicating that the effectiveness of decellularization is sufficient. In accordance with the aforementioned criteria, the amount of double-stranded DNA present in 1 mg of ECM after decellularization should not exceed 50 ng. Additionally, the length of DNA fragments should be less than 200 base pairs, and no nuclear structures should be observable in immunofluorescence or histochemical staining using 4',6-diamino-2-phenylindole (DAPI) or haematoxylin & eosin (HE). The amount and length of DNA residues in the decellularized ECM can be easily determined by visualizing gel electrophoresis following polymerase chain reaction (PCR) with agents such as propidium iodide or ethidium bromide. It is essential that all three detection methods for evaluating decellularization efficiency correlate with one another (Crapo et al., 2011).

In order to ensure the safety and efficacy of ECM derived from decellularization, it is essential to assess its sterility prior to its incorporation into in vitro culture systems or in vivo transplantation studies. While chemical and biological agents may be used to remove endotoxins and pathogenic structures from the tissue, it is crucial to select an appropriate sterilization method that does not compromise the integrity of the ECM in terms of micro and nano-architectural features. Certain sterilization techniques, such as the application of acids, ethylene oxide, and gamma irradiation, may cause damage to the extracellular matrix. Therefore, it is critical to carefully evaluate the sterilization agent used to avoid compromising the structural properties of the extracellular matrix. Supercritical carbon dioxide, a recently developed method, presents an alternative to traditional ECM sterilization techniques. This method has been found to result in minimal alterations to the extracellular matrix, making it a promising option compared to other sterilization techniques (de Wit et al., 2023).

Peracetic acid (PAA) sterilization, a recently implemented technique for the sterilization of tissue scaffolds, exhibits a remarkable ability to penetrate even mold and bacterial spores, ensuring highly effective sterilization of biological materials. In recent years, PAA has emerged as a widely used sterilization method in tissue engineering experiments involving natural scaffolds, as it can provide highly effective and rapid sterilization without promoting bacterial or fungal growth even at low concentrations. PAA's effectiveness is not hindered by low material densities, making it ideal for preserving the microarchitectural structure of the extracellular matrix. Furthermore, the use of PAA offers researchers a cost-effective, efficient, and convenient sterilization procedure (Yoganarasimha et al., 2014; Kajbafzadeh et al., 2013).

3. Decellularization Methods

3.1. Whole Organ Perfusion Method

In light of the advancements made in decellularization, whole organ perfusion systems are a relatively modern method. By preserving the extracellular matrix's 3-D structure very well, perfusion systems that utilize the organ's vascular system facilitate highly efficient decellularization. Perfusion systems have been effectively employed in the decellularization of organs, including the heart (Zubarevich et al., 2023), liver (Tomofuji et al., 2023), lung (Narciso et al., 2022), and kidney (Diedrich et al., 2024).

The process of whole organ perfusion decellularization is highly dependent on the selection of the vessel to which the peristaltic systems are connected. It is imperative that the chosen vessel is able to ensure the complete removal of cells from the tissue or organ being decellularized. The vessel must also be of a size that is compatible with the perfusion system used. A thorough understanding of the histological properties of the tissue being used is essential, as it is from this tissue that the ECM will be derived. Additionally, the device used in the process must be capable of preventing the entry of air and foam into the tissue, as the presence of air in the tissue can obstruct the vascular system and negatively impact the desellularization process. It is recommended that detergents, such as SDS and Triton X-100, be used as agents for perfusion decellularization, as they are commonly used and have been shown to be effective (Uygun et al., 2010, Singh et al., 2023).

3.2. Pressure Gradient Method

The utilization of a pressure gradient across tissue is particularly advantageous in enzyme-based desellularization applications, as it ensures superior preservation of the tissue's microarchitecture (Prasertsung et al., 2008). This was demonstrated through the measurement of hydroxyproline content, which revealed that flow with temperature modifications resulted in less collagen degradation compared to agitated flow. Bladder decellularization, for instance, employs the pressure gradient method (Schmitt et al., 2016). During this procedure, it was previously established that collagen type 1, collagen type 4, laminin, and GAG were not adversely affected by the decellularization process.

3.3. Soaking in Liquid and Shaking Method

Decellularization of tissues and organs can be achieved through the use of a liquid containing decellularization agents, which can be applied by immersion or agitation. The corneal decellularization method (Hao et al., 2023). However,

it is important to note that this method may not remove all cells from the tissue, and certain tissues may be more suitable for decellularization than others. Ideal tissues for decellularization are those that are sparsely populated with cells, possess a well-developed vascular system, and lack impediments to the diffusion of decellularization fluid. This method can be used alone for certain tissues, or in conjunction with other decellularization methods for more complex tissues. To date, decellularization of various tissues has been achieved through the use of liquids containing decellularization agents, including skeletal muscle and tendons (terrie et al., 2024), blood vessels (Zhang et al., 2024), heart valves (Ramm et al., 2021), cartilage (Lee et al., 2023), esophagus (Godefroy et al., 2023), trachea (Bergman et al., 2024), bladder (Xiao et al., 2022), and cornea (Kang et al., 2023). The length of these processes depends on factors such as the degree of tissue agitation, the thickness and density of the tissue, and the type of decellularization agent utilized. Tissues like the bladder can be removed quite easily, while denser tissues like dermis, tendons, and trachea may necessitate a more lengthy procedure that could take days or even months. The employment of detergents, enzymes, and alcohol in various combinations is typical in these methods.

4. Decellularization Based Tissue Engineering in Medicine

Decellularization-based tissue engineering is a highly innovative approach in modern medicine that has significantly impacted the field of regenerative therapies. The process involves the isolation of cellular components from tissues, all while retaining the ECM, which subsequently acts as a platform for the reintroduction of cells and the reconstruction of tissue. The adaptability and promise of decellularization-based methods are demonstrated through a wide range of applications in various medical fields. Regarding the advantages of using decellularization-based methods, they offer several potential benefits over traditional tissue engineering approaches. For example, decellularized scaffolds can be used to regenerate a wide range of tissues and organs, including bone, cartilage, heart muscle, and skin. Decellularized dermis has the potential to revolutionize the field of skin regeneration. In the treatment of burns and chronic wounds, this deeper layer of skin functions as a scaffold, supporting the growth of new skin cells and promoting faster healing. Additionally, it can reduce the appearance of scars and constitute the deeper layer of skin (Dussoyer et al., 2022) Facial reconstruction may be accomplished by utilizing decellularized facial tissue, which can be employed to reconstruct various facial features, including noses and ears, in the event of injury or surgery (Vyas et al., 2023). The integration of decellularized meniscus tissue may prove advantageous in the restoration or substitution of damaged menisci within the knee joint, thereby enhancing joint functionality and mitigating discomfort (Huang et al., 2023).

In a similar manner, decellularized articular cartilage may be utilized for addressing cartilage deficiencies in joints, potentially deferring or obviating the need for joint replacement surgery altogether (Khakpour et al., 2023). In the context of bone regeneration, the utilization of decellularized bone matrix may serve to stimulate bone growth and fusion following spinal surgery (Ma et al., 2024). Furthermore, the employment of decellularized bone chips may address bone defects resulting from fractures, thereby facilitating bone healing. Decellularized heart valves and blood vessels represent a promising alternative to mechanical valves and grafts for cardiovascular repair and replacement. Decellularization involves removing cells from the tissue, leaving an acellular scaffold that can be repopulated with the patient's own cells. This approach offers a more biocompatible and potentially longer-lasting solution than mechanical valves or synthetic grafts (Chen et al., 2023). Furthermore, decellularized blood vessels can be utilized to restore blood flow and prevent complications in damaged vessels (Ho et al., 2022). Decellularized ECM has several potential applications currently under investigation, including the regeneration of liver and kidney tissues. This involves utilizing decellularized tissues for targeted and sustained drug delivery, as well as creating in vitro disease models to study conditions and test treatments. In dentistry, ECM derived from teeth has shown promising results in the regeneration of bone and dental tissues.

Additionally, because the scaffold is already formed and has the necessary structural properties to support cell growth, it can be used to speed up the tissue engineering process and reduce the need for expensive and time-consuming tissue culture. Decellularization has been applied in various medical fields. For example, decellularized heart valves are currently utilized in clinical settings to replace diseased or damaged valves. Similarly, decellularized bone matrices are employed to stimulate bone growth in orthopedic procedures. Furthermore, decellularized tissues have been used to create new skin for burn victims and to repair damaged corneas. The applications of decellularization are extensive and continue to be explored in regenerative medicine. This technique has enabled the development of innovative approaches for tissue reconstruction while also addressing the shortage of donor organs for transplantation. The potential of decellularization in addressing organ shortages in transplantation is a promising area of ongoing research. Tissue engineering based on decellularization holds great potential in resolving various medical issues. The potential of this technology is quite promising and holds great promise for the future of healthcare. Tissue engineering based on decellularization is a rapidly advancing field with the potential to revolutionize various aspects of medicine. Additional research is expected to lead to even more innovative applications of this technology in the near future.

5. References

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CHAPTER 6

RELATIONSHIP OF FETAL STEM CELLS WITH IMMUNE SYSTEM

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Introduction

Stem cells are cells that maintain their cellular identity during their proliferation, differentiate and divide asymmetrically to renew themselves. Microchimerism (Mc) is defined as the presence of a small group of cells originating from another individual and genetically different from the cells of the host individual. During pregnancy, stem cell exchange takes place physiologically between the mother and the fetus via the placenta (1). In this way, two cases of microchimerism occur, namely FMc and maternal microchimerism (MMc). This cell traffic can be unidirectional or bidirectional. In one-way transfer, nutrients, water, electrolytes, oxygen, hormones and immunoglobulins pass from mother to fetus, while carbon dioxide and catabolism products pass from fetus to mother (2). In the bidirectional transition, cells and DNA material exchange occurs between the mother and the fetus (2). In mammals with placenta only, this reciprocal transfer of cells results in a higher rate of fetal cell transfer to the maternal body (3). FMc is defined as the presence and continuity of fetal cells in maternal tissues, and MMc is defined as the presence and continuity of maternal cells in fetal tissues. Although Mc is accepted as a physiological event, it has raised doubts about McCs existence and role in diseases in recent years (4-8). Although McC is accepted as a physiological event, the presence of McC in diseases in recent years has raised doubts about the role of these cells. FMcCs have been the subject of research since the early 1900s. In 1893, he first identified fetal cells in the lungs of women with eclampsia. Later, in 1960-1970, fetal hematopoitic cells were reported to be found in healthy and sick women (9). In 1981, the discovery of fetal cells located in mother mouse tissues encouraged research on this subject.

It has been claimed that FMcCs may be associated with cooperation or conflict of interest between male-female and mother-fetus (1). Some researchers have suggested that transfer of fetal cells has conflicting interests between male and female, giving them an adaptive and selective advantage (10). However, it shows that stem cell exchange between fetus and mother leads to cooperation between mother and fetus (1). FMc can guarantee fetal survival and improve maternal health, thereby increasing the health/life of both (11). These cells can cause postnatal changes in maternal physiology, lactation, thermoregulation, maternal affection, and neural plasticity, and only increase fetal fitness (12). However, it suggests that FMcCs may have a possible neutral function and be a pregnancy byproduct or residue with no biological value (1). High rates of FMcCs have been identified in maternal placental pathologies, fetal aneuploidies, preeclampsia, premature births or miscarriages, and placental complications (1). FMcCs have been found in maternal tumor sites such as colorectal cancer, breast cancer, thyroid cancer, melanoma, cervical cancer, lung cancer, bladder cancer, pancreatic cancer, and lymphoma, and it

has been suggested that they may have contradictory roles (17-21). It has been suggested that these cells may be beneficial, harmful or neutral for maternal physiopathology (20). FMc may have a beneficial, protective and regenerative role for maternal health. They participate in tissue repair, regeneration, cell therapy and maintenance of maternal internal balance (1). For example, the presence of FMcCs in inflamed maternal tissues suggests that they are involved in angiogenesis and healing processes (21). In lung cancer, women with one pregnancy and two or more pregnancies have a better prognosis than women and men who have never given birth. This suggests that FMcCs may suppress maternal tumor development (22). The presence of FMcCs are also associated with a lower risk of breast and bladder cancer (23). Conversely, FMc may also have a detrimental role on maternal health. FMc was found in many women with post-pregnancy autoimmune diseases (24). Since the embryo is a semi-allogeneic organism that can be rejected by the mother's immune system, they were considered alloimmune diseases (25). Other studies include women with cancer (13), women with autoimmune diseases such as systemic sclerosis, rheumatoid arthritis (14) or Sjogren's syndrome, and It has been found frequently in non-autoimmune pathologies such as hepatitis C (1,14).

The Effects on immune system and autoimmune diseases

Autoimmune diseases are a heterogeneous group of diseases and are characterized by a pathological response to one's own tissue (26). Since most autoimmune diseases are common in women, the frequency of some autoimmune diseases increases after pregnancy and resembles chronic graft versus host disease, it was thought that Mc may play a role in the pathogenesis of these diseases. The hypothesis that FMcCs play a role in autoimmune diseases was first put forward in 1996 (27). Genetic factors, environmental factors, toxic conditions, immunological suppression, trauma, preeclampsia and other diseases can be counted among the possible determinants of McCs migration (26). It is possible that some factors affecting the so-called maternal tolerance are directing chimeric cell traffic. Pregnancy is a physiological condition characterized by the tolerance of the maternal immune system against the antigens expressed by the fetüs (28). During normal pregnancy, the semiallogeneic embryo performs strategies and organizes to evade the attacks of the maternal immune system. This situation, which is called maternal tolerance, is predominantly an event related to the placenta and the cells called trophoblast in it (28). As a result, for the continuation of a successful pregnancy, either the maternal immune system is prevented from perceiving the fetal tissues as foreign or the attachment of maternal immune system cells to fetal cells in order to develop an immune reaction is prevented. The fetus, which is in immunological balance during the intrauterine life period, may be exposed to some negativities. Any systemic disease of the mother, toxins, drugs, and especially often infections may adversely affect the balance between mother

and fetus and move microchimeric cell traffic from normal to abnormal (29).

FMcCs were found in 31% of bone marrows, 39% of thymuses, and 15% of spleens at 14-17 weeks of gestation in mouse models. FMcCs in bone marrow and spleen expressed IgM and CD19. This showed that these cells could be B lymphocyte precursor cells. FMcCs localized in the thymus and spleen expressed CD3, CD4 and CD8. This suggested that these cells might be functional T lymphocyte precursor cells with antigen-specific allogeneic activity (expression of T cell receptors+ (TCR+)) (30,31). Presence and survival of FMcCs in the bone marrow in the first 3 months after birth expressed ITGAM, ENG, and PTPRC. It was reported that the presence of FMc in the blood 3 months after birth, these cells remained alive 17-18 days after birth and continued for up to 3 months, and their number decreased significantly in the days after birth (32). FMcCs were identified in 5 of the six metastatic lymph nodes (83.3% of cases). 50% of these cells expressed the CD45 marker (33). In monkeys, 3 years after birth, 50% less frequency of chimeric cells was found in the lymph node and spleen of females. These cells expressed CD34 and may be a source of stem cells. This suggests their capacity to contribute to different cell lineages (34). FMH was found in the spleens and lymph nodes of two women, and these cells expressed 90% of the CD45 marker. The possible relationship between FMcCs and autoimmune diseases has been suggested because of its high incidence in post-pregnancy women with this type of pathology. FMcCs have been found in women with systemic sclerosis or scleroderma, rheumatoid arthritis, systemic lupus erythematosus, Sjogren's syndrome, primary biliary cirrhosis, Grave's disease/hyperthyroidism, and Hashimoto's or hypothyroidism (14, 35-37). FMcCs can trigger Graft-versus-host disease (GVHD) by activating and regulating maternal immune responses. It may even participate in the suppression of immune tolerance, which is necessary for the continuation of pregnancy. However, the exact mechanisms in this regard are unknown (15). Fetal cells can differentiate into active T lymphocytes by developing an autoimmune response against maternal tissues (GVHD reaction). This response includes cytotoxic or helper fetal T lymphocytes in the presence of FMcCs.

Systemic Sclerosis

An association has been found between systemic sclerosis or scleroderma (SSc) and FMc. A higher number of fetal cells were found in SSc compared to controls (38). These cells have been reported to carry lymphocyte markers such as FMcCs, CD3, CD19, CD14 and CD56/16 and can persist up to 38 years after birth (39). In addition, higher frequency of fetal lymphocyte cells (CD3+) were found in the blood of women with SSc (46%) compared to healthy women (4%). These cells (56%) in the inflamed skin tissues of women with the disease and were able to act as functional T lymphocytes specific to allogeneic antigen (maternal) (40). FMcCs may be involved in the pathogenesis of the disease

by triggering a host reaction to a vaccine (41). FMcCs were found in women with SSc, particularly in lung, skin, spleen, lymph nodes, and adrenal gland tissues. However, it was not found in samples of women who died of pancreatic or non-autoimmune pathologies (42). These cells can cause autoreactivity by producing high concentrations of interferon-gamma and interleukin 4 when reacted with maternal antigens MHC (Major Histocompatibility Complex), which supports the hypothesis stated above (43). In other studies, FMcCs were detected only in the blood of women with SSc compared to controls (41). It has been suggested here that fetal cells are not the cause of maternal autoimmune diseases, but may be one of the factors that increase the ability to develop these pathologies, partly because they are foreign cells and contribute to maternal inflammation and autoimmunity (44). It has been reported that male DNA levels are found to be significantly higher in the blood of female patients with systemic sclerosis compared to the control group (45). In autopsies of patients with systemic sclerosis, cells carrying Y chromosome were found most frequently in spleen sections and less frequently in lymph node, lung, adrenal gland and skin sections (42). In patients with systemic sclerosis, maternal DNA was shown in 66.7% of the patients, and the frequency of maternal microchimerism was found to be higher in women with systemic sclerosis (72%) than in controls (22%) (46). It has been reported that patients diagnosed with systemic sclerosis before pregnancy have a more severe clinical course than patients diagnosed with post-pregnancy (40).

Autoimmune Thyroiditis

The more frequent occurrence or exacerbation of thyroid disorders in women, especially in the postpartum period, makes its relationship with microchimerism worth investigating. Therefore, it was investigated whether there is a relationship between FMcCs and thyroid disorders. As a matter of fact, while fetal cells were found in the thyroid sections of patients with thyroid disorders, they were never found in controls. It has been reported that FMcCs are not limited to inflammatory thyroid disorders, but are present individually or in clusters in all thyroid disorders (48). In a patient with progressive goiter, fully differentiated male thyroid follicles were observed, closely attached to the thyroid and indistinguishable from the remaining thyroid tissue. According to these findings, it was concluded that there is a relationship between thyroid diseases and FMcCs. It has also been suggested that fetal stem cells may have the capacity to differentiate into mature thyroid follicles under appropriate environmental and developmental factors in their mothers (48). It is well known that Hashimoto's disease, also defined as chronic autoimmune thyroiditis, is more common in middle-aged women and has a tendency to exacerbate after delivery. FMcCs were identified 12–46 years after birth in approximately 50% of these female patients. Here, it has been suggested that fetal microchimeric cells located in the thyroid tissue of the mother cause chronic inflammation

and hormone secretion by causing an intrathyroid graft-versushost reaction, and thus may play a role in the etiology of Hashimoto's thyroiditis (49). Contrast to women with adenoma (0%), FMcCs were subsequently found only in thyroid biopsies (19%) of women with Graves' disease (37). A higher FMcCs rate was found in thyroid biopsies of women with Hashimoto's thyroid (60%) and Graves' disease (40%) compared to thyroid biopsies (22%) of women with follicular adenoma. All thyroid biopsies (100%) of women with Hashimoto's thyroid had a low frequency of chimeric cells (15 out of 4900 fetal cells per 100,000 maternal cells, absent in healthy women). FMcCs were detected in 1 (6%) of 18 nodular goiters analyzed with a frequency of 182 fetal cells per 100,000 maternal cells (18).

Systemic Lupus Erythematosus

A relationship between FMcCs and systemic lupus erythematosus (SLE) was defined. However, no significant increase in circulating FMc was found in women with SLE (0%) compared to controls (20%) (45). A higher frequency of FMcCs were detected in the blood of women with SLE (68%) compared to healthy women (33%). A higher frequency of FMcCs were also discovered in women who had more children. This suggested that these cells could proliferate in maternal tissue (50). Similar results were obtained in kidney samples from women with SLE (26%), and these cells were located in the renal glomeruli compared to controls (55%) (51). FMcCs were identified in the blood of healthy and sick women with and without boys. A higher rate of FMcCs were found in the blood of women with male children. This was achieved when women with SLE (26%) were compared with SSc (22%) and controls (16%). It was found that women without sons had higher FMcCs counts than those with SSc (33%), SLE (23%), and controls (0%). The exact origin of these chimeric cells is unknown. No significant difference was found in the frequency or number of FMcCs in the blood of women with SLE (50%) and healthy women (50%). Regarding fetal cell count, 2.4 cells per 100,000 maternal cells were detected in the blood of women with SLE and 2.5 cells in healthy female samples (47). The autopsy of a female patient who died from complications of SLE showed Y chromosome-bearing cells in all histologically abnormal tissues, but not in normal tissues (53). At the same time, the McCs ratio in SLE cases was the same (50%) as in the control group (47). The presence of maternal microchimerism has been demonstrated in tissue and blood in children with neonatal lupus and congenital heart block, suggesting that this may have a role in the pathogenesis (54).

Rheumatic Joint Inflammation

It detected higher fetal DNA levels in the third trimester of pregnancy in women with rheumatoid arthritis (RA) who had recovered. A possible relationship between the DNA level and the pathogenicity of the disease has

been reported in women suffering from RA during pregnancy (36). Compared to healthy women (6%), DERAA -/- detected more fetal cells in the blood of women with RA (53%). It has been suggested that these cells may contribute to the pathogenesis of the disease by increasing the autoimmunity of mothers with RA by activating CD4+ T lymphocytes against maternal joint antigens (55).

Sjogren's Syndrome

Women with Sjogren's syndrome (SS) (33%) were not found to be significantly increased in circulating FMcCs compared to controls (20%) (56). FMcCs were not found in the salivary glands of women with SS nor in controls (57). FMcCs were identified for the first time in salivary gland biopsies (36%) and bronchoalveolar lavage fluid samples (22%) from women with SS. However, these cells were not found in blood samples (58). A higher frequency of fetal cells was observed in salivary gland biopsies of women with SS compared to controls (13%). However, no significant differences were found in the blood samples of these patients (33%) compared to controls (25%) (54).

Primary Biliary Cirrhosis

Primary biliary cirrhosis; it has attracted attention in terms of microchimerism because it is common in women and because chronic graft versus host disease resembles liver involvement. Most liver biopsies of patients with biliary cirrhosis show male DNA. Although FMc was found to be higher than controls, no difference was found between primary biliary cirrhosis and other liver diseases in terms of microchimerzyme (47). FMCs were first identified in liver biopsies of women (70%) and controls (72%) with primary biliary cirrhosis (PBC) (47). No fetal cells could be found in liver biopsies of women with PBC or chronic hepatitis C. He then detected a higher percentage of FMcCs in the blood of PBCs (45%) compared to controls (25%). However, no significant difference was found between the liver tissues of women with this male chromosome pathology and controls. While we found chimeric cells expressing the CD45 lymphocyte marker in liver biopsy (42%) of women with PBC, these cells were not detected in liver biopsies of women with chronic hepatitis C or alcoholic liver disease. In the blood of a healthy woman (6%), 1 chimeric cell was defined for every 106 maternal cells of FMCs and it was suggested that chimeric cells may be involved in the pathology (60).

CONCLUSION

During pregnancy, reciprocal cell exchange and exchange occur between the mother and the fetus, and fetal cells are seen in animal models and humans after birth. FMcCs, cardiomyocytes, neurons, endothelial cells, hematopoietic cells and lymphocytes proliferate in maternal tissues and differentiate into special stem cells. The short or long-term stay of these cells in the mother's body

and whether they are exposed to inappropriate effects has led to a great debate. In other words, the functional role of the mother in the pathophysiology has been the subject of debate. By interfering with events such as FMcCs tissue repair, angiogenesis or neurogenesis, they can be a source of progenitor cells that have a beneficial effect on maternal health. However, it may also have a harmful function by activating the immune response in the host, contributing to the emergence of autoimmune diseases. FMcCs have been associated with autoimmune tolerance and tissue repair, as well as with autoimmune diseases and cancer. In addition, it should be known that different pregnancy and delivery complications such as preeclampsia, third trimester bleeding and cesarean section may change the functional fate of FMcCs. Its investigations in different organs, tissues and species are important to understand the role of FMcCs in human health and disease. Understanding the properties of FMc, understanding the development of different pathologies such as autoimmune diseases, may be key to develop effective and specific therapeutic strategies. At the same time, today the presence of FMcCs and fetal DNA in the central nervous system in cases of postpartum psychosis, and the indicators of the immunological response likely to develop against the current microchimeric state (cytokines, autoantibodies, changes in white blood cell distribution, etc.) should be investigated.

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