Current Researches in Health Sciences-II

Editor: Dr. Enes Karaman • Dr. Gözde Özge Önder

ÖZGÜR YAYINLARI

Current Researches in Health Sciences-II

Editors

Dr. Enes Karaman

Dr. Gözde Özge Önder

ÖZGÜR YAYINLARI

Published by
Özgür Yayın-Dağıtım Co. Ltd.
Certificate Number: 45503

15 Temmuz Mah. 148136. Sk. No: 9 Şehitkamil/Gaziantep
+90.850 260 09 97
+90.532 289 82 15
www.ozguryayınlari.com
info@ozguryayinlari.com

Current Researches in Health Sciences-II

Editors: Dr. Enes Karaman • Dr. Gözde Özge Önder

Language: English
Publication Date: 2023
Cover design by Mehmet Çakır
Cover design and image licensed under CC BY-NC 4.0
Print and digital versions typeset by Çizgi Medya Co. Ltd.

ISBN (PDF): 978-975-447-651-4

DOI: https://doi.org/10.58830/ozgur.pub128

Suggested citation:
Karaman, E. (ed.) & Önder, G.Ö. (ed.) (2023). *Current Researches in Health Sciences-II*
DOI: https://doi.org/10.58830/ozgur.pub128. License: CC-BY-NC 4.0

The full text of this book has been peer-reviewed to ensure high academic standards. For full review policies, see https://www.ozguryayinlari.com/

ÖZGÜR YAYINLARI

Preface

Nowadays, at the point reached in the field of health, a lot of information is rapidly emerging, changing and needs to be known. In addition, it is very important for many people to access this up-to-date information quickly. In this context, it is necessary to follow up-to-date information. Although the information mentioned in the book are current issues, each of them is supported with appropriate visuals to make it easier for the readers to understand. In addition, the chapters have been kept as short as possible in accordance with the learning objective.

We would like to thank and present our respects to the authors of the chapters who contributed to the preparation of this book, believing that it will contribute to the students of medicine, dentistry and biology, as well as to those related to the field of health, since this book is easy to understand.

Dr. Enes Karaman

Dr. Gözde Özge Önder

Contents

Chapter 1

Exploring the Potential Therapeutic Approaches of Mesenchymal Stem/Stromal Cells (MSCs) in the Treatment of Vaginal Candidiasis

Mesude Bicer[1]

Abstract

Over the past few decades, there has been significant progress in understanding MSC therapy and its antimicrobial effects, leading to a substantial body of literature in this field. MSC-based therapy has emerged as a charming option for treatment modalities, serving as a cellular rehabilitative therapy for various diseases, particularly inflammatory conditions. Despite several clinical trials examining MSC-based therapies to struggle bacterial infections, there are currently insufficient studies specifically focused on vaginal candidiasis. The feasibility of autologous MSC and their targeted delivery to specific cells has resulted in their extensive utilization across various treatment fields. Although there are existing limitations, the transplantation of MSCs represents a remarkable and inspiring approach in the scope of medical science, necessitating further data collection to explore their potential in addressing vaginal candidiasis. Further efforts are warranted to improve efficient therapy using MSCs in therapeutic approaches. Depending upon the findings in pre-clinical experiments, it is crucial to further investigate the antifungal activities of MSCs and conduct translational studies to appreciate their clinical applications. This concise chapter aims to promote such endeavours in the remedial approaches of MSC for the treatment of vaginal candidiasis.

1.Introduction

Candida spp. are widely recognized as the predominant etiological agents responsible for fungal infections, ranging from severe invasive forms to less critical mucocutaneous manifestations. Within *Candida* spp., *Candida*

1 Dr. Öğretim Görevlisi, Abdullah Gül University, Bioengineering Department, mesude.bicer@agu.edu.tr, ORCID ID: 0000-0001-7089-5661

https://doi.org/10.58830/ozgur.pub128.c687

albicans holds a prominent position as the most prevalent causative organism (Richards et al. 2000). Notably, *Candida albicans* exhibits a dual nature, acting as a commensal microorganism in individuals with normal health status while posing a substantial risk for morbidity and mortality in human populations (Quindós 2014). This dimorphic yeast species demonstrates a commensal nature by inhabiting the skin, gastrointestinal tract and reproductive tracts of humans. Non-*C. albicans* species also have recognition receptors that are capable of colonizing various mucocutaneous surfaces in the human body (Sobel 2006). Furthermore, mucosal candidiasis, including vaginal candidiasis, is very common diseases. It has been estimated that a remarkable proportion of women, more than 75%, experience vulvovaginal candidiasis at least once in their lifetime (Aguin and Sobel 2015). In addition, approximately 5–10% of women suffer from recurrent episodes of vaginal candidiasis (Weissenbacher et al. 2009). While *Candida albicans* is the most primarily identified agent in invasive candidiasis, it has been a rise in the incidence of candidiasis caused by non-*Candida* species, including *Candida krusei* and *Candida glabrata*, *Candida parapsilosis* (Puig-Asensio et al. 2014). Additionally, *Candida auris* has become universally common among nosocomial pathogens (Chowdhary et al. 2018). The pathogenesis and diagnostic approaches for *candida* colonization are influenced by the host's immune status and display numerous variations depending on the clinical indications of the disease. In terms of therapeutic interventions, the range of antifungal agents accessible for the therapy of patients with invasive fungal illnesses is restricted. Despite the advancement of current antifungal drugs, the treatment options for systemic fungal diseases are constrained, leading to unfavourable outcomes in many cases (Scriven et al. 2017). These emerging *Candida* species often exhibit diminished susceptibility to commonly employed antifungal agents utilized for the treatment of such infections. Examples of these drugs include fluconazole, micafungin, echinocandins, anidulafungin, and caspofungin (Arendrup and Patterson 2017; Chowdhary et al. 2018). Vaginal candidiasis is still an unresolved problem in society. Various factors contributing to the inefficacy of these drugs have been identified, including several molecular resistance mechanisms (Arendrup 2014; Dominique Sanglard 2016). Notably, these resistance mechanisms are closely linked to elevated resistance against antifungal drugs and the host's immune-related factors, consequently leading to treatment failure (Muzny and Schwebke 2015). Novel antifungal agents are urgently needed to address the considerable clinical challenges caused by therapy-resistant fungal candidiasis (McCarthy and Walsh 2017). In recent times, increasing attention has been given to the study of Mesenchymal Stem/Stromal Cells

(MSCs) and their possibilities to treat fungal candidiasis, particularly in cases where conventional antifungal drugs have proven unsuccessful. MSCs possess notable anti-inflammatory and immunomodulatory mechanisms that can contribute to the resolution of fungal infectious episode (Schmidt et al. 2017), although their precise role in the context of vaginal infections remains incompletely understood. Thus, this chapter focuses on elucidating the therapeutic properties of MSCs and their antifungal activity against vaginal candidiasis.

2. Pathogenesis, Epidemiology and Antifungal Resistance of *Candida* species from Vaginal Candidiasis

2.1. The Pathogenesis

When *Candida* is present in the vaginal region without concurrent immunosuppression or mucosal damage, it typically does not manifest any disease-related symptoms and is hereby referred to as fungal alignment. On the contrary, vulvovaginal candidiasis can be characterized by the presence of *Candida* spp. accompanied by signs and symptoms of inflammation, indicating causative contagious agents. More than ten years ago, vulvovaginal candidiasis was divided into two groups: uncomplicated and complicated cases, which have gained international acceptance and verification (Pappas et al. 2009). Uncomplicated vulvovaginal candidiasis refers to sporadic occurrences, primarily caused by *C. albicans,* in immunocompetent women. On the other part, complicated vulvovaginal candidiasis encompasses severe cases of the infection associated with factors such as pregnancy, immunosuppression and uncontrolled diabetes (Sobel et al. 1998). Women who suffer from recurrent episodes of vulvovaginal candidiasis constitute a distinct subgroup within the healthy individuals, setting them apart from those who have sporadic cases of vulvovaginal candidiasis. In comparison to women with chronic vaginal symptoms unrelated to recurrent candidiasis, it has been observed that the symptoms experienced by women with recurrent vulvovaginal candidiasis have the most significant adverse effects on their occupational and social functioning (Nyirjesy et al. 2006).

During the initial phases of fungal pathogenesis, particularly in the case of *Candida albicans*, it has been demonstrated that these pathogens exploit host immune cells, impairing the early induction of proinflammatory cytokines, thereby diminishing their expression. Regardless of how, the immune response becomes intensified in the later phases of *Candida* diseases. A recent investigation focusing on *C. albicans* elucidated that pathogenic fungi downregulate the host immune response during the infections (Halder et

al. 2020). This study indicated that *C. albicans* complies with monocytes through the interaction between its β-glucan and the C3 receptor. By utilizing this involvement to the monocytes, the fungal pathogens induce to secrete proinflammatory cytokines containing transforming growth factor (TGF)-β. These TGF-β-carrying proteins enable the pathogens to dampen the immune response and exert anti-inflammatory effects. Furthermore, the fungal pathogens can suppress the activation of these proteins by means of secreting TGF- β (Netea et al. 2002; Halder et al. 2020). This mechanism allows the fungal pathogens to suppress the immune response in the host, favouring their own survival and persistence.

2.2. Epidemiology

Estimating the global incidence of invasive candidiasis poses challenges due to variations in diagnostic criteria and categorization methods employed. Many factors influence the incidence of this condition, including patients' age, overall health status, the prevalence of immunodeficiency disorders, frequency of organ transplantations, extent of major surgeries and utilization of cancer chemotherapy. It is significant to give due consideration to factors such as genetics, immunity, behavior, nutrition and others. In fact, the scarcity of population- based surveys initiatives significantly limits our understanding of the epidemiological surveillance of fungal candidiasis worldwide (Lamoth et al. 2018). The incidence of *Candida* species observed in females diagnosed with vaginal candidiasis exhibits significant variability across different geographic locations and studied populations. Characteristically, a single *Candida* species is described. However, in a small proportion of women (approximately 2% to 5%) with both complicated and uncomplicated vulvovaginal candidiasis, more than one species has been detected within the self-similar vaginal culture (Richter et al. 2005). Examining Australia, Europe and the United States of America, *C. albicans* is the most prevalent pathogens defined in patients with vulvovaginal candidiasis, accounting for approximately 76% to 89% of cases. This is followed by *C. glabrata,* which represents approximately 7% to 16% of cases. Non-*C. albicans* species collectively constitute 11% to 24% of vulvovaginal candidiasis cases within these countries and regions (Spinillo et al. 1997; Holland et al. 2003; Richter et al. 2005).

Numerous studies have indicated an upward trend in the incidence of invasive candidiasis within the United States of America when compared to Australia, Latin America, Europe and Canada. Population-based studies in the United States of America have reported incidences ranging from 9.5 to 26.2 cases of invasive candidiasis per 100,000 participants (Cleveland et al. 2015).

In contrast, most European countries including Iceland (Asmundsdottir et al. 2013), France (Bitar et al. 2014), Sweden (Ericsson et al. 2013), Norwegian (Hesstvedt et al. 2015) and Finland (Poikonen et al. 2010), have reported lower incidences of 2.9 to 5.7 cases per 100,000 population. Interestingly, Spain and Denmark have both reported incidences exceeding 8 cases of candidemia per 100,000 populations (Arendrup et al. 2011; Puig-Asensio et al. 2014). Australia and Canada exhibit candidemia rates that are similar to those observed in European surveillance, with incidences of nearly 3 cases per 100,000 participants (St-Germain et al. 2008; Chapman et al. 2017). Furthermore, certain Asian and African countries have indicated a higher prevalence of fungal species, particularly *C. glabrata*, in cases of vulvovaginal candidiasis. The distribution of *Candida* sp. in China closely resembles that observed in the United States. (Holland et al. 2003; Richter et al. 2005). Higher rates of non-*C. albicans* species have also been observed in specific populations such as HIV-infected women (Spinillo et al. 1997), postmenopausal women, and women with uncontrolled diabetes, regardless of HIV infection (de Leon et al. 2002). Interestingly, an association has been observed between increasing age and a higher proportion of other *Candida* species in females with vulvovaginal candidiasis (Holland et al. 2003). *C. glabrata* is the most commonly isolated among all species. These findings underscore the prominence of identifying *Candida* species and their sensibilities in high-risk females with both *C. albicans* and non-*C. albicans* vulvovaginal candidiasis in order to ensure influential treatment strategies.

2.3. Antifungal Resistance of *Candida* species from Vaginal Candidiasis

The treatment of *Candida* species presents a distinct challenge due to their inherent resistance mechanisms, necessitating substantially higher concentrations of antifungal drugs compared to planktonic cells (Barantsevich and Barantsevich 2022). These species have developed resistance through various mechanisms, including nutrient sensing, glucose starvation, and heightened oxidative stress responses, enabling their survival in the presence of antifungal components that trigger the concentration of reactive oxygen species (ROS) (Lopes and Lionakis 2022; Brown 2023). *Candida* isolates obtained from vaginal samples exhibit some resistance patterns that are resilient to antifungal agents in patients with candidiasis. Specially, a remarkable proportion of *C. glabrata* strains display resistance to fluconazole, particularly in cases where patients have indwelling catheters, resulting in fungal infections characterized by *C. glabrata* embedded in biofilms. Instead of the resistance to fluconazole *Candida* cells related

to biofilm often illustrate a high level of resistance to azoles, including amphotericin B (AmB) (Sobel et al. 2000).

Fluconazole is commonly preferred as a first-choice antifungal therapy to combat mucosal candidiasis, caused by *Candida albicans* and *Candida parapsilosis*. However, resistance to fluconazole is widely distributed among various *Candida* species, such as *Candida auris, Candida krusei* and *Candida glabrata*. These strains display high level of minimum inhibitory concentrations (MICs) for fluconazole ($\geq$64mg/L), indicating resistance to the antifungal agents (Chowdhary et al. 2016). Besides, these species can also exhibit resistance to other antifungal classes, including azoles, amphotericin B and even echinocandins (Arendrup and Patterson 2017; Chowdhary et al. 2018). The progress of antifungal resistance in *Candida* species is subject to various molecular mechanisms, depending on target gene mutation, enhancement of target expression, impaired intracellular conversion of drugs, increased activity of efflux pumps and decreased uptake of antifungal drugs (Jensen et al. 2015; D. Sanglard 2016). These alternatives may support the improvement of novel antifungal drugs, the identification of new applications for established drugs, the synergistic combination of existing drugs, or the advancement of biological therapies targeting virulence factors on *Candida* metabolism (McCarthy and Walsh 2017).

It is pivotal to address the growing challenge posed by candidiasis, which has infectious agents to exhibit resistance to conventional anti-fungal drugs. Despite the development in understanding of the mechanisms and treatment strategies against *candida* infections, there is still a need to develop effective alternative strategies to combat fungal pathogens (McCarthy and Walsh 2017). In this context, MSCs and their anti-fungal activity could provide emerging evidence as a therapeutic option for the treatment of vaginal candidiasis.

3. MSCs and Their Anti-fungal Activity

MSCs can be obtained from a variety of origins such as placenta, adipose tissue, Wharton's jelly, bone marrow, uterus, umbilical cord, dermis, amniotic fluid, peripheral blood, periosteum, skeletal muscle and dental pulp (Vizoso et al. 2017). In accordance with the "International Society for Cellular Therapy" , it has been defined that MSCs exhibit the following characteristics: (i) adherence to plastic surfaces (ii) expression of specific stem cell markers (e.g., CD29, CD44, CD73, CD90 and CD105), while lacking expression for hematopoietic markers (CD45 and CD14), endothelial markers (CD31 and CD34) and also HLA-DR surface

molecules, and (iii) the ability to be speciliazed in vitro into chondroblastic, osteoblastic and adipocytic cells (Dominici et al. 2006). MSCs have a specific talent for differentiating into multiple cell lineages and displaying widely immunomodulatory characteristics towards the congenital and acquired immune system. These properties are modulated by the release of soluble factors, including interleukins (IL) and interferons (IFN) (Zhang et al. 2020; Oh et al. 2021). Both clinical and experimental studies have supported the immunosuppressive capabilities of MSCs to treat a variety of invasive autoimmune illnesses. MSCs have been shown to downregulate the immune cells, such as B and T lymphocytes, natural killer (NK) cells and dendritic cells (Aggarwal and Pittenger 2005). MSCs can also secrete soluble proteins including interleukin-10 (IL-10), tumor necrosis factor- α (TNF-α), prostaglandin E2 (PGE2), indoleamine 2,3-dioxygenase (IDO), and interleukin-17, by which these proteins potentiate their antimicrobial effects (Yang et al. 2013; Alcayaga-Miranda et al. 2017).

Up to the present, stem cell-based therapy has shown promise in the treatment of immune diseases. While significant progress has been made in utilizing MSCs to address infectious diseases and their associated complications, there is limited research focusing on their potential as a host response against fungal infections (Keshtkar et al. 2022). Several cytokines that are expressed at high levels have been identified to possess antifungal potentials, including Chemokine (C-C motif) ligand (CCL- 5 and CCL-6), IL-6, IL-8 and IL-17. Among them, IL-17 plays an important role in mucocutaneous syndrome to tackle *Candida albicans* (Ling et al. 2015). Although the conventional mechanism of cytokine action involves cell signaling based on immune system, supporting document suggests that cytokines can be directly exerted to disrupt fungal pathogens. Notably, a study has shown that IL-17 can attach to the receptor of *Candida albicans,* leading to the prevention of fungal growth (Zelante et al. 2012). Moreover, Li et al. have observed that IL-17 exhibits a direct inhibitory effect on the proliferation of various eukaryotic cells, including neural stem cells, leading to a significant decrease in the number of neural precursor cells. Although purely and simply hypothesis, it is plausible that same inhibitory effect may occur against *Candida* cells. Thus, apart from its known proinflammatory function through immune system activation, IL-17 might also be exerted to restrain the proliferation of *Candida* cell (Li et al. 2013). In the context of adipose stem cells, specifically human umbilical cord-derived MSCs (hUCESCs), studies have shown elevated levels of IL-17. This heightened interleukin production with antifungal properties is not a characteristic observed in all MSCs, but rather a distinct feature of hUCESCs, likely

developed as an evolutionary response to *Candida* strains in the vagina, particularly in the conversion part of the cervix (Schneider et al. 2016). Consequently, the use of stem cells other than conventional drugs for the treatment of vaginal candidiasis may potentially be quite effective.

4. The Therapeutic Applications of MSCs against Vaginal Candidiasis

The NIH Clinical Trial Database currently contains over 1000 registered clinical trials focused on MSC therapy. Among these trials, approximately 47.1% (491 trials) are specifically targeting immune disorders. Within this subgroup, there are 2 trials focused on fungal infections and 5 trials conducted on infected individuals with vaginal diseases (source: https://ClinicalTrials.gov/ ; accessed on 8 June 2023). The trials addressing fungal and vaginal diseases primarily involve the transplantation of autologous or allogeneic MSC through local injection to treat some diseases including fistulas, rectovaginal fistulas, and Crohn's disease of vulva. Allogeneic MSC are more commonly used due to their ability to be expanded in large quantities and fully characterized before administration, offering greater convenience in terms of cell dose availability. MSCs possess the capability to react to immune response in the host and also release various soluble factors, leading to immunomodulatory properties. As a result, the therapeutic potential of MSCs has evolved from primarily focusing on their regenerative effects to encompassing their anti-inflammatory properties, as well as other notable attributes such as angiogenesis promotion, anti-oxidative stress effects, anti-tumoral and antimicrobial activities (Fernández-Francos et al. 2021).

Certain sources of MSC have gained attention for their potential in specific therapeutic indications. For instance, MSC sourced from reproductive tissues have shown promise in terms of their anti-tumor activity (Schneider et al. 2016). hUCESCs have been recognized for their immunomodulatory properties (Eiró et al. 2014), while MSC derived from dental pulp have demonstrated potential in addressing neurological disorders (Apel et al. 2009). The regenerative effects of MSC have been primarily attributed to paracrine signaling (Bluguermann et al. 2013), whereby MSCs also release a range of growth factors, which comprise vascular endothelial growth factor, platelet-derived growth factor, fibroblast growth factor as well as transforming growth factor (TGF-β1 and TGF-α), among others. These factors contribute to the increased proliferation of endothelial cells as well as their proliferation (Park et al. 2018; Ahangar et al. 2020). In addition to, MSCs have been reported to encourage the survival and reinforce the

regeneration of epithelial cells in the colon, by means of EVs including growth factors and cytokines (Shi et al. 2019; Sendon-Lago et al. 2021).

The immune response of the human body includes innated and acquired immune responses to combat infections. Within the scope of innate immunity, antimicrobial peptides (AMPs) play a crucial role by exerting antimicrobial effects and modulating the immune system, thereby reducing pathogen virulence (de Oca 2013). MSC have been found to directly contribute to antimicrobial activity through the release of AMPs. These peptides can induce the destruction of membrane integrity in microbial cells and interact with peculiar intracellular adhesion molecules to combat microbial agents (Alcayaga-Miranda et al. 2017; Marrazzo et al. 2019; Yagi et al. 2020). Several AMPs have been identified as effective against *Candida*, including human beta-Defensins (HBD), histatins, cathelicidin LL-37 Peptide (Edgerton et al. 1998; Joly et al. 2004; López-García et al. 2005). LL-37, in particular, exhibits a wide range of antimicrobial effect against both bacterial and fungal diversity as well as viral diseases (López-García et al. 2005; Peter et al. 2007; Wang et al. 2019). Multiple investigations have demonstrated the talent of LL-37 to disrupt the cellular membrane and lipid vesicles of certain bacteria (Henzler-Wildman et al. 2004). LL-37 has also been demonstrated to interact with the cell wall of *C. albicans* (Tsai et al. 2011). These findings match a study by den Hertog et al., which indicated that LL-37 can interfere in planktonic *C. albicans* to disrupt the plasma membrane and the cell wall of fungal cells (Hertog et al. 2006). Another study showed an efficient antifungal activity of LL-37 against *C. albicans* (López-García et al. 2005). HBD also possess fungicidal activity against *C. albicans* (Joly et al. 2004). In the presence of *C. albicans,* an increase in HBD-2 level was observed in the female genital tract (Kotani et al. 2020). These findings have shown a positive correlation between the presence of Lactobacillus and *Candida* spp., and the activity of HBD-2. Moreover, HBD-2 has shown efficacy in decreasing the amount of fungus in a mouse model of vaginal candidiasis (Liao et al. 2017).

Emerging clinical research has indicated the significant involvement of MSCs in antimicrobial activity. MSC therapy has also shown efficacy in facilitating prompt control in patients with invasive Aspergilllus infections, under the favour of hematopoietic stem cell transplantation (Ozdoğu et al. 2014). Investigations have indicated the efficiency of human cathelicidin LL-37 and its parts LL13-37 and LL17-37, in which this AMP displays comparable potency to restrain the growth of C. albicans. However, the death of C. albicans cells may also be attributed to other intracellular targets as well as LL13-37 fragments (Wong et al. 2011). In the case of patients

with agonising neutrophilic bronchial asthma, implementation of MSCs has been shown as a promising treatment by reducing Aspergillus-induced inflammation and improving diseases symptoms with the help of detention of the Th_{17} signaling pathway (Lathrop et al. 2014). Collectively, subsets of MSCs displaying the aforementioned characteristics hold potential to be valuable *candida*tes for addressing and combating infectious diseases. Further validations of these findings through additional research may open new avenues for innovative therapeutic strategies aimed at addressing vaginal candidiasis.

5. Conclusion

Vaginal candidiasis is a type of infectious diseases that is closely associated with the pathogenic mechanism of microorganism and is susceptible to a wide range of infectious pathogens due to its unique microbiome. Within this context, the follow-up of MSC-based therapy has the potential to influence the development, progression, prevention and containment of the disease. MSCs also play a remarkable role in regulating the host immune system in microbial infections. Furthermore, MSCs can be employed in the diagnosis of infectious diseases. Considering the significance of addressing vaginal infectious diseases and the convenience of using cell-based therapies, MSCs can be regarded as a viable and accessible option for the treatment of vaginal candidiasis. However, further extensive studies are needed in the future to explore their full potential and efficacy in this context.

The pathogenesis of *Candida* species is complex, posing challenges in finding an effective therapeutic strategy that comprehensively deals with various clinical applications. The therapeutic potential of MSCs has evolved over time, transitioning from autologous to allogeneic MSC applications and from the initial focus on their remedial effects to encompass anti-inflammatory, anti-tumor, antimicrobial and anti-oxidative stress properties. These advancements in understanding open up new possibilities for developing therapeutic strategies for vaginal candidiasis. MSCs have demonstrated the ability to counteract fungal infections, suggesting their potential as a defence mechanism against vaginal infections. Incorporating nanotechnology-based advancements can further optimize MSC-based therapeutic applications in clinical practice. These advancements pave the way for designing innovative therapeutic approaches against the diverse and intricate therapeutic targets associated with vaginal candidiasis using cell-based therapies.

References

Aggarwal S, Pittenger MF (2005) Human mesenchymal stem cells modulate allogeneic immune cell responses. Blood 105: 1815-1822

Aguin TJ, Sobel JD (2015) Vulvovaginal Candidiasis in Pregnancy. Current Infectious Disease Reports 17: 30

Ahangar P, Mills SJ, Cowin AJ (2020) Mesenchymal Stem Cell Secretome as an Emerging Cell-Free Alternative for Improving Wound Repair. International journal of molecular sciences 21: 7038

Alcayaga-Miranda F, Cuenca J, Khoury M (2017) Antimicrobial Activity of Mesenchymal Stem Cells: Current Status and New Perspectives of Antimicrobial Peptide-Based Therapies. Frontiers in Immunology 8:

Apel C, Forlenza OV, de Paula VJR, Talib LL, Denecke B, Eduardo CP, Gattaz WF (2009) The neuroprotective effect of dental pulp cells in models of Alzheimer's and Parkinson's disease. Journal of Neural Transmission 116: 71-78

Arendrup MC (2014) Update on antifungal resistance in Aspergillus and *Candida*. Clinical Microbiology and Infection 20: 42-48

Arendrup MC, Patterson TF (2017) Multidrug-Resistant *Candida*: Epidemiology, Molecular Mechanisms, and Treatment. The Journal of Infectious Diseases 216: S445-S451

Arendrup MC, Bruun B, Christensen JJ, Fuursted K, Johansen HK, Kjældgaard P, Knudsen JD, Kristensen L, Møller J, Nielsen L, Rosenvinge FS, Røder B, Schønheyder HC, Thomsen MK, Truberg K (2011) National Surveillance of Fungemia in Denmark (2004 to 2009). Journal of Clinical Microbiology 49: 325-334

Asmundsdottir LR, Erlendsdottir H, Gottfredsson M (2013) Nationwide study of candidemia, antifungal use, and antifungal drug resistance in Iceland, 2000 to 2011. J Clin Microbiol 51: 841-848

Barantsevich N, Barantsevich E (2022) Diagnosis and Treatment of Invasive Candidiasis. Antibiotics 11: 718

Bitar D, Lortholary O, Le Strat Y, Nicolau J, Coignard B, Tattevin P, Che D, Dromer F (2014) Population-based analysis of invasive fungal infections, France, 2001-2010. Emerg Infect Dis 20: 1149-1155

Bluguermann C, Wu L, Petrigliano F, McAllister D, Miriuka S, Evseenko DA (2013) Novel aspects of parenchymal–mesenchymal interactions: from cell types to molecules and beyond. Cell Biochemistry and Function 31: 271-280

Brown AJP (2023) Fungal resilience and host–pathogen interactions: Future perspectives and opportunities. Parasite Immunology 45: e12946

Chapman B, Slavin M, Marriott D, Halliday C, Kidd S, Arthur I, Bak N, Heath CH, Kennedy K, Morrissey CO, Sorrell TC, van Hal S, Keighley C, Goeman E, Underwood N, Hajkowicz K, Hofmeyr A, Leung M, Macesic N, Botes J, Blyth C, Cooley L, George CR, Kalukottege P, Kesson A, McMullan B, Baird R, Robson J, Korman TM, Pendle S, Weeks K, Liu E, Cheong E, Chen S (2017) Changing epidemiology of *candida*emia in Australia. J Antimicrob Chemother 72: 1103-1108

Chowdhary A, Voss A, Meis JF (2016) Multidrug-resistant *Candida* auris: 'new kid on the block' in hospital-associated infections? J Hosp Infect 94: 209-212

Chowdhary A, Prakash A, Sharma C, Kordalewska M, Kumar A, Sarma S, Tarai B, Singh A, Upadhyaya G, Upadhyay S, Yadav P, Singh PK, Khillan V, Sachdeva N, Perlin DS, Meis JF (2018) A multicentre study of antifungal susceptibility patterns among 350 *Candida* auris isolates (2009–17) in India: role of the ERG11 and FKS1 genes in azole and echinocandin resistance. Journal of Antimicrobial Chemotherapy 73: 891-899

Cleveland AA, Harrison LH, Farley MM, Hollick R, Stein B, Chiller TM, Lockhart SR, Park BJ (2015) Declining incidence of candidemia and the shifting epidemiology of *Candida* resistance in two US metropolitan areas, 2008-2013: results from population-based surveillance. PLoS One 10: e0120452

de Leon EM, Jacober SJ, Sobel JD, Foxman B (2002) Prevalence and risk factors for vaginal *Candida* colonization in women with type 1 and type 2 diabetes. BMC Infect Dis 2: 1

de Oca EPM (2013) Antimicrobial peptide elicitors: New hope for the post-antibiotic era. Innate Immunity 19: 227-241

Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, Deans RJ, Keating A, Prockop DJ, Horwitz EM (2006) Minimal criteria for defining multipotent mesenchymal stromal cells. The International Society for Cellular Therapy position statement. Cytotherapy 8: 315-317

Edgerton M, Koshlukova SE, Lo TE, Chrzan BG, Straubinger RM, Raj PA (1998) *Candida*cidal Activity of Salivary Histatins: IDENTIFICATION OF A HISTATIN 5-BINDING PROTEIN ON *Candida albicans* *. Journal of Biological Chemistry 273: 20438-20447

Eiró N, Sendon-Lago J, Seoane S, Bermúdez MA, Lamelas ML, Garcia-Caballero T, Schneider J, Perez-Fernandez R, Vizoso FJ (2014) Potential therapeutic effect of the secretome from human uterine cervical stem cells against both cancer and stromal cells compared with adipose tissue stem cells. Oncotarget 5: 10692-10708

Ericsson J, Chryssanthou E, Klingspor L, Johansson AG, Ljungman P, Svensson E, Sjölin J (2013) *Candida*emia in Sweden: a nationwide prospective observational survey. Clin Microbiol Infect 19: E218-221

Fernández-Francos S, Eiro N, Costa LA, Escudero-Cernuda S, Fernández-Sánchez ML, Vizoso FJ (2021) Mesenchymal Stem Cells as a Cornerstone in a Galaxy of Intercellular Signals: Basis for a New Era of Medicine. International journal of molecular sciences 22: 3576

Halder LD, Jo EAH, Hasan MZ, Ferreira-Gomes M, Krüger T, Westermann M, Palme DI, Rambach G, Beyersdorf N, Speth C, Jacobsen ID, Kniemeyer O, Jungnickel B, Zipfel PF, Skerka C (2020) Immune modulation by complement receptor 3-dependent human monocyte TGF-β1-transporting vesicles. Nature Communications 11: 2331

Henzler-Wildman KA, Martinez GV, Brown MF, Ramamoorthy A (2004) Perturbation of the Hydrophobic Core of Lipid Bilayers by the Human Antimicrobial Peptide LL-37. Biochemistry 43: 8459-8469

Hertog ALd, Marle Jv, Veerman ECI, Valentijn-Benz M, Nazmi K, Kalay H, Grün CH, Hof Wvt, Bolscher JGM, Amerongen AVN (2006) The human cathelicidin peptide LL-37 and truncated variants induce segregation of lipids and proteins in the plasma membrane of *Candida albicans*. 387: 1495-1502

Hesstvedt L, Gaustad P, Andersen CT, Haarr E, Hannula R, Haukland HH, Hermansen NO, Larssen KW, Mylvaganam H, Ranheim TE, Sandven P, Nordøy I, Kanestrøm A, Grub C, Onken A, Thielsen C, Skaare D, Tofteland S, Sønsteby LJ, Hjetland R, Hide R, Vik E, Kümmel A, Åsheim S (2015) Twenty-two years of *candida*emia surveillance: results from a Norwegian national study. Clin Microbiol Infect 21: 938-945

Holland J, Young ML, Lee O, S CAC (2003) Vulvovaginal carriage of yeasts other than *Candida albicans*. Sex Transm Infect 79: 249-250

Jensen RH, Astvad KM, Silva LV, Sanglard D, Jørgensen R, Nielsen KF, Mathiasen EG, Doroudian G, Perlin DS, Arendrup MC (2015) Stepwise emergence of azole, echinocandin and amphotericin B multidrug resistance in vivo in *Candida albicans* orchestrated by multiple genetic alterations. J Antimicrob Chemother 70: 2551-2555

Joly S, Maze C, McCray PB, Guthmiller JM (2004) Human β-Defensins 2 and 3 Demonstrate Strain-Selective Activity against Oral Microorganisms. Journal of Clinical Microbiology 42: 1024-1029

Keshtkar S, Kaviani M, Soleimanian S, Azarpira N, Asvar Z, Pakbaz S (2022) Stem Cell-Derived Exosome as Potential Therapeutics for Microbial Diseases. Frontiers in Microbiology 12:

Kotani H, Koshizuka T, Matsubara K, Nishiyama K, Sugiyama T, Suzutani T (2020) Relationship Between Human β-Defensin 2 and the Vaginal Environment. Japanese Journal of Infectious Diseases 73: 214-220

Lamoth F, Lockhart SR, Berkow EL, Calandra T (2018) Changes in the epidemiological landscape of invasive candidiasis. J Antimicrob Chemother 73: i4-i13

Lathrop MJ, Brooks EM, Bonenfant NR, Sokocevic D, Borg ZD, Goodwin M, Loi R, Cruz F, Dunaway CW, Steele C, Weiss DJ (2014) Mesenchymal stromal cells mediate Aspergillus hyphal extract-induced allergic airway inflammation by inhibition of the Th17 signaling pathway. Stem Cells Transl Med 3: 194-205

Li Z, Li K, Zhu L, Kan Q, Yan Y, Kumar P, Xu H, Rostami A, Zhang G-X (2013) Inhibitory effect of IL-17 on neural stem cell proliferation and neural cell differentiation. BMC Immunology 14: 20

Liao H, Liu S, Wang H, Su H, Liu Z (2017) Efficacy of Histatin5 in a murine model of vulvovaginal candidiasis caused by *Candida albicans*. Pathogens and Disease 75:

Ling Y, Cypowyj S, Aytekin C, Galicchio M, Camcioglu Y, Nepesov S, Ikinciogullari A, Dogu F, Belkadi A, Levy R, Migaud M, Boisson B, Bolze A, Itan Y, Goudin N, Cottineau J, Picard C, Abel L, Bustamante J, Casanova J-L, Puel A (2015) Inherited IL-17RC deficiency in patients with chronic mucocutaneous candidiasis. Journal of Experimental Medicine 212: 619-631

Lopes JP, Lionakis MS (2022) Pathogenesis and virulence of *Candida albicans*. Virulence 13: 89-121

López-García B, Lee PHA, Yamasaki K, Gallo RL (2005) Anti-Fungal Activity of Cathelicidins and their Potential Role in *Candida albicans* Skin Infection. Journal of Investigative Dermatology 125: 108-115

Marrazzo P, Crupi AN, Alviano F, Teodori L, Bonsi L (2019) Exploring the roles of MSCs in infections: focus on bacterial diseases. J Mol Med (Berl) 97: 437-450

McCarthy MW, Walsh TJ (2017) Drugs currently under investigation for the treatment of invasive candidiasis. Expert Opin Investig Drugs 26: 825-831

Muzny CA, Schwebke JR (2015) Biofilms: An Underappreciated Mechanism of Treatment Failure and Recurrence in Vaginal Infections. Clinical Infectious Diseases 61: 601-606

Netea MG, Stuyt RJL, Kim S-H, Van der Meer JWM, Kullberg BJ, Dinarello CA (2002) The Role of Endogenous Interleukin (IL)-18, IL-12, IL-1β, and Tumor Necrosis Factor-α in the Production of Interferon-γ Induced by *Candida albicans* in Human Whole-Blood Cultures. The Journal of Infectious Diseases 185: 963-970

Nyirjesy P, Peyton C, Weitz MV, Mathew L, Culhane JF (2006) Causes of chronic vaginitis: analysis of a prospective database of affected women. Obstet Gynecol 108: 1185-1191

Oh S, Jang AY, Chae S, Choi S, Moon J, Kim M, Spiekerkoetter E, Zamanian RT, Yang PC, Hwang D, Byun K, Chung W-J (2021) Comparative analysis on the anti-inflammatory/immune effect of mesenchymal stem cell therapy for the treatment of pulmonary arterial hypertension. Scientific Reports 11: 2012

Ozdoğu H, Yeral M, Boğa C, Kozanoğlu I (2014) Use of mesenchymal cells to modulate immune suppression and immune reconstruction in a patient with aplastic anemia complicated by invasive sino-orbital aspergillosis. Turk J Haematol 31: 181-183

Pappas PG, Kauffman CA, Andes D, Benjamin DK, Jr., Calandra TF, Edwards JE, Jr., Filler SG, Fisher JF, Kullberg BJ, Ostrosky-Zeichner L, Reboli AC, Rex JH, Walsh TJ, Sobel JD (2009) Clinical practice guidelines for the management of candidiasis: 2009 update by the Infectious Diseases Society of America. Clin Infect Dis 48: 503-535

Park S-R, Kim J-W, Jun H-S, Roh JY, Lee H-Y, Hong I-S (2018) Stem Cell Secretome and Its Effect on Cellular Mechanisms Relevant to Wound Healing. Molecular Therapy 26: 606-617

Peter B, Lilian W-J, Kristina B, Birgitta A, Johan S (2007) The Antimicrobial Peptide LL-37 Inhibits HIV-1 Replication. Current HIV Research 5: 410-415

Poikonen E, Lyytikäinen O, Anttila VJ, Koivula I, Lumio J, Kotilainen P, Syrjälä H, Ruutu P (2010) Secular trend in candidemia and the use of fluconazole in Finland, 2004-2007. BMC Infect Dis 10: 312

Puig-Asensio M, Padilla B, Garnacho-Montero J, Zaragoza O, Aguado JM, Zaragoza R, Montejo M, Muñoz P, Ruiz-Camps I, Cuenca-Estrella M, Almirante B (2014) Epidemiology and predictive factors for early and late mortality in *Candida* bloodstream infections: a population-based surveillance in Spain. Clinical Microbiology and Infection 20: O245-O254

Puig-Asensio M, Padilla B, Garnacho-Montero J, Zaragoza O, Aguado JM, Zaragoza R, Montejo M, Muñoz P, Ruiz-Camps I, Cuenca-Estrella M, Almirante B (2014) Epidemiology and predictive factors for early and late mortality in *Candida* bloodstream infections: a population-based surveillance in Spain. Clin Microbiol Infect 20: O245-254

Quindós G (2014) Epidemiology of *candida*emia and invasive candidiasis. A changing face. Revista Iberoamericana de Micología 31: 42-48

Richards MJ, Edwards JR, Culver DH, Gaynes RP (2000) Nosocomial infections in combined medical-surgical intensive care units in the United States. Infect Control Hosp Epidemiol 21: 510-515

Richter SS, Galask RP, Messer SA, Hollis RJ, Diekema DJ, Pfaller MA (2005) Antifungal susceptibilities of *Candida* species causing vulvovaginitis and epidemiology of recurrent cases. J Clin Microbiol 43: 2155-2162

Sanglard D (2016) Emerging Threats in Antifungal-Resistant Fungal Pathogens. Frontiers in Medicine 3:

Sanglard D (2016) Emerging Threats in Antifungal-Resistant Fungal Pathogens. Front Med (Lausanne) 3: 11

Schmidt S, Tramsen L, Schneider A, Schubert R, Balan A, Degistirici Ö, Meisel R, Lehrnbecher T (2017) Impact of human mesenchymal stromal cells on antifungal host response against Aspergillus fumigatus. Oncotarget 8: 95495-95503

Schneider J, Eiró N, Pérez-Fernández R, Martínez-Ordóñez A, Vizoso F (2016) Human Uterine Cervical Stromal Stem Cells (hUCESCs): Why and How they Exert their Antitumor Activity. Cancer Genomics Proteomics 13: 331-337

Scriven JE, Tenforde MW, Levitz SM, Jarvis JN (2017) Modulating host immune responses to fight invasive fungal infections. Current Opinion in Microbiology 40: 95-103

Sendon-Lago J, Rio LG-d, Eiro N, Diaz-Rodriguez P, Avila L, Gonzalez LO, Vizoso FJ, Perez-Fernandez R, Landin M (2021) Tailored Hydrogels as Delivery Platforms for Conditioned Medium from Mesenchymal Stem Cells in a Model of Acute Colitis in Mice. Pharmaceutics 13: 1127

Shi X, Chen Q, Wang F (2019) Mesenchymal stem cells for the treatment of ulcerative colitis: a systematic review and meta-analysis of experimental and clinical studies. Stem Cell Research & Therapy 10: 266

Sobel JD (2006) The emergence of non-albicans *Candida* species as causes of invasive candidiasis and candidemia. Curr Infect Dis Rep 8: 427-433

Sobel JD, Faro S, Force RW, Foxman B, Ledger WJ, Nyirjesy PR, Reed BD, Summers PR (1998) Vulvovaginal candidiasis: epidemiologic, diagnostic, and therapeutic considerations. Am J Obstet Gynecol 178: 203-211

Sobel JD, Kauffman CA, McKinsey D, Zervos M, Vazquez JA, Karchmer AW, Lee J, Thomas C, Panzer H, Dismukes WE (2000) Candiduria: a randomized, double-blind study of treatment with fluconazole and placebo. The National Institute of Allergy and Infectious Diseases (NIAID) Mycoses Study Group. Clin Infect Dis 30: 19-24

Spinillo A, Capuzzo E, Gulminetti R, Marone P, Colonna L, Piazzi G (1997) Prevalence of and risk factors for fungal vaginitis caused by non-albicans species. Am J Obstet Gynecol 176: 138-141

St-Germain G, Laverdière M, Pelletier R, René P, Bourgault AM, Lemieux C, Libman M (2008) Epidemiology and antifungal susceptibility of bloodstream *Candida* isolates in Quebec: Report on 453 cases between 2003 and 2005. Can J Infect Dis Med Microbiol 19: 55-62

Tsai P-W, Yang C-Y, Chang H-T, Lan C-Y (2011) Characterizing the Role of Cell-Wall β-1,3-Exoglucanase Xog1p in *Candida albicans* Adhesion by the Human Antimicrobial Peptide LL-37. PLOS ONE 6: e21394

Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R (2017) Mesenchymal Stem Cell Secretome: Toward Cell-Free Therapeutic Strategies in Regenerative Medicine. International journal of molecular sciences 18: 1852

Wang G, Narayana JL, Mishra B, Zhang Y, Wang F, Wang C, Zarena D, Lushnikova T, Wang X (2019) Design of Antimicrobial Peptides: Progress Made with Human Cathelicidin LL-37. *Antimicrobial Peptides: Basics for Clinical Application,* (Matsuzaki K, ed.) p. ^ pp. 215-240. Springer Singapore, Singapore.

Weissenbacher TM, Witkin SS, Gingelmaier A, Scholz C, Friese K, Mylonas I (2009) Relationship between recurrent vulvovaginal candidosis and immune mediators in vaginal fluid. European Journal of Obstetrics and Gynecology and Reproductive Biology 144: 59-63

Wong JH, Ng TB, Legowska A, Rolka K, Hui M, Cho CH (2011) Antifungal action of human cathelicidin fragment (LL13-37) on *Candida albicans*. Peptides 32: 1996-2002

Yagi H, Chen AF, Hirsch D, Rothenberg AC, Tan J, Alexander PG, Tuan RS (2020) Antimicrobial activity of mesenchymal stem cells against Staphylococcus aureus. Stem Cell Research & Therapy 11: 293

Yang R, Liu Y, Kelk P, Qu C, Akiyama K, Chen C, Atsuta I, Chen W, Zhou Y, Shi S (2013) A subset of IL-17+ mesenchymal stem cells possesses anti-*Candida albicans* effect. Cell Research 23: 107-121

Zelante T, Iannitti RG, De Luca A, Arroyo J, Blanco N, Servillo G, Sanglard D, Reichard U, Palmer GE, Latgè J-P, Puccetti P, Romani L (2012) Sensing of mammalian IL-17A regulates fungal adaptation and virulence. Nature Communications 3: 683

Zhang B, Tian X, Hao J, Xu G, Zhang W (2020) Mesenchymal Stem Cell-Derived Extracellular Vesicles in Tissue Regeneration. Cell Transplantation 29: 0963689720908500

Chapter 2

Hemoptysis

Elif Guliyev[1]

Abstract

Composed of two Greek words ("heima"-blood and "ptysis"-spitting), hemoptysis is a symptom that alarms the patient. If the hemoptysis is not massive, there is time to investigate the underlying disease. Massive hemoptysis is life-threatening. It has serious mortality and morbidity. In this article, the latest developments in the hemoptysis approach will be discussed.

Hemoptysis is the expectoration of blood or mixed secretions from the lower respiratory tract. The amount may be small or in amounts that can cause death. Sputum mixed with mucus, thick, sticky, blackish, and sour, is called bloody sputum (hemopteic krasha).

Life-threatening bleeding is defined as massive hemoptysis. Massive hemoptysis, which constitutes 5-15% of all hemoptysis, requires urgent intervention. For massive bleeding, different amounts are reported as 200-1000 ml bleeding in 24 hours, more than 200 ml in 2 hours, and more than 600 ml in 24 hours in different sources. Definitions for this non-standardized amount of bleeding do not always reflect the clinical characteristics of the patient. For example, the acute situation caused by 200 mL blood expectoration in the last 10 minutes, and 200 mL expectoration spread throughout the day cause different clinical pictures and require different interventions. For this reason, today, the definition is made by considering the clinical characteristics of the patient.

Any amount of hemoptysis leading to a life-threatening condition such as respiratory failure or hypotension is recommended to be defined as massive hemoptysis (life-threatening hemoptysis).

1 Dr., Kocaeli University, Faculty of Medicine, Department of Pulmonary Diseases, elifkrsl@gmail.com, Orcid: 0000-0002-1501-3360

https://doi.org/10.58830/ozgur.pub128.c507

Pathophysiology

Perfusion of the lungs; 95% is supplied from the pulmonary arteries (right ventricle) and 5% from the bronchial arteries (the descending aorta between T3-T8, most often from the T5-T6 level (80%), phrenic arteries, subclavian arteries, intercostal arteries). 95% of pulmonary blood circulation is provided by the pulmonary artery (functional role) and its branches, which have a low-pressure system.

Since the bronchial arteries (nutritional role) are supplied with blood from the systemic arteries, blood pressure is high and this high pressure increases the risk of massive bleeding. At the same time, the bronchial arteries are the vessels that supply the airways and are affected by airway lesions. Often (40%) there is one bronchial artery on the right and two on the left. Various anatomical variations have been described in normal healthy individuals (Figure-1). They may also have aberrant origins from other systemic arteries (20%)

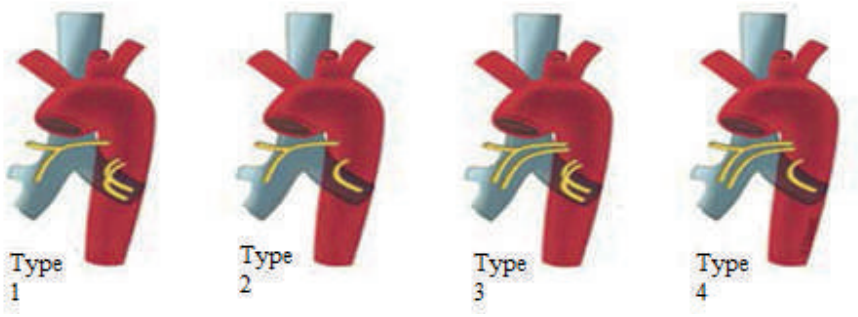


Figure 1- Bronchial artery variations

Type 1: 1 right Bronchial artery 2 left Bronchial arteries of the same origin

Type 2: 1 right Bronchial artery and 1 left Bronchial artery of the same origin

Type 3: 2 right Bronchial arteries and 2 left Bronchial arteries of the same origin

Type 4: 2 right Bronchial arteries of the same origin and 1 left Bronchial artery,

Bleeding in patients presenting with the complaint of hemoptysis; originates from 90% bronchial arteries, 5% pulmonary arteries, 5% non-bronchial systemic arteries, and very rarely pulmonary-bronchial veins and

capillaries. Pulmonary venous bleeding is usually mild and may occur in left heart failure, mainly due to pulmonary venous hypertension.

Bleeding due to the bronchial venous system can occur with proliferating submucosal bronchial veins, often as a complication of mitral stenosis, may be life-threatening, and may constitute an indication for surgical intervention.

Hemoptysis occurs with the formation of capillary anastomoses between the pulmonary artery and systemic bronchial arteries, pulmonary thromboembolism, vasculitis, suppression of pulmonary circulation due to hypoxic vasoconstriction, and an increase in bronchial flow. Hemoptysis develops as a result of the widening of the vessels and thinning of their walls due to excessive flow increase in these anastomoses. On the other hand, the change in vascular anatomy as a result of chronic inflammatory lung diseases causes the bronchial arteries to expand and fold, and new collaterals are formed with the clarification of normal anastomoses between the bronchial arteries and pulmonary veins, and the release of angiogenic growth factors, supporting neovascularization and pulmonary vascular remodeling. Hemoptysis develops as a result of an increase in collaterals and enlargement and fragility of vessels.

Causes of Hemoptysis

The vast majority of cases of hemoptysis occur in adults. Although the average age varies according to the underlying cause, hemoptysis due to lung cancer is usually seen between the ages of 40-60. It is seen twice as often in men than in women.

The most common causes of hemoptysis are pulmonary tuberculosis, bronchial cancer, bronchiectasis, pneumonia, vasculitic syndromes, and pulmonary infarction. In our country, Fidan et al. and Ünsal et al., bronchiectasis, tuberculosis, and lung cancer were reported as the three most common causes. On the other hand, up to 50% of cases of unknown-cryptogenic hemoptysis are reported worldwide.

In pulmonary tuberculosis, massive bleeding may occur with the rupture of Rasmussen aneurysms in the cavity wall. Aspergillus infection is the most common fungal agent that settles in the cavity wall and causes hemoptysis in our country. In pulmonary edema; The patient has hemoptysis in the form of pink foamy sputum, and the patient has severe dyspnea, orthopnea, and heart failure findings. The causes of hemoptysis are listed in Table 1.

Bleeding seen in bronchiectasis originates from the bronchial artery and can be serious. It can also be traumatic or iatrogenic hemoptysis. In

alveolar hemorrhages; While hemoptysis is seen immunologically, periodic hemoptysis "catamenial hemoptysis" may occur in the menstrual cycle due to ectopic endometrium in women. Despite all possibilities, the cause of hemoptysis cannot be determined in 50% of the cases. The prognosis is generally good in idiopathic hemoptysis, but it may recur.

Table 1- Conditions causing hemoptysis

Infections: Tuberculosis Fungal infections Lung abscess Bronchiectasis/Bronchitis	**Neoplasm:** Bronchogenic carcinoma Pulmonary metastases Carcinoid Medication (VEGF etc.)	**Vascular/itis:** Pulmonary embolism AV malformations Wegener's granulomatosis Left heart failure
Coagulopathy: Anticoagulant therapy Platelet dysfunction Hemophilia DIC	**Iatrogenic:** Endoscopic biopsy CT guided biopsy Swan Ganz catheterization	**Cardiac** Mitral stenosis left heart failure Tricuspid endocarditis
Medicines Aspirin Anticoagulation penicillamine Solvents Cocaine	**Trauma** Thoracic traumas bronchial rupture fat embolism Tracheal-innominate artery fistula	**Other** foreign body broncho-lithiasis bronchopleural fistula Bronchial telangiectasia amyloidosis pneumoconiosis Catamenial hemoptysis

Diagnosis / Differential Diagnosis

An important step in diagnosis; is to distinguish whether the hemoptysis is a true hemoptysis or a pseudo hemoptysis. Pseudohemoptysis, that is, non-pulmonary blood expectoration, is considered as bleeding from the upper respiratory tract, esophagus, or stomach. Table 2 shows the distinction between hemoptysis and pseudo hemoptysis.

Upper respiratory tract bleeding usually originates from the nose, nasopharynx, throat, and mouth. In this type of bleeding, inhalations of bloody secretions cause a cough reflex and may give the impression of hemoptysis in the form of coughing up blood. Intraoral bleeding is usually caused by psoriasis of the gums. Here the blood is mixed with saliva.

Since hemoptysis and pseudo hemoptysis are not always easily differentiated, a detailed history should be taken from all patients and a careful physical examination should be performed.

Table 2- Hemoptysis-pseudo-hemoptysis distinction

Clinical feature	**HEMOPTYSIS**	**PSEUDHEMOPTYSIS**
origin of blood	Respiratory tract	Oral cavity, larynx, esophagus, stomach,
Cough	More often	less often
respiratory symptoms	More often	less often
Esophagogastric symptoms	less often	More often
Alcohol use, liver disease	less often	More often
nausea, vomiting	less often	More often
Hematemesis, melena	less often	More often
The color of expectorated blood	bright red	brown or black
Intensity of expectoration	clotted or liquid	coffee grounds
Foaminess of expectoration	Generally	rare or absent
pH	alkaline	Acid
alveolar macrophage in sputum	yes	None
Food particles in the expectorator	None	Var

How much bleeding was in the anamnesis, is the bleeding fresh red, mixed with sputum, is it the first time or has it happened before, is there any bleeding from the nose, or gingival, are there signs of infection such as fever, at night sweats, weight loss, joint pain Do you have signs of chronic and/or systemic disease such as blood in the urine, rash, smoking, history of pulmonary, cardiac, renal or rheumatic disease, family or own hematological disease, history of tuberculosis, use of anticoagulant and antiplatelet drugs history, cocaine or other substance use, history of deep vein thrombosis or pulmonary embolism and risk factors, chemical exposure should be questioned.

In the physical examination, signs of respiratory failure such as tachypnea, cyanosis, and use of accessory respiratory muscles should be evaluated first.

Vital signs, condition of the patient, signs of bleeding in the upper respiratory tract, skin bruising, rash and telangiectasia (hereditary hemorrhagic telangiectasia or vasculitis, etc.), venous engorgement, peripheral edema, liver congestion, cachexia, lymphadenomegaly, abnormal heart sounds on auscultation, wheezing, rales should be examined for localized rhonchi.

Diagnostic tests;

- Radiological Imaging
  - Chest X-ray - Computed tomography
- Complete blood count, platelet
- Complete urine
- Renal functions
- Looking for ARB
- Serological tests
- Ventilation perfusion scintigraphy
- Echocardiography
- Bronchial arteriography
- Pulmonary angiography
- Bronchoscopy (Fiberoptic, Rigid)

Bidirectional chest radiography is the first examination to be requested. Lung X-ray; 33-82% may indicate the localization of bleeding, and 35% may indicate the underlying cause (tuberculosis or tumor). If the etiology cannot be identified, contrast-enhanced CT should be performed. Compared to traditional angiography, it is a more accurate choice if multidetector CT can distinguish bronchial and non-bronchial systemic arteries more accurately.

According to whole blood, coagulation, and inflammation parameters, routine biochemistry, and patient findings, tests such as d-dimer and autoantibodies should be requested. In case of insufficient thoracic CT, bronchoscopy should be performed. If there is no abnormality in CT, recurrent hemoptysis, or high-risk malignancy is suspected, if there is CT abnormality, therapeutic bronchoscopy should be performed to stop bleeding as well as for diagnostic purposes. Rigid bronchoscopy has advantages such as superior imaging, airway safety, adequate aspiration or cleaning of blood, clots, and debris, and better use of various therapeutic intervention devices. On the other hand, the fiberoptic bronchoscope is easy to find, can be used at the bedside, and can be easily applied to the intubated patient.

Treatment

Treatment management in hemoptysis is based on the amount of bleeding and the vital stability of the patient. Massive hemoptysis is a medical emergency that needs immediate evaluation and treatment. Death from hemoptysis is usually due to asphyxia, not blood loss. In addition to the severity of bleeding, lung reserve also plays a role in hemoptysis to such an extent that it obstructs the conductive airways of the patient and causes asphyxia and respiratory failure. Even a small amount of hemoptysis can cause asphyxia in a patient with chronic lung disease and limited lung reserve.

In the first evaluation; Whether there is a life-threatening situation or a life-threatening situation at any time, comorbidities and treatments that may be associated with hemoptysis should be evaluated.

First things to do in massive hemoptysis;

1. To prevent aspiration into the unaffected lung,

2. To stop the bleeding,

3. To treat the disease that causes bleeding.

In massive hemorrhages, the cases should be followed up in the intensive care unit. In order to prevent aspiration into the unaffected lung, the patient should be laid on his side with the bleeding side down, oxygen support should be provided, vital parameters should be monitored (saturation, blood pressure, arterial, etc.), and vascular access should be established.

All blood and secretions expected by the patient should be collected in order to monitor the patient's vital and hematological findings and determine the rate of blood and volume lost.

Volume loss should be completed and corrected if there is a coagulation disorder (blood, fresh frozen plasma transfusion, etc.).

Medical treatment;

-Tranexamic acid (transamine); is a synthetic lysine derivative that inhibits plasmin activation. Although it does not contribute to clot formation, it prevents clot disintegration. It is commercially available in 250 mg IV and 250-500 mg oral forms. It is used as 3X1 1 g IV, 3X500 mg orally, and 3X500 mg nebules with 5 cc saline. It is used in hepatic insufficiency, but the dose should be adjusted in renal insufficiency.

-codeine sulfate; 30-60 mg IM every 4-6 hours can be administered to suppress the cough reflex.

- In cases with high anxiety and agitation, midazolam 2-4 mg iv or 5 mg diazepam every 6 hours may be useful for sedation.

intubation;

If bleeding continues and asphyxia develops; The patient is intubated with an 8.5 mm or larger tube so that a bronchoscopy can be performed when necessary.

- If the lesion is in the trachea, selective intubation is performed.

- While left single-lumen intubation is recommended for right system bleeding,

- In left system bleeding, right single lumen intubation is not recommended as it will prevent right upper lobe aeration. In cases of left system bleeding, placement of the endotracheal tube in the trachea and obstruction of the left main bronchus with a balloon catheter from the outer part of the endotracheal tube or through the tube in front of the vocal cords are recommended.

bronchoscopy;

-Rigid bronchoscopy is preferred. Because with rigid bronchoscopy, aspiration of blood and clots can be achieved better, it also helps localization of the bleeding site, the major airways can be seen better and adequate ventilation can be provided. The disadvantage is that it requires general anesthesia.

-Fiberoptic bronchoscopy, which can be performed at the bedside in lower grade bleeding, can be selected to evaluate subsegment anatomy and especially upper lobe orifices.

Recently, it has been stated that while the patient is intubated with a double-lumen endotracheal tube and oxygenation is provided from one lumen of the tube using fiberoptic bronchoscopy, the bleeding site can be detected with FOB from the other lumen, and aspiration can be performed if necessary. This view has not found much support today.

If the chest X-ray is normal or if the bleeding side cannot be determined due to aspiration to the other side, an emergency bronchoscopy can be performed;

1- The bleeding site is determined

2- The formed coagulum is cleaned

3-Bleeding can be controlled by irrigating the bleeding area with adrenaline or cold water.

If the blood comes from the right bronchial system, single lung intubation can be performed on the healthy side with a Fogarty catheter under the guidance of a bronchoscope. This type of application is not recommended on the right side as it may obstruct the upper lobe bronchus. Wrong-side intubation or slippage of the tube can result in death.

Repetitive bronchial lavage with cold (+40 °C) isotonic liquid, administered topically with a bronchoscope, can stimulate local vasoconstriction and accelerate hemostasis. Adrenaline in 1/20.000 dilution (1 ml adrenaline + 19 ml saline), thrombin, and thrombin/fibrinogen solutions are also applied topically.

When bleeding cannot be stopped with these measures;

1- If the bleeding site is known; When the balloon-buffered catheter pushed through the bronchoscope with the endobronchial balloon tamponade method is inflated and fixed in the proximal part of the bleeding segment or subsegment bronchus, aspiration into the intact lung areas is prevented. The balloon should be deflated after 24 hours to prevent complications such as ischemia and post-obstructive pneumonia.

2- If the bleeding site is unknown; Bleeding should be tried to be controlled by performing unilateral lung ventilation with double-lumen catheters.

In intubated patients with massive hemoptysis, electrocautery, argon plasma coagulation, and Nd-YAG laser therapy, as well as photo resection and vaporization, can control bleeding and intervene in the endobronchial lesion.

However, in practice, bleeding can be controlled by irrigation of the bleeding segment with adrenaline solution or cold water without the need for many of these methods. The methods used in the treatment of massive hemoptysis are shown in Table 3.

Table 3- Bronchoscopic methods in massive hemoptysis

Topical treatments	Endobronchial treatments
Washing with cold saline (40) Adrenaline lavage (1:20,000 1-2 ml) Tranexamic acid Vasopressin Ornipressin, Terlipressin Thrombin-fibrinogen complex Oxidized regenerated cellulose	Fogarty balloon tamponade Endobronchial blocker Silicone plugs The thermal ablative technique (laser, APC, Electrocautery, cryotherapy) Endotracheal intubation

Bronchial Artery Embolization (BAE);

It is a minimally invasive and effective method. Especially in hemoptysis due to bronchial artery bleeding, bronchial artery embolization with polyvinyl alcohol, particles N-butyl cyanoacrylate glue, gelatin sponges, triacryl gelatin microspheres, and metallic coils selectively to the bleeding site is recommended. The full anatomy of the bronchial circulation should be determined by performing pulmonary angiography before the procedure.

6.5% spinal artery ischemia is the most important complication. Due to the recurrence risk of up to 30%;

1- In hemoptysis that cannot be stopped by conventional methods,

2- In those with bilateral disease,

3- It is applied in cases with limited respiratory functions and who cannot tolerate surgery.

Since massive hemoptysis mostly originates from the bronchial arteries, it is not recommended for bleeding originating from the pulmonary artery.

Radiotherapy;

-Compression due to edema in the perivascular tissue,

- Vascular thrombosis-forming effect is utilized.

It may be an option when surgical resection is contraindicated and BAE cannot be performed. It may be particularly useful in hemoptysis caused by tumors such as pulmonary angiosarcoma or hemangioendothelioma.

Surgical;

Indications

If the cause of massive hemoptysis is a localized lesion

• If complete resection is possible

• Failure to perform BAE or the continuation of bleeding despite being performed

• If it is thought that it would be a waste of time to try excessive bleeding and embolization that will disrupt hemodynamics.

• These are types of hemoptysis in which embolization is ineffective, such as pulmonary artery rupture and mycetoma.

Segmentectomy, lobectomy, or pneumonectomy can be performed by surgical thoracotomy. Up to 50% mortality has been reported in cases undergoing surgical intervention due to massive hemoptysis.

RESOURCES

1- Li H, Ding X, Zhai S, Gao K. A retrospective study on the management of massive hemoptysis by bronchial artery embolization: risk factors associated with recurrence of hemoptysis. *BMC Pulm Med*. 2023;23(1):87. doi:10.1186/s12890-023-02371-1

2-Carvalho Silva1 M, Filipe Cruz1 J. An undescribed cause of hemoptysis. *J Bras Pneumol*. Published online December 16, 2022:e20220410. doi:10.36416/1806-3756/e20220410

3-McDonaldDM. Angiogenesis and Remodeling of Airway Vasculature in Chronic Inflammation. *Am J Respir Crit Care Med*. 2001;164(supplement_2):S39-S45. doi:10.1164/ajrccm.164.supplement_2.2106065

4-Cauldwell EW, Siekert RG. The bronchial arteries; an anatomic study of 150 human cadavers. Surg Gynecol Obstet. 1948 Apr;86(4):395-412.

5-Zheng Z, Zhuang Z, Yang M, et al. Bronchial artery embolization for hemoptysis: A systematic review and meta-analysis. *Journal of Interventional Medicine*. 2021;4(4):172-180. doi:10.1016/j.jimed.2021.08.003

6-Moen CA, Burrell A, Dunning J. Does tranexamic acid stop haemoptysis?: Table 1: *Interact CardioVasc Thorac Surg*. 2013;17(6):991-994. doi:10.1093/icvts/ivt383

7-Prey B, Francis A, Williams J, Krishnadasan B. Evaluation and Treatment of Massive Hemoptysis. *Surgical Clinics of North America*. 2022;102(3):465-481. doi:10.1016/j.suc.2021.11.002

8-Sakr l., Dutau H. Massive hemoptysis: an update on the role of bronchoscopy in diagnosis and management. Respiration . 2010;80(1):38-58. doi: 10.1159/000274492. Epub 2010 Jan 8.

9-Abdulmalak C, Cottenet J, Beltramo G, Georges M, Camus P, Bonniaud P, Quantin C. Haemoptysis in adults: a 5-year study using the French nationwide hospital administrative database. Eur Respir J 2015 Aug;46(2):503-11. doi: 0.1183/09031936.00218214. Epub 2015 May 28.

10-Fidan A, Ozdoğan S, Oruç O, Salepci B, Ocal Z, Cağlayan B. Hemoptysis: a retrospective analysis of 108 cases. Respiratory Medicine 2002; 96(9): 677-680. doi: 10.1053/rmed.2002.1359.

11- Yoon W, Kim JK, Kim YH, Woong T Chung TW, Kang HK. Bronchial and nonbronchial systemic artery embolization for life-threatening hemoptysis: a comprehensive review. Radiographics 2002;22:1395. doi: 10.1148/rg.226015180.

11-Ittrich H, Bockhorn M, Klose H, Simon M. The Diagnosis and Treatment of Hemoptysis. Dtsch Arztebl Int 2017; 114:371–81. DOI: 10.3238/arztebl.2017.0371.

12-Cutshall DM, Inman BL, Myers M. Treatment of Massive Hemoptysis with Repeated Doses of Nebulized Tranexamic Acid. *Cureus*. Published online September 26, 2022. doi:10.7759/cureus.29625

13-Fartoukh M, Voiriot G, Hadad S, et al. Severe haemoptysis. In: Heunks L, Demoule A, Windisch W, eds. *Pulmonary Emergencies*. European Respiratory Society; 2016:132-150. doi:10.1183/2312508X.10001916

14-Khalil A, Fedida B, Parrot A, Haddad S, Fartoukh M, Carette MF. Severe hemoptysis: From diagnosis to embolization. *Diagnostic and Interventional Imaging*. 2015;96(7-8):775-788. doi:10.1016/j.diii.2015.06.007

15-Rand AD, Blaikley J, Booton R,3 Chaudhuri N, Gupta V, Khalid S, et al. British Thoracic Society guideline for diagnostic flexible bronchoscopy in adults. Du Rand IA, et al. Thorax 2013;68:i1–i44. doi:10.1136/thoraxjnl-2013-203618.

16-Davidson K, Shojaee S. Managing Massive Hemoptysis. Chest. 2020 Jan;157(1):77-88. doi: 10.1016/j.chest.2019.07.012. Epub 2019 Jul 30.

Chapter 3

The Effect of Sexual Myths on Quality of Sexual Life, Marital Satisfaction and Self-Esteem in Married Women with Physically Disabled

Türkan Akyol Güner[1]

Abstract

Physical disability constitutes a large proportion of the population both in the world and in Turkey. However, the problems related to marriage life, sexuality and sexual life of this population have not been adequately studied. The aim of this study is to evaluate the effect of sexual myths on the sexual quality of life, marital satisfaction and self-esteem in married women with physical disability. This descriptive and cross-sectional study was conducted between May 2022 - March 2023. The data of the study were collected by face-to-face interviews with physically disabled married women registered with the Disabled Associations in Zonguldak. The study was completed with 266 physically disabled married women who met the inclusion criteria. "Personal Charateristics Form", "Sexual Myths Scale", "Sexual Quality of Life Scale-Female", "Marital Satisfaction Scale" and "Rosenberg Self-Esteem Scale" were used for the data collection. SPSS was used to analyze the data. In the study, the level of belief in sexual myths of disabled married women was found to be above the medium level, and their sexual life quality, marital satisfaction and self-esteem were found to be low level. In addition, It was determined that there was a negative and strong relationship between belief in sexual myths and quality of sexual life ($r=-0.71$; $p<0.001$); there was a negative and moderate relationship between belief in sexual myths and marital satisfaction ($r=-0.51$; $p<0.05$) and self-esteem ($r=-0.58$; $p<0.05$). This study showed that married women with disability experience problems in their sexual lives. It is thought that the data obtained from the study will contribute to the literature.

1 Assit. Prof., Zonguldak Bülent Ecevit University, Faculty of Health Sciences
E-mail: akyol_turkan@hotmail.com, ORCİD ID: 0000-0003-0138-0669

https://doi.org/10.58830/ozgur.pub128.c509

1. Introduction

Disabled is defined as a person who has lost his physical, mental, spiritual, emotional, and social abilities in various degrees as a result of any disease or accident, from birth or later, and cannot fulfill the requirements of normal life (WHO Global Disability Action Plan 2014–2021; Global Status Report on Disability and Development Prototype 2015). About 15% of the world's population and 12.29% of Turkey's population are people with disability (Turkey and Disabled People in the World, 2020). Of these, 8.8% are individuals with physical disabilities and it has been reported that women have a higher rate of physical disability (Disabled&Elderly, 2017). Physical disability is defined as a disability that does not affect life functions much (WHO Global Disability Action Plan 2014–2021; Global Status Report on Disability and Development Prototype 2015). A physically disabled is a person who has inadequacy, deficiency, and loss of function in the musculoskeletal system. A significant population is affected by the problems of people with a physical disability, who are known to be outnumbered among disability types (Disabled And Elderly Statistics Bulletin, 2022).

People with physical disabilities face many health and social problems throughout their lives. Although these problems are in various and different areas, the main problems are related to their married life or sexual life (Hanson, 1983). The sexual life of the physically disabled is an issue that is not emphasized much, and there are various sexual myths in societies, especially about the sexuality of women with disability. False information, exaggerated beliefs, fabrications, and superstitions that people think are true about sexual matters are called "sexual myths" (Torun et al., 2011; Keçe, 2019). There are some sexual myths about disabled women that both they and society believe. These myths; "Disabled women cannot marry, - Disabled women do not need sex even if they get married, - Disabled women are not sexually attractive, - Disabled women have excessive sexual desire, - Social needs of disabled women are more important than sex, - Disabled young girls do not need sexual education, - Disabled women cannot fully have sex, - Disabled women should not have children, - Disabled women may not be able to have sexual intercourse, Disabled women should be grateful in sexual relations" (Basson, 1998; Mythbusting, 2022).

The state or level of belief in sexual myths, which arise due to many individual and social factors, varies from person to person with a disability, and especially women are more affected by this situation. Due to society's perspective and pressure on the sexuality of disabled people, women with a

disability believe these sexual myths too much and face many difficulties in meeting their sexual needs (Torun et al., 2011). However, disabled women also have rights, sexual feelings, desires, needs, and problems, just like non-disabled women (Law Students For Reproductive Justice Women With Disabilities, 2009). In general, this humane need is prejudiced by society and it is assumed that they cannot have sexual intercourse even if they are married (Hershey, 2000). This situation negatively affects the sexual life quality and marital satisfaction of especially married disabled women (Ivy and Memphis, 2007; Litzinger and Gordon, 2005).

Marriage satisfaction is the satisfaction of spouses in their relationships, happiness in marriage, and positive or negative expression of harmony between spouses. In other words, marital satisfaction is one of the quality indicators of marriage and sexuality (Spanier, 1976; Tezer, 1996). It has also been reported in studies that marital satisfaction affects marital happiness and support exchange between spouses and is very important for the successful execution of the marriage process (Ivy and Memphis, 2007; Litzinger and Gordon, 2005; Celenk and Van de Vijver, 2013; Kudiaki, 2002). In addition, most of the studies have shown that the sexual quality of life is positively related to marital satisfaction (Ivy and Memphis, 2007; Litzinger and Gordon, 2005). If physically disabled women have problems in their sexual life and marital satisfaction, as well as their already difficult social lives, their sexual worth decreases, and they may also have to cope with psychological problems such as a lack of self-confidence and low self-esteem (Earle, 2001; Taleporos and McCabe, 2001). For disabled people, sexual satisfaction and marital satisfaction can be significant indicators of their social integration, quality of life, and self-esteem (Lee and Oh, 2012).

Self-esteem is the individual's perception of herself/himself as a resourceful, valuable person and is considered a positive personality trait. Self-esteem is a function of respect for self and others, confidence, and self-efficacy (Rosenberg, 1965). It is defined as an individual's attitude towards self-importance, competence, and evaluation of achievement (Coopersmith, 1967). Accordingly, self-esteem, which is the sum of an individual's positive and negative attitudes towards herself/himself, is the combination of competence, personal worth, and body image. The feelings of both inadequacy and frustration in disabled people affect their self-esteem (Kassinove and Tafrate, 2002; Yatkın, 2013). The individual's realization of his/her worth and having positive feelings towards himself/herself can be considered an important indicator of mental health. Low self-esteem can cause many problems related to sexuality, especially sexual interest/arousal

disorder. Communication problems between spouses can cause feelings of insignificance, low libido, and sexual dissatisfaction. Self-esteem and self-confidence of disabled people increase in direct proportion to satisfaction with sexual life (Earle, 2001). Information on disability and sexuality in the literature is also scarce and limited. It has been reported that studies should be carried out on these issues to reveal the problems experienced by disabled individuals in their sexual lives and to find solutions. In addition, sexual myths are among the important factors that can affect the general public health, as they affect the sexual process and the sexual quality of life (Taleporos and McCabe, 2001; Wiegerink et al., 2006; Cumurcu et al., 2012). In the literature review, no study was found that evaluated the effects of sexual myths on the sexual quality of life, marital satisfaction, and self-esteem in married women with disability.

The aims of this study were:

1. To determine the effect of sexual myths on the sexual quality of life in married women with physical disabilities.
2. To determine the effect of sexual myths on marital satisfaction in married women with physical disabilities.
3. To determine the effect of sexual myths on self-esteem in married women with physical disabilities.
4. To determine the sociodemographic factors affecting sexual myth, sexual quality of life, marital satisfaction, and self-esteem in physically disabled married women.

2. Methods

2.1. Design and Participants

This descriptive and cross-sectional study was conducted with physically disabled married women living in Zonguldak between May 2022 - March 2023. Zonguldak province is the 21st province with the highest number of the disabled population in Turkey. The data of the study were collected through face-to-face interviews with physically disabled married women registered with the three Disability Associations in Zonguldak. The study's universe consists of 301 physically disabled married women who were members of associations. The sample of the study was determined as 170 women from the study population with a 95% confidence interval and a 5% margin of error. OpenEpi, Version 3 program was used for sample calculation (http://www.openepi.com). The purposive sampling method

was used to reach sampling in the study. The study was completed with 266 physically disabled married women who met the inclusion criteria. Between the ages of 18 and 65, married, a woman, physically disabled, literate, and willing to participate in the study were the inclusion criteria.

2.2. Data Collection Process

"Personal Characteristics Form", "Sexual Myths Scale", "Sexual Quality of Life Scale-Female", "Marital Satisfaction Scale" and "Rosenberg Self-Esteem Scale" were used for the data collection.

2.2.1. Personal Characteristics Form

The form consisted of eight questions prepared by the researcher by examining the literatüre (Litzinger and Gordon, 2005; Celenk and Van de Vijver, 2013; Taleporos and McCabe, 2001). This form included questions about the personal characteristics, disability, and sexual lives of disabled women.

2.2.2. The Sexual Myths Scale (SMS)

This scale was developed to determine whether individuals have sexual myths or not by Gölbasi et al. (Golbasi et al., 2016). The scale has a total of 28 items under eight sub-scales. The scale is a 5-point Likert-type scale, which is scored from 1 to 5. Total scores range from 28 to 140, where higher scores indicate an increase in the likelihood of having a sexual myth. Cronbach alpha of the scale was found to be 0.91. In this study, Cronbach's alpha was determined to be 0.90.

2.2.3. Sexual Quality of Life-Female (SQOL-F)

The sexual quality of life of women was measured using the Turkish version (Tuğut and Gölbaşı, 2010). of the SQOL-F, which was developed by Symonds et al. (Symonds et al., 2005). The sexual quality of life of women for the past 4 weeks was investigated using this scale, which can be used as a valid and reliable measurement tool for women aged 18-65. For the scale, consisting of 18 items, the scores of items 1, 5, 9, 13, and 18 were reversed before calculating the scale items, which were scored between 1 and 6. The total score to be obtained from the scale was converted to 100. The formula [(raw score of the scale - 18) x 100/90] was used for this conversion. High scores indicate a good sexual quality of life (Tuğut and Gölbaşı, 2010). Cronbach's alpha value of the Turkish version of the scale was 0.83. In this study, Cronbach's alpha value of the scale was 0.82."

2.2.4. Marriage Life Scale (MLS)

The scale was developed by Tezer (1996) to measure the general satisfaction level of spouses regarding their marital relationship. The scale is composed of 10 items. The participants assessed to what extent each item defined them, by using a 5-point Likert scale (1 = I do not agree, 5 = I agree). The highest point on the scale is 50, and the lowest point is 10. A high score on the scale indicates a high level of marital satisfaction. The Cronbach Alpha coefficient of the scale was 0.91. In this study, Cronbach's alpha value of the scale was 0.89 (Tezer, 1996).

2.2.5. Rosenberg Self-Esteem Scale (RSES)

The scale developed by Rosenberg (1965) has 12 subdimensions. In this study, the Self-Esteem sub-dimension (10 items) was used (Rosenberg, 1965). It was adapted into Turkish by Çuhadaroğlu in 1986 (Çuhandaroğlu, 1986). The score that can be obtained from the scale ranges from "0" to "6". High scores indicate low self-esteem. The Cronbach Alpha coefficient of the scale was 0.75. In this study, Cronbach's alpha value of the scale was 0.78.

2.3. Statistical analysis

Statistical analysis of the data was performed using SPSS 22.0 (IBM Corporation, Armonk, NY, USA) package program. The conformity of the data to the normal distribution was assessed with Kolmogorov-Smirnov and Shapiro-Wilk tests. Percentage, mean±SD, t-test, One-Way Analysis of Variance (ANOVA), Kruskall Wallis H Test, Mann Whitney U, and Tukey test were used in data analysis. Pearson Moments Multiplication Correlation analyses were used to evaluate the relationship between variables. In the calculation of the correlation strength, the following ranges were taken as a reference: very weak correlation (r = 0–0.25), weak correlation (r = 0.26–0.49), moderate correlation (r = 0.50–0.69), strong correlation (r = 0.70–0.89), and very strong correlation (r = 0.90–1.0) (Gürbüz & Şahin, 2014). $p < 0.05$ value was considered to be significant.

2.4. Ethical standards

This study was performed in line with the principles of the Declaration of Helsinki. Ethical approval was obtained from the Zonguldak Bülent Ecevit University Human Researches Ethics Commission (Date/Number: 13.05.2022-165693). Then, permission was obtained from the Zonguldak Branch Office of the Turkish Disabled Association for disabled associations where the study was conducted (E-46751649- E-2180878-105754). The

participants were informed about the purpose and benefits of the study and were asked to sign the "Informed Consent" form. Permission for the use of the scales was obtained from the responsible authors via e-mail.

3. Results

The descriptive characteristics of married women with physical disabled were given in Table 1. Accordingly, 42.9% were in the 31-40 age range, 49.6% were married between 6-10 years, 66.2% were primary school graduates, 46.6% had one child, 78.2% were unemployed, and the majority of them had moderate income conditions. 49.6% of disabled women had sexual intercourse 1 time per month or less and 74.4% were congenitally disabled.

Table 1. Descriptive characteristics of married women with a physical disabled (N=266)

Variables	n(%)
Age	
20-30 years	90 (33.8)
31-40 years	114 (42.9)
41 and above	62 (23.3)
Duration of marriage	
1-5 year	62 (23.3)
6-10 year	132 (49.6)
11 and above	72 (27.1)
Education status	
Primary education	176 (66.2)
High school	52 (19.5)
Undergraduate	38 (14.3)
Number of children	
0 child	62 (23.3)
1 child	124 (46.6)
2 and above child	80 (30.1)
Working status	
Yes	58 (21.8)
No	208 (78.2)
Income status	
Good	38 (14.3)
Middle/Poor	228 (85.7)
Frequency of sexual intercourse	
1-2 times a week	92 (34.6)
3 times or more a week	42 (15.8)
1 time per month or less	132 (49.6)
Disability status	
Congenital	198 (74.4)
Later	68 (25.6)

The scales mean scores of the disabled married women were given in Table 2. According to the scale evaluation, it was determined that the level of belief in sexual myths of disabled married women was above the moderate level (88.56±19.61). Moreover, their sexual life quality (46.28±5.12), marital satisfaction (26.26±12.47), and self-esteem (3.20±1.64) were found to be low levels.

Table 2. Mean scores of scales

Mean±SD	Min-Max
88.56±19.61	28-120
46.28±5.12	21-100
26.26±12.47	10-50
3.20±1.64	0-6

SMS: Sexual Myths Scale; **MLS**: Marital Life Scale

SQOL-F: Sexual Quality of Life Questionnaire-Female

RSES: Rosenberg Self-Esteem Scale

The comparison of the mean scores of the scales according to the descriptive characteristics of the physically disabled married women was given in Table 3. A statistically significant difference was found between the mean SMS scores of disabled women according to age (p=0.012), education status (p=0.001), working status (p=0.003), income status (p=0.017), and frequency of sexual intercourse (p=0.047). It was determined that the level of belief in sexual myths was higher among disabled women who were over 41 years old, graduated from primary school, did not work, had a medium or low income, and had sexual intercourse once a month or less (Table 3).

A statistically significant difference was found between the mean SQLQ-F scale scores of disabled married women according to age (p=0.041), duration of marriage (p=0.020), education status (p=0.036), frequency of sexual intercourse (p=0.045) and disability status (p=0.047). According to this; It was determined that the sexual quality of life was higher and statistically significant for those between the ages of 20-30, those with a marriage duration of 1-5 years, those with an undergraduate degree, those who had sexual intercourse 1-2 times a week, and those with congenital disability (Table 3).

A statistically significant difference was found between the mean MLS scores of disabled women according to age (p=0.007), education status (p=0.041), income status (p=0.031), frequency of sexual intercourse (p=0.027), and disability status (p=0.041). It was determined that

the marital satisfaction of disabled women between 20-30 years of age, undergraduate graduates, good income, having sexual intercourse 1-2 times a week, and later having a disability were higher and more significant. When the mean scores of the RSES were examined, it was determined that the self-esteem of disabled women was lower and more significant as their age increased, educational status, income status, and frequency of sexual intercourse decreased (Table 3).

Table 3. Mean scores of scales according to descriptive characteristics of women with a physical disabled

	SMS	**SQOL-F**	**MLS**	**RSES**
	Mean±SD	**Mean±SD**	**Mean±SD**	**Mean±SD**
Age				
20-30 years[1]	72.11±8.27	46.97±11.17	28.28±2.75	2.41±1.48
31-40 years[2]	80.07±7.93	43.70±12.35	26.52±3.48	3.06±1.45
41 and above[3]	88.67±11.02	40.05±7.89	25.61±4.18	3.58±1.87
Statistics	**F=18.422; p=0.012; 3>1***	**F=3.567; p=0.041; 1>3***	**F=10.217; p=0.007; 1>3***	**KW=10.624; p=0.003; 1>3***
Duration of marriage				
1-5 years[1]	84.61±4.79	49.97±3.28	27.62±5.28	3.21±1.85
6-10 year[2]	86.05±6.92	47.01±6.07	26.12±6.92	3.22±1.67
11 and above[3]	84.17±5.34	45.87±5.01	25.37±6.08	3.20±1.08
Statistics	F=5.791; P=0.736	**F=4.110 p=0.020; 1>3***	F=4.927; p=0.131	KW=10.145; p=0.101
Education status				
Primary education[1]	93.01±8.40	41.97±13.41	24.48±7.03	3.37±1.87
High school[2]	86.52±7.38	46.85±12.95	26.57±6.12	3.04±1.84
Undergraduate[3]	62.90±9.25	48.10±11.96	27.91±9.01	2.91±1.24
Statistics	**F=23.812; P=0.001; 1>3***	**F=3.958; p=0.036; 3>1***	**F=2.980; p=0.041; 3>1***	**KW=10.627; p=0.040; 3>1***
Number of children				
0 child[1]	84.27±9.01	46.04±11.14	25.98±4.61	3.12±1.65
1 child[2]	85.58±5.92	45.57±10.05	22.57±3.80	3.23±1.91
2 and above child[3]	85.97±8.12	45.92±15.45	21.93±3.92	3.21±1.54
Statistics	F=4.928; p=0.692	F=0.617; p=0.541	F=4.845; p=0.057	KW=4.453; P=0.384
Working status				
Yes	80.01±4.18	45.03±13.76	26.91±6.01	2.66±1.30
No	88.12±6.57	45.43±8.57	26.20±5.87	3.47±1.64
Statistics	**t=0.129; p=0.003**	t=1.627; p=0.523	t=0.191; p=0.071	**U=103.52; p=0.021**
Income status				
Good	81.71±1.62	46.06±14.01	26.78±5.58	2.84±1.15
Middle/Poor	87.30±3.58	44.65±13.01	23.18±6.21	3.45±1.58

Statistics	**t=0.189; p=0.017**	t=1.629; p=0.601	**t=1.017; p=0.031**	**U=104.50; p=0.021**
Frequency of sexual intercourse				
1-2 times a week[1]	82.17±7.28	46.03±14.14	28.12±11.78	2.34±1.45
3 times a week or more[2]	86.21±5.32	44.65±13.09	26.01±9.78	2.74±1.56
1 time per month or less[3]	87.98±4.27	40.04±12.78	23.47±5.31	3.37±1.45
Statistics	**F=10.272; p=0.047; 3>1***	**F=5.758; p=0.045;1>3**	**F=3.928; p=0.027; 1>3***	**KW =9.324; p=0.004; 1>3***
Disability status				
Congenital	84.01±5.11	46.06±8.10	22.87±3.12	3.29±1.65
Later	83.21±4.75	43.35±6.84	25.78±2.85	3.02±1.67
Statistics	t=1.042; p=0.103	**t=1.871; p=0.0**47	**t=1.875; p=0.041**	U=-92.12; p=0.007

t=Independent Sample t-test**; F:** ANOVA**; KW:** Kruskal Wallis H test **U:** Mann Whitney U test**, *:** Tukey test

SMS: Sexual Myths Scale; **SQOL-F:** Sexual Quality of Life Questionnaire-Female; **MLS:** Marital Life Scale

RSES: Rosenberg Self-Esteem Scale

In Table 4, the correlation between the level of belief in sexual myths, quality of sexual life, marital satisfaction, and self-esteem of physically disabled married women was examined. Accordingly, there was a negative and strong correlation between the level of belief in sexual myths and the quality of sexual life($r=-0.71$; $p<0.001$); It was determined that there was a negative and moderate relationship between belief in sexual myths and marital satisfaction ($r=-0.51$; $p<0.05$) and self-esteem ($r=-0.58$; $p<0.05$). It was determined that there was a moderate and positive correlation between the quality of sexual life and marital satisfaction ($r=0.62$; $p<0.001$) and self-esteem ($r=0.50$; $p<0.05$), and a moderate and positive correlation between marital satisfaction and self-esteem ($r=0.63$; $p<0.001$).

Table 4. The relationship between sexual myths, quality of sexual life, marital satisfaction and self-esteem in women with physical disabled

	SMS	**SQOL-F**	**MLS**	**RSES**	
SMS	1.00				
SQOL-F	-0.71**	1.00			
MLS	-0.51*	0.62**	1.00		
RSES	-0.58*	0.50*	0.63**	1.00	
Pearson Moments Multiplication Correlation				***p<0.05**	****p<0.001**

SMS: Sexual Myths Scale; **SQOL-F**: Sexual Quality of Life Questionnaire Female

MLS: Marital Life Scale; **RSES:** Rosenberg Self-Esteem Scale

The regression table shows the effects on sexual myths of sexual quality of life, marital satisfaction, and self-esteem in physically disabled married women (Table 5). According to this; Sexual quality of life, marital satisfaction, and self-esteem were determined to be effective factors in sexual myths (F=30.994; p=0.000). These results are also consistent with the correlation analysis results.

Table 5. Regression table of the effects on sexual myths of sexual quality of life, marital satisfaction, and self-esteem

Dependent Variable	Independent Variable	β	t	p	Adj. R2	F
	Constant		8.748	0.000*	0.499	30.994
Sexual Myths	**Sexual Quality of Life**	-0.453	-6.476	0.000*		
	Marital Satisfaction	-0.378	-5.855	0.018**		
	Self-Esteem	-0.320	-4.980	0.026**		

***p<0.001;**
****p<0.05** **R=Regression coefficient**

4. Discussion

Physical disability constitutes a large proportion of the population both in the world and in Turkey. However, the problems related to married life, sexuality, and sexual life of this population have not been adequately studied. Considering the importance of the subject for disabled individuals, it has been reported that it is important to conduct new studies to find solutions to the problems (Taleporos and McCabe , 2001; Wiegerink et al., 2006). This study, which was planned for these reasons, it was aimed to evaluate the effect of sexual myths on the sexual quality of life, marital satisfaction, and self-esteem in physically disabled married women. *The reason why married women were chosen as a criterion in the study is that false beliefs about sexuality are more common in women and they need more sexual health information. This situation has more negative effects on marital satisfaction and self-esteem* (Basson, 1998; Hershey, 2000; Taleporos and McCabe, 2001).

Sex myths, which are one of the issues that affect sexuality and sexual satisfaction, cause sexuality to be complicated. The most important reasons for the emergence of sexual myths are the inability to talk about sexuality, the value judgments of society, and the insufficient number of scientific studies on sexuality (Mythbusting, 2022; Taleporos and McCabe, 2001). In the study, it was determined that the physically disabled married women had a belief in sexual myths above the moderate level according to the scale evaluation. In

a study conducted with disabled individuals, it was determined that disabled women were more exposed to sexual myths and this reduced their sexual quality of life (McKenzie, 2012). In similar studies, it was determined that disabled people had sexual myths and this situation reduced their sexual life satisfaction (Taleporos and McCabe, 2001; Wiegerink et al., 2006). In the study, it was observed that the level of belief in sexual myths was higher in those who were older, less educated, unemployed, low-income, and had less sexual intercourse. As a result of a study conducted by Taleporos and McCabe on the sexual lives of people with a physical disability, it was determined that age, educational status, and frequency of sexual intercourse affect the level of belief in sexual myths (Taleporos and McCabe, 2001). There have not been many studies on sexual myths in people with disability. This situation limits the discussion of the study results. However, the results of the study were found to be similar to the results of the literature.

Sexuality constitutes an important dimension of the quality of life for people (Earle, 2001). People with physical disabilities struggle with many sexual problems related to their physical limitations. This situation causes a decrease in the sexual quality of life in people with disability (Taleporos and McCabe, 2001). In the study, when the sexual quality of life of married women with a physical disability was examined, it was determined that it was at a low level according to the scale evaluation. As a result of a study conducted on the sexual quality of life of disabled individuals by Wiegerink et al., it was reported that the sexual quality of life of disabled people was low (Wiegerink et al., 2006). In similar studies, it has been reported that disabled individuals experience some difficulties in sexual relations and their sexual quality of life was low (Earle, 2001; Taleporos and McCabe, 2001; Glass and Soni, 1999). These results are similar to the results of the study. Different from the results of this study, some studies showed that disabled women had a normal sexual life like their same sex and did not experience great difficulties and that physically disabled women got used to sexual life more easily. Disabled women were reported to have higher sexual satisfaction, higher sexual esteem, and lower levels of sexual depression than men. It was stated that this was because women paid less attention to their genital functions (Drench, 1992; Tepper et al., 2001; Silvers, 1996).

In the study, it was determined that age, duration of the marriage, educational status, frequency of sexual intercourse, and disability status affect the sexual quality of life in disabled women. In the study, the sexual quality of life of women with a congenital physical disability was found to be higher. It has been reported that people with long-term physical disabilities experience more positive feelings about their sexuality over time (McCabe

and Taleporos, 2003). This situation can be interpreted as the sexual adaptation process of women with a physical disability getting better as they get used to and accept their disability. As a result of a study examining the sexual quality of life of disabled people, it was seen that the duration of the marriage and educational status affect the quality of sexual life, similar to this study's results (Taleporos and McCabe, 2002).

When each individual reaches a certain maturity, society expects her/him to marry, establish a family and have children. However, when it comes to the marriage of disabled people, it is strange for them to get married. It is thought that disabled women cannot be good wife, a good mothers, cannot fulfill their domestic responsibilities, and have no sex life. Marriage and family life of disabled people is an issue that is not emphasized. When the literature is examined, it has been seen that the studies on the marriages and marital satisfaction of disabled individuals are very limited and it is recommended to conduct similar studies (Orbuch et al., 1996). In addition, there is no study in the literature examining the marital satisfaction of disabled people according to different variables. The results of this study are therefore important. Studies have mainly focused on families with disabled children and parents. It was found that married women with a physical disability who participated in this study had a low level of marital satisfaction. In addition, it was determined that age, education level, income status, frequency of sexual intercourse, and disability status affect marital satisfaction. In similar studies, it was determined that people with physical disabilities had low marital satisfaction (Lee and Oh, 2012). In addition, similar to the results of the study, it was determined that age, education level, income status, and sexual satisfaction affect marital satisfaction in physically disabled people (Orbuch et al., 1996; Bradbury et al., 2000; Fincham and Beach, 2010).

It is stated that being disabled from birth is an important factor in reducing marital satisfaction in disabled people (Lobentanz et al., 2004). In the study, it was determined that the marital satisfaction of disabled women whose disability occurred later was higher. As a result of a study conducted on 1217 disabled married people in the United States, it was determined that the subsequent occurrence of physical disability increases marital satisfaction (Yorgason et al., 2008). In the results of similar studies, it was reported that congenital physical disability reduces marital satisfaction (Lobentanz et al., 2004; Nosek et al., 1985).

Self-esteem, which is the sum of an individual's positive and negative attitudes towards herself/himself, is the combination of social competence,

personal worth, and body image. The feeling of frustration and labeling in disabled people affect self-esteem (Rosenberg, 1965; Yatkın, 2013). In addition, organ deficiency is thought to have a significant effect on leading to low self-esteem. In the study, it was determined that the self-esteem of married women with a physical disabilities was low. Similar to the results of the study, in a study conducted with physically disabled people, it was determined that the self-esteem of disabled people was lower than that of non-disabled people (Franzoı et al., 1989). In the study, it was observed that age, education level, income status, employment status, and frequency of sexual intercourse affected the self-esteem of disabled women. In similar studies, it was determined that self-esteem was higher in disabled people with high educational and economic status and working (Akpınar and Şahin, 2016; Dökmen and Kışlak, 2004). It can be thought that economic comfort, having a job and social security increase social status, and increase self-esteem by making individuals feel more comfortable and safe.

Disabled people struggle with many sexual problems related to the physical limitations they experience. These situations can cause a decrease in sexual relations and sexual worthiness in disabled people (Taleporos and McCabe, 2001). In the study, it was determined that there was a significant relationship between the frequency of sexual intercourse and self-esteem, and it was determined that those who had more frequent sexual intercourse had higher self-esteem. In a similar study, it was seen that the frequency of sexual intercourse and sexual life satisfaction were directly proportional to self-esteem (Earle, 2001).

According to the study results; No statistically significant difference was found between self-esteem and disability status. Similarly, in the study of Balcı and Şahin, no significant difference was found between self-esteem and disability status (Balcı and Şahin, 2016). In Yatkın's study, it was observed that being physically disabled, whether congenital or later, did not affect self-esteem (Yatkın, 2013). These results are similar to this study's results.

5. Conclusion

People with a physical disability may experience numerous difficulties in their sexual lives as in other areas. Experiencing sexual problems in addition to physical problems can cause problems in psychosocial life and marital relations. Identifying sexual problems in people with physical disabilities determines how we can help them. This is important that increase the quality of life of disabled people. It should not be forgotten that people with disability are as normal as people who do not have regular sexual life

and have children, and they should be supported. The data obtained from this study will make it possible to evaluate the marriages of married disabled women and will provide new information on marriage and sexual problems. It is thought that this information will be a source for education programs on disability and sexuality and will guide experts working in this field.

Implications

To prevent the sexual problems of disabled people; Awareness should be created by planning education on this issue in society; Educated people should be trained to provide professional counseling to the families of disabled people on sexual issues; Health professionals should also be provided with in-service training on sexual problems and coping strategies for the disabled people. It is recommended to carry out studies involving large groups to understand what types of sexual problems are experienced according to the type of physical disability.

Limitations

The lack of studies on sexual difficulties and marital relationships among disabled persons limited the discussion of the study's findings. Another weakness of the study is that this study group is special, the number of married women with disabilities registered with the disabled association is low, and the total population does not participate.

Acknowledgments

I'd want to thank the disabled women and the presidents of the associations where the survey was performed for their help.

Disclosure statement

The author have no competing interest to disclose.

Funding

This study is not supported by any funding.

References

Akpınar, R.B., & Şahin, G. (2016). Anger and Anger Expression Styles, Self-Esteem and Life Satisfaction of Individuals with Orthopedic Handicap. *Journal of Nursology,* 19, 0-0. doi.org/10.17049/ahsbd.38376

Basson, R. (1998). Sexual health of women with disabilities. *Canadian Medical Association Journal.* 159(4), 359-362.

Bradbury, T., Fincham, F.D., & Beach, S.R.H. (2004). Research on the nature and determinants of marital satisfaction: A decade in review. *Journal of Marriage and Family,* 62(4), 964–980. doi.org/10.1111/j.1741-3737.2000.00964.x

Celenk, O., & Van de Vijver, F.J. (2013). What makes couples happy? Marital and life satisfaction among ethnic groups in the Netherlands. *Journal of Cross-Cultural Psychology.* 44(8), 1273-1291. doi:10.1177/0022022113486003

Coopersmith, S. (1967). The Antecedents of self-esteem. Published by W. H. Freeman and Co Ltd, New York.

Cumurcu, B., Karlıdağ, R., & Almış, B.H. (2012). Sexuality in the physically disabled. *Current Approaches in Psychiatry*, 4(1), 84-98.

Çuhandaroğlu, F. (1986). Self-esteem in adolescents. Unpublished Specialization Thesis. Hacettepe University Faculty of Medicine, Department of Psychiatry, Ankara.

Disability - Draft WHO global disability action plan 2014–2021: *Better health for all people with disability - Report by the Secretariat.* Sixty-Seventh World Health Assembly, A67/16; 2014. http://www.who. int/disabilities/action plan/en

Disabled and elderly statistics bulletin. (2022). https://aile.gov.tr/media/129311/eyhgm_istatistik_bulteni_kasim_22.pdf.

Disabled&Elderly services, family and social policies ministry of Turkey. (2017). http://www.gsdrc.org/wpcontent/uploads/2017/09/179-Disability-prevalence-and-trends.pdf

Dökmen, Z.Y., & Kışlak, Ş.T. (2004). Comparision of Handicapped and Non-handicapped University Students' Demographical and Psychological Characteristics and Problems. *Kriz Dergisi,* 12, 33-47. doi.org/10.1501/Kriz_0000000207

Drench, M. (1992). Impact of altered sexuality and sexual function in spinal cord injury: A review. *Sexuality and Disability,* 10, 3-14. doi.org/10.1007/BF01102244

Earle, S. (2001). Disability, facilitated sex and the role of the nurse. *Journal of Advanced Nursing.* 36(3), 433-440. doi:10.1046/j.1365-2648.2001.01991.x

Fincham, F.D., & Beach, S.R.H. (2010). Marriage in the new millennium: A decade in review. *Journal of Marriage and Family,* 72(3), 630–649. doi.org/10.1111/j.1741-3737.2010.00722.x

Franzoı, S.L., Kessenıch, J.J., & Sugrue, P.A. (1989). Gender diffrences in the experience of body awareness. An experimental sampling study. *Sex Roles,* 21, 499-515. doi.org/10.1007/BF00289100

Glass, C., & Soni, B. (1999). Toolbox: Sexual problems of disabled patients. *The Western Journal of Medicine,* 171, 107-109.

Global Status Report on Disability and Development Prototype. (2015). http://www.un.org/esa/socdev/documents/disability/2016/GlobalStatusReportonDisabilityandDevelopment.pdf

Golbasi, Z., Evcili, F., Eroglu, K., & Bircan, H. (2016). Sexual Myths Scale (SMS): development, validity and reliability in Turkey. *Sexuality and Disability,* 34(1), 75-87.

Gürbüz, S., & Şahin, F. (2014). Research methods in social sciences. *Seçkin Publishing,* Turkey, 100-130.

Hanson, L. (1983). Designing with care: A guide to the adaptation of the built environment for disabled persons. New York, United Nations.

Hershey, L. (2000). Women with disabilities: health, reproduction, and sexuality. Global women's ıssues and knowledge, pp. 58-80. Routledge Press.

Ivy, A.U., & Memphis, U.S. (2007). The influence of a surrender style of coping and empathy on marital and sexual satisfaction. *Dissertation Abstracts International*. 67 (9-B), 5407.

Kassinove, H., & Tafrate, R.C. (2002). Anger management: The complete treatment guidebook for practitioners. pp. 270-271. California: Impact Publishers.

Keçe, C. (2019). Bed legends (3rd Edition), Yagci S (Ed), pp. 11-20. Compass publishing, Ankara.

Kudiaki, Ç. (2002). The relationship between sexual satisfaction and marital adjustment (Unpublished Master's Thesis). Ankara University, Ankara.

Law students for reproductive justice women with disabilities. (2009). http://lsrj.org/documents/09_Women_with_Disabilities.pdf.

Lee, E-K.O., & Oh, H. (2012). Marital satisfaction among adults with disabilities in South Korea. *Journal of Disability Policy Studies.* 23(4), 215–224. doi.org/10.1177/104420731246766

Litzinger, S., & Gordon, K.C. (2005). Exploring relationships among communication, sexual satisfaction and marital satisfaction. *Journal of Sex &Marital Therapy*. 31, 409-424.

Lobentanz, I.S., Asenbaum, S., Vass, K., Sauter, C., Klösch, G., Kollegger, H., & Zeitlhofer, J. (2004). Factors influencing quality of life in multiple sclerosis patients: Disability, depressive mood, fatigue and sleep quality. *Acta Neurologica Scandinavica,* 110 (1), 6–13. doi.org/10.1111/j.1600-0404.2004.00257.x

McCabe, M.P., & Taleporos, G. (2003). Sexual esteem, sexual satisfaction, and sexual behavior among people with physical disability. *Archives of Sexual Behavior,* 32(4), 359-369. doi: 10.1023/a:1024047100251.

McKenzie, J.A. (2013). Disabled people in rural South Africa talk about sexuality. *Culture, Health & Sexuality,* 1–15.

Mythbusting. (2022). http://www.sexualityanddisability.org/sexuality/mythbusting.aspx

Nosek, M.A., Fuhrer, M.J., & Potter, C. (1995). Life satisfaction of people with physical disabilities: Relationship to personal assistance, disability status, and handicap. *Rehabilitation Psychology,* 40(3), 191–202. doi: 10.1037/0090-5550.40.3.191

Orbuch, T.L., House, J.S., Mero, R.P., & Webster, P.S. (1996). Marital quality over the life course. *Social Psychology Quarterly,* 59 (2), 162–171. doi. org/10.2307/2787050

Rosenberg, M. (1965). Society and the adolescent self image. Princeton University Press.

Silvers, B. (1996). Sexuality, early-onset physical disability, and sense of coherence (Doctoral dissertation). Alameda, California School of Professional Psychology.

Spanier, G.B. (1976). The measurement of marital quality. *Journal of Sex &Marital Therapy.* 5, 288– 300. doi: 10.1080/00926237908403734.

Symonds, T., Boolell, M., & Quirk, F. (2005). Development of questionnaire on sexual quality of life in women. *Journal of Sex & Marital Therapy,* 31(5), 385-397.

Taleporos, G., & McCabe, M. (2002). The impact of sexual esteem, body esteem, and sexual satisfaction on psychological well-being in people with physical disability. *Sexuality and Disability,* 20(3), 176–183. doi. org/10.1023/A:1021493615456

Taleporos, G., & McCabe, M.P. (2001). Physical disability and sexual esteem. *Sexuality and Disability.* 19, 131-148. doi.org/10.1023/A:1010677823338

Tepper, M.S., Whipple, B., Richards, E., & Komisaruk, B.R. (2001). Women with complete spinal cord injury: A phenomenological study of sexual experiences. *Journal of Sex & Marital Therapy,* 27, 615-623.

Tezer, E. (1996). Conflict-handling behavior toward spouses and supervisors. *The Journal of Psychology.* 130, 281-292. doi.org/10.1080/00223980.1996.9915009

Tezer, E. (1996). Satisfaction from marital relationship: Marital life scale. *Turkish Psychological Counseling and Guidance Association*, 2, 1–7. Retrieved from https://dergipark.org.tr/tr/pub/tpdrd/issue/21427/229707

Torun, F., Torun, S.D., & Özaydın, A.N. (2011). Men's belief in sexual myths and factors effecting these myths. *Düşünen Adam The Journal of Psychiatry And Neurological Sciences*. 24, 24-31. doi: 10.5350/DAJPN2011240103

Tuğut, N., & Gölbaşı, Z. (2010). A validity and reliability study of Turkish version of the Sexual Quality of Life Questionnaire-Female. *Cumhuriyet Medical Journal,* 32, 172-180.

Turkey and in the world disabled people. (2020). https://ey-der.com/ana-sayfa/turkiyevedunyadaengelliler/#:~:text=Bu%20nedenle%2C%20d%-C3%BCnyadaki%20%E2%80%9Cen%20b%C3%BCy%C3%BCk,siyse%2050% 2D64%20ya%C5%9F%20aras%C4%B1nda%E2%80%A6.

Wiegerink, D.J., Roebroeck, M.E., Donkervoort, M., Stam, H.J., & Cohen-Kettenis, P.T. (2006). Social and sexual relationships and young adults with cerebral palsy: a review. *Clinical Rehabilitation,* 20(12), 1023-1031. doi: 10.1177/0269215506071275

Yatkın, Ş. (2013). Visually and orthopedically handicapped adolescents' self-esteem levels to comprehend the effect of social attitudes towards them. *Black Sea Journal of Social Sciences,* 9(5), 3-12.

Yorgason, J.B., Booth, A., & Johnson, D. (2008). Health, disability, and marital quality: Is the association different for younger versus older cohorts? *Research on Aging,* 30(6), 623–648. doi.org/10.1177/0164027508322570

Chapter 4

Artificial Intelligence (AI) and Ethics in Medicine at a Global Level: Benefits and Risks

Sadi Elasan[1]

Yusuf Ateş[2]

Abstract

Technological developments in medicine have created significant transformations in healthcare services and offered more effective diagnosis and treatment options for patients. Among these advances, artificial intelligence (AI) plays a pivotal role in a variety of medical applications, from disease diagnosis and treatment planning to clinical research and patient care optimization. However, the rapid development of artificial intelligence in medicine also raises ethical challenges and concerns, including patient privacy, data security, inequality and societal impacts. This study examines the potential benefits and risks associated with the global use of artificial intelligence in medicine. The study presents examples and features of global AI-based medical applications, including data-driven diagnosis and treatment, disease prediction and early warning systems, personalized care and treatment planning, drug development and discovery, telemedicine and remote healthcare. Discussions of confidentiality, fairness, integrity, transparency, patient autonomy, responsibility and accountability, change management, social acceptance are emphasized, emphasizing the importance of ethical rules and guidelines in the use of AI in medicine. An analysis of global publication trends in the study of AI and ethics in medicine is also presented, providing insights into the most influential countries and networks of collaboration. As a result, AI has enormous potential in medicine and offers numerous benefits, including better access to healthcare, improved diagnosis and treatment, customized care, resource efficiency, disease prevention and early detection. However, risks related to data security, privacy, inequality and ethical considerations must be addressed. Also, careful management, data security, ethical practices and protection of human factors are vital in leveraging the full potential of AI in medicine.

1 Assoc. Prof., Van Yüzüncü Yıl University, sadielasan@yyu.edu.tr, 0000-0002-3149-6462

2 Teacher, Repuclic of Türkiye Ministry of National Education, Van Fevzi Geyik Primary School, kayramyagizz@gmail.com

https://doi.org/10.58830/ozgur.pub128.c508

1. INTRODUCTION

Technological developments in medicine and health provide the transformation of health services with more effective diagnosis and treatment options for patients. Emerging technologies such as artificial intelligence (AI) play an important role in medicine. AI can perform operations such as data analytics, learning, prediction, suggestion, and decision-making by giving computer systems human-like capabilities. In medicine, AI offers a range of applications, from disease diagnosis and treatment planning to clinical research to optimizing patient care.

However, the rapid development of AI in medicine also raises many ethical challenges and concerns. The use of AI in medicine brings with it several ethical issues such as patient privacy, data security, inequality, ethical values and societal impacts. Therefore, it is important to evaluate the benefits and risks of the use of AI in medicine at a global level.

This section will examine the potential benefits and risks of the use of AI in medicine at the global level. First, an overview of the concepts of AI and medicine will be presented, and the potential and limitations of AI in medicine will be discussed. Then, the relationship between ethics and AI will be addressed, and ethical issues arising from the use of AI in medicine will be discussed.

1.1. Artificial Intelligence and Medicine: Definitions and Concepts

Today, artificial intelligence (AI) technology in the field of medicine creates revolutionary changes in the diagnosis, treatment and care processes of patients. AI is a discipline that gives computer systems human-like abilities and is used in many medical applications, thanks to its ability to perform complex operations such as data analytics, learning, prediction, and decision-making.

The concepts of Artificial Intelligence and medicine provide a fundamental basis for understanding the use of Artificial Intelligence in medicine. Artificial Intelligence refers to a general description of artificial intelligence technology and enables computer systems to have human-like capabilities. Medicine, on the other hand, is a science and application field that covers diagnosis, treatment and care processes related to human health. The use of Artificial Intelligence in medicine interacts with many different aspects of the medical field.

However, the use of Artificial Intelligence in medicine also involves some risks. Risks such as data security and privacy, ethical and legal issues, reliance on

technology, and human factors can affect the use of AI in medicine. Risks such as completely relying on AI's decisions, ignoring the human factor, violating ethical rules and compromising patients' privacy must be carefully considered.

In conclusion, Artificial Intelligence holds great potential in the medical field. However, risks such as ethical, legal, safety and human factors must also be considered. In the future, the use of Artificial Intelligence in medicine is expected to increase and more research and regulations are required in this area. Artificial Intelligence can enable patients to benefit from healthcare services in a more effective and optimized way in the medical field, but it needs to be carefully managed and used correctly.

1.2. The Potential and Limitations of Artificial Intelligence in Medicine

The potential and limitations of AI in medicine include:

Potentials:

• Can use big data analytics and machine learning for disease diagnosis and prediction.

• In treatment planning, it can offer optimized treatment recommendations based on patients' genetic profiles and clinical data.

• It can help make more effective decisions by analysing medical data in data analytics and research.

Limitations:

• Data security and privacy is an important issue and medical data must be stored securely.

• Decision-making processes and outcomes of AI are controversial and should be used in conjunction with clinical assessments by human physicians.

• Issues such as ethical issues, patient confidentiality, data use and liability should be considered.

1.3. The Relationship Between Ethics and Artificial Intelligence

The relationship between ethics and artificial intelligence is extremely important in terms of the impact of artificial intelligence technologies on people's lives, social structure and cultural values. This relationship stems from the fact that AI technologies should be used by ethical principles. Ethical values, human rights, social norms and societal expectations are important factors to consider in the use of AI technologies.

The observance of fundamental principles such as fairness, transparency, reliability, confidentiality, responsibility and human-centeredness ensures the ethical compliance of artificial intelligence systems. However, there are also challenges and controversies with artificial intelligence systems. For example, issues such as data bias, algorithm errors, discrimination, security risks, and unemployment concerns make the ethical relevance of AI systems questionable.

However, the relationship between ethics and artificial intelligence also presents several challenges and controversies. For example, issues such as the risks of discrimination and inequality related to the use of AI systems, privacy violations during data collection and use, and the interference of AI systems in human decisions can trigger ethical discussions.

Therefore, it is important to develop standards and guidelines for the ethical use of AI technologies and to implement these standards in an ethically acceptable manner. It is also important to raise public awareness of the ethically correct use of AI technologies and to involve various stakeholders in the development of AI technologies.

1.4. Global AI-Based Medical Data Collection and Use Practices: Examples and Features

• Data-Driven Diagnosis and Treatment: AI can help diagnose and treat diseases by analysing medical data using big data analytics and machine learning techniques. For example, in image-based diagnostic methods (e.g., radiology, pathology), artificial intelligence can automatically analyse images to detect medical conditions such as cancer, brain damage, and fractures, helping doctors make quick and accurate diagnoses.

• Disease Prediction and Early Warning: AI can use patients' health data to predict disease risk and develop early warning systems. For example, by analysing a patient's electronic health records and clinical data, AI can predict the risk of diseases such as diabetes, hypertension and heart disease and alert doctors to monitor patients at an early stage and take appropriate action.

• Treatment Planning and Personalized Care: AI can plan treatments and provide personalized treatment options using patients' genetic, clinical and other health data. For example, in cancer treatment, AI can analyse a patient's genetic profile, tumour characteristics, and other factors to determine the most effective treatment plan and guide doctors.

• Drug Development and Discovery: AI can accelerate drug development processes and identify potential new drug candidates in drug discovery.

Using big data analytics and AI algorithm methods, AI can select the most promising candidates from thousands of potential drug molecules and identify them for laboratory testing, thereby accelerating drug development processes.

• Telemedicine and Remote Healthcare: AI can help diagnose and treat patients in telemedicine and remote healthcare. Artificial intelligence can play an important role in remote patient monitoring, diagnosis and treatment processes, especially in remote areas, places with transportation difficulties or in emergencies. For example, AI-powered telemedicine systems can assist healthcare professionals in remote diagnosis, treatment planning and patient monitoring by analysing patients' symptoms, medical data, and test results.

1.5. Features of AI-based medical applications are as follows:

• Speed and Accuracy: AI can produce fast and accurate results using big data analytics and machine learning techniques. Especially in image-based diagnostic methods, artificial intelligence can obtain faster and more accurate results than humans.

• Learning and Development: AI can perform better over time. Machine learning algorithms can continuously analyse data and improve themselves to produce more accurate results.

• Objectivity: AI can produce objective results without being affected by human factors. Medical decisions can be based on objective data rather than emotions and prejudices.

• Big Data Analytics: AI can analyse large amounts of data that are difficult for humans to analyse, which can lead to new insights and discoveries.

• Limitations and Challenges: AI-based medical applications also have limitations and challenges. For example, the lack of transparency and interpretability in AI models can lead to trust issues between healthcare professionals and patients. Potential biases in AI models can also lead to ethical and legal issues. Therefore, the development of transparent, interpretable and unbiased AI models is crucial for the successful adoption of AI-based medical practices.

1.6. Ethical Rules and Guidelines: The Use of Artificial Intelligence in Medicine

The use of artificial intelligence (AI) in medicine is regulated by ethical rules and guidelines. Below are some ethical rules and guidelines:

• Confidentiality and privacy policy: Confidentiality and privacy of data are important in the use of AI. Data must be protected and processed by legal regulations.

• Fair use: AI models should not discriminate and their results should be used fairly and equally for all patients.

• Accuracy and reliability: AI models should produce accurate and reliable results.

• Transparency and explain ability: The decision-making processes and results of AI models should be explainable.

• Patient autonomy: Patient preferences and consent are important.

• Training and certification: Health professionals who will use AI should have appropriate training and certification.

• Responsibility and accountability: The use of AI should be documented and evaluated.

• Change management: Change management strategies should be used for the use of AI.

• Social acceptance and ethical discussion: There should be social acceptance and ethical discussions about the use of AI.

2. MATERIAL

A systematic data collection method, search strategy and network analysis software were used to ensure the reliability of our study and the accuracy of the results. Global publication trends in artificial intelligence and ethics in medicine were identified by examining the most influential countries.

In this study, global post-pandemic heart disease studies conducted between 1999-2023 (last access date: 25 April 2023) using the "Web of Science Core Collection (WOS, Clarivate Analytics, Philadelphia, PA, USA)" database were examined. As a result of searches made using the keywords "Artificial Intelligence, Ethics, Medicine" in the database, 602 suitable studies were found.

3. RESULTS

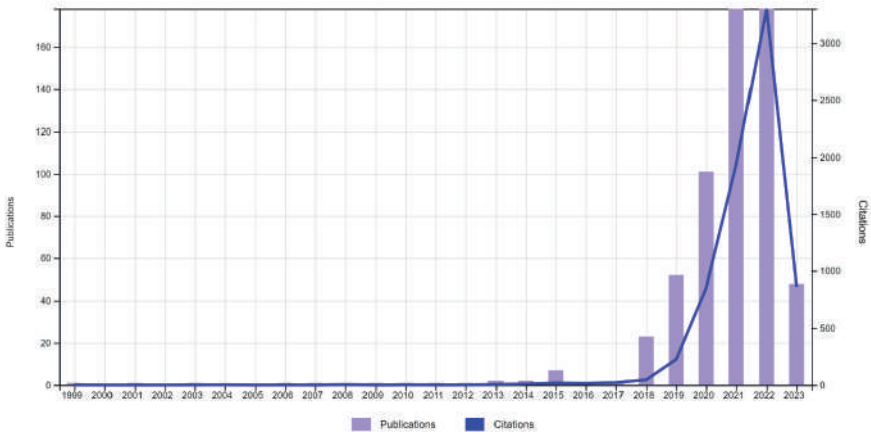


Figure 1. Frequency of publications and citations by year

In this study, 602 published articles were taken from the WOS database. Articles were cited 5786 times (without self-citations). The average number of citations per article is 12. H index is 41. Especially since 2018, both the number of citations and the number of articles showed an increasing trend. In 2023, it is estimated that the number of broadcasts will peak. The distribution of publications and citations is shown in Figure 1.

The USA ranks first in the number of articles published (n=200; 33%), followed by the UK (n=93; 16%); Germany (n=84; 14%) and Canada (n=80; 13%) followed. There were broadcasts from a total of 72 countries around the world, including these first 4 countries, and Turkey ranked 11th. Countries with 12 or more publications are listed in Table 1.

Table 1. Countries with at least 10 publications.

Research Areas	Record Count	% of 602
USA	200	33.223
England	93	15.449
Germany	84	13.953
Canada	80	13.289
Netherlands	57	9.468
China	54	8.970
Australia	50	8.306
France	36	5.980
Switzerland	35	5.814
Italy	33	5.482
Turkey	24	3.987
Spain	19	3.156
Belgium	17	2.824
Singapore	17	2.824
India	16	2.658
Sweden	16	2.658
Austria	13	2.159
Ireland	12	1.993
Showing 18 out of 72 entries (least 12 publications)		

Network analysis

In this study, the "collaboration network" was analyzed using VOSviewer (version 1.6.19, Leiden University, The Netherlands) to identify global trends in "Artificial Intelligence and Ethics in Medicine" studies and important topics of research in this field. These analyzes were performed using text mining and data visualization (bubbles maps and other graphical) methods to ensure the accuracy and reliability of the study.

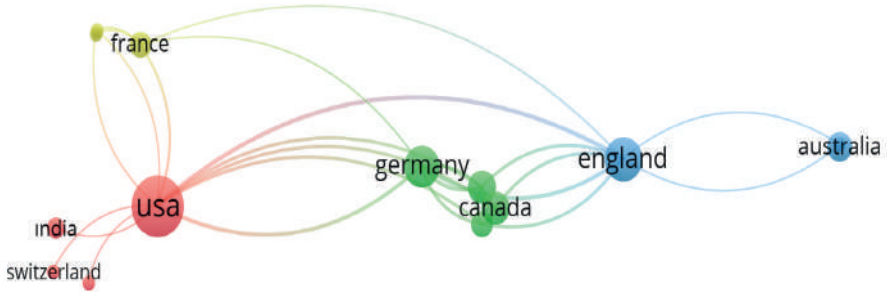


Figure 2. International collaboration network map. (Collaboration between countries is shown by lines, with thickness indicating strength, and circle/text size indicating the level of int. collaboration)

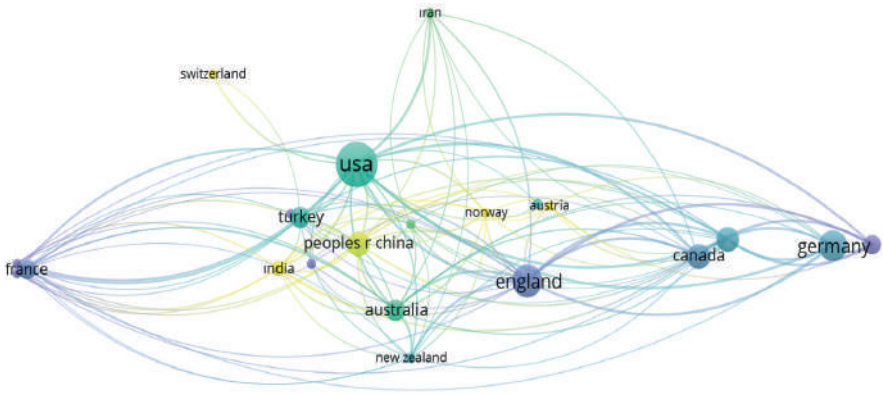


Figure 3. Bibliographic coupling analysis for country. (The relatedness of items was determined based on the number of references the share)

In this study, he examined the worldwide trends and publication trends on the subject of "Artificial Intelligence (AI) and Ethics in Medicine at a Global Level: Benefits and Risks".

The results can be used to guide research in this area and provide a roadmap for research in Artificial Intelligence and Ethics in Medicine at a Global Level. In conclusion, the findings reveal global trends in "Artificial Intelligence and Ethics in Medicine at the Global Level".

4. CONCLUSION

4.1. Potential Benefits of Artificial Intelligence in Medicine: Implications for Patients, Healthcare and Society

Artificial intelligence offers many benefits in the medical field. These are better access to patient's health, higher sensitivity in diagnosis and treatment, customized treatment and care, efficiency and rational use of resources, disease prevention and early diagnosis, health care planning and resource allocation, training and improvement in practice. While these benefits enable patients to receive better health care, they increase the efficiency of health services and provide a more rational use of resources. AI could also potentially be instrumental in disease prevention and early detection and assist in healthcare planning and resource allocation processes. Artificial intelligence, which also offers potential benefits in medical education and practice, can help medical students and professionals improve their skills.

4.2. Potential Risks of Artificial Intelligence in Medicine: Data Security, Privacy, Inequality and Ethical Issues

The use of AI in medicine can lead to potential risks such as data security, privacy, inequality and ethical issues. It emphasizes the need for data security and privacy, protection of health data, and precautions against unauthorized access risks. Inequality indicates that the use of artificial intelligence may increase health inequalities in some regions or population groups. Finally, ethical issues point out that the use of artificial intelligence affects issues such as transparency, accountability, fairness and responsibility. Therefore, it is important that the use of artificial intelligence in medicine is carried out correctly and ethically.

4.3. Trends and Future Prospects in Artificial Intelligence and Medicine at a Global Level

Artificial intelligence is developing rapidly in medicine and has the potential to provide many benefits. Artificial intelligence will be used more widely in processes such as image analysis, diagnosis, treatment planning and disease follow-up. Artificial intelligence, which will enable patients to take an active role, can help them predict disease risks and make informed health decisions. There is also the potential to provide more effective and accessible healthcare to people living in low-income and rural areas. However, it is important to pay attention to data security, ethics and accountability issues. Artificial intelligence can be used in surgical robots, health consultants, personalized treatment plans and drug discovery processes. However, it is also important to protect the human factor and ethical values. As a result, artificial intelligence in medicine can enable patients to receive faster and more accurate diagnoses and treatment, increase the efficiency of healthcare services and improve public health. However, it is important not to ignore data security, ethical problems and the human factor in this process.

REFERENCES

Abdullah, Y. I., Schuman, J. S., Shabsigh, R., Caplan, A., & Al-Aswad, L. A. (2021). Ethics of artificial intelligence in medicine and ophthalmology. *Asia-Pacific journal of ophthalmology (Philadelphia, Pa.)*, *10*(3), 289.

Currie, G., Hawk, K. E., & Rohren, E. M. (2020). Ethical principles for the application of artificial intelligence (AI) in nuclear medicine. *European Journal of Nuclear Medicine and Molecular Imaging*, *47*, 748-752.

Davenport, T., & Kalakota, R. (2019). The potential for artificial intelligence in healthcare. Future Healthc J 6 (2): 94–98.

Guan, J. (2019). Artificial intelligence in healthcare and medicine: promises, ethical challenges and governance. *Chinese Medical Sciences Journal*, *34*(2), 76-83.

Hamet, P., & Tremblay, J. (2017). Artificial intelligence in medicine. *Metabolism*, *69*, S36-S40.

Hindocha, S., & Badea, C. (2022). Moral exemplars for the virtuous machine: the clinician's role in ethical artificial intelligence for healthcare. *AI and Ethics*, *2*(1), 167-175.

Jackson, B. R., Ye, Y., Crawford, J. M., Becich, M. J., Roy, S., Botkin, J. R., ... & Pantanowitz, L. (2021). The ethics of artificial intelligence in pathology and laboratory medicine: principles and practice. *Academic Pathology*, *8*, 2374289521990784.

Karimian, G., Petelos, E., & Evers, S. M. (2022). The ethical issues of the application of artificial intelligence in healthcare: a systematic scoping review. *AI and Ethics*, *2*(4), 539-551.

Keskinbora, K. H. (2019). Medical ethics considerations on artificial intelligence. *Journal of clinical neuroscience*, *64*, 277-282.

LaChat, M. R. (1986). Artificial intelligence and ethics: an exercise in the moral imagination. *Ai Magazine*, *7*(2), 70-70.

Martinho, A., Kroesen, M., & Chorus, C. (2021). A healthy debate: Exploring the views of medical doctors on the ethics of artificial intelligence. *Artificial Intelligence in Medicine*, *121*, 102190.

Mörch, C. M., Atsu, S., Cai, W., Li, X., Madathil, S. A., Liu, X., ... & Ducret, M. (2021). Artificial intelligence and ethics in dentistry: a scoping review. *Journal of dental research*, *100*(13), 1452-1460.

Racine, E., Boehlen, W., & Sample, M. (2019, September). Healthcare uses of artificial intelligence: Challenges and opportunities for growth. In *Healthcare management forum* (Vol. 32, No. 5, pp. 272-275). Sage CA: Los Angeles, CA: SAGE Publications.

Saheb, T., Saheb, T., & Carpenter, D. O. (2021). Mapping research strands of ethics of artificial intelligence in healthcare: a bibliometric and content analysis. *Computers in Biology and Medicine*, *135*, 104660.

Solanki, P., Grundy, J., & Hussain, W. (2023). Operationalising ethics in artificial intelligence for healthcare: A framework for AI developers. *AI and Ethics*, *3*(1), 223-240.

Chapter 5

Childhood Hypertension

Gizem Gencan[1]

Abstract

A significant public health issue is the rising prevalence of hypertension (HT) among children and adolescents. According to studies, controlling and preventing HT in children will result in adequate early treatment and a favorable prognosis, which will lessen the burden of adult cardiovascular disease. HT is defined as systolic or diastolic blood pressure at or above the 95th percentile (P) for age, sex, and height at least three times. When children are initially examined, their blood pressure is normal, and blood pressure measurements begin from age three if there are no risk factors for hypertension. Blood pressure should be monitored yearly in children three years and older.

Children typically experience primary HT. Renal parenchymal illnesses (60–80%), renovascular diseases (10%), and aortic coarctation (2%) are the most frequent causes. In young patients with HT, screening tests (complete urinalysis, hemogram, electrolytes, urea, creatinine, calcium, phosphorus, uric acid, lipid panel, urinary and renal doppler ultrasonography, eye exam, echocardiography, thyroid function tests, renin, and aldosterone) should be carried out. Additional required tests are ordered in response to the patient's new symptoms.

Both medical procedures and lifestyle modifications are part of HT treatment. Recommendations for food and exercise are non-drug therapy. The most widely prescribed medications include calcium channel blockers, vasodilators, diuretics,- blockers, and angiotensin-converting enzyme (ACE) inhibitors. Because of their adverse effects, -blockers are not the first choice. It is advised to use just one medicine for treatment if possible. If, despite raising the maximum dose, blood pressure cannot be controlled by a single medication, a second medication is administered. HT treatment aims to reduce or prevent the risk of cardiovascular disease and damage to target organs in both the early and late stages.

1 Yozgat Bozok University Faculty of Medicine Research and Training Hospital, Department of Pediatrics

https://doi.org/10.58830/ozgur.pub128.c561

1. Introduction

A significant public health issue is the rising prevalence of hypertension (HT) among children and adolescents. Research indicates that preventing and managing hypertension in pediatric patients can result in timely and effective treatment, leading to favorable outcomes and ultimately reducing the prevalence of cardiovascular disease in the adult population.

Hypertension (HT) is defined as having a systolic or diastolic blood pressure equal to or greater than the 95th percentile (P) for an individual's age, sex, and height, and this must be observed on at least three distinct occasions. During the initial assessment of pediatric patients, blood pressure levels are typically within the normal range. However, if there are no identifiable risk factors for hypertension, blood pressure monitoring may commence at the age of three years. It is recommended that blood pressure be assessed every year in children who are three years of age or older. Measuring blood pressure during each medical follow-up is recommended for individuals with hypertension risk factors, such as obesity, diabetes mellitus, and kidney diseases. In the case of children below the age of three, it is recommended to measure blood pressure during each follow-up visit if there exists a risk of hypertension due to factors such as prematurity, low birth weight, umbilical catheterization during the neonatal period, recurrent urinary tract infections, renal pathologies, and other related conditions.

In 2017, the American Academy of Pediatrics (AAP) revised its clinical practice guidelines to provide a more precise definition of hypertension and prehypertension in children.

Blood Pressure Staging (2017 – AAP)

Blood Pressure Stage	Children aged 1-13	Children >13 years old
Normal Blood Pressure	-Systolic and Diastolic Blood Pressure <90 P	-Blood Pressure <120/80 mmHg
High Blood Pressure (prehypertension)	-95 P> Systolic and/or Diastolic Blood Pressure ≥ 90 P, or -95P>Blood Pressure>120/80 mmHg (whichever is lower) (Patient's Blood Pressure is >120/80 mmHg, and this value is classified as High Blood Pressure even if the patient is <90P)	- Systolic Blood Pressure between 120-129 mmHg and Diastolic Blood Pressure < 80 mmHg
Stage 1 HT	95P+12 mmHg > Systolic and/or Diastolic Blood Pressure ≥95P or Blood Pressure between 130/80 – 139/89 mmHg (whichever is lower)	Blood Pressure 130/80 – 139/89 mmHg
Stage 2 HT	Systolic and/or Diastolic Blood Pressure ≥ 95P +12 mmHg, or Blood Pressure ≥ 140/90 mmHg (whichever is lower)	Blood Pressure ≥ 140/90 mmHg
Isolated systolic hypertension	Systolic Blood Pressure ≥ 95P and Diastolic Blood Pressure <90P	≥140/<90

2. Blood Pressure Measurement Method

The procedure involves obtaining a measurement on the right upper arm using an appropriate cuff while the individual is seated (or supine for newborns). Before the measurement, the individual should rest for at least 5 minutes. In cases where automatic oscillometric devices indicate elevated blood pressure readings, verifying the results using android devices is recommended.

Age Appropriate Cuff (Cuff) Sizes

	Arm circumference (cm)	Cuff width (cm)	Cuff length (cm)
Newborn	10	4	8
Infant	6-15	5	15
Child	16-21	8	21
Adolescent	22-26	10	24
Adult	27-34	13	30
Older adult	35-44	16	38

3. Causes of Hypertension

The prevalence of primary hypertension is highest among pediatric patients. Renal parenchymal diseases account for most cases at 60-80%, then renovascular diseases at 10%, and aortic coarctation at 2%. Additional etiologies encompass endocrine etiologies, pharmacological utilization, intraventricular hemorrhage, bronchopulmonary dysplasia, essential hypertension, and white coat hypertension.

4. Diagnostic Approach to Hypertension in Children

Screening tests should be performed in the first step in pediatric patients with HT. Afterward, necessary further tests are requested according to the additional symptoms of the patient.

4.1. Screening Tests

- Complete urinalysis (for proteinuria)
- Henogram
- Electrolytes, urea, creatinine, calcium, phosphorus, uric acid
- Lipid panel (triglyceride, cholesterol)
- Urinary ultrasonography (USG) and renal Doppler USG
- Eye examination (for HT retinopathy)
- Echocardiography (ECO) (for left ventricular hypertrophy and aortic coarctation)
- Hormone levels: fT4, TSH, Renin, Aldosterone

4.2. Advanced Tests

- Fasting insulin and glucose (if obesity)

• 24 h urinary protein excretion and creatinine clearance (if there is renal pathology)

• Spot urine Na, K (in case of monogenic HT)

• Blood gases (in case of monogenic HT)

• 24-hour urine VMA, HVA and 5HIAA (in Pheochromocytoma clinic)

• MIBG scintigraphy (if urine metanephrines are high)

• DMSA / MAG3 or DTPA (if there is an anomaly in urinary USG)

• Captopril scintigraphy (if renal artery stenosis is suspected)

• Renal angiography (DSA) or MR angiography (less invasive than DSA)

5. Hypertension Treatment

Targets in the treatment of HT;

• To reduce or prevent the risk of cardiovascular disease and target organ damage in the early and late stages.

• The objective is to decrease blood pressure levels to less than 95 P for primary hypertension cases without any associated organ damage while considering factors such as gender, age, and height. Additionally, for individuals 13 years or older, the aim is to lower blood pressure levels to below 130/80 mmHg.

• In order to achieve a reduction in blood pressure levels for individuals with chronic kidney disease (CKD), diabetes mellitus (DM), or hypertension (HT) accompanied by target organ damage, it is recommended to maintain a blood pressure level below 90 P. If proteinuria is present, the target blood pressure level should be further reduced to below 75 P.

The management of HT encompasses pharmacological interventions and modifications to one's daily habits and behaviors. Non-pharmacological interventions encompass dietary and physical activity guidelines. A recommended dietary approach involves restricting salt intake to less than 2300 mg per day while increasing consumption of minerals such as potassium and magnesium, as well as folic acid and fiber. Additionally, a diet high in unsaturated and low overall fat is advised. Physical activity is believed to have advantageous effects on hypertension and risk factors associated with cardiovascular diseases. It is recommended that patients engage in exercises other than weight lifting. Furthermore, engaging in competitive sports within unregulated Stage II HT is not permissible.

Drug Treatment of Hypertension

The pharmacological agents most frequently employed in clinical practice include ACE inhibitors, calcium channel blockers, vasodilators, diuretics, and β-blockers. Beta-blockers are not the preferred initial option due to their adverse effects. It is advisable, if feasible, to pursue a monotherapy approach for treatment. In cases where the maximum dosage of a single drug fails to regulate blood pressure, the addition of a second drug is considered. Typically, the administration of ACE inhibitors in combination with diuretics or vasodilators in conjunction with diuretics (or infrequently β-blockers) is observed.

The "ACD" strategy is a practical and reasonable approach for the long-term treatment of children with hypertension.

"ACD" Strategy; "A"; ACE inhibitors (ACEI) (Enalapril,..) and angiotensin receptor blockers (ARB) (losartan,..).

"C"; Calcium channel blockers. Dihydropyridines (nifedipine, amlodipine) and non-dihydropyridines (verapamil, diltiazem; concomitant use with β-blockers is contraindicated (ventricular dysfunction or AV block)

"D"; Diuretics (furosemide, hydrochlorothiazide [HCTZ])

"B"; Beta-blockers (propanolol or atenolol) alone or in combination (B + D or B + C) are not preferred in most hypertensive children.

If more than one drug will be used, "A" category drugs can be combined with "C" or "D" group drugs (A + C or A + D, or A + C + D).

6. Hypertensive Crisis Management and Treatment

Hypertensive emergency: The presence of severe symptomatic hypertension, characterized by a rapid increase in blood pressure, along with one or more instances of target organ damage, is observed. Clinical manifestations such as seizure or encephalopathy, papilledema, retinal hemorrhage or exudate, and indications of heart failure or renal failure may be observed.

Hypertensive urgency: Severe hypertension or rapid increase in blood pressure but no target organ damage or significant symptoms.

Other findings may be present (findings suggesting the underlying cause of hypertension):

- Hematuria, proteinuria, and edema (glomerulonephritis)

• Ataxia, focal neurological deficit, lethargy, coma (intracranial mass or intracranial trauma)

• Decreased femoral pulses or lower extremity blood pressure (Coarctation of the aorta)

• High-dose sympathomimetic use (cocaine, amphetamine,..)

• Pregnancy (eclampsia)

• Abdominal murmur (renovascular disease)

6.1. Diagnostic Evaluation:

• Extremely high blood pressure should be confirmed (measurement technique, cuff size, and location should be checked)

• Blood pressure should be measured from all four extremities (especially in infants and small children)

• Other causes of severe hypertension should be excluded (primary head trauma, intracranial mass, aortic coarctation, use of sympathomimetics..)

• Blood tests: CBC, reticulocyte count; serum electrolytes (Ca, BUN, Cr,.) should be checked

• Urine tests: TIT, urine culture, pregnancy test, and urine drug screening should be done.

• Other diagnostic tests: ECG, Tele-AC X-ray (heart failure), ECHO, and Cranial CT (if there is evidence of trauma or intracranial mass) should be evaluated.

6.2. Treatment

- It is imperative to assess the patient's airway and respiration and ensure the airway is secured, including intubation.
- It is imperative to initiate vascular access.
- To measure blood pressure, it is recommended to insert an intra-arterial catheter if feasible. Alternatively, frequent blood pressure measurements can be taken using the auscultation or oscillometric method.
- In cases of hypertensive emergency, it is recommended to initiate intravenous therapy and to avoid reducing blood pressure by more than 25% of the intended total decrease within the initial 8-hour period.

- The clinical situation determines whether intravenous or oral therapy is appropriate for managing hypertensive urgency.
- It is advisable to prioritize medications that exhibit a swift antihypertensive response.

Resources:

Simone G, Mancusi C, Hanssen H. Et al. Hypertension in children and adolescents. Eur Heart J. 2022 Sep 14;43(35):3290–3301. doi: 10.1093/eurheartj/ehac328.

Lurbea E, RoseicE.A, Cruickshankd J.K, Dominiczak A.Et al. 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. J Hypertens 34:1887–1920 Copyright 2016 Wolters Kluwer Health, Inc. All rights reserved.

Baştuğ F, Dursun İ, Tülpar S, Yel S, Nalçacıoğlu H, Kısaarslan A, Pınarbaşı A.S, Şahin N, Çiçek S. Çocuk Nefroloji El Kitabı, Klinik Pratik Yaklaşımlar. Çocuk Nefroloji Derneği, Kayseri 2018; 33-53.

Lurbe, Empar; Agabiti-Rosei, Enrico; Cruickshank, J. Kennedy; Dominiczak, Anna; Erdine, Serap; Hirth, Asle; Invitti, Cecilia; Litwin, Mieczyslaw; Mancia, Giuseppe; Pall, Denes; Rascher, Wolfgang; Redon, Josep; Schaefer, Franz; Seeman, Tomas; Sinha, Manish; Stabouli, Stella; Webb, Nicholas J.; Wühl, Elke; Zanchetti, Alberto (2016). 2016 European Society of Hypertension guidelines for the management of high blood pressure in children and adolescents. Journal of Hypertension, 34(10), 1887–1920.

Phadke K, Goodyer P, Bitzan M. Hypertension. Manual of Pediatric Nephrology. Springer 2014; 315-349

Hypertension: in Avner E.D, Harmon W.E, Niaudet P, Yoshikawa N, Emma F, Goldstein S.L (eds) Pediatric Nephrology (7th ed) Springer-Verlag Berlin Heidelberg 2016 pp. 1905-2099

Tümer N, özkaya N. Yenidoğan döneminde hipertansiyon tanı ve tedavi adü tıp fakültesi dergisi; 2(1): 33-38

Seliem WA, Falk MC, Shadbolt B, Kent AL. Antenatal and postnatal risk factors for neonatal hypertension and infant follow-up. Pediatr Nephrol. 2007;22(12):2081-7.

nmalFlynn J. Etiology, clinical features and diagnosis of neonatal hypertension. 2014 [cited 2015, Mar 29.]. www.uptodate.com.

Tej K Mattoo, MD, DCH, FRCP. Evaluation of hypertension in children and adolescents. 2017 www.uptodate.com.

Flynn JT, Kaelber DC, Baker-Smith CM, et al. Clinical Practice Guideline for Screening and Management of High Blood Pressure in Children and Adolescents. Pediatrics 2017; 140.

Seeman T, Hamdani G, Mitsnefes M. Hypertensive crisis in children and adolescents. Pediatr Nephrol` ; Jun 28, 2018.

Chapter 6

Current Treatment Methods for Carpometacarpal Joint Fractures and Dislocations

Eşref Selçuk[1]

Abstract

Carpometacarpal (CMC) joint fractures and dislocations are rare hand injuries often resulting from a fall onto a flexed wrist. Treatment options vary as there is no consensus due to these injuries' rarity. The CMC joints connect the hand and carpal bones, with each joint playing a distinct role, making the injuries examined in three groups.

These joints have a robust design due to strong ligaments and unique articulations. Extensor tendons contribute to hand stability, and injuries to these structures can have varying clinical consequences. Despite the absence of a universally recognized categorization method, fractures and dislocations are generally categorized based on displacement direction, joint count, and injury nature.

Diagnosing carpometacarpal (CMC) joint fractures and dislocations involves detailed examination and imaging, including X-rays, CT scans, and MRIs. These injuries are treated to restore the hand's anatomy and functionality. Treatment varies widely and may include conservative measures, open reduction, or internal fixation, with no consensus due to the rarity of such injuries.

Complications include metacarpophalangeal joint stiffness, which can lead to contractures. Despite the severity of these injuries, both surgical and non-surgical treatments often result in positive outcomes, with delayed treatment, inaccurate articular restoration, and secondary fracture displacement leading to poorer outcomes.

Key to successful treatment is early detection and a comprehensive approach including potential surgical intervention and rehabilitation.

1 Lect. Dr.; Trakya University, Department of Orthopedics and Traumatology, Türkiye. trkesref@hotmail.com ORCID No: 0000-0002-1657-1110

https://doi.org/10.58830/ozgur.pub128.c629

1. INTRODUCTION

Fractures and dislocations of the Carpometacarpal (CMC) joint are infrequently observed injuries of the hand. [1]. These injuries can occur as isolated dislocations or as complex fracture dislocations. They typically result from a fall onto a flexed wrist[2]. Since these injuries are rare and often undiagnosed, there is no consensus on their treatment. Some authors opt for closed reduction and follow-up, while others favor open reduction and internal fixation[2–5]. The literature mostly consists of case series[6].

The CMC joint not only provides a connection between the hand and the carpal bones but also plays a distinct role in each joint in the hand. Therefore, it's often examined in three separate groups. These are classified as the first CMC (thumb, preaxial), second and third CMC (central), and fourth and fifth CMC (postaxial or ulnar) injuries[1]. This chapter will focus on 2.-5. CMC joint fractures and dislocations.

1.1.Anatomy

The carpometacarpal (CMC) joints, which connect the carpals of the wrist and the metacarpals of the fingers, have significant clinical importance due to their relevance in understanding various hand pathologies, such as fractures and degenerative joint diseases. These joints showcase a notable stability, which can be attributed to their unique anatomical configuration. The scant number of reported dislocations or fractures in these joints in the literature could well be due to this robust design[7].

Integral to this joint stability are the strong dorsal, palmar, and interosseous ligaments. An interesting pattern to note is the decreased stability provided by these ligaments as one moves radially to ulnarly from the second to the fifth CMC joint. This decrease in stability manifests as augmented mobility in the 4.-5. CMC joints, which, regrettably, results in enhanced fracture instability at these locations [8,9].

Further contributing to the hand's anatomical intricacy are particular tendons: the extensor carpi radialis longus, brevis, and ulnaris. These tendons are notably instrumental in the onset of avulsion fractures at the 2.-3.-5. metacarpals[10].

Taking a closer look at the unique articulations within the hand, one can appreciate the critical role that the CMC joints play in maintaining each ray's longitudinal arch. For instance, the trapezoid is connected to the 2. metacarpal, the capitate to 3.metacarpal, and the hamate to the 4.-5. metacarpal. This setup provides the distal carpal row a greater degree of

rigidity compared to the more flexible proximal row, thereby striking a fine balance between stability and mobility within the hand [11].

The crucial role of ligaments, the connective tissue that links bones together, cannot be overstated. They provide a critical contribution to joint integrity. Specifically, the dorsal ligaments are stronger and more distinct than the palmar ligaments. However, the palmar ligaments, despite being less prominent, bring substantial support to their respective joints[8,11]. The interosseous ligaments connect the third and fourth metacarpals to the capitate and hamate.

The 2.-3. metacarpals establish a stable core structure in the hand, backed by complex bony articulations and ligaments.Key tendons, such as the extensor carpi radialis longus and brevis, contribute to wrist extension and grip strength. Notably, the 2.-3. CMC joints have limited flexibility, mostly in flexion-extension. This rigidity makes these structures susceptible to fractures under high-energy loads, such as falls, punches, or athletic collisions. These injuries can be compounded by the contraction of key extensor muscles, leading to possible bone fragment displacement and varied clinical consequences depending on the injury's specifics.

The base of the fourth metacarpal, presenting five distinct shapes, may form a joint exclusively with the hamate or additionally with the capitate. Its stability is derived from both ligaments and the support of adjacent metacarpals, making isolated fractures very rare due to lack of muscular insertions. The fifth metacarpal base, however, can become unstable due to its ulnar slope and absence of a supportive structural pillar. Notwithstanding, sturdy ligaments and tendons provide additional support, and these tendons can also generate deforming force during a fracture.

Both the 4.-5. CMC joints provide more flexibility than the 2.-3. CMC joints, allowing for flexion-extension, radial-ulnar deviation, and pronation-supination, which is crucial for grasping and palmar cupping. Their fractures are usually the result of axial loading, often associated with dislocations, and can be instigated by different types of trauma. Uncontested extensor forces acting on the base of the fifth metacarpal can cause a shift of the fractured piece towards the body and in the direction of the ulnar back side, necessitating proper adjustment and treatment to prevent further complications.

The synovial membrane forms an essential part of joint anatomy. It encapsulates the joint, reducing friction by secreting synovial fluid, a lubricating liquid that allows the smooth gliding of articulating bones

Blood supply to the CMC joints, pivotal for their normal functioning, arises from a combination of several arteries. The 3.-4. metacarpals are nourished by carpal arches and deep palmar arteries, while the 5. metacarpal depends on the ulnar artery.

Innervation of the CMC joints, which communicate with the central nervous system, primarily stems from ulnar nerve, the median nerve, and the posterior interosseous nerves.

2. FRACTURE TYPE

Fractures and dislocations of the CMC joint can be classified according to direction of displacement, joint count, and nature of the injury. Nonetheless, a universally agreed-upon classification system for these injuries does not exist. Studies have reported that dorsal dislocations are more common, while volar dislocations are less frequently observed. Rarely, complex dislocations known as divergent complex injuries can occur, accompanied by torsional forces.

Nalebuff has categorized isolated 5. CMC joint dislocations as volar radial and volar ulnar dislocations[12]. Additionally, Cain has classified 5th CMC joint fractures and dislocations based on the absence of the hamate bone's involvement[13].

A modification to the classification was proposed by Garcia-Elias, which includes the involvement of the radial two lesser digits[14]. This classification system describes three subtypes: trans-metacarpal injuries, carpometacarpal injuries, and trans-carpal injuries.

3. DIAGNOSIS

Diagnosing and evaluating hand injuries, particularly those involving the carpometacarpal joint (CMCJ), requires a detailed examination and imaging [15]. When assessing hand injuries, it is important to be thorough as swelling can conceal deformities. Recognizing dorsal and volar deformities is particularly significant.

The Indian greeting known as 'namaste' test can serve as a valuable technique to assess potential finger shortening. It is crucial to perform a meticulous evaluation of neurovascular status to identify any possible nerve injury[16]. Swelling can lead to median nerve involvement, which may necessitate carpal tunnel release[17]. Motor examination is critical for identifying ulnar nerve involvement[18].

It is crucial to evaluate the possible detachment of specific muscles and the thumb. In addition to the clinical examination, imaging plays a vital role in the diagnosis and evaluation of hand injuries.

Plain radiographs from different angles should be included in the examination (figure 1). Radiographic assessment should be systematic and should focus on IC and CMC joints. Several features need to be evaluated on X-rays, including Gilula's arcs, parallel articular surfaces, absence of surface overlap, uniform joint space, and metacarpal cascade lines.

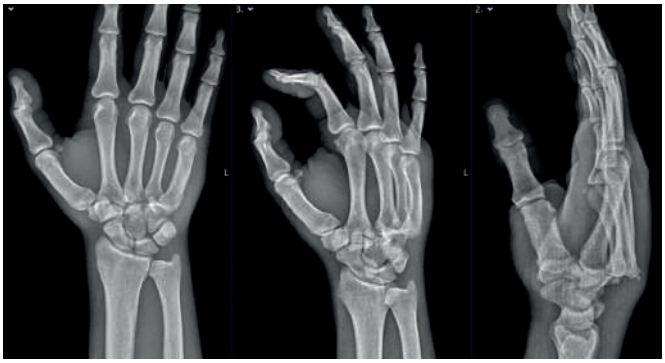


Figure 1. Plain radiographs from different angles, 3-5. CMC fracture dislocation

If clinical signs warrant it, further evaluation with 3D CT or MRI should be taken into account (figure 2). CT scans are preferred for surgical planning and provide better visualization of cortical breaches [5]. On the other hand, MRI excels at detecting purely trabecular fractures.

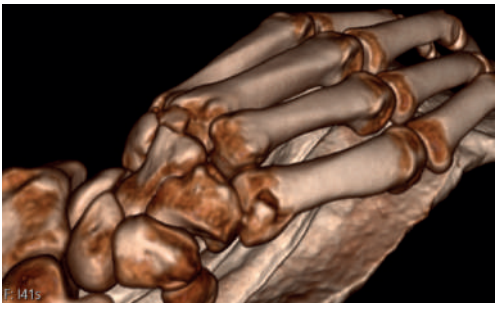


Figure 2. 3D CT 3-5.CMC fracture dislocation

4. TREATMENT

The aim in the fractures-dislocations of the CMC joint is to restore disrupted anatomy (figure 3). A painless, stable hand that can perform normal functions is targeted. Since these cases are rarely seen, there is no common consensus in treatment.

Several factors are considered in the evaluation of second and third carpometacarpal joint fractures and dislocations. The fitting of the joint surface, the attachment site of extensor carpi radialis longus and brevis relative to the fracture are important for surgical indication. However, some authors have expressed the opposite view due to partial immobility of this joint.

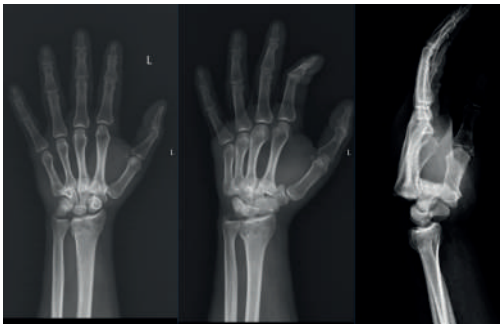


Figure 3. Disrupted anatomy of 2-5 CMC joints

In the literature, there are cases followed by conservative treatment and open reduction (figure 4). Since the ligaments cannot be evaluated accurately without directly seeing the injured area in surgery, many authors suggest open reduction & K-wire [2,19]. Based on the fracture type, cases using tension band, screw, or suture anchor have also been reported.

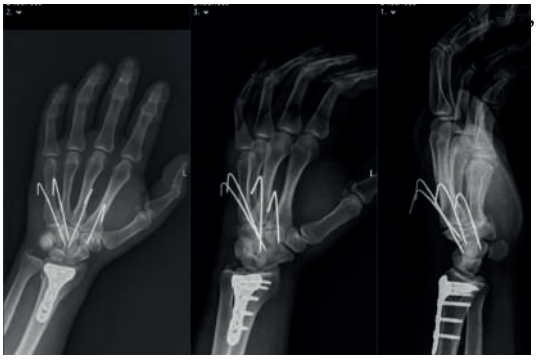


Figure 4. Open reduction, k wire fixation of 2-5. CMC joint

There isn't a widely agreed-upon treatment strategy for fractures of the bases of the 4.-5. metacarpals. Utilizing a compact dorsal arm splint for conservative immobilization is an alternative considered for clinical situations involving non-displaced fractures and well-aligned joints after closed reduction, allowing for fracture union to take place. However, in the case of significant displacement, fragmentation, or complete tear, closed reduction and percutaneous K wire or open reduction and internal fixation are recommended.

Some studies indicate that conservative management can be effective for fractures involving the joint at the base of the 5. Metacarpal [8]. However, it is stated that osteoarthritis development can occur with conservative treatment in displaced intra-articular fractures and surgical intervention may be required in some cases. Other studies show that even after surgical treatment, some patients may experience sustained pain.

There is a limited body of literature concerning fractures at the base of the 4. metacarpal. Typically, these fractures are found in conjunction with 5. CMC dislocations or hamate fractures (figure 5). Open reduction and internal fixation are advised in the surgical treatment of hamato-metacarpal fracture dislocations (figure 6).

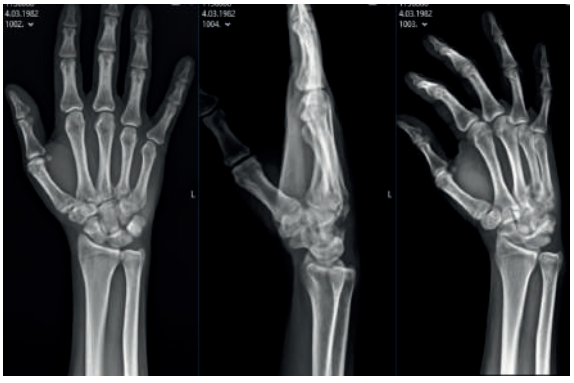


Figure 5. Hamate fracture with 4-5. CMC fracture dislocation

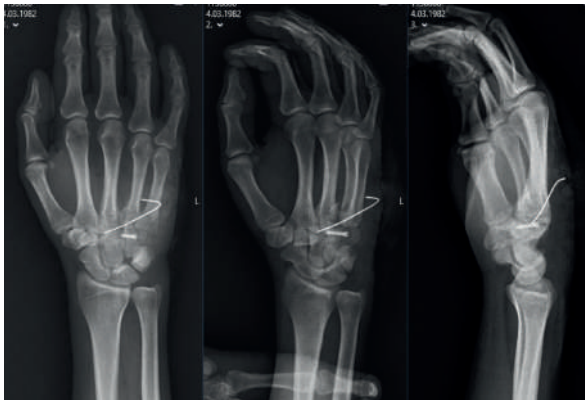


Figure 6. Open reduction, internal fixation and k wire

The treatment protocol of the 4.-5. metacarpal base fractures is still a disputed issue. While conservative treatment is generally preferred for minimally displaced fractures, surgical intervention should be considered in cases of significant displacement or comminution.

Currently, there are only a few studies that compare the functional outcomes of closed and open treatment [16]. There is evidence suggesting that obtaining a precise reconstruction of the original anatomical structure through meticulous open reduction and internal fixation of intra-articular fragments using small K-wires may result in superior functional outcomes relative to closed reduction and stabilization with K-wires. It is crucial to assess each case on an individual basis and tailor the treatment decision based on patient-specific factors.

5. COMPLICATION

A common complication of CMC joint fracture dislocation is metacarpophalangeal (MCP) joint stiffness, potentially leading to extension contractures. This issue, resulting from factors like swelling-induced MCP joint extension, postoperative patient comfort habits, and interossei muscle injuries, is multifaceted.

Prompt surgical intervention is recommended to release hematoma, reduce hand swelling, and prevent ischemic injury to the interossei muscles. Throughout the treatment of the CMC joint using K-wires, the MCP joint is maintained in full flexion to prevent any tethering of the skin or soft tissues. K-wires are inserted from both the radial and ulnar aspects of the hand to further mitigate the risk of tendon tethering. Postoperatively,

patients diligently elevate their hand and engage in supervised therapy that specifically targets flexion of the MCP joint. If stiffness persists, early-stage rubber band traction is utilized, with established contractures potentially requiring surgical release.

6. PROGNOSIS AND CONCLUSION

Regardless of the severity of CMC joint fracture dislocations, positive results are typically attained through the utilization of both non-surgical and surgical treatment approaches. Outcomes are enhanced by the hand's ability to compensate for loss in motion through adjacent joints. However, poor outcomes are tied to delayed treatment, inaccurate articular restoration, concurrent neurological injuries, and secondary fracture displacement.

Various treatments such as CMC joint arthrodesis, interposition arthroplasty, and CMC joint resection have been successfully employed. Anatomical reduction with adequate stabilization is key to satisfactory results. The most favorable results observed in individuals undergoing open reduction and K-wire.

Fractures occurring at the bases of the 2.-3. metacarpals typically exhibit improved outcomes when treated through open reduction and internal fixation. Conversely, fractures involving the base of the 4. metacarpal, though infrequent, tend to demonstrate more positive long-term results with open reduction and internal fixation. The management of fractures at the base of the 5. metacarpal remains a subject of debate, as both surgical and non-surgical approaches frequently result in persistent pain for patients.

In conclusion, early detection and a comprehensive approach to treatment, which could include surgical and non-surgical management and rehabilitation, often lead to favorable outcomes in CMC joint fracture dislocations[20].

7.REFERENCE

[1] Hammert WC, Boyer MI, Bozentka DJ, Calfee RP. ASSH Manual of Hand Surgery. Wolters Kluwer Health; 2012.

[2] Bushnell BD, Draeger RW, Crosby CG, Bynum DK. Management of Intra-Articular Metacarpal Base Fractures of the Second Through Fifth Metacarpals. Journal of Hand Surgery 2008;33. https://doi.org/10.1016/j.jhsa.2007.11.019.

[3] Mueller JJ. Carpometacarpal dislocations: Report of five cases and review of the literature. J Hand Surg Am 1986;11:184–8. https://doi.org/https://doi.org/10.1016/S0363-5023(86)80048-X.

[4] Frick L, Mezzadri G, Yzem I, Plotard F, Herzberg G. Luxations carpométacarpiennes fraîches des doigts longs. Étude à propos de 100 cas. Chir Main 2011;30:333–9. https://doi.org/10.1016/j.main.2011.06.010.

[5] Büren C, Gehrmann S, Kaufmann R, Windolf J, Lögters T. Management algorithm for index through small finger carpometacarpal fracture dislocations. European Journal of Trauma and Emergency Surgery 2016;42:37–42. https://doi.org/10.1007/s00068-015-0611-z.

[6] Hartwig RH, Louis DS, Arbor A, From M. THE JOURNAL OF BONE AND JOINT SURGERY Multiple Carpometacarpal Dislocations A REVIEW OF FOUR CASES. n.d.

[7] Bentley G. European Surgical Orthopaedics and Traumatology: The EFORT Textbook. Springer Berlin Heidelberg; 2014.

[8] Bushnell BD, Draeger RW, Crosby CG, Bynum DK. Management of Intra-Articular Metacarpal Base Fractures of the Second Through Fifth Metacarpals. Journal of Hand Surgery 2008;33. https://doi.org/10.1016/j.jhsa.2007.11.019.

[9] Hammert WC, Boyer MI, Bozentka DJ, Calfee RP. ASSH Manual of Hand Surgery. Wolters Kluwer Health; 2012.

[10] Büren C, Gehrmann S, Kaufmann R, Windolf J, Lögters T. Management algorithm for index through small finger carpometacarpal fracture dislocations. European Journal of Trauma and Emergency Surgery 2016;42:37–42. https://doi.org/10.1007/s00068-015-0611-z.

[11] Bhardwaj P, Sivakumar BS, Vallurupalli A, Pai M, Sabapathy SR. Fracture dislocations of the carpometacarpal joints of the fingers. J Clin Orthop Trauma 2020;11. https://doi.org/10.1016/j.jcot.2020.05.039.

[12] Nalebuff EA. Isolated anterior carpometacarpal dislocation of the fifth finger: Classification and case report. Journal of Trauma - Injury, Infection and Critical Care 1968;8. https://doi.org/10.1097/00005373-196811000-00017.

[13] Cain JE, Shepler TR, Wilson MR. Hamatometacarpal fracture-dislocation: Classification and treatment. Journal of Hand Surgery 1987;12. https://doi.org/10.1016/S0363-5023(87)80064-3.

[14] Garcia-Elias M, Bishop AT, Dobyns JH, Cooney WP, Linscheid RL. Transcarpal carpometacarpal dislocations, excluding the thumb. Journal of Hand Surgery 1990;15. https://doi.org/10.1016/S0363-5023(09)90011-9.

[15] Frick L, Mezzadri G, Yzem I, Plotard F, Herzberg G. Luxations carpométacarpiennes fraîches des doigts longs. Étude à propos de 100 cas. Chir Main 2011;30:333–9. https://doi.org/10.1016/j.main.2011.06.010.

[16] Cates R, Rhee P, Kakar S. Multiple Volar Carpometacarpal Dislocations: Case Report/Review of the Literature. J Wrist Surg 2016;05. https://doi.org/10.1055/s-0036-1580602.

[17] Weiland AJ, Lister GD, Villarreal-Rios A. Volar fracture dislocations of the second and third carpometacarpal joints associated with acute carpal tunnel syndrome. Journal of Trauma - Injury, Infection and Critical Care 1976;16. https://doi.org/10.1097/00005373-197608000-00014.

[18] Gore DR. Carpometacarpal dislocation producing compression of the deep branch of the ulnar nerve. J Bone Joint Surg Am 1971;53. https://doi.org/10.2106/00004623-197153070-00016.

[19] Gehrmann S V., Kaufmann RA, Grassmann JP, Lögters T, Schädel-Höpfner M, Hakimi M, et al. Fracture-dislocations of the carpometacarpal joints of the ring and little finger. Journal of Hand Surgery: European Volume 2015;40:84–7. https://doi.org/10.1177/1753193414562706.

[20] Lawlis JF, Gunther SF, D4 M, Washington DC. THE JOURNAL OF BONE AND JOINT SURGERY Carpometacarpal Dislocations LONG-TERM FoLLoww uP*. n.d.

Chapter 7

The Impact of Biochemical Alterations in the Tumor Microenvironment on Cancer Progression and Treatment

Seyhan Turk[1]

Abstract

The tumor microenvironment (TME) plays a critical role in cancer progression and treatment response. Recent studies have revealed that biochemical alterations within the TME can significantly influence tumor behavior and therapeutic outcomes.

Alterations in the TME, such as changes in pH, hypoxia, and nutrient availability, have been shown to promote cancer cell survival and growth. Acidic pH conditions within the TME enhance tumor invasiveness and metastasis while conferring resistance to conventional therapies. Hypoxia, caused by insufficient oxygen supply, not only promotes genetic instability and immune evasion but also induces resistance to radiation and certain chemotherapeutic agents. Additionally, nutrient deprivation within the TME can activate survival pathways in cancer cells, leading to treatment resistance.

Understanding the biochemical alterations in the TME has led to the development of novel therapeutic approaches. Strategies to modulate the TME, such as targeting angiogenesis, reversing immunosuppression, and normalizing the microenvironment, have shown promise in preclinical and clinical studies. Combining conventional therapies with agents targeting the TME holds potential to overcome treatment resistance and improve patient outcomes.

In conclusion, the biochemical alterations within the TME significantly impact cancer progression and treatment response. Recognizing these alterations and their influence on therapeutic outcomes is crucial for developing effective treatment strategies. Continued research in this area is vital to unravel the complexity of the TME and identify novel therapeutic targets for improving cancer patient outcomes.

1 Lecturer Dr. Hacettepe University, Faculty of Parmacy, epartment of Biochemistry
seyhan.turk@hacettepe.edu.tr, ORCID ID: 0000-0003-3843-4173

https://doi.org/10.58830/ozgur.pub128.c626

Introduction

The tumor microenvironment consists of various cellular and non-cellular components surrounding the tumor. Biochemical changes in the tumor microenvironment may have important effects on cancer progression and treatment (Arneth, 2020). Biochemical changes in the tumor microenvironment can promote tumor growth and invasion. Factors secreted by tumor cells and stromal cells within the microenvironment can stimulate cell proliferation, angiogenesis, and tissue remodeling, allowing the tumor to expand and invade neighboring tissues (Denton et al., 2018).

Biochemical factors in the microenvironment, such as cytokines and chemokines, can recruit immunosuppressive cells, including regulatory T cells and myeloid-derived suppressor cells, which reduce the anti-tumor immune response (Denton et al., 2018; Wu et al., 2015). The altered composition of extracellular matrix components, increased interstitial pressure, and impaired blood supply can limit the delivery and effectiveness of chemotherapy drugs and radiation therapy (Wu et al., 2015; Multhoff et al., 2012). In addition, the microenvironment may provide survival signals to cancer cells, protecting them from the cytotoxic effects of treatment (Hinshaw et al., 2019).

The tumor microenvironment undergoes angiogenic remodeling, characterized by the formation of new blood vessels to meet the increased nutrient and oxygen demands of the growing tumor (Fukumura et al., 2007). Biochemical factors secreted by cancer cells, such as vascular endothelial growth factor (VEGF), stimulate angiogenesis and aggregation of endothelial cells (Nicosia, 1998; Byrne et al., 2005). This facilitates the establishment of an extensive network of blood vessels within the tumor.

1. Oncogenes and Tumor Suppressor Genes

Oncogenes are genes that, when mutated or activated, promote cell growth and division (Feldman et al., 1991). Tumor suppressor genes normally regulate cell growth and division and may prevent cancer development (Jones et al., 2009). Changes in these genes can upset the balance between cell proliferation and cell death (Ryan et al., 2001).

Proto-oncogenes are the main regulators of biological processes and are found in normal cells. It can act as proto-oncogenes, growth factors, signal transducers, and nuclear transcription factors. The genomes of mammals and birds contain several proto-oncogenes that regulate normal cell differentiation and proliferation (Müller, 1986). Changes in these genes that affect the regulation of their behavior or the structure of their encoded

proteins can emerge as oncogenes in cancer cells. When such oncogenes are produced, they promote cell proliferation and play a crucial role in the pathogenesis of cancer. There are two categories of physical mutations that result in the activation of proto-oncogenes: those that cause differences in the structure of the encoded protein and those that cause dysregulation of protein expression (Jan et al., 2019; Abel et al., 2009). Point mutations of RAS proto-oncogenes and chromosomal translocations producing chimeric genes such as Philadelphia translocation (BRC-ABL) are examples of mutations that affect structure (Abel et al., 2009; Bataille et al., 2017; Kurebayashi, 2001; Klinakis et al., 2006).

Activation of proto-oncogenes results in their conversion to oncogenes; To date, 50 to 60 oncogenes have been identified (Lee et al., 2010). Each proto-oncogene promoter allows the gene to respond to a variety of physiological signals. Depending on the metabolic requirements of the cell, a proto-oncogene may be expressed at very low levels; however, under certain conditions, the expression of the gene can be significantly induced (Lee et al., 2010).

The activation mechanisms of proto-oncogenes are as follows:

a) Chromosomal translocation of a proto-oncogene from a non-replicating location to an adjacent location where it can be replicated (chromosomal translocation of the MYC oncogene in human Burkitt lymphoma).

An example of a chromosomal translocation involving the MYC oncogene is the t(8;14) translocation commonly found in Burkitt lymphoma. In this translocation, a portion of chromosome 8 containing the MYC gene fuses with a portion of chromosome 14, resulting in dysregulation of MYC expression. MYC oncogene plays a crucial role in cell cycle regulation, cellular growth and differentiation. Deregulated expression of MYC due to the t(8;14) translocation leads to constitutive activation of the MYC signaling pathway. This pathway is involved in various cellular processes and is involved in tumor progression. MYC levels are tightly regulated under normal conditions. However, MYC is overexpressed in the presence of the t(8;14) translocation (Quatrin et al., 2021). The displaced MYC gene is now under the control of regulatory elements from the immunoglobulin heavy chain gene (IgH) on chromosome 14, resulting in increased MYC expression. The overexpressed MYC protein forms a heterodimer with its partner protein Max. This MYC-Max complex binds to specific DNA sequences called E-boxes in the promoters of target genes, leading to their activation. Target genes regulated by MYC are involved in cell proliferation,

metabolism and apoptosis (Lüscher et al., 2012; Nie et al., 2012). One of the primary functions of MYC is to promote cell cycle progression from G1 phase to S phase. MYC activates the expression of genes involved in cell cycle regulation, such as cyclins and cyclin-dependent kinases (CDKs), which direct cell division. MYC also supports metabolic reprogramming to support the increased energy demands of rapidly dividing cancer cells. It increases nutrient availability for cancer cell growth by activating genes involved in glucose uptake, glycolysis and glutamine metabolism. Normally, MYC regulates cell death and apoptosis. However, in the context of MYC translocation, its dysregulated expression may impair apoptosis and allow cancer cells to escape programmed cell death. In addition, MYC overexpression can trigger cellular senescence, a state of irreversible growth arrest. The dysregulated MYC signaling pathway promotes uncontrolled cell proliferation, genomic instability, and resistance to cell death mechanisms. These factors contribute to tumor growth, metastasis, and progression in Burkitt's lymphoma and potentially other cancers associated with MYC dysregulation (Rohrberg et al., 2020).

b) Point mutation of a proto-oncogene in which the substitution of a single base by another base results in the substitution of an amino acid in the oncoprotein (a point mutation at codon 12 of the RAS oncogene).

It is a well-known genetic change found in several types of cancer, including colorectal cancer, lung cancer, and pancreatic cancer. This mutation affects the RAS gene, specifically one of three isoforms: KRAS, NRAS, or HRAS. The mutation results in the substitution of a single nucleotide that results in an amino acid change in the protein product of the RAS gene (Miyakura et al., 2002). The most common mutation at codon 12 is the replacement of glycine (G) with valine (V), aspartic acid (D), cysteine (C), or arginine (R). This substitution disrupts the intrinsic GTPase activity of the RAS protein, preventing it from hydrolyzing GTP to GDP, which is essential for normal RAS function and regulation. The mutated RAS protein is locked in its active GTP-bound state, leading to sustained activation of downstream signaling pathways involved in cell growth and survival. RAS is an important upstream regulator of the mitogen-activated protein kinase (MAPK) pathway (Gerber et al., 2022; Farr et al., 1988). The mutated RAS protein lacks efficient GTPase activity and disrupts normal down-regulation of RAS signaling. This leads to sustained activation of downstream effectors even in the absence of growth factor stimulation. Constitutive activation of RAS signaling promotes uncontrolled cell growth, survival, and escape of growth inhibitory signals. It also contributes to increased angiogenesis,

invasion, and metastasis and ultimately promotes tumor progression (Sparmann et al., 2004).

c) Gene amplification by including multiple copies of an oncogene results in increased oncoprotein production (c-MYC in neuroblastoma).

Neuroblastoma is a childhood cancer that arises from immature nerve cells called neuroblasts. It is characterized by abnormal growth of these cells in the adrenal glands, abdomen, chest, or spinal cord (David et al., 1989). Amplification or overexpression of the c-MYC oncogene is commonly observed in neuroblastoma. This can occur through a variety of mechanisms, including gene amplification, chromosomal rearrangements, or dysregulation of transcriptional control elements. Dysregulation of c-MYC contributes to the uncontrolled cell proliferation, survival, and differentiation observed in neuroblastoma. Dysregulated c-MYC in neuroblastoma affects various cellular processes and signaling pathways. Overexpression of c-MYC in neuroblastoma leads to increased cell proliferation and decreased apoptosis, promoting tumor growth. c-MYC also affects the balance between cell differentiation and apoptosis. Normally, c-MYC expression is downregulated during cell differentiation. However, dysregulated c-MYC expression in neuroblastoma inhibits differentiation and contributes to tumor progression by promoting cell survival. c-MYC stimulates the production of pro-angiogenic factors (Hatzi et al., 2002). In neuroblastoma, dysregulated c-MYC can enhance angiogenesis. Similar to other cancers, dysregulated c-MYC in neuroblastoma drives metabolic reprogramming to meet the energy demands of rapidly dividing cells. It promotes glucose uptake, glycolysis and glutamine metabolism, ensuring tumor cell growth and survival. c-MYC dysregulation may contribute to genomic instability by leading to the accumulation of additional genetic changes in neuroblastoma cells. Genomic instability is a hallmark of cancer and can further increase tumor progression and heterogeneity. Understanding the role of dysregulated c-MYC in neuroblastoma is crucial for developing targeted therapies. Efforts are being made to develop drugs that specifically inhibit c-MYC or target downstream pathways affected by c-MYC dysregulation. By targeting c-MYC and its associated signaling pathways, the researchers aim to disrupt neuroblastoma cell growth and improve patient outcomes (Nisar et al., 2020).

d) Combining a gene that promotes transcription (promoter gene) near the proto-oncogene causes overexpression of the gene (mechanism of retrovirus carcinogenicity).

Retroviruses are a family of RNA viruses that have the ability to integrate their viral DNA into the host cell's genome. They can cause carcinogenicity

(cancer development) through several mechanisms. Retroviruses can integrate their viral DNA into the host cell's genome, usually near or within the genes involved in cell growth regulation. This integration can disrupt the normal regulation of these genes, leading to uncontrolled cell growth and potentially their transformation into cancer cells. Viral integration can activate oncogenes or contribute to carcinogenesis by inactivating tumor suppressor genes. Some retroviruses carry oncogenes, which are genes that can induce cancer development. These viral oncogenes are derived from cellular genes captured during previous infections and incorporated into the viral genome. When the retrovirus infects a host cell, the viral oncogene can be expressed and contribute to cellular transformation by altering normal cell growth and survival pathways (Sahu et al., 2022). Retroviral infection can suppress the immune system and allow cells to multiply that would otherwise be eliminated by immune surveillance. This immunosuppression can create a conducive environment for cancer development and progression. Retroviral infection can lead to chronic inflammation, which is known to play a role in promoting tumor growth and progression. Inflammation produces reactive oxygen species and inflammatory mediators, which can damage DNA, promote cell proliferation, and create an environment that promotes cancer growth. Immortalization and Telomere Retroviruses can induce cellular immortalization by activating telomerase, an enzyme that lengthens telomeres, the protective ends of chromosomes. Telomerase activation allows cells to transcend the natural limits in cell division and continue to proliferate, which is a characteristic feature of cancer cells. It is important to note that retroviruses have varying levels of carcinogenic potential. For example, certain retroviruses such as human T-cell lymphotropic virus type 1 (HTLV-1) and human immunodeficiency virus (HIV) have been strongly associated with certain types of cancer, such as adult T-cell leukemia/lymphoma and AIDS (Romanish et al., 2010; Fan, 1994).

2. Genetic Mutations

Mutations in genes play a crucial role in the development of cancer. Oncogenes, tumor suppressor genes, and other genes involved in DNA repair, cell cycle regulation, and apoptosis (programmed cell death) can undergo mutations (Grandér, 1998).

Cancer is a genetic disease while many factors can contribute to cancer development, genetic mutations are the driving force behind the onset and progression of most cancers (Aranda-Anzaldo, 2001).

Driver Mutations: Driver mutations are changes in certain genes that give cancer cells a growth advantage. These mutations directly contribute to the development and progression of cancer. Driver mutations can occur in oncogenes (genes that promote cell growth) or tumor suppressor genes (genes that prevent uncontrolled cell growth). Examples of commonly mutated oncogenes include KRAS, EGFR, and BRAF, while tumor suppressor genes such as TP53 and PTEN are frequently mutated in various cancers (Temko et al., 2018; Li, 2016).

Passenger (Passanger) Mutations: Passenger mutations are genetic changes that occur during cancer development but do not directly contribute to tumor growth. These mutations are a result of the genomic instability and chaotic nature of cancer cells. Although passenger mutations do not drive cancer progression, they can be used to trace a tumor's evolutionary history and provide insight into its cellular diversity (Bozic et al., 2016).

Germline Mutations: Germline mutations are inherited genetic changes found in every cell of an individual's body. These mutations are passed on from parents and may predispose individuals to an increased risk of developing certain types of cancer. For example, mutations in the BRCA1 and BRCA2 genes significantly increase the risk of breast and ovarian cancer (Iau et al., 2001).

Somatic Mutations: Somatic mutations are acquired genetic changes that occur in certain cells throughout a person's life. These mutations are not inherited and are usually caused by exposure to environmental factors such as radiation, chemicals, and tobacco smoke. Somatic mutations accumulate over time and can lead to the development of cancer. For example, exposure to UV radiation from the sun can cause mutations in skin cells, increasing the risk of skin cancer (Martincorena et al., 2015).

Different types of mutations can leave certain patterns or signatures in the cancer genome. These mutation signatures can provide insight into the underlying causes of genetic mutations and help identify potential carcinogens. For example, exposure to tobacco smoke leaves a distinct mutational signature characterized by certain types of DNA changes (Alexandrov et al., 2016).

3. Cell Signaling Pathways

Various signaling pathways control cell growth, survival, and proliferation. Changes in these pathways can lead to uncontrolled cell division and evasion of cell death mechanisms. For example, the Ras-Raf-MAPK pathway and the PI3K-AKT-mTOR pathway are frequently dysregulated in cancer. Cell

signaling pathways play a crucial role in normal cellular processes, including cell growth, proliferation, differentiation and survival. However, when these signaling pathways become dysregulated, it can lead to the development and progression of cancer (Harvey, 2019).

Oncogenic mutations or changes can occur in components of signaling pathways such as receptor tyrosine kinases (RTKs), downstream signaling molecules, or transcription factors. These mutations can trigger uncontrolled cell growth and proliferation, leading to constitutive activation of signaling pathways. Examples include mutations in the EGFR gene in lung cancer or the BRAF gene in melanoma that result in hyperactive MAPK signaling (Lundby et al., 2019). Many cancers take advantage of growth factor signaling pathways, such as the epidermal growth factor receptor (EGFR) pathway, to promote cell survival and proliferation. Dysregulation of growth factor receptors or their downstream effectors can lead to sustained activation of the pathway, promoting tumor growth. Targeting these pathways with specific inhibitors has become a successful therapeutic strategy in some cancers (Yewale et al., 2013). The PI3K/AKT/mTOR pathway is frequently dysregulated in cancer. Activation of this pathway supports cell survival, growth and metabolism. Mutations in components of the PI3K pathway or upstream regulators such as loss of PTEN lead to increased signaling along this pathway, contributing to uncontrolled cell growth and resistance to therapies (Martelli et al., 2011). The Wnt/β-Catenin pathway plays a crucial role in embryonic development and tissue homeostasis. Dysregulation of this pathway can occur through mutations in components of the Wnt pathway or stabilization of β-catenin. Abnormal activation of the Wnt/β-Catenin pathway promotes cell proliferation and is associated with a variety of cancers, including colorectal cancer (Bian et al., 2020). Notch signaling pathway regulates cell fate determination, differentiation and tissue development. Dysregulation of Notch signaling has been associated with numerous cancers, including leukemia, breast cancer, and pancreatic cancer. Abnormal activation of Notch signaling can disrupt normal cellular differentiation, leading to uncontrolled cell growth and tumorigenesis (Yin et al., 2010). The Hedgehog pathway is involved in embryonic development and tissue homeostasis. Mutations in components of the Hedgehog pathway, such as Flattened (SMO) or Patched (PTCH), can cause aberrant activation of the pathway, contributing to a variety of cancers, including basal cell carcinoma and medulloblastoma (Skoda et al., 2018).

4. Metabolism

Cancer cells exhibit altered metabolism compared to normal cells. They often rely on glycolysis (the breakdown of glucose) even in the presence of oxygen, known as the Warburg effect. This metabolic shift provides cancer cells with the necessary building blocks for rapid proliferation. Cancer cells exhibit different metabolic changes compared to normal cells, a phenomenon known as "metabolic reprogramming". These changes in metabolism are essential to support increased energy demands, rapid proliferation and survival of cancer cells.

Cancer cells often rely on glycolysis, which is the breakdown of glucose, even in the presence of oxygen (aerobic conditions); this is a less efficient way of producing energy in mitochondria compared to oxidative phosphorylation. This metabolic switch enables cancer cells to produce ATP and the building blocks necessary for cell growth and proliferation more quickly. Intermediate products of glycolysis can also be directed to support other biosynthetic pathways needed for cell division (Lincet et al., 2015). Cancer cells have an increased demand for an amino acid, glutamine. Glutamine acts as a carbon source for the synthesis of nucleotides, lipids and non-essential amino acids that support the high biosynthetic needs of cancer cells. Additionally, glutamine metabolism contributes to the production of antioxidants and helps cancer cells manage oxidative stress (Desideri et al., 2015). Cancer cells exhibit enhanced lipid biosynthesis to provide membrane building blocks and support cell growth. They regulate de novo fatty acid synthesis and increase lipid intake. Lipids also play a role in signaling pathways and may promote cancer cell survival and migration. Rapidly dividing cancer cells require a large supply of nucleotides for DNA and RNA synthesis. Cancer cells regulate pathways involved in nucleotide biosynthesis to meet this demand. Increased nucleotide synthesis also provides an opportunity for therapeutic targeting of cancer metabolism (Robinson et al., 2020; Mashima et al., 2009). Some cancers rely on glycolysis, while others exhibit enhanced mitochondrial metabolism. This includes increased oxidative phosphorylation, fatty acid oxidation and tricarboxylic acid (TCA) cycle activity. Mitochondrial metabolism is crucial in certain types of cancer to provide ATP, biosynthetic precursors and maintain redox balance. Cancer cells often have increased reactive oxygen species (ROS) levels due to their altered metabolism and high proliferation rate. To manage elevated ROS, cancer cells upregulate antioxidant defense systems such as the glutathione pathway and thioredoxin system to maintain redox homeostasis and promote cell survival (Alberghina et al., 2012).

These metabolic changes give cancer cells a selective advantage that supports their growth, survival, and ability to adapt to the tumor microenvironment. Targeting cancer cell metabolism has emerged as an exciting area of cancer research with the aim of developing treatments that specifically disrupt the metabolic weaknesses of cancer cells while sparing normal cells (Vander Heiden, 2011).

5. Angiogenesis

Tumor growth and progression requires the development of new blood vessels, a process known as angiogenesis. Cancer cells have the ability to secrete various factors that promote angiogenesis to ensure adequate blood flow to support increased nutrient and oxygen demands.

Angiogenic factors such as vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF), platelet-derived growth factor (PDGF), and angiopoietins are produced and released by cancer cells. These factors stimulate the growth and migration of endothelial cells, the blood vessel building blocks (Carmeliet et al., 2011). Angiogenic factors released by cancer cells bind to specific receptors on the surface of endothelial cells, activating signaling pathways that promote cell proliferation, migration and formation of new blood vessels. This process allows endothelial cells to invade surrounding tissue and form capillary shoots (Carmeliet et al., 2011). Remodeling of the extracellular matrix is crucial for the formation of new blood vessels. Cancer cells and stromal cells in the tumor microenvironment secrete proteolytic enzymes such as matrix metalloproteinases (MMPs), which degrade the extracellular matrix, creating a pathway for endothelial cells to migrate and form new vasculature (Gálvez et al., 2001). Endothelial cells proliferate and form sprouts that lengthen and connect with nearby vessels to form a network of new blood vessels. This process is called neovascularization or angiogenesis. Newly formed blood vessels support the growth and survival of the tumor by supplying the growing tumor with oxygen, nutrients, and growth factors (Gálvez et al., 2001).

The tumor microenvironment, composed of stromal cells, immune cells, and components of the extracellular matrix, plays a crucial role in regulating angiogenesis. Cancer cell interactions with surrounding stromal cells, including cancer-associated fibroblasts and immune cells, can modulate the production of angiogenic factors and influence the angiogenic response (Payne et al., 2011). Targeting angiogenesis has been a successful therapeutic strategy in cancer treatment. Drugs that inhibit angiogenesis such as anti-angiogenic antibodies (eg, bevacizumab) and small molecule inhibitors (eg,

tyrosine kinase inhibitors that target VEGF receptors) have been developed and are used in combination with other therapies to limit blood flow to tumors to reduce their growth and improve patient outcomes (Petrovic, 2016).

6. Epigenetic Changes

Epigenetic modifications include changes in DNA and associated proteins without altering the underlying genetic sequence. These modifications can have profound effects on gene expression and play a crucial role in numerous biological processes, such as cancer (Zheng et al., 2008).

DNA methylation is a prevalent epigenetic modification involving the addition of a methyl group to DNA molecules, typically at cytosine residues within CpG dinucleotides. DNA methylation can influence gene expression by preventing transcription factors from binding to regulatory regions of genes, thereby suppressing gene expression. Hypermethylation of tumor suppressor gene promoter regions may result in their inactivation, thereby fostering the development of cancer. In contrast, hypomethylation at particular genomic regions can result in oncogene activation or genomic instability (Kulis et al., 2010). Histones are proteins that DNA wraps around and forms a structure called chromatin. Various chemical modifications such as methylation, acetylation, phosphorylation and ubiquitination can occur in histone proteins. These modifications can regulate gene expression by altering the DNA's accessibility to transcriptional machinery. For instance, acetylation of histones is generally associated with gene activation, whereas methylation may be associated with either gene activation or suppression, depending on the site and degree of methylation. Frequently observed in cancer, abnormal histone modifications cause dysregulation of gene expression and promote tumor growth and progression (Zhao et al., 2019). Non-coding RNAs such as microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) are emerging as crucial players in epigenetic regulation. miRNAs can bind to messenger RNAs (mRNAs) and block their translation or regulate gene expression by promoting their degradation. Deregulated expression of miRNAs has been implicated in a variety of cancers. On the other hand, lncRNAs can interact with DNA, RNA and proteins by affecting gene expression and chromatin organization. Altered expression of specific lncRNAs has been associated with cancer development and progression (Morlando et al., 2018). Chromatin remodeling complexes can alter the structure and accessibility of chromatin by affecting gene expression. These complexes use energy to reposition, remove or replace histones, allowing for changes in gene accessibility and transcriptional regulation. Dysregulation

of chromatin remodeling complexes can lead to abnormal gene expression patterns and contribute to cancer development (Längst et al., 2015).

Conclusion

Biochemical changes in the tumor microenvironment can affect cancer cell metabolism. Hypoxia, a common feature of solid tumors, leads to altered metabolic pathways, including increased glycolysis and dependence on alternative energy sources. These metabolic adaptations provide survival advantages to cancer cells and support their growth and proliferation.

Understanding the effects of biochemical changes in the tumor microenvironment is crucial to developing effective cancer treatments. Targeting specific components and signaling pathways within the microenvironment has the potential to improve treatment outcomes by inhibiting tumor growth, overcoming therapy resistance, and enhancing anti-tumor immune responses. Treatments that modulate the tumor microenvironment, such as immunotherapies and anti-angiogenic agents, are being actively explored as promising strategies in cancer therapy.

References

1. Arneth B. Tumor Microenvironment. Medicina. 2020;56(1):15.
2. Denton AE, Roberts EW, Fearon DT. Stromal Cells in the Tumor Microenvironment. In: Owens BMJ, Lakins MA, editors. Stromal Immunology. Cham: Springer International Publishing; 2018. p. 99-114.
3. Wu AA, Drake V, Huang HS, Chiu S, Zheng L. Reprogramming the tumor microenvironment: tumor-induced immunosuppressive factors paralyze T cells. Oncoimmunology. 2015;4(7):e1016700.
4. Multhoff G, Vaupel P. Radiation-induced changes in microcirculation and interstitial fluid pressure affecting the delivery of macromolecules and nanotherapeutics to tumors. Frontiers in Oncology. 2012;2.
5. Hinshaw DC, Shevde LA. The Tumor Microenvironment Innately Modulates Cancer Progression. Cancer Research. 2019;79(18):4557-66.
6. Fukumura D, Jain RK. Tumor microvasculature and microenvironment: Targets for anti-angiogenesis and normalization. Microvascular Research. 2007;74(2):72-84.
7. Nicosia RF. What Is the Role of Vascular Endothelial Growth Factor-Related Molecules in Tumor Angiogenesis? The American Journal of Pathology. 1998;153(1):11-6.
8. Byrne AM, Bouchier-Hayes DJ, Harmey JH. Angiogenic and cell survival functions of Vascular Endothelial Growth Factor (VEGF). Journal of Cellular and Molecular Medicine. 2005;9(4):777-94.
9. Feldman SR, Yaar M. Oncogenes: The Growth Control Genes. Archives of Dermatology. 1991;127(5):707-11.
10. Jones RG, Thompson CB. Tumor suppressors and cell metabolism: a recipe for cancer growth. Genes Dev. 2009;23(5):537-48.
11. Ryan KM, Phillips AC, Vousden KH. Regulation and function of the p53 tumor suppressor protein. Current Opinion in Cell Biology. 2001;13(3):332-7.
12. Müller R. Proto-oncogenes and differentiation. Trends in Biochemical Sciences. 1986;11(3):129-32.
13. Jan R, Chaudhry GE. Understanding Apoptosis and Apoptotic Pathways Targeted Cancer Therapeutics. Adv Pharm Bull. 2019;9(2):205-18.
14. Abel EL, Angel JM, Kiguchi K, DiGiovanni J. Multi-stage chemical carcinogenesis in mouse skin: Fundamentals and applications. Nature Protocols. 2009;4(9):1350-62.
15. Bataille P, Reynard A, Ducarme G. Spontaneous heterotopic triplets - A review of literature. J Gynecol Obstet Hum Reprod. 2017;46(8):657-9.
16. Kurebayashi J. Biological and clinical significance of HER2 overexpression in breast cancer. Breast Cancer. 2001;8(1):45-51.

17. Klinakis A, Szabolcs M, Politi K, Kiaris H, Artavanis-Tsakonas S, Efstratiadis A. Myc is a Notch1 transcriptional target and a requisite for Notch1-induced mammary tumorigenesis in mice. Proc Natl Acad Sci U S A. 2006;103(24):9262-7.
18. Lee EY, Muller WJ. Oncogenes and tumor suppressor genes. Cold Spring Harb Perspect Biol. 2010;2(10):a003236.
19. Quatrin M, Pasti C, Romano S, Iarossi B, Giménez V, Schuttenberg V, et al. A new dual translocation of chromosome 14 in a pediatric Burkitt lymphoma/leukemia patient: t(8;14) and t(14;15). Cancer Genetics. 2021;258-259:131-4.
20. Lüscher B, Vervoorts J. Regulation of gene transcription by the oncoprotein MYC. Gene. 2012;494(2):145-60.
21. Nie Z, Hu G, Wei G, Cui K, Yamane A, Resch W, et al. c-Myc Is a Universal Amplifier of Expressed Genes in Lymphocytes and Embryonic Stem Cells. Cell. 2012;151(1):68-79.
22. Rohrberg J, Van de Mark D, Amouzgar M, Lee JV, Taileb M, Corella A, et al. MYC Dysregulates Mitosis, Revealing Cancer Vulnerabilities. Cell Reports. 2020;30(10):3368-82.e7.
23. Miyakura Y, Sugano K, Fukayama N, Konishi F, Nagai H. Concurrent Mutations of K-ras Oncogene at Codons 12 and 22 in Colon Cancer. Japanese Journal of Clinical Oncology. 2002;32(6):219-21.
24. Gerber M, Goel S, Maitra R. In silico comparative analysis of KRAS mutations at codons 12 and 13: Structural modifications of P-Loop, switch I&II regions preventing GTP hydrolysis. Computers in Biology and Medicine. 2022;141:105110.
25. Farr CJ, Saiki RK, Erlich HA, McCormick F, Marshall CJ. Analysis of RAS gene mutations in acute myeloid leukemia by polymerase chain reaction and oligonucleotide probes. Proceedings of the National Academy of Sciences. 1988;85(5):1629-33.
26. Sparmann A, Bar-Sagi D. Ras-induced interleukin-8 expression plays a critical role in tumor growth and angiogenesis. Cancer Cell. 2004;6(5):447-58.
27. David R, Lamki N, Fan S, Singleton EB, Eftekhari F, Shirkhoda A, et al. The many faces of neuroblastoma. RadioGraphics. 1989;9(5):859-82.
28. Hatzi E, Murphy C, Zoephel A, Ahorn H, Tontsch U, Bamberger A-M, et al. N-myc oncogene overexpression down-regulates leukemia inhibitory factor in neuroblastoma. European Journal of Biochemistry. 2002;269(15):3732-41.
29. Nisar S, Hashem S, Macha AM, Yadav KS, Muralitharan S, Therachiyil L, et al. Exploring Dysregulated Signaling Pathways in Cancer. Current Pharmaceutical Design. 2020;26(4):429-45.

30. Sahu S, Singh B, Rai AK. Human endogenous retrovirus regulates the initiation and progression of cancers (Review). Mol Clin Oncol. 2022;17(4):143.
31. Romanish MT, Cohen CJ, Mager DL. Potential mechanisms of endogenous retroviral-mediated genomic instability in human cancer. Seminars in Cancer Biology. 2010;20(4):246-53.
32. Fan H. Retroviruses and Their Role in Cancer. In: Levy JA, editor. The Retroviridae. Boston, MA: Springer US; 1994. p. 313-62.
33. Grandér D. How do mutated oncogenes and tumor suppressor genes cause cancer? Medical Oncology. 1998;15(1):20-6.
34. Aranda-Anzaldo A. Cancer Development and Progression: A Non-Adaptive Process Driven by Genetic Drift. Acta Biotheoretica. 2001;49(2):89-108.
35. Temko D, Tomlinson IPM, Severini S, Schuster-Böckler B, Graham TA. The effects of mutational processes and selection on driver mutations across cancer types. Nature Communications. 2018;9(1):1857.
36. Li X. Dynamic changes of driver genes' mutations across clinical stages in nine cancer types. Cancer Medicine. 2016;5(7):1556-65.
37. Bozic I, Gerold JM, Nowak MA. Quantifying Clonal and Subclonal Passenger Mutations in Cancer Evolution. PLOS Computational Biology. 2016;12(2):e1004731.
38. Iau PTC, Macmillan RD, Blamey RW. Germ line mutations associated with breast cancer susceptibility. European Journal of Cancer. 2001;37(3):300-21.
39. Martincorena I, Campbell PJ. Somatic mutation in cancer and normal cells. Science. 2015;349(6255):1483-9.
40. Alexandrov LB, Ju YS, Haase K, Van Loo P, Martincorena I, Nik-Zainal S, et al. Mutational signatures associated with tobacco smoking in human cancer. Science. 2016;354(6312):618-22.
41. Harvey AJ. Overview of Cell Signaling Pathways in Cancer. In: Badve S, Kumar GL, editors. Predictive Biomarkers in Oncology: Applications in Precision Medicine. Cham: Springer International Publishing; 2019. p. 167-82.
42. Lundby A, Franciosa G, Emdal KB, Refsgaard JC, Gnosa SP, Bekker-Jensen DB, et al. Oncogenic Mutations Rewire Signaling Pathways by Switching Protein Recruitment to Phosphotyrosine Sites. Cell. 2019;179(2):543-60.e26.
43. Yewale C, Baradia D, Vhora I, Patil S, Misra A. Epidermal growth factor receptor targeting in cancer: A review of trends and strategies. Biomaterials. 2013;34(34):8690-707.

44. Martelli AM, Evangelisti C, Chappell W, Abrams SL, Bäsecke J, Stivala F, et al. Targeting the translational apparatus to improve leukemia therapy: roles of the PI3K/PTEN/Akt/mTOR pathway. Leukemia. 2011;25(7):1064-79.

45. Bian J, Dannappel M, Wan C, Firestein R. Transcriptional Regulation of Wnt/β-Catenin Pathway in Colorectal Cancer. Cells. 2020;9(9):2125.

46. Yin L, Velazquez OC, Liu Z-J. Notch signaling: Emerging molecular targets for cancer therapy. Biochemical Pharmacology. 2010;80(5):690-701.

47. Skoda AM, Simovic D, Karin V, Kardum V, Vranic S, Serman L. The role of the Hedgehog signaling pathway in cancer: A comprehensive review. Biomolecules and Biomedicine. 2018;18(1):8-20.

48. Lincet H, Icard P. How do glycolytic enzymes favour cancer cell proliferation by nonmetabolic functions? Oncogene. 2015;34(29):3751-9.

49. Desideri E, Ciriolo MR. Glutamine Addiction of Cancer Cells. In: Rajendram R, Preedy VR, Patel VB, editors. Glutamine in Clinical Nutrition. New York, NY: Springer New York; 2015. p. 99-111.

50. Robinson AD, Eich M-L, Varambally S. Dysregulation of de novo nucleotide biosynthetic pathway enzymes in cancer and targeting opportunities. Cancer Letters. 2020;470:134-40.

51. Mashima T, Seimiya H, Tsuruo T. De novo fatty-acid synthesis and related pathways as molecular targets for cancer therapy. British Journal of Cancer. 2009;100(9):1369-72.

52. Alberghina L, Gaglio D, Gelfi C, Moresco R, Mauri G, Bertolazzi P, et al. Cancer cell growth and survival as a system-level property sustained by enhanced glycolysis and mitochondrial metabolic remodeling. Frontiers in Physiology. 2012;3.

53. Vander Heiden MG. Targeting cancer metabolism: a therapeutic window opens. Nature Reviews Drug Discovery. 2011;10(9):671-84.

54. Carmeliet P, Jain RK. Molecular mechanisms and clinical applications of angiogenesis. Nature. 2011;473(7347):298-307.

55. Gálvez BG, Matıas-Román S, Albar JP, Sánchez-Madrid F, Arroyo AG. Membrane Type 1-Matrix Metalloproteinase Is Activated during Migration of Human Endothelial Cells and Modulates Endothelial Motility and Matrix Remodeling*. Journal of Biological Chemistry. 2001;276(40):37491-500.

56. Payne SJ, Jones L. Influence of the tumor microenvironment on angiogenesis. Future Oncology. 2011;7(3):395-408.

57. Petrovic N. Targeting Angiogenesis in Cancer Treatments: Where do we Stand? Journal of Pharmacy & Pharmaceutical Sciences. 2016;19(2):226-38.

58. Zheng YG, Wu J, Chen Z, Goodman M. Chemical regulation of epigenetic modifications: Opportunities for new cancer therapy. Medicinal Research Reviews. 2008;28(5):645-87.

59. Kulis M, Esteller M. 2 - DNA Methylation and Cancer. In: Herceg Z, Ushijima T, editors. Advances in Genetics. 70: Academic Press; 2010. p. 27-56.

60. Zhao Z, Shilatifard A. Epigenetic modifications of histones in cancer. Genome Biology. 2019;20(1):245.

61. Morlando M, Fatica A. Alteration of Epigenetic Regulation by Long Noncoding RNAs in Cancer. International Journal of Molecular Sciences. 2018;19(2):570.

62. Längst G, Manelyte L. Chromatin Remodelers: From Function to Dysfunction. Genes. 2015;6(2):299-324.

Chapter 8

Orexinergic System and Antinociception

Ercan Özdemir[1]

Abstract

Orexin-A and orexin-B neuropeptides are specifically synthesized and secreted in hypothalamic neurons. It is known that the orexinergic system participates in pain modulation as well as its roles in various physiological processes such as feeding, stress processing, endocrine and cognitive functions. Orexin ligands activate orexin type-1 (OXR1) and orexin type-2 (OXR2) receptors, each with a different distribution. Orexins are mediators that play important roles in regulating pain perception at the spinal and supraspinal levels. These regulatory roles of orexins have been demonstrated in mechanical, thermal, and chemical models of pain. Periaqueductal gray (PAG) area of the central nervous system plays an important role in the pain modulation of orexins. Furthermore, the orexinergic system locus seroleus (LC) and paragigantocellular lateralis (LPGi) are associated areas and play an important role in chronic neuropathic pain, stress pain and migraine pain as well as opioid analgesic activity. In addition, it has been expressed that the antinociceptive effects of orexins are mediated by endocannabinoids. This review summarizes studies investigating the antinociceptive effects of orexin in various types of pain, including migraine, neuropathic pain, visceral and orofacial pain, and its effects on opioid analgesia and tolerance.

1. Introduction

Orexin-A and orexin-B neuropeptides that make up the orexin (hypocretin) system are produced by enzymatic reactions from prepro-orexin in the lateral hypothalamus. Each of these neuropeptides exert their effects via two G-protein coupled receptors (GPCR) called orexin-1 (OX1) and orexin-2 (OX2). Orexin-A activates both OX1 and OX2 receptors with similar affinity, while orexin-B activates only OX2 receptors (1).

1 Prof. Dr., Department of Physiology, Faculty of Medicine, Sivas Cumhuriyet University, eozdemir3368@gmail.com, eozdemir@cumhuriyet.edu.tr., ORCID: 0000-0001-8231-1053

https://doi.org/10.58830/ozgur.pub128.c688

Orexinergic neurons are located especially in the dorsal medial hypothalamus, perifornical area, and lateral parts of the lateral hypothalamus (2, 3). These neuropeptides have been reported to be involved in a wide variety of physiological functions such as arousal (4), feeding (2), neuroendocrine processes (5), and autonomic control (6). There are two types of G-protein coupled receptors for orexin ligands that are distributed differently in the central nervous system (7). These receptors are orexin type-1 (OX1R) and orexin type-2 receptors. OX1R is selective for orexin-A, while OX2R binds both orexin neuropeptides (2). The widespread distribution of orexin receptors in the central nervous system explains the multifaceted contribution of the endogenous orexinergic system to homeostatic regulation in the brain (2). Abundant evidence suggests that both OX1 and OX2 receptors are expressed on the soma at pre- and post-synaptic terminals, as well as in the medial and lateral hypothalamic areas (8).

Numerous studies have shown that orexins have antinociceptive effects in the brain and spinal cord in different types of pain, including mechanical (tail-pressure), thermal (hot-plate, tail-flick, claw-retraction), chemical (formalin, capsaicin, and abdominal) (9). Orexin receptors can be detected in many brain structures known to be involved in the transmission of pain signals (10). It has been reported that Orexin-A exerts antinociceptive effects in both the brain and spinal cord. In contrast, it is stated that orexin-B has little antinociceptive effect (11). The periaqueductal gray (PAG) area in the central nervous system is an important site in pain regulation by orexins. The analgesic supraspinal mechanism involves retrograde inhibition of GABA release in the ventrolateral PAG. In addition, orexins interact with endocannobinoids to produce analgesia (12).

The purpose of this section is to elucidate the role of orexin, a hypothalamic neuropeptide, modulation of pain transmission, opioid analgesia and tolerance.

2. Mechanisms of Analgesic Effect of Orexin

A large body of evidence provides important insights into the possible mechanisms of orexin's analgesic action. Orexin increases antinociceptive activity in the ventral tegmental area (VTA) by activation of orexinergic receptors in the nucleus accumbens (NAc) in rats (13, 14). Functions related to nutrition, neuroendocrine functions, sensory information and nociception are probably formed as a result of mutual interactions of orexins and neurokinin-1 receptor. This is evidenced by the direct anatomical contact between orexin-A and neurokinin-1 receptors in the marginal layer of the

dorsal horn (15). Another analgesic mechanism of action with Orexin-A is retrograde inhibition by GABA secretion by increasing the production of 2-arachidonoylglycerol (2-AG), an endocannabinoid, in the ventrolateral PAG (16). In addition, a study with orexin-B suggested that activation of glycinergic and purinergic neurotransmission at the spinal level enhanced the analgesic effect of orexin (17).

3. Orexinergic System and Neuropathic Pain

Orexinergic neurons play an important role in chronic neuropathic pain as well as stress pain, headache, migraine pain (Table 1). It is a treatment-resistant chronic pain condition that results from any lesion or disease of the somatosensory nervous system and has adverse effects on quality of life. The role of the orexinergic system in neuropathic pain has been demonstrated in various animal models. The effect of orexin-A on neuropathic pain was evaluated in rats with neuropathic pain created by sciatic nerve ligation. Orexin-A administered intrathecally 7 days after sciatic nerve injury reduced the level of mechanical allodynia and neuropathic pain induced by partial sciatic nerve ligation (18).

It reveals that orexins can reduce heat-induced hyperalgesia in rats with chronic constriction damage to the sciatic nerve. Orexin-A administration inhibited hyperalgesia caused by sciatic nerve damage, while orexin-A antiserum antagonizes this effect (19). In addition, orexin-A inhibits Ca2+ flow through the L-type Ca2+ channel in dorsal root ganglion (DRG) neurons of rat segmental spinal nerve ligation with its effect on high K+-induced [Ca2+]i increase. This indicates an important effect of orexin for nociceptive modulation. In addition, nifedipine and lidocaine potently block high K+-induced depolarization [Ca2+] increase in rats with spinal nerve ligation (20). Furthermore, neuropharmacological evidence indicates that OXR2 mediates its inhibitory effects on KCl-induced increases in [Ca2+]i in C-fiber neurons in a model of sciatic nerve ligation-induced hyperalgesia in rats (21). In a neuropathic pain model in rats, orexin-A exerts its antinociception effect through injection of the cholinergic agonist carbachol and stimulation of the posterior hypothalamus, partially mediated by the OXR1 in the dorsal horn of the spinal cord (22). Intrathecal administration of orexin-A to animals significantly reduced the occurrence of mechanical allodynia experimentally induced by sciatic nerve ligations (18). In addition, intrathecal administration of orexin-A blocked hyperalgesia, a form of neuropathic pain, in streptozotocin-induced diabetes mellitus mice (23). Moreover, orexin neurons show that they modulate the sensations of pain and itching in the opposite direction, namely pain relief and itching

exacerbation (24). In addtion, orexin-A/OX1R signaling has been shown to play an important role in the prevention of central poststroke pain in mice through activation of the descending pain control system (25).

Table 1. Summary of studies on pain modulation of the orexinergic system

Orexin A/Orexin B	**OXR1/ OXR2**	**Pain models**	**Antinociceptive activity**	**Ref.**
Orexin A/Orexin B (i.c.v. and i.t.)	OXR1/ OXR2	Tail-flick, hot-plate test, in mice	Enhanced antinociception	(9)
Orexin A (i.c.v.)	OXR1 agonist	Tail-flick test, in rats	Inhibitory effects of pain on RVM	(26)
Orexin A (intra-PAG injection)	OXR1 agonist	Formalin test, in rats	Decreases nociception	(27)
Orexin A (intra-LPGi)	OXR1	Formalin test, in rats	Decreases nociception	(28)
Orexin A (i.c.v. and i.t.)	OXR1	Sciatic nerve ligation (neuropathic pain model) in rats	Alleviates mechanical allodynia	(18)
Orexin A (i.v.) Orexin B (i.v.)	OXR1 OXR2	Model of trigeminovascular nociception in rats	Orexin A has antinociception Orexin B has no antinociception	(29)
Orexin A	OXR1	Model of trigeminovascular nociception in rats	Inhibit A-fibre responses to electrical stimulation	(30)
Orexin A antagonist	OXR1 (SB-334867)	Formalin test, in rats	Attenuate swim- and restraint stress-induced antinociception	(10)

icv, intrcerebroventricularis; it, intratechalis; LPGi, paragigantocellularis lateralis; RVM, rostral ventral medulla

4. Orexinergic System and Stress Induced Analgesia

The stress-induced analgesic effect is an important form of the defensive behavioral reaction to fight-or-flight (31). Acute and chronic stress affect the orexinergic system, causing changes in both pain threshold and nociceptive behaviors (32). Evidence suggests that chronic stress activates orexin neurons and thereby inhibits pain transmission In addition, stressful conditions in experimental animals appear to increase orexin levels, resulting in improved animal performance and blocking of pain signals (33). Recent evidence has shown that the interaction between corticotropin-releasing factor (CRF) and orexinergic neurons may be physiopathologically related to the control

of stress-related behaviors. Activation of the orexinergic system modulates the activity of CRF neurons via OX2R (7). Many evidences suggest that OX1R responds to both pain and stressful stimuli and is therefore likely to be involved in stress-induced analgesia (34).

During stress, hypothalamic orexin neurons are activated and orexins are released. Increasing 2-arachidonoylglycerol (2-AG) activates the orexin receptor type-1 in the lateral PAG, resulting in analgesia. Stress analgesia has been demonstrated in a mouse model where the orexin and nociceptin/orphanin FQ systems coordinately regulate nociception (31). In an experimental study, the paw thermal nociceptive test showed that restraint of immobilization of the rat increased the pain threshold by 20.5%. Injection of nociceptin/orphanin into the perifornical area of the lateral hypothalamus in rats significantly reduced stress-induced analgesia. It has been reported that the formation of stress-induced analgesia is mediated by direct inhibition of the orexinergic system in the perifornical area (35). Intracerebroventricular injection of the selective orexin receptor type-1 antagonist SB 334867 reduced the analgesic effect of restraint stress in the formalin test. Similarly, blocking of orexin-1 receptors with SB-334867 in rats resulted in a reduction in antinociceptive behaviors induced by swimming and restraint stress in the formalin test (10). Therefore, it can be stated that the orexin receptor type-1 mediates an opioid-independent stress-induced analgesia. In one study, prior administration of the OXR1 antagonist SB 334867 resulted in a reduction in the antinociceptive effects of restraint stress in animals (36).

In addition, another study demonstrated the role of orexin receptors in antinociception induced by swimming or restraint stress. This study showed that exposure to 6 minutes of swimming stress combined with 30 minutes of restraint stress can significantly reduce formalin-induced nociception in rats. The analgesic effect caused by restraint stress or swimming stress was fully inhibited by the orexin-1 receptor antagonist SB-334867 (10).

5. Orexinergic System and Headache

Migraine is defined as a diffuse, chronic, debilitating neurovascular disorder associated with sensory sensitivity, usually manifested by severe unilateral headaches. Cluster headache, on the other hand, is a disease that starts with recurrent unilateral pain attacks, is severe, and is often associated with autonomic symptoms (37). In general, cluster headache and migraine are expressed as the two main primary headache disorders. The basic mechanism in the occurrence of migraine includes the activation of the trigeminovascular system. Orexin-A prevented electrical stimulation-

induced vasodilation, which was prevented by SB-334867 pretreatment. In addition, orexin-A blocks the prejunctional release of calcitonin gene-related peptide (CGRP), which is very important in migraine, from trigeminal neurons (38).

Furthermore, it has been determined that the activation of the hypothalamus has an important role in the physiopathology of cluster headache. Cluster headache manifests itself with circadian or seasonal attacks and shows some features such as changes in hormone levels (37). Orexin neurons are highly organized in the hypothalamus and show broad projections to areas involved in nociception and autonomic regulation. Given these features of the orexinergic system, orexins are likely to play an important role in the pathogenesis of cluster headache and migraine. Injection of orexin-A into the posterior hypothalamic region of the rat reduces A- and C-fiber type nerve responses to dural electrical stimulation in the trigeminal nucleus caudalis and harmful thermal stimulation of the facial receptive field (39). Intravenous administration of orexin-A has been shown to inhibit neurogenic dural vasodilation through OXR1 activation. This effect acts on the trigeminovascular system, causing partial blocking of calcitonin gene-related peptide (CGRP) release (29). In another study, intravenous injection of orexin-A was shown to block type-A nerve fiber responses to dural electrical stimulation through activation of OXR1 (30).

Although many studies support that the orexinergic system has a very important role in the pathophysiology of migraine, the results of a clinical study show that the orexin receptor antagonist fluorexant does not have sufficient analgesic effect as a potential therapeutic approach in migraine (40).

6. Orexinergic System and Visceral Pain

Visceral sensation is one of the main functions of the gastrointestinal tract and is controlled by the central nervous system. The modulatory role of orexins secreted from the brain in visceral sense indicates that the orexinergic system may play a role in the pathophysiology of irritable bowel syndrome (41). According to a study, intracisternal injection of orexin-A caused an increase in the threshold volume of the abdominal withdrawal reflex due to colonic distension. At the same time, the threshold volume was not changed by the intracisternal OXR1 antagonist SB334867, while centrally administered SB334867 completely blocked the morphine-induced analgesic effect against colonic enlargement (42). The results obtained in this study show that orexin-A can play a modulatory role by being secreted from the central nervous system to prevent pain caused by colonic distension.

Furthermore, dopaminergic signaling pathways have also been found to play an important role in orexin-induced central analgesic activity against colonic distension (43). In addition, prior administration of the D1 dopamine receptor antagonist SCH23390 to animals prevented the analgesic effect from centrally injected orexin-A for colonic distension (44). Moreover, it has been demonstrated that the adenosine signaling system also plays an important role in visceral antinociception regulated by the orexinergic system. Evidence suggests that subcutaneous administration of theophylline, an adenosine antagonist, or 1,3-dipropyl-8-cyclopentylxanthine, an adenosine A1 receptor antagonist, against colonic distension caused by the centrally injected A1 agonist N(6)-cyclopentyladenosine (CPA) or orexin-A. demonstrated that it inhibits antinociceptive activity (44).

5. The Role of the Orexinergic System on Morphine Analgesia and Tolerance

Opioid drugs such as morphine are frequently used in the clinic for the treatment of chronic and severe pain. On the other hand, long-term use of these drugs often causes tolerance to analgesic effects (45). There are several possible explanations for the development of morphine tolerance, including activation of an intracellular signaling pathway such as nitric oxide (NO) and mammalian rapamycin target (mTOR), apoptosis in dorsal ganglion neurons, ghrelin and opioid receptor desensitization, and endocannabinoid receptor induction (46-49). Numerous studies have shown that OXR1 antagonist administration causes a decrease in the development of morphine-induced tolerance (50-52) (Table 2).

Co-administration of OXR1 antagonist SB-334867 with morphine inhibits the development of opioid tolerance. Therefore, these data suggest that OXR1 plays an important role in the development of tolerance to morphine (51).

Table 2. The role of the orexinergic system on morphine analgesia and tolerance

Orexin A/ Orexin B	**OXR1/ OXR2**	**Pain models**	**Morphine analgesia and tolerance**	**Ref.**
Orexin A antagonist	OXR1 antagonist (SB-334867)	Tail-flick, hot-plate test, in rats	Attenuates morphine tolerance	(52)
Orexin A antagonist	OXR1 blockade (SB-334867)	The electrical activity of LC neurons was studied using single unit recording in rats	Prevents morphine tolerance	(53)
Inhibits orexin A secretions (Yokukansan)	No receptor blockade	Hot-plate test, in rats	Attenuates morphine tolerance	(54)
No effect	OXR1/OXR2 antagonist suvorexant	Tail-flick test in mice	Reduces morphine tolerance and dependence	(55)
No effect	OXR1 antagonist (SB-334867)	The electrical activity of LC neurons was studied using single unit recording in rats	Attenuates morphine tolerance	(56)
Orexin B	OXR2 agonist	Tail-flick, hot-plate test, in rats	Attenuates morphine tolerance	(52)
No effect	OXR1 antagonist, icv (SB-334867)	The electrical activity of LPGi neurons was studied using single unit recording in rats	Prevents morphine tolerance	(57)
No effect	OXR1 antagonist, icv (SB-334867	Transparent cylindrical plexiglas test chamber, in rats	Decreases naloxone precipitated morphine withdrawal signs.	(58)
No effect	OXR1 antagonist, icv (SB-334867	Transparent cylindrical plexiglas test chamber, in rats	Decreases naloxone precipitated morphine withdrawal signs.	(59)
No effect	OXR1 antagonist, icv (SB-334867	The warm-water tail immersion test, in rats	Attenuates morphine tolerance and dependence	(60)
No effect	OXR1 antagonist, icv (SB-334867	Tail-flick test, in rats	Attenuates morphine analgesic tolerance	(51)

icv, intrcerebroventricularis; LC, locus coeruleus; LPGi, paragigantocellularis lateralis

In addition, orexins play a modulatory role in the analgesic effect of met-enkephalin in locus coeruleus (LC) neurons. The nucleus locus coeruleus is one of the important central nervous system regions in the analgesic activity of opioids and the development of tolerance to morphine. However, they are detected in high concentrations in LC neurons of the orexin receptor type-1 (61). Spinal administered orexin-A has been shown to reduce mechanical hyperalgesia caused by repeated intradermal injections of the mu opioid receptor agonist DAMGO via OX1R (62). Inhibition of OXR1 with SB-334867 induced a significant alleviation of the development of morphine dependence and behavioral symptoms induced by naloxone administration in rats. In addition, long-term blockade of OXR1 may reduce formalin-induced nociception (63).

In one study, intracerebroventricular administration of the OXR1 antagonist SB-334867 to rats demonstrated tolerance to the nociceptive effect of morphine and a reduction in naloxone-induced withdrawal symptoms. Therefore, orexins may play a role in morphine analgesic tolerance and dependence via OXR1 receptors (60). In another study, when the selective orexin receptor-1 antagonist SB-334867 was administered to rats in the locus cereleus, morphine reduced analgesic tolerance and the development of physical dependence (59). In an electrophysiological study, it was shown that blockade of orexin type-1 receptors prevented the development of morphine tolerance in the lateral paragigantocellularis nucleus of rats (57). Similarly, blocking the orexin type-1 receptor in the lateral paragigantocellularis nucleus in rats has been shown to reduce naloxone-induced morphine withdrawal symptoms (58). In two studies at different times, the central inhibition of orexin type-1 receptors with an OXR1 antagonist showed significant reductions in activation of locus coeruleus (LC) neurons in morphine-dependent rats with naloxone (56, 64). In an electrophysiological study, it was demonstrated that OX1R blockade by SB-334867 in rats prevented the development of morphine tolerance in LC neurons (53). Administration of suvorexant, an OXR1 antagonist, to mice reduces morphine-induced tolerance and dependence. In addition, with repeated administrations, morphine increases tolerance and dependence in mouse brain through elevation of NMDA, p-ERK and CREB protein levels. Suvorexant prevents opioid tolerance and addiction by blocking orexin receptors in the brain and lowering p-ERK and CREB protein levels (55).

Bilateral administration of the OX1 receptor antagonist into the nucleus accumbens (NAc) showed that morphine sensitization acquisition was reduced, but the OXR2 antagonist produced similar effects only at the

maximal dose. These findings suggest that the OX1 and OX2 receptors are in the NAc and play a role in acquiring morphine sensitivity (65). Yokukansan, a traditional herbal (Kampo) drug consisting of seven components, has been shown to reduce morphine tolerance through inhibition of orexin-A secretion (54). In an experimental study, it was reported that orexin-A may participate in the expression of naloxone-induced morphine withdrawal syndrome by partially reducing the activity of neurons carrying $GABA_A$ receptors (66). In addition, co-administration of OX1R antagonist SB-334867 and OX2R agonist orexin-B with morphine to rats decreased morphine tolerance in tail-flick and hot-plate antinociceptive tests (52).

6. Conclusion

Numerous evidences confirm that orexinergic neurons show antinociceptive activity at both spinal and supraspinal levels in various types of pain, such as neuropathic pain, migraine pain, stress pain, and headache. According to the literature, orexin-A shows its antinociceptive activity by OX1 receptors and this activity is more than orexin-B. Although many mechanisms have been suggested, the antinociceptive action mechanisms of orexins have not been fully elucidated. Ventrolateral PAG is an important site of pain modulation of orexinergic neurons and plays a role in supraspinal regulation. Retrograde GABA secretion has an important role in this supraspinal mechanism. In addition, clinical studies investigating the role of the orexinergic system in pain processing are mostly related to the treatment of migraine, cluster headache, chronic neuropathic pain, and stress pain. The results of the studies showed that orexin receptors, especially OXR1, play an important role in morphine analgesic tolerance and dependence.

References

1. Boss C, Roch C. Recent trends in orexin research–2010 to 2015. Bioorg Med Chem Lett. 2015;25:2875–87.
2. Sakurai T, Amemiya A, Ishii M, Matsuzaki I, Chemelli RM, Tanaka, et al. Orexins and orexin receptors: a family of hypothalamic neuropeptides and G protein coupled receptors that regulate feeding behavior. Cell. 1998;92:573-585.
3. Peyron C, Tighe DK, Van Den Pol AN, Lecea L De, Heller HC, Sutcliffe JG, Kilduff TS. Neurons containing hypocretin (orexin) project to multiple neuronal systems. J Neurosci. 1998;18:9996-10015.
4. Chemelli RM, Willie JT, Sinton CM, Elmquist JK, Scammell T, Lee C, et al. Narcolepsy in orexin knockout mice: molecular genetics of sleep regulation. Cell. 1999;98:437-51.
5. Taylor MM, Samson WK. The other side of the orexins: endocrine and metabolic actions. Am J Physiol Endocrinol Metab. 2003;284;E13-E17.
6. Date Y, Ueta Y, Yamashita H, Yamaguchi H, Matsukura, S., Kangawa, K., et al. Orexins, orexigenic hypothalamic peptides, interact with autonomic, neuroendocrine and neuroregulatory systems. Proc Natl Acad Sci. U.S.A. 1999;96:748-753.
7. Marcus J, Aschkenasi C, Lee C. Differential expression of orexin receptors 1 and 2 in the rat brain. J Comp Neurol. 2001;435:6–25.
8. Van den Pol AN, Gao XB, Obrietan K, Kilduff TS, Belousov AB. Presynaptic and postsynaptic actions and modulation of neuroendocrine neurons by a new hypothalamic peptide, hypocretin/orexin. J Neurosci. 1998;18:7962-7971.
9. Mobarakeh JI, Takahashi K, Sakurada S, Nishino S, Watanabe H, Kato M, Yanai K. Enhanced antinociception by intracerebroventricularly and intrathecally-administered orexin A and B (hypocretin-1 and -2) in mice. Peptides. 2005;26(5):767-77.
10. Heidari-Oranjaghi N, Azhdari-Zarmehri H, Erami E, Haghparast A. Antagonism of orexin-1 receptors attenuates swim- and restraint stress-induced antinociceptive behaviors in formalin test. Pharmacol Biochem Behav. 2012;103(2):299-307.
11. Cady RJ, Denson JE, Sullivan LQ, Durham PL. Dual orexin receptor antagonist 12 inhibits expression of proteins in neurons and glia implicated in peripheral and central sensitization. Neuroscience. 2014;269:79–92.
12. Razavi BM, Hosseinzadeh H. A review of the role of orexin system in pain modulation. Biomed Pharmacother. 2017;90:187-193.
13. Haghparast A, Yazdi-Ravandi S, Razavi Y, Haghparast A, Goudarzvand M. Orexin A induced antinociception in the ventral tegmental area involves D1

and D2 receptors in the nucleus accumbens. Pharmacol Biochem Behav. 2014;126:1–6.

14. Kang X, Tang H, Liu Y, Yuan Y, Wang M. Research progress on the mechanism of orexin in pain regulation in different brain regions. Open Life Sci. 2021;20;16(1):46-52.
15. Nazli M. Immunohistochemical localisations of orexin-A and the neurokinin 1 receptor in the rat spinal cord. Acta Vet. (Beogr.) 2004;54:311–318.
16. Ho YC, Lee HJ, Tung LW, Liao YY, et al. Activation of orexin 1 receptors in the periaqueductal gray of male rats leads to antinociception via retrograde endocannabinoid (2-arachidonoylglycerol)-induced disinhibition. J Neurosci. 2011;31:14600–14610.
17. Grudt T, van den Pol A, Perl E. Hypocretin-2 (orexin-B) modulation of superficial dorsal horn activity in rat. J Physiol. 2002;538:517–525.
18. Yamamoto T, Saito O, Shono K, Aoe T, Chiba T. Anti-mechanical allodynic effect of intrathecal and intracerebroventricular injection of orexin-A in the rat neuropathic pain model. Neurosci Lett. 2003;347:183–186.
19. Suyama H, Kawamoto M, Shiraishi S, Gaus S, Kajiyama S, Yuge O. Analgesic effect of intrathecal administration of orexin on neuropathic pain in rats. In Vivo. 2004,18:119–124.
20. Kimura M, Ishikawa M, Kawai Y, Shibutani K, Uchida R, Eguchi Y, Kuboyama N. Effects of lidocaine and orexin on [Ca2+]i in dosal root ganglion neuron of rat segmental spinal nerve ligation model. Japan Pharmacol Ther. 2014;42:723–734.
21. Yamaguchi M, Ishikawa M, Aono Y, Saigusa T. OX_2 receptors mediate the inhibitory effects of orexin-A on potassium chloride-induced increases in intracellular calcium ion levels in neurons derived from rat dorsal root ganglion in a chronic pain model. Neuropsychopharmacol Rep. 2020;40(1):30-38.
22. Jeong Y, Holden J. The role of spinal orexin-1 receptors in posterior hypothalamic modulation of neuropathic pain. Neuroscience. 2009;159:1414–1421.
23. Kajiyama S, Kawamoto M, Shiraishi S, Gaus S, Matsunaga A, Suyama H, et al. Spinal orexin-1 receptors mediate anti-hyperalgesic effects of intrathecally-administered orexins in diabetic neuropathic pain model rats. Brain Res. 2005;1044(1):76–86.
24. Kaneko T, Kuwaki T, Kashiwadani H. Hypothalamic orexinergic neurons modulate pain and itch in an opposite way: pain relief and itch exacerbation. J Physiol Sci. 2022;22;72(1):21.

25. Matsuura W, Nakamoto K, Tokuyama S. Involvement of descending pain control system regulated by orexin receptor signaling in the induction of central post-stroke pain in mice. Eur J Pharmacol. 2020;874:173029.
26. Azhdari-Zarmehri H, Semnanian S, Fathollahi Y, Pakdel FG. Tail flick modification of orexin-a induced changes of electrophysiological parameters in the rostral ventromedial medulla. Cell J. 2014;16(2):131-40.
27. Azhdari Zarmehri H, Semnanian S, Fathollahi Y, Erami E, Khakpay R, Azizi H, Rohampour K. Intra-periaqueductal gray matter microinjection of orexin-A decreases formalin-induced nociceptive behaviors in adult male rats. J Pain. 2011;12(2):280-7.
28. Erami E, Azhdari-Zarmehri H, Ghasemi-Dashkhasan E, Esmaeili MH, Semnanian S. Intra-paragigantocellularis lateralis injection of orexin-A has an antinociceptive effect on hot plate and formalin tests in rat. Brain Res. 2012a;1478:16-23.
29. Holland P, Akerman S, Goadsby P. Orexin 1 receptor activation attenuates neurogenic dural vasodilation in an animal model of trigeminovascular nociception. J Pharmacol Exp Ther. 2005;315:1380–1385.
30. Holland P, Akerman S, Goadsby P. Modulation of nociceptive dural input to the trigeminal nucleus caudalis via activation of the orexin 1 receptor in the rat. Eur J Neurosci. 2006;24:2825–2833.
31. Xie X, Wisor J, Hara J, Crowder T, LeWinter R, Khroyan T. Hypocretin/orexin and nociceptin/orphanin FQ coordinately regulate analgesia in a mouse model of stress-induced analgesia. J Clin Invest. 2008;118:2471–2481.
32. Azhdari-Zarmehri H, Mohammad-Zadeh M, Shabani M. The role of hypocretin/orexin in stress-induced analgesia, J. Kerman Uni Med Sci. 2015;22:205–17.
33. Watanabe S, Kuwaki T, Yanagisawa M, Fukuda Y, Shimoyama M. Persistent pain and stress activate pain-inhibitory orexin pathways. NeuroReport. 2005;16:5–8.
34. Sofi-Abadi M, Heidari-Oranjaghi N, Ghasemi E, Esmaeili MH, Haghdoost-Yazdi H, Erami E, et al. Assesment of orexin receptor 1 in stress attenuated nociceptive behaviours in formalin test. Physiol Pharmacol 2011;12:188–93.
35. Gerashchenko D, Horvath TL, Xie X. Direct inhibition of hypocretin/orexin neurons in the lateral hypothalamus by nociceptin/orphanin FQ blocks stress induced analgesia in rats. Neuropharmacology. 2011;60:543–549.
36. Bonaventure P, Yun S, Johnson PL, Shekhar A, Fitz SD, Shireman BT, et al. A selective orexin-1 receptor antagonist attenuates stress-induced hyperarousal without hypnotic effects. J Pharmacol Exp Ther. 2015;352(3):590-601.

37. Holland P, Goadsby P. Cluster headache hypothalamus, and orexin. Curr Pain Headache Rep. 2009;13:147–154.

38. Roohbakhsh A, Alavi MS, Azhdari-Zarmehri H. The orexinergic (hypocretin) system and nociception: An update to supraspinal mechanisms. Curr Med Chem. 2018;25(32):3917-3929.

39. Bartsch T, Levy MJ, Knight YE, Goadsby PJ. Differential modulation of nociceptive dural input to [hypocretin] orexin A and B receptor activation in the posterior hypothalamic area. Pain 2004;109:367–78.

40. Chabi A, Zhang Y, Jackson S, Cady R, Lines C, Herring WJ, Connor KM, Michelson D. Randomized controlled trial of the orexin receptor antagonist filorexant for migraine prophylaxis. Cephalalgia 2015;35:379–388.

41. Longstreth GF, Thompson WG, Chey WD, Houghton LA, Mearin F, Spiller RC. Functional bowel disorders, Gastroenterology. 2006;130:1480–1491.

42. Okumura T, Nozu T, Kumei S, Takakusaki K, Miyagishi S, Ohhira M. Antinociceptive action against colonic distension by brain orexin in conscious rats. Brain Res. 2014;1598:12–17.

43. Okumura T, Nozu T, Kumei S, Takakusaki K, Miyagishi S, Ohhira M. Involvement of the dopaminergic system in the central orexin-induced antinociceptive action against colonic distension in conscious rats. Neurosci Lett. 2015;605:34–38.

44. Okumura T, Nozu T, Kumei S, Takakusaki K, Miyagishi S, Ohhira M. Adenosine A1 receptors mediate the intracisternal injection of orexin-induced antinociceptive action against colonic distension in conscious rats. J Neurol Sci. 2016;15:106–110.

45. Andrade AG, Vandermaelen R. Morphine tolerance and dependence in the locus coeruleus: single cell studies in brain slices. Eur J Pharmacol. 1983;91:161–69.

46. Ozdemir E, Bagcivan I, Durmus N, Altun A, Gursoy S. The nitric oxide-cGMP signaling pathway plays a significant role in tolerance to the analgesic effect of morphine. Can J Physiol Pharmacol. 2011;89(2):89-95.

47. Baser T, Ozdemir E, Filiz AK, Taskiran AS, Gursoy S. Ghrelin receptor agonist hexarelin attenuates antinociceptive tolerance to morphine in rats. Can J Physiol Pharmacol. 2021;99(5):461-467.

48. Avci O, Ozdemir E, Taskiran AS, Inan ZDS, Gursoy S. Metformin prevents morphine-induced apoptosis in rats with diabetic neuropathy: a possible mechanism for attenuating morphine tolerance. Naunyn Schmiedebergs Arch Pharmacol. 2022;395(11):1449-1462.

49. Ozdemir E. The role of the cannabinoid system in opioid analgesia and tolerance. Mini Rev Med Chem. 2020;20(10):875-885.

50. Nazemi S, Azhdari-Zarmehri H, Haghdoost-Yazdi H. Role of orexin in the tolerance and physical dependence to morphine. J Babol Univ Med Sci. 2014;16:54–61.
51. Ranjbar-Slamloo Y, Azizi H, Fathollahi Y, Semnanian S. Orexin receptor type-1 antagonist SB-334867 inhibits the development of morphine analgesic tolerance in rats. Peptides. 2012;56–59.
52. Ozdemir E, Baser T, Taskiran AS. Blockade of orexin receptor type-1 by SB-334867 and activation of orexin receptor type-2 attenuate morphine tolerance in rats. Physiol Int. 2022;109(4):457-474.
53. Abdollahi H, Ghaemi-Jandabi M, Azizi H, Semnanian S. The role of orexin type-1 receptors in the development of morphine tolerance in locus coeruleus neurons: An electrophysiological perspective. Brain Res. 2016;1646:91-97.
54. Katayama A, Kanada Y, Tsukada M, Akanuma Y, Takemura H, Ono T, Suga H, Mera H, Hisamitsu T, Sunagawa M. Yokukansan (Kampo medicinal formula) prevents the development of morphine tolerance by inhibiting the secretion of orexin A. Integr Med Res. 2018;7(2):141-148.
55. Esmaili-Shahzade-Ali-Akbari P, Hosseinzadeh H, Mehri S. Effect of suvorexant on morphine tolerance and dependence in mice: Role of NMDA, AMPA, ERK and CREB proteins. Neurotoxicology. 2021;84:64-72.
56. Fakhari M, Azizi H, Semnanian S. Central antagonism of orexin type-1 receptors attenuates the development of morphine dependence in rat locus coeruleus neurons. Neuroscience. 2017;363:1-10.
57. Ghaemi-Jandabi M, Azizi H, Semnanian S. Blockade of orexin type 1 receptors inhibits the development of morphine tolerance in lateral paragigantocellularis nucleus: an electrophysiological approach. Brain Res. 2014;1578:14-22.
58. Ahmadi-Soleimani SM, Ghaemi-Jandabi M, Azizi H, Semnanian S. Orexin type 1 receptor antagonism in lateral paragigantocellularis nucleus attenuates naloxone precipitated morphine withdrawal symptoms in rats. Neurosci Lett. 2014;558:62-6.
59. Mousavi Y, Azizi H, Mirnajafi-Zadeh J, Javan M, Semnanian S. Blockade of orexin type-1 receptors in locus coeruleus nucleus attenuates the development of morphine dependency in rats. Neurosci Lett. 2014;578:90-4.
60. Erami E, Azhdari-Zarmehri H, Rahmani A, Ghasemi-Dashkhasan E, Semnanian S, Haghparast A. Blockade of orexin receptor 1 attenuates the development of morphine tolerance and physical dependence in rats. Pharmacol Biochem Behav. 2012b;103(2):212-9.
61. Mohammad Ahmadi Soleimani S, Azizi H, Mirnajafi-Zadeh J, Semnanian S. Orexin type 1 receptor antagonism in rat locus coeruleus prevents the

analgesic effect of intra-LC met-enkephalin microinjection. Pharmacol Biochem Behav. 2015;136:102–106.

62. Youn DH, Jun J, Kim TW, Park K. Spinal orexin A attenuates opioid-induced mechanical hypersensitivity in the rat. Korean J Pain. 2022;35(4):433-439.
63. Kourosh-Arami M, Joghataei MT, Komaki A, Gholami M, Najafi Z, Lavaie M. Persistent effects of the orexin-1 receptor antagonist SB-334867 on naloxone precipitated morphine withdrawal symptoms and nociceptive behaviors in morphine dependent rats. Int J Neurosci. 2022;132(1):67-76.
64. Aghajani N, Pourhamzeh M, Azizi H, Semnanian S. Central blockade of orexin type 1 receptors reduces naloxone induced activation of locus coeruleus neurons in morphine dependent rats. Neurosci Lett. 2021;755:135909.
65. Assar N, Mahmoudi D, Mousavi Z, Zarrabian S, Haghparast A. Role of orexin-1 and -2 receptors within the nucleus accumbens in the acquisition of sensitization to morphine in rats. Behav Brain Res. 2019;373:112090.
66. Davoudi M, Azizi H, Mirnajafi-Zadeh J, Semnanian S. The blockade of $GABA_A$ receptors attenuates the inhibitory effect of orexin type 1 receptors antagonist on morphine withdrawal syndrome in rats. Neurosci Lett. 2016;617:201-6.

Chapter 9

Advancing Medical Frontiers: Unveiling the Potential of Artificial Placenta in Perinatal Medicine

Rauf Melekoğlu[1]

Ayşe Şebnem Erenler[2]

Abstract

Advancements in medical technology have led to the development of artificial placenta systems, which aim to support the survival and development of extremely premature infants. The placenta plays a vital role in fetal development by facilitating nutrient exchange and waste removal. Premature birth poses significant challenges in neonatal care, with preterm infants facing increased risks of morbidity and mortality. Respiratory failure is a major concern due to the underdeveloped lungs of preterm infants. Artificial placenta models have been designed to mimic fetal and utero-placental physiology, offering potential solutions to these challenges.

This abstract reviews the history and components of artificial placenta systems, highlighting the importance of pumpless arterio-venous (AV) circuits, low-resistance oxygenators, umbilical access, and immersion in sterile fluid. The development of these components has led to improved survival rates and stability in experimental models. However, challenges such as cardiac afterload, optimization of circuit design, and prevention of infection and inflammation remain to be addressed.

Promising artificial placenta models have been developed by research groups at the University of Michigan, Tohoku University (Sendai, Japan), and the University of Western Australia (Perth). These models have demonstrated

1 Inonu University, Faculty of Medicine, Department of Obstetrics and Gynecology, 44280,Malatya, TURKİYE, rauf.melekoglu@inonu.edu.tr, ORCID:https://orcid.org/0000-0001-7113-6691

2 Malatya Turgut Ozal University, Faculty of Medicine, Department of Medical Biology,44210, Malatya, TURKİYE, serenler44@gmail.com, ORCID:https://orcid.org/ 0000-0002-1786-5022

https://doi.org/10.58830/ozgur.pub128.c689

increased survival times, stable hemodynamics, and successful organ maturation. The Philadelphia Children's Hospital model, known as EXTEND AW, has shown particular success, with lambs surviving up to 28 days and exhibiting normal organ development and neurological maturation.

Despite these advancements, there are still challenges to overcome before artificial placenta technology can be implemented in clinical practice. These include reducing oxygenator surface area, improving hemocompatibility, optimizing nutrition and amniotic fluid composition, standardizing patient selection criteria, and developing efficient cannulation techniques.

In conclusion, artificial placenta technology is a promising field with the potential to revolutionize neonatal care. With further advancements and research, artificial placenta systems may offer a solution to the challenges faced by extremely premature infants, improving their chances of survival and long-term health outcomes.

Fetal and placental anatomy

The human gestation period, ranging approximately 266 days or thirty-eight weeks from fertilization to delivery, is a remarkable process of prenatal development. During the early stages of pregnancy, the embryonic period unfolds, encompassing the formation of crucial organ systems that lay the foundation for the growing individual. This period concludes at the end of the eighth week, marking the transition into the fetal period. Throughout the remainder of pregnancy, the focus shifts towards the maturation of organ systems and overall growth (Fig 1). The placenta, a complex and highly specialized organ composed of both embryonic/fetal and maternal components, plays a pivotal role in supporting the developing fetus. It serves as a nexus for nutrient exchange, providing essential sustenance for fetal development, while also facilitating the removal of metabolic waste products. The intricate interplay between the embryo/fetus and the maternal organism through the placenta ensures the survival and thriving of the growing life within the womb (1).

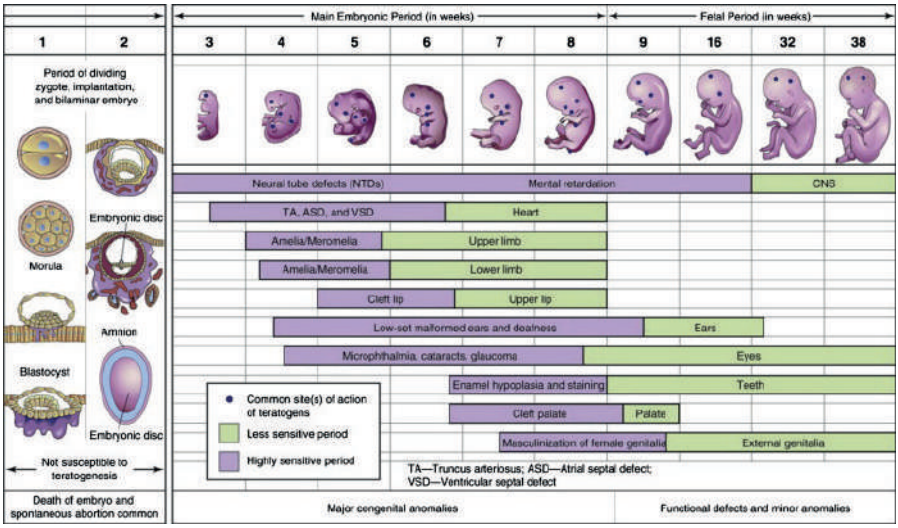


Figure 1. A depiction of the various embryonic and fetal stages during intrauterine development (2).

Placenta

The placenta is a remarkable fetomaternal organ that plays a critical role in supporting the developing fetus throughout pregnancy. It consists of two distinct components: the fetal part, which originates from the chorionic sac (chorion frondosum), and the maternal part, derived from the endometrium (decidua basalis). Together, the placenta and the umbilical cord form a sophisticated transport system, facilitating the exchange of substances between the mother and the fetus (3).

The placenta serves multiple essential functions, ensuring the well-being and development of the growing fetus:

Protection: The placenta acts as a protective barrier, shielding the fetus from potentially harmful substances and pathogens present in the maternal circulation.

Nutrition: It plays a crucial role in providing the fetus with vital nutrients, including oxygen, glucose, amino acids, and lipids, necessary for its growth and development.

Respiration: The placenta facilitates the exchange of respiratory gases, allowing the transfer of oxygen from the maternal bloodstream to the fetal circulation, while carbon dioxide and other waste products are eliminated.

Excretion: Metabolic waste products produced by the fetus, such as urea and bilirubin, are eliminated through the placenta, preventing their accumulation and ensuring a stable internal environment.

Hormone, Cytokine, and Growth Factor Production: The placenta is a remarkable endocrine organ, producing a variety of hormones, cytokines, and growth factors essential for maintaining pregnancy, regulating maternal physiological adaptations, and supporting fetal development.

The intricate functions of the placenta make it a vital interface between the mother and the developing fetus, enabling the exchange of substances necessary for growth, metabolism, and overall well-being. Its role extends far beyond simple nutrient transfer, highlighting its significance in ensuring a successful and healthy pregnancy (Fig 2).

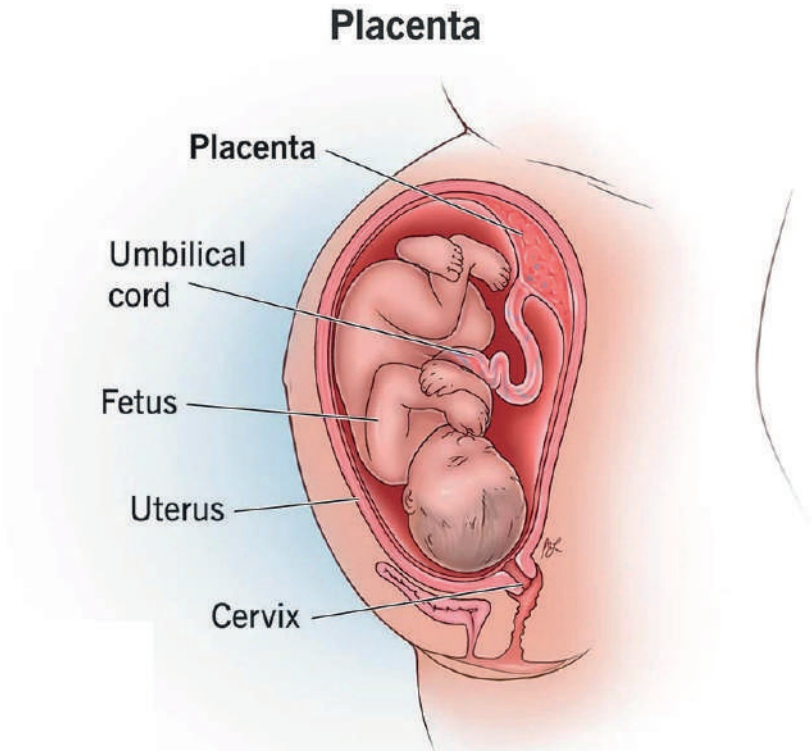


Figure 2. An illustrative depiction showcasing the fetus, placenta, and adjacent anatomical structures (4).

Amniotic Fluid: Unveiling Its Significance in Embryonic Development and Maternal-Fetal Interactions

Amniotic fluid, a clear and yellowish fluid that surrounds and protects the fetus within the uterus, plays a crucial role in supporting fetal growth, movement, and development (Fig 3). It serves multiple functions, including creating space for fetal growth, facilitating absorption of nutrients for growth and maturation, providing a protective barrier against external trauma, and exhibiting antibacterial properties due to the presence of cytokines, antibacterial peptides, and chemokines. Furthermore, amniotic fluid aids in the dilation of the cervix during delivery, contributing to the birthing process (5).

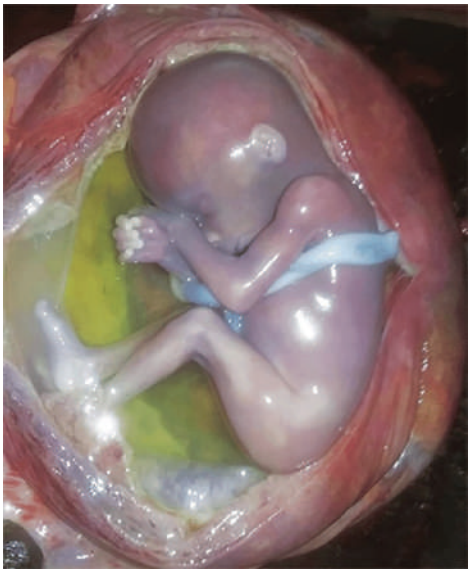


Figure 3. The fetal development stage characterized by the presence of amniotic fluid surrounding the fetus (6).

Preterm birth

Preterm birth, which occurs before 37 weeks of gestation, presents significant challenges in neonatal care. The nine-month gestation period is commonly divided into trimesters for convenience. However, current technology does not support the viability of fetuses born before approximately 22 weeks of gestation. Survival rates for infants born between 22 and 28 weeks of gestation have improved, but a substantial proportion still experience significant morbidity that affects their long-term survival. For instance, mortality rates for infants born at 23-25 weeks of gestation range from 74% to 28%, while morbidity rates range from 92% to 80% (7).

Prematurity, encompassing all newborn babies born before 37 weeks of gestation, represents a major concern in neonatal health. It is the leading cause of neonatal death and the most common cause of prenatal hospitalization, accounting for more than one-third of all infant deaths (7). Prematurity is associated with a range of complications, including acute respiratory distress syndrome (RDS), bronchopulmonary dysplasia, periventricular and intraventricular bleeding, cerebral palsy, retinopathy of prematurity (ROP), and necrotizing enterocolitis (7).

Prematurity- Respiratory stress

Respiratory failure poses a significant challenge in the care of very early preterm newborns due to the impaired gas exchange resulting from the structural and functional immaturity of their lungs (8).

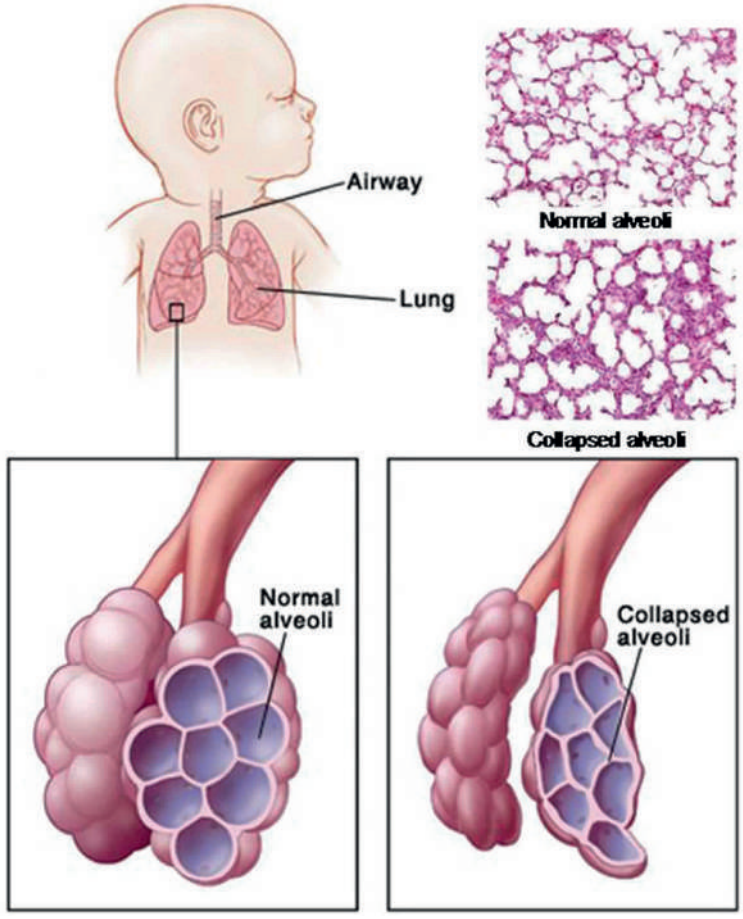


Figure 4. A schematic representation depicting the normal and collapsed alveoli within the lung of an infant (9).

The premature initiation of gas ventilation in the lungs hinders lung development. Extremely premature infants at the limit of viability (22-24 weeks) have lungs in the late canalicular phase of pulmonary development, characterized by a limited number of fully formed alveoli and a thick alveolar/capillary interface (Fig 4). At this stage, the underdeveloped lungs are ill-equipped for efficient gas exchange, leading to respiratory failure. Additionally, the immaturity of the premature lungs makes them more susceptible to oxidative stress, infection, and inflammatory damage, further compromising oxygenation and contributing to the development of bronchopulmonary dysplasia, a chronic respiratory disease associated with iatrogenic damage. It is worth noting that despite advancements in neonatal care, such as minimally invasive neonatal ventilation, exogenous surfactant administration, and prenatal corticosteroid therapy, these interventions cannot fully prevent severe respiratory failure due to the structural and functional immaturity of the premature lungs (10).

Understanding the challenges posed by the immature lungs of very early preterm newborns and their susceptibility to respiratory failure and related complications is crucial for healthcare professionals in providing optimal care and developing innovative strategies to improve outcomes for these vulnerable infants.

Artificial Placenta – History and Components

The concept of an artificial placenta, which can support the survival and development of extremely premature infants, has been a topic of fascination and inspiration in both scientific and fictional Works (Fig 5). Extracorporeal life support (ECLS) refers to the preservation of life through an external assist device, while extracorporeal membrane oxygenation (ECMO) involves the direct transfer of air or oxygen into the blood via a gas-permeable membrane. The idea of using extracorporeal oxygenation to treat respiratory failure in severely premature infants emerged shortly after the development of primitive oxygenator technology in the 1950s (11).

Early experiments in the field of artificial placenta research date back to 1958 when Westin et al. cannulated the umbilical vessels of previable human fetuses and connected them to a heated perfusion chamber with an oxygenator, extending their survival for up to 12 hours (12). Subsequent studies in fetal lambs by Callaghan and colleagues further advanced the understanding of artificial placenta technology. Over the years, various research groups have explored different models of artificial placenta, refining circuit configurations, pumps, oxygenators, vascular access methods, and fluid containers to improve oxygen delivery and enhance survival rates (13).

During the 1970s, advancements in neonatal care, including positive pressure mechanical ventilation, prenatal maternal steroids, and exogenous surfactant administration, reduced the focus on artificial placenta research (14,15). However, the recognition of the limitations of these treatments, advancements in oxygenator technology in the 1990s, and increased interest in fetal and utero-placental physiology reignited the exploration of artificial placenta technology. The research shifted from solely emphasizing long-term survival to also evaluating safety, fetal well-being, and specific organ maturation, with the aim of eventual clinical implementation (16).

An artificial placenta should encompass several key components to effectively support fetal development and function. These include:

• Maintenance of fetal circulation configuration and open major fetal shunts to prevent neonatal transmission.

• Blood oxygenation and maintenance of correct hemoglobin saturation without lung aeration.

• Hemodynamic stability, including appropriate heart rate and blood pressure responses to fetal conditions.

• Proper hydration and elimination of excess fluid to maintain fluid balance and electrolyte composition.

• Adequate kidney function for the elimination of metabolic nitrogenous wastes.

• Endocrine support to ensure the appropriate hormonal environment for fetal development (17).

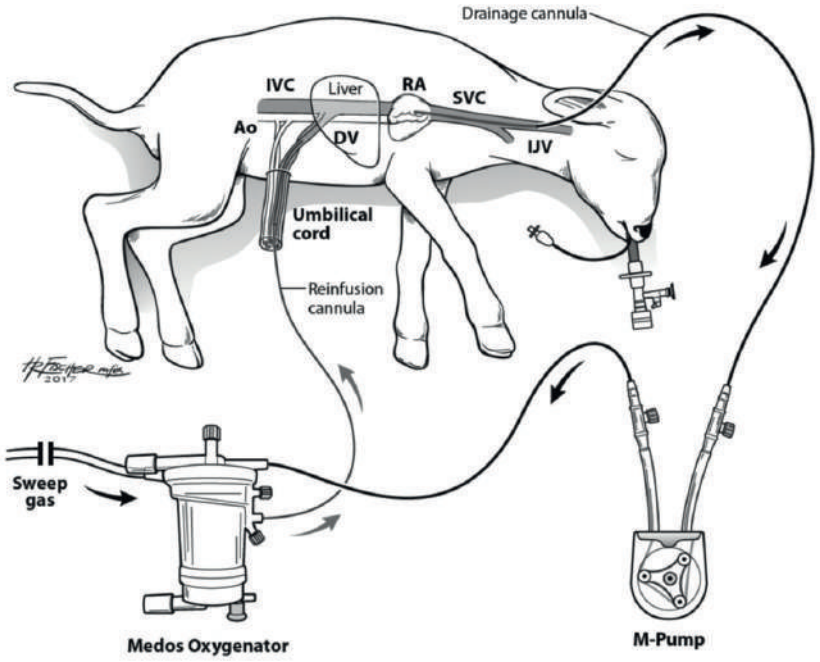


Figure 5. Artificial placenta setup: Blood is drained from the right jugular vein using a collapsible-tubing roller pump and directed to an oxygenator/heat exchanger, after which it is returned through the umbilical vein. The second umbilical vein is used for intravenous fluid and medication administration, while an umbilical arterial line is inserted for hemodynamic monitoring and blood gas sampling. The lamb's lungs are kept filled with amniotic fluid by clamping the endotracheal tube. Key anatomical landmarks include the aorta (Ao), ductus venosus (DV), internal jugular vein (IJV), inferior vena cava (IVC), right atrium (RA), and superior vena cava (SVC) (18).

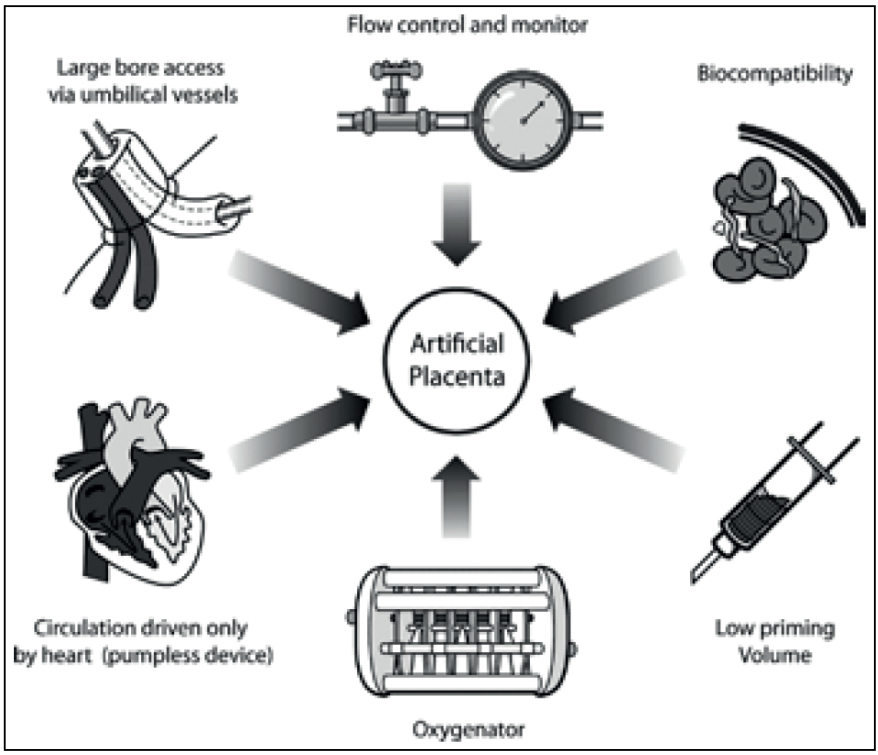


Figure 6. The essential components and key features necessary for the development of an artificial placenta device (19).

Pumpless arterio-venous circuit

An ideal artificial placental circuit, designed to mimic fetal and utero-placental physiology, should feature a "pumpless" arterio-venous (AV) configuration that allows for autoregulation and blood flow driven by the fetal cardiac beat and arterial pressure as the driving force through the oxygenator. Early development of artificial placenta technology focused on AV configurations (20-22).

However, early models required the inclusion of pumps to overcome the high resistance of the oxygenator and achieve physiological fetal flows (Fig 6). Survival rates in pump-assisted models were systematically limited due to cardiac decompensation, resulting in circulatory depression and fetal hydrops. Both centrifugal and roller pumps caused suction that impeded the natural pumping function of the fetal heart, leading to imbalances in preload and afterload that strained the cardiac function. The fetal heart is highly

sensitive to such imbalances, especially in the presence of high-resistance oxygenators and pumped circuits (23).

The umbilical artery (UA) and umbilical vein (UV) configuration presents challenges due to spasm, and preserving vascular integrity is crucial in this model. Two main configurations were utilized to connect the fetal blood vessels to the artificial placental circuit. The arteriovenous (AV) configuration simulated the natural placenta by using the umbilical vessels, with fetal blood being pumped by the heart from the umbilical arteries (UA) to the umbilical vein (UV). The venovenous (VV) configuration involved connecting the artificial placenta to an oxygenator with the exit through a catheterized jugular vein and return via the UV. To address load imbalances, open-top reservoirs were incorporated into the circuit to convert pulsatile flow to laminar flow and regulate pressure and flows back to the right heart. The addition of reservoirs improved neonatal survival rates in the circuit but introduced risks of air-blood interaction, increased initial volume, and did not effectively prevent progressive cardiac decompensation. The inclusion of a hemodialyzer improved fluid and electrolyte balance and circuit survival but failed to prevent progressive circulatory failure and was subsequently disregarded in later versions of the model (24). In pumpless systems, increased cardiac afterload remained the limiting factor due to supraphysiological resistance resulting from limitations in oxygenator technology and other circuit components (25).

Further advancements in artificial placenta technology are necessary to overcome the challenges associated with cardiac afterload and optimize the circuit's performance. These advancements require improvements in oxygenator technology and other circuit components to achieve physiological resistance levels and ensure adequate blood flow, while also addressing issues related to fluid and electrolyte balance, air-blood interaction, and circuit stability. Overcoming these challenges will bring us closer to the development of a functional and effective artificial placenta for supporting the survival and development of extremely premature infants.

Low resistance oxygenators

In the early models of artificial placenta, film oxygenators with high surface area, large feed volume, and high resistance were utilized. These oxygenators allowed for effective oxygenation through direct blood-air contact but posed challenges in terms of biological compatibility and increased the risk of infection (26). To address these concerns, membrane oxygenators became the new standard in the 1970s. These oxygenators employed flat and

helical gas-permeable membranes for gas exchange, eliminating the need for direct blood-air contact. In recent artificial placenta models, hollow fiber oxygenators with resistances equivalent to physiological placental resistances have been implemented, significantly reducing the cardiac afterload required for pumpless circulation (27).

Continuous efforts are being made to further optimize the design of oxygenators in artificial placenta systems. One approach is to reduce the surface area and initial volume of the oxygenators. Decreasing the surface area helps minimize platelet and complement activation, improving biocompatibility (28). Additionally, researchers are exploring the use of alternative microfluidic oxygenators, which have the potential to further reduce feed volumes and enhance the efficiency of gas exchange. These microfluidic oxygenators hold promise for improving the performance and compactness of artificial placenta systems (29).

By advancing oxygenator technology and exploring innovative designs, researchers aim to optimize gas exchange efficiency, minimize adverse reactions, and improve overall compatibility in artificial placenta models. These advancements are crucial for the development of a functional and safe artificial placenta that can effectively support the survival and development of extremely premature infants (Fig 7).

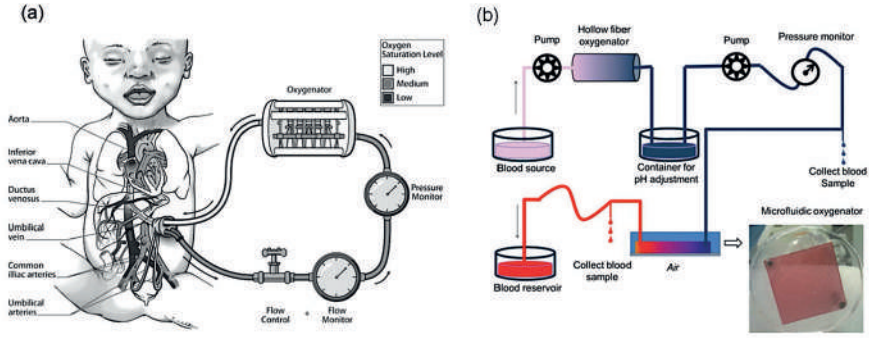


Figure 7. The implementation of a microfluidic oxygenator for the artificial placentaconcept, and the arrangement of the experimental setup for in vitro gas exchange testing (29).

Umbilical access

In the pursuit of an ideal artificial placenta model that closely mimics fetal and utero-placental physiology, the use of umbilical vessels for vascular

access has been extensively explored. Various configurations utilizing cervical, femoral, and umbilical vessels have been tested over time. Comparisons have shown that cannulation of the umbilical artery and umbilical vein (UA/UV) provides superior flow distribution, particularly to vital organs such as the heart and brain (30).

To optimize flow dynamics, the principles of the Hagen-Poiseuille law are considered. By using short end-to-end cannulas (approximately 2 cm) and larger umbilical arteries, the resistance within the circuit can be minimized, allowing for physiological flows in animal models with UA/UV cannulation (31). The avoidance of cervical vessel cannulation offers additional advantages, including the protection of important vessels, reduced risk of embolic events in the brain, and decreased likelihood of complications related to decannulation, thereby minimizing the need for fetal sedation or paralysis. However, one of the challenges associated with umbilical vascular access is vasospasm, which, if severe, can result in circulatory arrest and subsequent fetal demise (32). To mitigate this risk, humidification of the umbilical vessels and the administration of vasodilators such as papaverine, both topically and intravenously, have been proposed (33).

To ensure the integrity of the umbilical vessels and prevent trauma, erosion, turbulence, and obstruction, the use of very short umbilical cannulas that do not extend deep into the vessels has been recommended. The curvature of human umbilical vessels makes intra-abdominal advancement of cannulas impractical (34).

In summary, optimizing the vascular access in artificial placenta models is crucial for ensuring adequate and physiological blood flow distribution. Cannulation of the umbilical artery and umbilical vein has shown promising results, although challenges such as vasospasm need to be addressed for successful implementation. By refining the cannulation technique and addressing associated complications, researchers aim to develop an artificial placenta model that effectively supports fetal circulation and development.

Immersion in sterile fluid

An important aspect of artificial placental models is the immersion of the fetus in a sterile and warm liquid environment, typically maintained at around 39.0°C. This immersion serves several crucial purposes in supporting fetal development and well-being. Firstly, it provides insulation, protecting the fetus from external factors such as sound, mechanical pressure, infection, and injury. Additionally, the presence of amniotic fluid helps regulate the

fetus's body temperature, preventing excessive heat loss or fluid imbalance. It also plays a role in moistening the umbilical vessels and creating a space that allows for normal fetal breathing and swallowing movements.

One significant advantage of keeping the fetal lungs fluid-filled is that it delays the neonatal hemodynamic transition caused by ventilation. This helps maintain the integrity of the fetal circulation and supports ongoing pulmonary development. Ventilation of immature lungs can lead to complications such as oxygen toxicity, inflammatory damage to delicate lung tissue, and arrest of lung development. On the other hand, the presence of fluid in the airways and glottic resistance stimulates pulmonary maturation. Similarly, the presence of amniotic fluid in the gastrointestinal tract has been shown to enhance the development of the intestinal mucosa and support overall growth.

While the benefits of a liquid immersion environment are evident, researchers have also acknowledged the potential risks associated with fetal sepsis in open or semi-closed bath models. Due to their immature immune systems, fetuses are more vulnerable to bacterial invasion through the lungs or skin. Infection can trigger a systemic inflammatory response that affects brain and lung development and often leads to fetal death. To mitigate this risk, the transition to a closed and sterile environment with continuous amniotic fluid exchange has been implemented. This approach helps eliminate pneumonia and reduces the risk of amniotic fluid contamination and fetal bacteremia, while also minimizing the need for prophylactic antimicrobials.

However, it is important to consider the potential drawbacks of fetal immersion, such as the physical and potential psychological barrier it may create between the fetus and prospective parents. The impact of this distance on parent-fetal bonds is a significant question that requires further investigation. Understanding whether prospective parents are accepting of this separation and how it may influence their emotional connection with the fetus is crucial in the development and implementation of artificial placental models.

In summary, the immersion of the fetus in a sterile and warm liquid environment has numerous advantages in supporting fetal development, including insulation, temperature regulation, maintenance of lung and gastrointestinal tract development, and prevention of infection. Balancing the benefits and potential drawbacks of fetal immersion is essential to optimize the design and acceptance of artificial placental models (Fig 8).

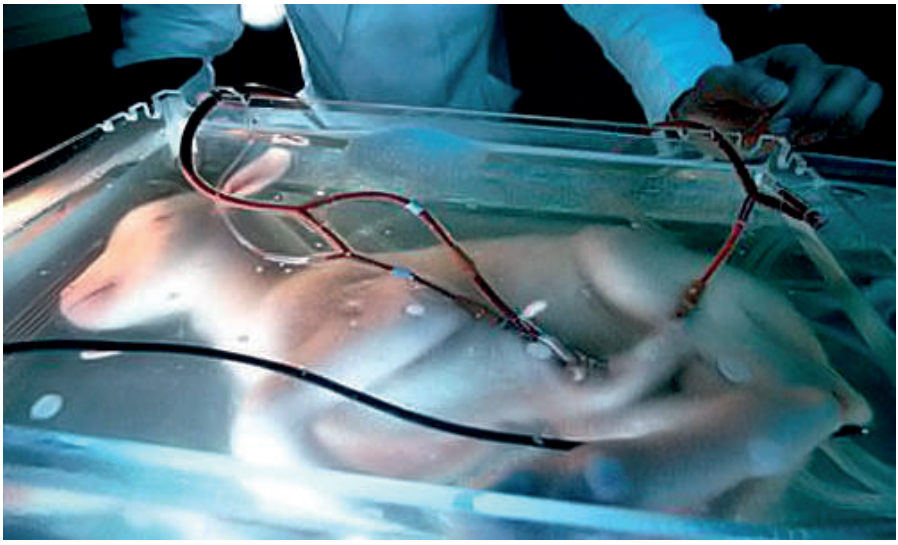


Figure 8. A fetal lamb supported by an artificial placenta system, receiving support and immersed in an amniotic fluid bath (40).

Feeding and medication

As the focus shifted towards long-term survival in artificial placental circuits, the need for improved nutrition became apparent. Dextrose or glucose infusions alone were insufficient, leading to the addition of amino acids, lipids, and vitamins to provide a total calorie target of 70-80 kcal/kg/day. This comprehensive nutritional approach aims to support optimal growth and organ maturation in the fetus (41).

Maintaining ductal patency is crucial for fetal circulation, and the continuous infusion of prostaglandins (PGE1/PGE2) has become a standard practice in all artificial placenta models. This helps prevent fatal ductal narrowing caused by increased oxygen tension (42). To counteract the thrombogenicity associated with the tubing and oxygenator, heparin is also continuously infused. However, compared to extracorporeal life support (ECLS) circuits, the heparinization requirements in artificial placental models may be reduced due to factors such as decreased surface area, priming volume, and the systemic inflammatory response (43).

In previous studies, intermittent sedation with midazolam and pentobarbital was utilized to manage periods of fetal agitation and reduce the risk of traumatic decannulation events. However, recent studies have questioned the effect of sedatives on fetal neurodevelopment, leading to

a cautious approach in their use. Additionally, the use of umbilical cord cannulation without direct contact to the fetus has decreased the need for sedation in some cases. It is crucial to avoid prolonged paralysis as it can negatively impact fetal muscle development and hinder spontaneous breathing after discontinuation of extracorporeal life support (44).

Repetitive phlebotomy for monitoring fetal well-being can lead to progressive anemia. Daily administration of erythropoietin has been shown to prevent this anemia, ensuring adequate oxygen-carrying capacity while minimizing the need for excessive blood sampling. To prevent bacteremia, prophylactic intravenous broad-spectrum antibiotics and antifungals are commonly administered in many artificial placenta models, and in some cases, they may be added to synthetic amniotic fluid. However, in sterile closed circuits, the need for these interventions is reduced unless contamination occurs (45).

Current and Successful Artificial Placenta Models: Design and Outcomes

The quest to replicate fetal and utero-placental physiology has driven the progress in artificial placenta development, aided by advancements in technology. Various models have emerged, each employing distinct approaches to recreate the intricate dynamics of fetal and utero-placental interactions. Over the past decade, the Michigan, Sendai-Perth, and Philadelphia groups have spearheaded research and analysis of three prominent artificial placenta models, each with its own level of success.

University of Michigan (USA): Veno-Venous Preterm Extracorporeal Life Support

In the pump-assisted veno-venous (VV) circuit with jugular vein/umbilical vein (JV/UV) cannulation, the use of a fluid-filled endotracheal tube replaced immersion in amniotic fluid to address concerns related to sepsis. This modification extended the mean survival of fetuses from 1 day to nearly 2 weeks, with the maximum reported survival reaching 17 days. Hemodynamic stability was achieved through the administration of vasopressors, while supraphysiological partial pressures of oxygen were necessary to ensure adequate oxygenation. However, the maintenance of ductal patency through prostaglandin E1 (PGE1) infusion proved to be inconsistent.

Although this artificial placenta model successfully rescued late premature lambs that had previously failed on postpartum mechanical ventilation, it does not fully align with the primary objective of artificial placental technology,

which is to delay lung ventilation and thereby preserve lung maturation. The fetal lambs used in the study were predominantly at the 118- and 130-day stages of gestation, corresponding to the early and late saccular phases of lung development, respectively. It is worth noting that borderline human fetuses at 22-24 weeks of gestation are in the late canalicular phase, which corresponds to approximately 100 to 110 days of gestation in lambs (46).

This discrepancy in lung development stages between the animal model and human fetuses highlights the need for further research and refinement in artificial placenta models to better emulate human fetal physiology. Adjustments and adaptations specific to the developmental stage of human fetuses will be crucial to optimize outcomes and fulfill the goal of preserving lung maturation in extremely premature infants.

Tohoku University, Sendai (Jap) and University of Western Australia, Perth (Aus): Ex-vivo Uterine Environment (EVE)

In 2012, a Japanese team made significant advancements in optimizing the pumpless arterio-venous (AV) circuit initially developed by the Michigan group. They achieved this by reducing the priming volume and introducing milrinone, an inotropic vasodilator drug. These modifications led to an increase in survival time from 4 hours to 30 hours. However, the model still faced limitations due to progressive circulatory failure caused by high circuit resistance .To address this issue, the researchers implemented a parallel configuration of two hollow fiber membrane oxygenators, effectively reducing the circuit resistance by half. This improvement resulted in a further increase in survival time to 1, 2, and eventually 7 days. External circuit flow regulators were necessary to stabilize hemodynamics and prevent high cardiac output failure. In order to mitigate the risks of bacteremia and systemic inflammatory response, the fluid bath was transformed into a closed, low-volume container, and continuous circulation of sterilized amniotic fluid was established. Prophylactic administration of meropenem and fluconazole was incorporated into the amniotic fluid, both intravenously and locally. Additionally, hydrocortisone was added to the artificial placenta model to suppress inflammatory responses, promote lung maturation, and prevent hypocortisolemic refractory hypotension. The use of corticosteroids successfully alleviated the inflammatory reaction, and the absence of infection was noted. However, analysis of the brains of the experimental subjects revealed evidence of brain white matter damage, likely attributed to systemic hypoperfusion or acute embolic events. These findings underscore the need for further optimization of the model in preparation for its potential clinical application. The maximum reported survival time achieved in this study was 7 days (47).

Further research and refinement are necessary to address the remaining challenges and ensure the safety and efficacy of the artificial placenta model. This includes developing strategies to enhance perfusion to vital organs and prevent adverse neurological outcomes, among other consideration

Philadelphia Children's Hospital (USA):

Extra-Uterine Environment for Neonatal Development (EXTEND)

In 2017, the Philadelphia team introduced the EXTEND AW model, which demonstrated promising outcomes in lambs aged 106 to 117 days. The lambs survived for up to 28 days within the artificial placenta model, followed by successful ventilation. The model utilized a pumpless, low-resistance, low-surface area heparin-coated arterio-venous (AV) circuit with umbilical artery/umbilical vein (UA/UV) cannulation. The circuit maintained stable hemodynamics without the need for external flow regulators, vasopressors, or corticosteroids. Refractory hypotension was effectively managed by providing physiological oxygen delivery and circuit flows.

The lambs were immersed in a closed, sterile fluid medium with continuous exchange of synthetic amniotic fluid, eliminating the need for prophylactic antimicrobials. Notably, the absence of bacteremia was observed throughout the study. The Philadelphia team reported long-term stable hemodynamics, somatic growth, and sustained organ maturation in the lambs. The lungs of the lambs in the EXTEND model progressed naturally from the canalicular to the saccular phase of lung development, without the use of corticosteroids or tracheal occlusion. Assessment of pulmonary function during mechanical ventilation showed comparable results to age-matched control lambs.

Brain analysis revealed normal cerebral maturation, with no evidence of white matter damage or intracranial hemorrhage. It is worth noting that fetal lambs are considered to be less prone to intracranial bleeding compared to human fetuses, as sheep experience earlier germinal matrix maturation. Neurodevelopmental maturation in the lambs within the artificial placenta model was demonstrated by the progressive consolidation of sleep/wake cycles, measured through ocular electromyography.

Transiently decreased cardiac contractility was observed during a one-week adaptation phase in the circuit, followed by normal cardiac function and contractility for the remainder of the study. No myocardial damage or inflammation was observed, and the heart development and structure in the circuit lambs were comparable to age-matched controls.

While specific assessments of liver and kidney function have not been reported yet, metabolic parameters reflecting kidney function remained stable. Mild increases in hepatic function tests and bilirubin levels were observed during the study period, which may be attributed to the absence of maternal elimination rather than indicating hepatic dysfunction (48)

The EXTEND AW model developed by the Philadelphia team demonstrates promising outcomes, including stable hemodynamics, normal organ development, and neurological maturation in lambs within the artificial placenta model. Further studies are needed to evaluate liver and kidney function, as well as to address any potential long-term effects and optimize the model for future clinical applications (Fig 9).

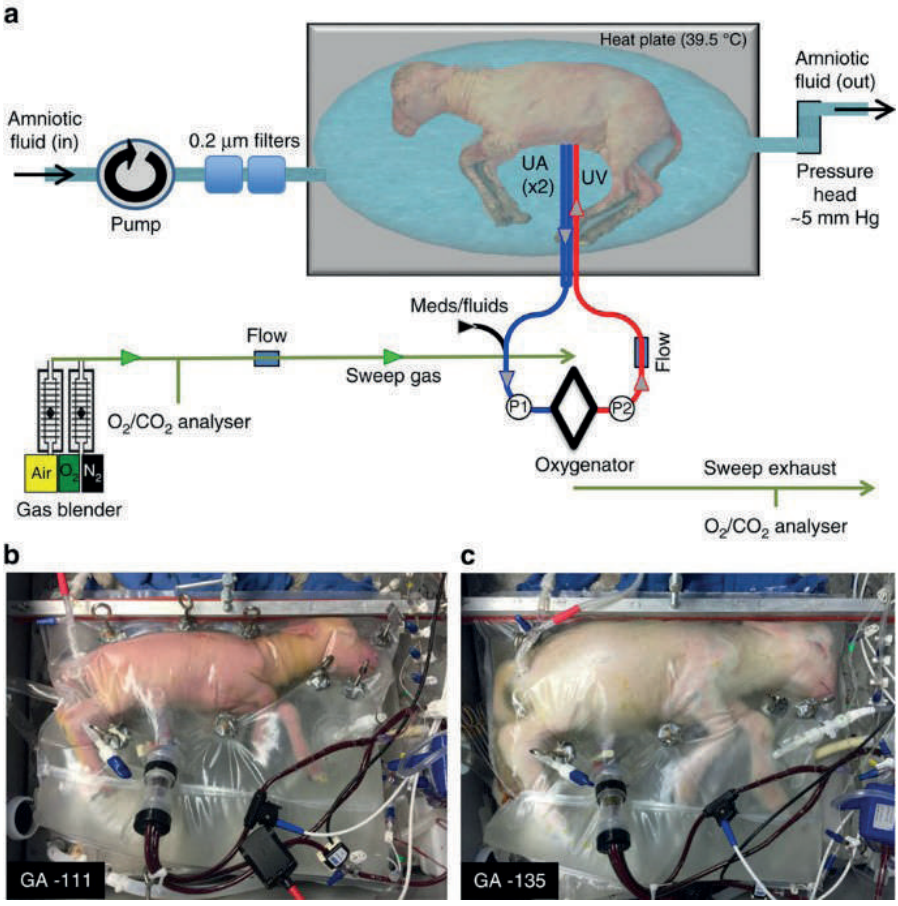


Figure 9.The UA/UV Biobag system design includes a pumpless, low-resistance oxygenator circuit, a closed fluid environment with continuous fluid exchange, and an umbilical vascular interface. The figure shows a representative lamb cannulated at 107 days of gestation and on day 4 of support, as well as the same lamb on day 28 of support, demonstrating somatic growth and maturation(49).

Challenges in clinical practice

Significant progress has been made in artificial placenta technology over the past decade, bringing it closer to clinical implementation. However, several challenges still need to be addressed.

One challenge is the reduction of oxygenator surface area and improvement of hemocompatibility through the use of new biomaterial coatings. This can help minimize the need for systemic anticoagulation, thereby reducing the risk of bleeding complications. Restoring placental alignment to allow for pulsatile flow from umbilical arteries to transition into laminar flow in the umbilical vein has not yet been achieved in recent artificial placenta models. Incorporating a closed, compliant pressure chamber into the system can prevent pulsatile flows from reaching the fetal right heart via the umbilical vein. Optimizing nutrition, growth factors, and amniotic fluid composition is essential for promoting proper somatic growth within the artificial placenta system. Exploring enteral nutrition strategies, as opposed to solely relying on parenteral nutrition (TPN), may be advantageous for the developing gut and reduce the risks associated with TPN-related cholestasis. Therefore, further investigation in this area is necessary. The widespread application of artificial placenta technology in emergency scenarios, such as premature rupture of membranes, requires standardized patient selection criteria and the development of a simple, fast, and effective cannulation technique. Advancements in these areas will contribute to the creation of an ideal artificial placenta model.

Conclusion

Artificial placenta technology is an evolving field of study that has captivated the scientific community for over six decades. Progress in circuit configuration, oxygenator technology, vascular access, fluid immersion, sterility, nutrition, and medical therapy has significantly advanced this technology, bringing it closer to mimicking utero-placental physiology. Recent experimental models have demonstrated improved survival rates and hemodynamic stability, allowing for safety assessments in preparation for future clinical applications. Despite these promising results, challenges remain along the path to clinical implementation. Furthermore, artificial placenta technology not only holds great clinical potential but also provides exciting opportunities for further research endeavors.

References

Nicolaides KH. First-trimester screening for chromosomal anomalies. Semin Perinatol. 2005; 29:190–194.

Lally M, Valentine-French S. Lifespan Development: A Psychological Perspective Second Edition. http://dept.clcillinois.edu/psy/LifespanDevelopment.pdf (2019)

Huppertz B. The anatomy of the normal placenta. J Clin Pathol. 2008 Dec;61(12):1296-302.

Cleveland clinic web site. Placenta. Figure. https://my.clevelandclinic.org/health/body/22337-placenta (2023).

Beall MH, van den Wijngaard JP, van Gemert MJ, Ross MG. Amniotic fluid water dynamics. Placenta. 2007;28(8-9):816-23.

Sanjay BB, Bimbisar BM. Homicide by smothering masqueradedas Accidental ElectrocutionInternational Journal of Education and Research in Health Sciences, July-September 2016;2(3):46-49

Fraser J, Walls M, McGuire W. Respiratory complications of preterm birth. BMJ. 2004; 329(7472): 962–965.

Chrıstıan l. Hermansen and Kevın N. Lorah. Respiratory Distress in the Newborn Am Fam Physician. 2007;76(7):987-994.

Health Jade Web Site. Infant respiratory distress syndrome. https://healthjade.net/infant-respiratory-distress-syndrome (2023)

Brion LP, Primhak RA, Ambrosio-Perez I. Diuretics acting on the distal renal tubule for preterm infants with (or developing) chronic lung disease. Cochrane Database Syst Rev 2003;(4)

Davisa R, Brynera B, Mychaliska GB. A paradigm shift in the treatment of extreme prematurity: the artificial placenta. Curr Opin Pediatr 2014;26(3):370-6.

Westin B, Nyberg R, Enhorning G. A technique for perfusion of the previable human fetus. Acta Paediatr 1958; 47:339–349.

Callaghan JC, Maynes EA, Hug HR. Studies on lambs of the development of an artificial placenta. Review of nine long-term survivors of extracorporeal circulation maintained in a fluid medium. Can J Surg 1965; 8:208–213.

Ballard PL, Granberg P, Ballard RA. Glucocorticoid levels in maternal and cord serum after prenatal betamethasone therapy to prevent respiratory distress syndrome. J Clin Investig 1975; 56:1548–1554. 8.

Liggins GC, Howie RN. A controlled trial of antepartum glucocorticoid treatment for prevention of the respiratory distress syndrome in premature infants. Pediatrics 1972; 50:515–525.

Awad JA, Cloutier R, Fournier L, et al. Pumpless respiratory assistance using a membrane oxygenator as an artificial placenta: a preliminary study in newborn and preterm lambs. J Invest Surg 1995; 8:21–30.

Stephen D. Artificial placenta: Analysis of recent progress Bird European Journal of Obstetrics & Gynecology and Reproductive Biology. 2017;208:61–70.

Church J, Perkins E, Coughlin M, Mychaliska GB. Perfluorocarbons Prevent Lung Injury and Promote Development during Artificial Placenta Support in Extremely Premature Lambs. Neonatology . 2018; 113(4):313-321.

Rochow N, Chan EC, Wen-I , Selvaganapathy PR. Artificial Placenta - Lung Assist Devices for Term and Preterm Newborns with Respiratory Failure. Int J Artf Organs. 2013;36:6.

Reoma JL, Rojas A, Kim AC, et al. Development of an artificial placenta I: pumpless arterio-venous extracorporeal life support in a neonatal sheep model. J Pediatr Surg. 2009;44(1):53-59.

Rochow N, Chan EC, Wu WI, et al. Artificial placenta–lung assist devices for term and preterm newborns with respiratory failure. Int J Artif Organs. 2013;36(6):377-391.

Partridge EA, Davey MG, Hornick MA, Flake AW. An Extrauterine environment for neonatal development: EXTENDING fetal physiology beyond the womb. Semin Fetal Neonatal Med. 2017;22(6): 404-409.

Kuwabara Y, Okai T, Imanishi Y, et al. Development of Extrauterine fetal incubation system using extracorporeal membrane oxygenator. Artif Organs. 1987;11(3):224-227.

Kuwabara Y, Okai T, Kozuma S, et al. Artificial placenta: long-term extrauterine incubation of isolated goat fetuses. Artif Organs. 1989; 13(6):527-531

Miura Y, Matsuda T, Funakubo A, et al. Novel modification of an artificial placenta: pumpless arteriovenous extracorporeal life support in a premature lamb model. Pediatr Res. 2012;72(5):490-494.

Gaylor JDS. Membrane oxygenators: current developments in design and application. J Biomed Eng. 1988;10(6):541-547.

Acharya G, Sonesson SE, Flo K, Rasanen J, Odibo A. Hemodynamic aspects of normal human feto-placental (umbilical) circulation. Acta Obstet Gynecol Scand. 2016;95(6):672-682.

Arens J, Schnoering H, Pfennig M, et al. The Aachen MiniHLM–a miniaturized heart-lung machine for neonates with an integrated rotary blood pump. Artif Organs. 2010;34(9):707-713.

Wen-I W, Niels Rochow, Emily C Chan,.Lung assist device: Development of microfluidic oxygenators for preterm infants with respiratory failure. 2013;13(13)

Fujimori K, Murata Y, Quilligan EJ, Nagata N, Hirano T, Sato A. Distribution of oxygenated blood flow at three different routes of extracorporeal membrane oxygenation in exteriorized fetal lambs. J Obstet Gynaecol Res. 2001;27(2):103-109.

Wallace JM, Bourke DA, Aitken RP, Leitch N, Hay WW. Blood flows and nutrient uptakes in growth-restricted pregnancies induced by overnourishing adolescent sheep. Am J Phys Regul Integr Comp Phys. 2002;282(4):1027-1036.

Alexander DP, Britton HG, Nixon DA. Survival of the foetal sheep at term following short periods of perfusion through the umbilical vessels. J Physiol. 1964;175(1):113-124.

Partridge EA, Davey MG, Hornick MA, et al. An extra-uterine system to physiologically support the extreme premature lamb. Nat Commun. 2017;8:15112.

Stehbens WE, Wakefield JSJ, Gilbert-Barness E, Zuccollo JM. Histopathology and ultrastructure of human umbilical blood vessels. Fetal Pediatr Pathol. 2005;24(6):297-315

Teitel DF, Iwamoto HS, Rudolph AM. Changes in the pulmonary circulation during birth-related events. Pediatr Res. 1990;27(4 Pt 1): 372-378.

Morton SU, Brodsky D. Fetal physiology and the transition to extrauterine life. Clin Perinatol. 2016;43(3):395-407

Miura Y, Usuda H, Watanabe S, et al. Stable control of physiological parameters, but not infection, in preterm lambs maintained on ex vivo uterine environment therapy. Artif Organs. 2017;41(10): 959-968

Partridge EA, Davey MG, Hornick MA, et al. An extra-uterine system to physiologically support the extreme premature lamb. Nat Commun. 2017;8:15112.

Felix R. De Bie, Marcus G. Davey, Abby C. Larson, Jan Deprest, Alan W. Flake. Artificial placenta and womb technology: Past, current, and future challenges towards clinical translation. Prenatal Diagnosis. 2021;41:145–158.

IEEE Engineering in Medicineand Biology Society. https://bioengineer.org/artificial-womb-born (2023).

Partridge EA, Davey MG, Hornick MA, et al. An extra-uterine system to physiologically support the extreme premature lamb. Nat Commun. 2017;8:15112.

Sakata M, Hisano K, Okada M, Yasufuku M. A new artificial placenta with a centrifugal pump: Long-term total extrauterine support of goat fetuses. J Thorac Cardiovasc Surg. 1998;115(5):1023-1031.

Miura Y, Matsuda T, Usuda H, et al. A parallelized Pumpless artificial placenta system significantly prolonged survival time in a preterm lamb model. Artif Organs. 2016;40(5)

Unno N, Kuwabara Y, Okai T, et al. Development of an artificial placenta: survival of isolated goat fetuses for three weeks with DE BIE ET AL. 155 umbilical arteriovenous extracorporeal membrane oxygenation. Artif Organs. 1993;17(12):996-1003.

Usuda H, Watanabe S, Saito M, et al. Successful use of an artificial placenta to support extremely preterm ovine fetuses at the border of viability. Am J Obstet Gynecol. 2019;221(1)

Bryner BS, Mychaliska GB. ECLS for preemies: the artificial placenta. Semin Perinatol. 2014 Mar;38(2):122-9.

Miura Y, Saito M, Usuda H, Woodward E, Rittenschober-Böhm J, Kannan PS, Musk GC, Matsuda T, Newnham JP, Kemp MW**.** Ex-Vivo Uterine Environment (EVE) Therapy Induced Limited Fetal Inflammation in a Premature Lamb Model. Plos One. 2015 Oct 16;10(10).

Emily A Partridge , Marcus G Davey , Matthew A Hornick , Alan W Flake . An EXTrauterine environment for neonatal development: EXTENDING fetal physiology beyond the womb. Semin Fetal Neonatal med. 2017;22(6):404-409.

Emily A. Partridge, Marcus G. Davey, Matthew A. Hornick, Patrick E. McGovern, et all. An extra-uterine system to physiologically support the extreme premature lamb.Nature Communications. 2017; 8:5112.

Chapter 10

Targeting the SH2 Domain of STAT3 Proteins in Breast Cancer Treatment

Busra Demir Cetinkaya[1]

Abstract

Stat proteins, transcription factors that convert extracellular stimuli into appropriate biological responses, are involved in many normal physiological cell processes, including proliferation, differentiation, apoptosis, angiogenesis, and immune system regulation. Irregular Stat activation is often associated with tumorigenesis. This situation has made the Stat pathway an interesting target for drug development studies in cancer treatment and has led to the development of various inhibitors targeting this pathway. Stat signal inhibitors are divided into two main groups as inhibitors with direct and indirect effects. Direct inhibitors target the SH domain, DNA binding domain, or N-terminal domain of the Stat3 protein; indirect inhibitors target upstream components of the Stat3 pathway, such as JAK2 and EGFR. It is known that Stat3 has a strong relationship with the formation of breast cancer and its permanent activation is most pronounced in breast cancer. In this study, primarily the components of the Stat signaling pathway, activation/inactivation and the functions of Stat3 were emphasized, the inhibitors that act by directly inhibiting the SH2 domain of Stat3 proteins in breast cancer cells were focused, and the results of the research examining the effects of these inhibitors on breast cancer cells were compiled.

Introduction

Signal converter and transcription activator (Stat) proteins are transcription factors that convert extracellular stimuli into appropriate biological responses (Catlett-Falcone, Dalton, & Jove, 1999). Stat proteins were first identified in 1994 as key proteins involved in cytokine signaling and interferon-related antiviral activity (Jr, Kerr, & Stark, 1994; Sadowski, Shuai, Jr, &

1 Assistant Professor; Department of Pharmaceutical Toxicology, Erzincan Binali Yıldırım University Faculty of Pharmacy, Erzincan, Turkey, e-mail address: ecz.busrademir@gmail.com, ORCID: 0000-0002-9926-107X

https://doi.org/10.58830/ozgur.pub128.c630

Gilman, 1993). Over time, they have been found to be involved in many normal physiological cell processes, including proliferation, differentiation, apoptosis, angiogenesis, and immune system regulation (Verhoeven et al., 2020). The Stat protein family consists of seven members, Stat1, Stat2, Stat3, Stat4, Stat5a, Stat5b, and Stat6 (Logotheti & Pützer, 2019). All Stat proteins consist of structurally and functionally conserved regions (Verhoeven et al., 2020). Although each is encoded by a separate gene (Furqan et al., 2013), they show high homology in their functional domains (Logotheti & Pützer, 2019). Structurally functional parts of Stat proteins; The N-terminal domain (ND), DNA binding domain, coiled-coil domain (CCD), Src-homology 2 (SH2) domain, and C-terminal transcriptional activation domain (TAD) each have an important function. ND mediates homo- and heterodimerization of Stat monomers with the highly conserved SH2 domain, which is the target of many Stat inhibitors. The DNA binding domain enables the DNA and Stat complex to form. CCD functions as a nuclear localization signal. The C-terminal transcription domain with highly conserved phosphorylated tyrosine (Y) and serine (S) residues recruits additional transcriptional activators and enhances the transcriptional activity of Stat (Verhoeven et al., 2020; Xin et al., 2020). Stat structural domains are shown in Figure 1.

Figure 1: Schematic representation of STAT structural domains

1. ACTIVATION of STAT SIGNALING PATHWAY

The activation process of Stats begins following the binding of cytokines, growth factors, and hormones to their receptors on the cell surface (Turkson & Jove, 2000). These receptors are receptor-related tyrosine kinases such as Janus kinase (JAK) or receptors with intrinsic tyrosine kinase activity such as Platelet-derived growth factor receptor (PDGFR), Epidermal growth factor recepor (EGFR), Fms-like tyrosine kinase 3 (FLT3). Stats are also known to be activated by constitutively active non-receptor protein tyrosine kinases (PTKs) such as c-Src Bcr-Abl and Breast tumor kinase (Brk) (Buettner, Mora, & Jove, 2002; Furqan et al., 2013; Weaver & Silva, 2007). With specific phosphorylation of Stat proteins by these tyrosine kinases (Furqan et al., 2013), the two Stat monomers form dimers via reciprocal phosphotyrosine-SH2 interactions, translocate to the nucleus, and bind to Stat-specific

DNA response elements of target genes to induce gene transcription (Turkson & Jove, 2000) and mediate processes related to cellular immunity, proliferation, apoptosis and differentiation (Logotheti & Pützer, 2019). However, the functionality of Stat proteins is not limited to forming dimers by phosphorylation. Unphosphorylated Stat dimers and tetramer/oligomer conformations also play a role in the functionality of some Stats (Moriggl et al., 2005; Park et al., 2016). Two nonphosphorylated Stat dimers can form tetramers with N-terminal oligomerization domains, stabilizing its binding to DNA (Y. Zhao et al., 2013). The regulation of the Stat signaling pathway is shown in Figure 2.

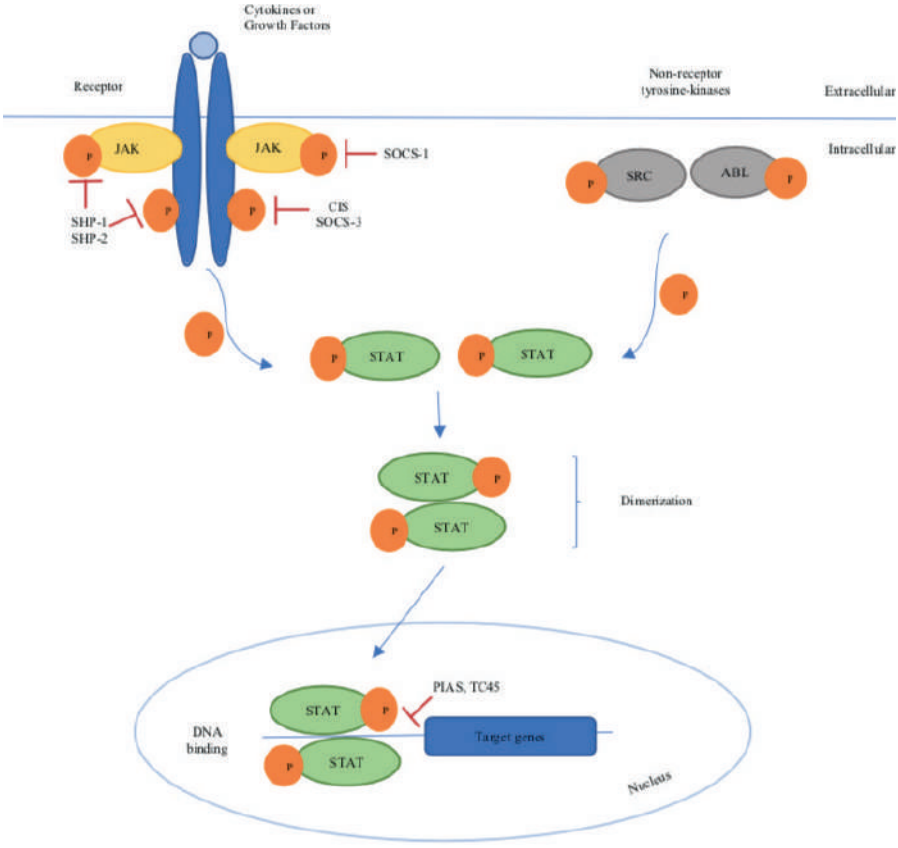


Figure 2: Regulation of STAT signaling pathway

2. NEGATIVE REGULATORS of STAT SIGNALING PATHWAY

The state of activation of stat proteins is transient. Activated Stat proteins are deactivated within hours and returned to the cytoplasm (Calò et al., 2003). Inactivation of Stat proteins is mediated by suppressor of cytokine signaling (SOCS), protein inhibitor of activated Stat (PIAS), and negative regulators including phosphatases (T. K. Kim & Maniatis, 1996). SOC is also known as JAK binding proteins (JABs) or cytokine-induced SH2 (CIS) proteins or Stat-induced Stat inhibitors (SSI) (Calò et al., 2003). The SOCS family consists of eight members, namely SOCS-1/JAB/SSI-1, SOCS-2, SOCS-3, SOCS-4, SOCS-5, SOCS-6, SOCS-7 and CIS (Lim & Cao, 2006). They inhibit Stat proteins by different mechanisms based on suppression of JAKs or competition for receptor binding (Lim & Cao, 2006). Namely; SOCS-1 suppresses JAK activity through direct interaction, while SOCS-3 inhibits Stat activation by first interacting with the phosphorylated receptor and then interacting with JAKs. CIS inhibits activation by competing with Stats for the same docking site on phosphorylated receptors (Lim & Cao, 2006). Some SOCS genes are transcriptionally regulated by Stats themselves, forming part of a classical negative feedback loop for cytokine signaling, indicating that Stats can negatively regulate their phosphorylation states (Calò et al., 2003; Desrivières et al., 2006). It is also known that SOCS proteins can cause receptor protein turnover mediated proteolytic degradation process via a ubiquitin-proteasome (Krebs & Hilton, 2001).

A second class of proteins that cause inactivation of Stat proteins is the protein inhibitor of activated Stat (PIAS). These proteins interact directly with Stat dimers in the nucleus to form protein complexes and block transcription. Namely; The resulting complexes cannot induce gene transcription because they do not bind to DNA or because nuclear co-repressor molecules are recruited into transcription complexes (Desrivières et al., 2006; Hodge, Hurt, & Farrar, 2005). The mammalian PIAS family, which consists of PIAS1, PIAS3, PIASy, PIASxa/ARIP3, and PIASxb/Miz1 have a certain degree of specificity towards Stat members; PIAS1 and PIASy are specific to Stat1, PIAS3 to Stat3 and Stat5, and PIASx to Stat4 (Liu & Shuai, 2003).

Another group of negative regulators of stat proteins is phosphatases. TC45, a nuclear tyrosine phosphatase in this group, deactivates phosphorylated Stats in the nucleus (Desrivières et al., 2006). There is evidence that TC45 is a related Stat phosphatase for Stat1 and Stat3, and has also been reported to

be involved in the regulation of cytoplasmic dephosphorylation of JAK1 and JAK3 (Ibarra-Sanchez et al., 2000; Simononic, Lee-Loy, Barder, Tremblay, & McGlade, 2002).

In addition to TC45, tyrosine phosphatases such as SHP1 and SHP2 are localized in the cytoplasm and have SH2 domains. These phosphatases disrupt JAK/Stat signaling by interacting with SH2 domains and phosphorylated tyrosine residues of JAKs and Stats (Desrivières et al., 2006). CD45, an active transmembrane molecule in hematopoietic cells, and PTP1B and TC-PTP phosphatases active in the cytoplasm of these cells are among the phosphatases responsible for the negative regulation of Stat proteins (Desrivières et al., 2006).

3. THE ROLE of STAT3 PROTEINS in the BREAST GLAND

Stat3 and Stat5 proteins are involved in fundamental changes in the mammary gland, including processes such as lactation and involution. Mice deficient in Stat3 have been found to die during early embryogenesis (Takeda et al., 1997). Mammary gland involution is a multi-step process in which the lactating gland morphologically returns to a state close to the pre-pregnancy state and a high degree of epithelial cell death and stromal rearrangement occurs (Groner & von Manstein, 2017; Stein, Salomonis, & Gusterson, 2007). Stat3 signaling induces epithelial cell death to clear differentiated milk-producing cells during involution (Groner & von Manstein, 2017). It has been shown that the disappearance of the lactation stimulus causes Stat3 phosphorylation to initiate involution, but the upregulation of pStat3 is not due to the decrease in lactogenic hormones, while Leukemia inhibitory factor (LIF) is the first activator of Stat3 during involution (Hughes & Watson, 2018; Kritikou et al., 2003; M. Li et al., 1997).

4. THE ROLE of STAT3 PROTEINS in BREAST CANCER

Stat3 is overactive in many types of cancer as a result of autocrine and paracrine stimulation by cytokines and growth factors such as interleukins (IL-6, IL-10, IL-12), interferons (IFNs), granulocyte colony stimulating factor (G-CSF or CSF3), prolactin (PRL), growth hormone (HGH), epidermal growth factor (EGF) , hepatocyte growth factor (HGF), essential fibroblast growth factor (FGF2), virus proteins (e.g., v-Src, v-Fps, v-Sis) or due to persistent activation via Intrinsic tyrosine kinase activities such as erb-b2 receptor tyrosine kinase 2 (ERBB2), epidermal growth factor receptor (EGFR), and hepatocyte growth factor receptor HGFR, non-receptor tyrosine kinases (such as c-Src and c-abl) or G protein-coupled receptor (Kortylewski, Jove, & Yu, 2005; Lim & Cao, 2006). It is known

that Stat3 is strongly associated with breast cancer formation and its permanent activation is most prominent in breast cancer (Groner & von Manstein, 2017).

Stat3 has been shown to be constitutively activated in approximately 70% of breast tumors (Alvarez et al., 2005). Although activated in all types of breast cancer, it has been most commonly associated with triple-negative breast cancer that lacks estrogen receptor (ER) or progesterone receptor (PR) expression and does not show Her2 amplification (L. Marotta et al., 2011; S.R Walker et al., 2009). It is an interesting paradox that Stat3 protein, which is involved in every stage of mammary gland development and has important roles in the basic changes in the mammary gland, has a strong relationship with mammary tumor formation (Groner & von Manstein, 2017; Sarah R. Walker, Xiang, & Frank, 2014). In normal breast cells, Stat3 is activated by Leukemia inhibitory factor (LIF) to promote involution with the abolition of the lactation stimulus (Hughes & Watson, 2018; Sarah R. Walker et al., 2014), whereas in many breast cancer cell lines, it is activated in an autocrine fashion by interleukin-6 (IL-6) produced by These cells (Lieblein et al., 2008; L. Marotta et al., 2011). Stat3, which is constantly in the activated state; can lead to malignant cell behavior by upregulating genes such as B-cell lymphoma 2 (BCL2), B-cell lymphoma-extra large (BCL-XL), Myeloid Cell Leukemia Sequence 1 (MCL1) that play a role in apoptosis, gene expression of cyclin D, the main target of transcriptional control of the cell cycle and other cell cycle and survival-related genes such as B -cell lymphoma 2 (BCL2), myc proto-oncogene (c-MYC). (Igelmann, Neubauer, & Ferbeyre, 2019). Recent studies have shown that Stat3 promotes the process of malignant transformation by activating genes involved in the Phosphoinositide 3-Kinase (PI3K) /AKT/ Mammalian Target of Rapamycin (mTOR) pathway, the Nuclear Factor Kappa-Light-Chain Enhancer of Activated B-Cells (NF-κB) pathway, and the cell cycle regulation pathway (Banerjee & Resat, 2016; Igelmann et al., 2019).

Oncostatin M (OSM), a member of the IL-6 cytokine family, can promote breast cancer progression by inducing upregulation of IL-6 and phosphorylation of stat3 (Ma, Qin, & Li, 2020; Tawara, Scott, Emathinger, Ide, et al., 2019; Tawara, Scott, Emathinger, Wolf, et al., 2019). In addition, while IL-35 inhibits conventional T (T-conv) cells and promotes breast cancer progression through Stat3 and Stat1 activation, IL-8 and growth-regulated oncogene (GRO) chemokines contribute to breast cancer progression by activating Stat3 (Hao). et al., 2018; Ma et al., 2020; Valeta-Magara et al., 2019). Stat3 is also known to contribute to breast cancer metastasis. Stat3 along with IL-6 has been shown to contribute to the malignant phenotype

of cancer cell by upregulating the expression of the EMT-inducing Twist, in part by promoting invasion and epithelial-mesenchymal transition (Lo et al., 2007; Sullivan et al., 2009; Sarah R. Walker). et al., 2014; Yadav, Kumar, Datta, Teknos, & Kumar, 2011). Stat3 is known to contribute to breast cancer metastasis by upregulating Matrix metallopeptidase 2 (MMP2), Matrix metallopeptidase 9 (MMP9), Snail, Slug, and vimentin (Kamran, Patil, & Gude, 2013; Z. Li et al., 2019; Ma et al., 2020). Stat3 is a protein that also affects the angiogenesis process of the tumor cell. It contributes to this process by up-regulating nodal factors of angiogenesis, particularly vascular endothelial growth factor (VEGF), hypoxia-inducible factor 1 alpha (HIF-1α), and Matrix metalloproteinase-2 (MMP-2) (Kortylewski et al., 2005). Stat3 is also known to localize to mitochondria, and mito-Stat3 is known to regulate mitochondrial metabolism and mitochondrial gene expression (Chueh, Leong, & Yu, 2010; Igelmann et al., 2019; Macias, Rao, Carbajal, Kiguchi, & DiGiovanni). , 2014; Sala et al., 2019; Wegrzyn et al., 2009; Q. Zhang et al., 2013). Recent evidence suggests that Stat3 may promote survival of breast cancer cells through its effects on mitochondrial function (Gough et al., 2009). These proteins are known to cause drug resistance as well as processes such as tumor initiation, cell cycle, survival, metastasis and angiogenesis. Cancer stem cells (CSCs), also called tumor initiating cells (TICs), are a group of specialized cancer cells found in tumors that have the ability to self-renew and specifically produce a variety of tumor cells. These cells are considered to be responsible for recurrence and metastasis and resistance to treatment (Gibbs et al., 2005). In breast cancer, Stat3 has been shown to be essential for the viability of cancer stem cells (Hirsch, Iliopoulos, & Struhl, 2013), it has been reported that a non-CSC population can be transformed into a CSC-like population through OCT-4 regulation of the IL-6/JAK1/Stat3 signaling pathway (S. Y. Kim et al., 2013). In addition, it has been found that the JAK2/Stat3 signaling pathway in breast cancer increases chemoresistance by increasing carnitine palmitoyltransferase 1B (CPT1B) and fatty acid beta oxidation (FAO) (Wang et al., 2018). It has been determined that the Src/Stat3 signaling pathway is involved in multidrug resistance in triple negative breast cancer cells (Tzeng et al., 2018).

5. INHIBITON of STAT3 PROTEINS in BREAST CANCER TREATMENT

Stat signaling inhibitors are divided into two main groups as inhibitors that act directly and indirectly. Direct inhibitors target the SH domain, DNA binding domain, or N-terminal domain of Stat3 protein (McMurray, 2006; Xiong, Yang, Shen, Zhou, & Shen, 2014), indirect inhibitors target

upstream components of the Stat3 pathway such as JAK2 and epidermal growth factor receptor (EGFR) (Thilakasiria et al., 2021). In this review, we focused on inhibitors that act by directly inhibiting SH2 domain of Stat3 proteins in breast cancer cells.

5.1. SH2 Domain Inhibitors or Dimerization Inhibitors

The SH2 domain plays a critical role both in mediating the activation of Stat3 through its interaction with phosphorylated tyrosine residues on the cytoplasmic portion of the receptors, and in forming dimers of the two Stat3 monomers through reciprocal phosphotyrosine-SH2 interactions (Turkson & Jove, 2000; Xiong et al., 2014). Stat dimers migrate to the nucleus and mediate processes related to cellular immunity, proliferation, apoptosis and differentiation by binding to Stat-specific DNA response elements of target genes to induce gene transcription (Turkson & Jove, 2000) (Logotheti & Pützer, 2019). Inhibition of the SH2 domain suppresses the phosphorylation and activation of the Stat3 protein, resulting in inhibition of the cellular processes it mediates (Xiong et al., 2014). Considering the mentioned functions of the SH2 domain, molecules capable of blocking the SH2 domain of Stat3 have been evaluated for the treatment of different tumors (Tolomeo & Cascio, 2021). It can be said that the SH2 domain inhibits both the activation and dimerization of Stat3 proteins and is important in terms of creating an effective treatment approach for cancer treatment by preventing the dimerization of proteins that escape activation (Berg, 2008). Compounds that inhibit the SH2 domain of Stat3 proteins can be grouped as peptide and peptidomimetic and non-peptidic chemical inhibitors (new series of small molecules) considering their chemical structures. The schematic representation of SH2 domain inhibitors studied in breast cancer cells is shown in Figure 3.

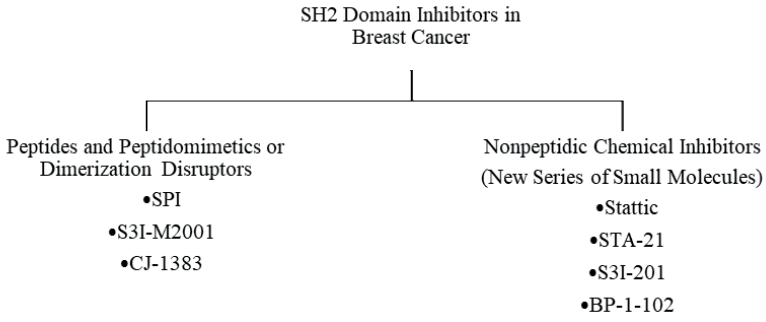


Figure 3: Schematic representation of SH2 domain inhibitors studied in breast cancer cells

5.1.1. Peptides and Peptidomimetics Targeting the STAT3 SH2 Domain in Breast Cancer

The SH2 domain inhibitor *SPI* is a 28-mer peptide derived from the SH2 domain of Stat3. It acts by strongly and selectively inhibiting the Stat3 SH2 domain interaction with the pTyr residue on the cytoplasmic tail of IL-6R (Xiong et al., 2014) (W. Zhao, Jaganathan, & Turkson, 2010). In a study by Zhao et al., SPI dose-dependently reduced cell viability and growth in MDA-MB-231 and MDA-MB-435 breast cancer cell lines with constitutively activated Stat3, and also induced apoptosis in MDA-MB-231 cells with constitutively activated Stat3 (W. Zhao et al., 2010).

Since peptidomimetics have better pharmacokinetic properties than peptides, peptidomimetic compounds have been developed by using the peptide XpYL compound as the basic scaffold (Furqan et al., 2013). *CJ-1383* is a cell permeable small molecule peptidomimetic targeting the SH2 domain (Chen et al., 2010; Thilakasiria et al., 2021). In the study of Chen et al., it was shown that CJ-1383 inhibited cellular Stat3 signaling and cell growth and induced apoptosis in a dose-dependent manner in MDA-MB-468 breast cancer cell line with constitutively activated Stat3 (Chen et al., 2010). *S3I-M2001* is an oxazole-based peptidomimetic of the Stat3 SH2 domain-binding phosphotyrosine peptide (K. A. Z. Siddiquee et al., 2007). The compound has been shown to inhibit Stat3-dependent transcription, transformation, survival and migration in both human and mouse cells by selectively disrupting Stat3 dimerization (K. A. Z. Siddiquee et al., 2007). In the study of Siddiquee et al., it was determined that S3I-M2001 inhibited the growth of human breast tumor xenografts (K. A. Z. Siddiquee et al., 2007).

5.1.2. Nonpeptidic Chemical Inhibitors Targeting the STAT3 SH2 Domain in Breast Cancer

The low cell penetration of phosphopeptides led to evaluation of the efficacy of a "new set of small molecules" for Stat3 SH2 domain inhibition (Xiong et al., 2014). These non-peptide molecules are cell permeable and have better physicochemical properties, unlike molecules derived from peptides or peptidomimetics (Yue & Turkson, 2009). Their mechanism of action is similar to peptidomimetics: by interacting with the Stat3-SH2 domain, they inhibit Stat3:Stat3 dimerization and thus nuclear translocation and transcriptional activity (Furqan et al., 2013).

Stattic (Stat three-inhibitory compound) was the first non-peptide inhibitor of Stat3 discovered (Schust, Sperl, Hollis, Mayer, & Berg, 2006).

Stattic inhibits the function of the SH2 domain of both unphosphorylated and phosphorylated Stat3, preventing Stat3 dimerization and binding to DNA (Berg, 2008) (Xiong et al., 2014). Stattic shows selective Stat3 inhibition; While it does not inhibit Stat1 and Stat5b in vitro, it has been shown to inhibit Stat3 (Berg, 2008). Stattic has been shown to induce apoptosis after permanently inhibiting the phosphorylation of Stat3 in breast cancer cell lines MDA-MB-231 and MDA-MB-435, which constitutively show Stat3 activation (Schust et al., 2006).*STA-21* is a natural deoxytetrangomycin, an angucycline antibiotic (Song, Wang, Wang, & Lin, 2005). It binds effectively to the SH2 domain of Stat3, effectively inhibiting Stat3 dimerization and abolishing its nuclear translocation. If we look at the results of the studies carried out in breast cancer cells in detail; STA-21 inhibited Stat3-dependent luciferase activity in MDA-MB-435s breast cancer cell line with constitutively activated Stat3 and showed high DNA binding activity in these cells. Another breast cancer cell line with constitutively active stat3 signaling, MDA-MB-468, also inhibited stat3 DNA binding activity and its donwstream antiapoptotic factors (Bcl-XL and cyclin D1), but phosphorylation of upstream regulators of Stat3 (P-JAK2, P-Src, P-EGFR) unaffected by STA-21.In the same study, the effects of STA-21 on cell growth and survival in breast cancer cell lines with constitutively active Stat3 activity as well as luciferase activity and DNA binding activity were investigated: MDA-MB-231, MDA-MB-435s, and MDA-MB-468 (that express persistently activated Stat3) significantly inhibited the survival of breast cancer cell lines, but showed minimal inhibitory effect on MCF-7 and MDA-MB-435 breast cancer cells (that have no constitutive Stat3 signaling) (Song et al., 2005). *S3I-201* (also known as NSC 74859) is a low molecular weight salicylic acid derivative and inhibits Stat3 dimerization by coupling the salicylic acid moiety with the pTyr binding site of the Stat3-SH2 domain (K. Siddiquee et al., 2007). S3I-201 significantly inhibited constitutive Stat3 activation in MDA-MB-231, MDA-MB-435 and MDA-MB-468 breast cancer cell lines with harbor constitutive Stat3 activation. In addition, treatment with S3I-201 in all three cell lines caused a decrease in the number of viable cells, while cell viability was not significantly affected in the MDA-MB-453 breast cancer cell line which do not harbor aberrant Stat3 activity. When it was examined whether the loss of cell viability caused by S3I-201 was mediated by apoptosis, it was determined that S3I-201 significantly induced apoptosis in the MDA-MB-435 breast cancer cell line with harbor constitutive Stat3 activation. In the same study, it was noted that S3I-201 caused a significant decrease in the expression of Stat3 target genes encoding Cyclin D1, Bcl-xL and Survivin in the MDA-MB-231 breast

cancer cell line with constitutive Stat3 activation. In the same study, it was determined that S3I-201 strongly inhibited tumor growth in human breast (MDA-MB-231) tumor-bearing mice (K. Siddiquee et al., 2007). *BP-102*, an analog of S3I-201, binds to the SH2 domain of Stat3, inhibiting Stat3-phospho-tyrosine (pTyr) peptide interactions and hence Stat3 activation by the same mechanism as S3I-201 (X. Zhang et al., 2012). In the study of Zhang et al., BP-1-102 suppressed cell proliferation, anchorage-dependent and independent growth, and colony numbers and also induced apoptosis in MDA-MB-231 breast cancer cells harboring aberrantly active Stat3. Overall, induction of Focal adhesion kinase (FAK) and paxillin phosphorylation and downregulation of E-cadherin are thought to contribute to Stat3-mediated malignant progression. Decreased phosphorylation of paxillin and FAK and increased expression of E-cadherin were seen in MDA-MB-231 cells treated with BP-1-102. To further investigate the effect of BP-1-102 on Stat3 cross-talks, the study examined its effect on the production of soluble factors by tumor cells: In culture medium from MDA-MB-231 cells treated with BP-1-102, granulocyte colony-stimulating factor (G-CSF), soluble intercellular adhesion molecule (sICAM) 1 and macrophage migration-inhibitory factor (MIF)/ glycosylation-inhibiting factor (GIF) levels were found to be lower, so it was concluded that BP-1-102 inhibited the production of soluble factors by tumor cells. Again in the same study, it was shown that BP-1-102 inhibited the growth of mouse xenografts of human breast (MDA-MB-231) tumor that harbor aberrantly active Stat3 as a result of intravenous and oral gavage administration without any significant changes in body weights or significant signs of toxicity such as loss of appetite, decreased activity or lethargy (X. Zhang et al., 2012).

6. CONCLUSION

Deregulated activation of the Stat pathway, which is involved in many normal physiological cell processes, including proliferation, differentiation, apoptosis, angiogenesis, and immune system regulation (Verhoeven et al., 2020), is frequently associated with tumorigenesis. It is known that Stat3 is strongly associated with breast cancer formation and its permanent activation is most prominent in breast cancer (Groner & von Manstein, 2017). Although activated in all types of breast cancer, it has been most associated with triple-negative breast cancer that lacks estrogen receptor (ER) or progesterone receptor (PR) expression and does not show Her2 amplification (L. L. C. Marotta et al., 2011; S.R Walker et al., 2009; Sarah R. Walker et al., 2014). The role of the Stat pathway in cancer development has made this pathway an interesting target for drug development in cancer

therapy and has led to the development of many inhibitors targeting this pathway. Stat signaling inhibitors are divided into two main groups as inhibitors that act directly and indirectly. Direct inhibitors target the SH domain, DNA binding domain, or N-terminal domain of Stat3 protein (McMurray, 2006; Xiong et al., 2014), indirect inhibitors target upstream components of the Stat3 pathway such as JAK2 and epidermal growth factor receptor (EGFR) (Thilakasiria et al., 2021). In our study, we focused on inhibitors that act by directly inhibiting the SH2 domain of Stat3 proteins in breast cancer cells. Considering their chemical structures, these compounds can be grouped as peptides (SPI), peptidomimetics (CJ-1383, S3I-M2001) and non-peptidic chemical inhibitors (new series of small molecules) (Stattic, STA-21, S3I-201, BP-1- 102). When the results of the studies investigating the effects of these compounds on breast cancer cells with constitutively active Stat3 signaling were examined, it was observed that the compounds showed anticarcinogenic effects such as inhibition of cell viability, migration, induction of apoptosis and inhibition of tumor growth in breast tumor xenograft models. Based on these results, it can be said that the SH2 domain of Stat3 is an important target for breast cancer treatment, worthy of further investigation, and that SH2 domain inhibitors can be used alone or in combination with existing chemotherapeutics, resulting in clinically significant results such as higher efficacy and less toxicity.

References

Alvarez, J. ., Febbo, P. ., Ramaswamy, S., Loda, M., Richardson, A., & Frank, D. . (2005). Identification of a genetic signature of activated signal transducer and activator of transcription 3 in human tumors. *Cancer Research*, *65*(12), 5054–5062.

Banerjee, K., & Resat, H. (2016). Constitutive activation of STAT3 in breast cancer cells: A review. *International Journal of Cancer, 138*(11), 2570–2578.

Berg, T. (2008). Signal Transducers and Activators of Transcription as Targets for Small Organic Molecules. *ChemBioChem*, *9*, 2039–2044.

Buettner, R., Mora, L., & Jove, R. (2002). Activated STAT signaling in human tumors provides novel molecular targets for therapeutic intervention. *Clinical Cancer Research*, *8*(4), 945–954.

Calò, V., Migliavacca, M., Bazan, V., Macaluso, M., Buscemi, M., Gebbia, N., & Russo, A. (2003). STAT proteins: from normal control of cellular events to tumorigenesis. *Journal of Cellular Physiology*, *197*(2), 157–168.

Catlett-Falcone, R., Dalton, W. S., & Jove, R. (1999). STAT proteins as novel targets for cancer therapy. *Current Opinion in Oncology*, *11*(6), 490.

Chen, J., Bai, L., Bernard, D., Nikolovska-Coleska, Z., Gomez, C., Zhang, J., … Wang, H. (2010). Structure-based design of conformationally constrained, cell-permeable STAT3 inhibitors. *ACS Medicinal Chemistry Letters*, *1*(2), 85–89.

Chueh, F. Y., Leong, K. F., & Yu, C. L. (2010). Mitochondrial translocation of signal transducer and activator of transcription 5 (STAT5) in leukemic T cells and cytokine-stimulated cells. *Biochemical and Biophysical Research Communications*, *402*(4), 778–783.

Desrivières, S., Kunz, C., Barash, I., Vafaizadeh, V., Borghouts, C., & Groner, B. (2006). The biological functions of the versatile transcription factors STAT3 and STAT5 and new strategies for their targeted inhibition. *Journal of Mammary Gland Biology and Neoplasia*, *11*, 75–87.

Furqan, M., Akinleye, A., Mukhi, N., Mittal, V., Chen, Y., & Liu, D. (2013). STAT inhibitors for cancer therapy. *Journal of Hematology & Oncology*, *6*(90).

Gibbs, C. ., Kukekov, V. ., Reith, J. ., Tchigrinova, O., Suslov, O. ., Scott, E. ., … Steindler, D. . (2005). Stem-like cells in bone sarcomas: Implications for tumorigenesis. *Neoplasia*, *7*, 967–976.

Gough, D. ., Corlett, A., Schlessinger, K., Wegrzyn, J., Larner, A. ., & Levy, D. . (2009). Mitochondrial STAT3 supports Ras-dependent oncogenic transformation. *Science*, *324*, 1713–1716.

Groner, B., & von Manstein, V. (2017). Jak Stat signaling and cancer: Opportunities, benefits and side effects of targeted inhibition. *Molecular and Cellular Endocrinology*, *451*, 1–14.

Hao, S., Chen, X., Wang, F., Shao, Q., Liu, J., Zhao, H., … Mao, H. (2018). Breast cancer cell–derived IL-35 promotes tumor progression via induction of IL-35-producing induced regulatory T cells. *Carcinogenesis*, *39*(12), 1488–1496.

Hirsch, H. A., Iliopoulos, D., & Struhl, K. (2013). Metformin inhibits the inflammatory response associated with cellular transformation and cancer stem cell growth. *Proceedings of the National Academy of Sciences*, *110*(3), 972–977.

Hodge, D. R., Hurt, E. M., & Farrar, W. L. (2005). The role of IL-6 and STAT3 in inflammation and cancer. *European Journal of Cancer*, *41*, 2502–2512.

Hughes, K., & Watson, C. J. (2018). The multifaceted role of STAT3 in mammary gland involution and breast cancer. *International Journal of Molecular Sciences*, *19*(6), 1695.

Ibarra-Sanchez, M., Simoncic, P., Nestel, F., Duplay, P., Lapp, W., & Tremblay, M. (2000). The T-cell protein tyrosine phosphatase. *Seminars in Immunology*, *12*(4), 379–386.

Igelmann, S., Neubauer, H. A., & Ferbeyre, G. (2019). STAT3 and STAT5 Activation in Solid Cancers. *Cancers*, *11*(1428).

Jr, J. E. D., Kerr, I. M., & Stark, G. R. (1994). Jak-STAT pathways and transcriptional ac- tivation in response to IFNs and other extracellular signaling proteins. *Science*, *264*(5164), 1415–1421.

Kamran, M. Z., Patil, P., & Gude, R. P. (2013). Role of STAT3 in cancer metastasis and translational advances. *BioMed Research International*.

Kim, S. Y., Kang, J. W., Song, X., Kim, B. K., Yoo, Y. D., Kwon, Y. T., & Lee, Y. J. (2013). Role of the IL-6-JAK1-STAT3-Oct-4 pathway in the conversion of non-stem cancer cells into cancer stem-like cells. *Cellular Signalling*, *25*(4), 961–969.

Kim, T. K., & Maniatis, T. (1996). Regulation of interferon-γ-activated STAT1 by the ubiquitin-proteasome pathway. *Science*, *273*(5282), 1717–1719.

Kortylewski, M., Jove, R., & Yu, H. (2005). Targeting STAT3 affects melanoma on multiple fronts. *Cancer and Metastasis Reviews*, *24*, 315–327.

Krebs, D., & Hilton, D. (2001). SOCS proteins: Negative regulators of cytokine signaling. *Stem Cells*, *19*, 378–387.

Kritikou, E. ., Sharkey, A., Abell, K., Came, P. ., Anderson, E., Clarkson, R. ., & Watson, C. . (2003). A dual, non-redundant, role for LIF as a regulator of development and STAT3-mediated cell death in mammary gland. *Development*, *130*, 3459–3468.

Li, M., Liu, X., Robinson, G., Bar-Peled, U., Wagner, K. ., Young, W. ., … Furth, P. A. (1997). Mammary-derived signals activate programmed cell death during the first stage of mammary gland involution. *Proceedings of the National Academy of Sciences*, *94*(7), 3425–3430.

Li, Z., Chen, Y., An, T., Liu, P., Zhu, J., Yang, H., … Yang, X. (2019). Nuciferine inhibits the progression of glioblastoma by suppressing the SOX2-AKT/STAT3-Slug signaling pathway. *Journal of Experimental & Clinical Cancer Research*, *38*(1), 1–15.

Lieblein, J. ., Ball, S., Hutzen, B., Sasser, A. ., Lin, H. ., Huang, T. ., … Lin, J. (2008). STAT3 can be activated through paracrine signaling in breast epithelial cells. *BMC Cancer*, *8*, 1–14.

Lim, C. P., & Cao, X. (2006). Structure, function, and regulation of STAT proteins. *Molecular BioSystems*, *2*, 536–550.

Liu, B., & Shuai, K. (2003). The PIAS protein family and Tc-PTP. In P. B. Sehgal, D. E. Levy, & T. Hirano (Eds.), *Signal Transducers and Activators of Transcription (STATs): Activation and Biology* (pp. 75–85). Kluwer Academic Publishers.

Lo, H. ., Hsu, S. ., Xia, W., Cao, X., Shih, J. ., Wei, Y., … Hung, M. . (2007). Epidermal growth factor receptor cooperates with signal transducer and activator of transcription 3 to induce epithelial-mesenchymal transition in cancer cells via up-regulation of TWIST gene expression. *Cancer Research*, *67*, 9066–9076.

Logotheti, S., & Pützer, B. M. (2019). STAT3 and STAT5 Targeting for Simultaneous Management of Melanoma and Autoimmune Diseases. *Cancers*, *11*(1448).

Ma, J., Qin, L., & Li, X. (2020). Role of STAT3 signaling pathway in breast cancer. *Cell Communication and Signaling*, *18*(33), 1–13.

Macias, E., Rao, D., Carbajal, S., Kiguchi, K., & DiGiovanni, J. (2014). Stat3 binds to mtDNA and regulates mitochondrial gene expression in keratinocytes. *Journal of Investigative Dermatology*, *134*(7), 1971–1980.

Marotta, L. ., Almendro, V., Marusyk, A., Shipitsin, M., Schemme, J., Walker, S. ., … Lee, H, K. (2011). The JAK2/STAT3 signaling pathway is required for growth of CD44 + CD24- stem cell-like breast cancer cells in human tumors. *The Journal of Clinical Investigation*, *121*(7), 2723–2735.

Marotta, L. L. C., Almendro, V., Marusyk, A., Shipitsin, M., Schemme, J., Walker, S. R., … Kornelia Polyak. (2011). The JAK2/STAT3 signaling pathway is required for growth of CD44 + CD24- stem cell-like breast cancer cells in human tumors. *The Journal of Clinical Investigation*, *121*(7), 2723–2735.

McMurray, J. S. (2006). A New Small-Molecule Stat3 Inhibitor. *Chemistry & Biology*, *13*(11), 1123–1124.

Moriggl, R., Sexl, V., Kenner, L., Duntsch, C., Stangl, K., Gingras, S., … Beug, H. (2005). Stat5 tetramer formation is associated with leukemogenesis. *Cancer Cell*, *7*(1), 87–99.

Park, H. ., Li, J., Hannah, R., Biddie, S., Leal-Cervantes, A. ., Kirschner, K., … Green, A. . (2016). Cytokine-induced megakaryocytic differentiation is regulated by genome-wide loss of a uSTAT transcriptional program. *The EMBO Journal*, *35*(6), 580–594.

Sadowski, H. B., Shuai, K., Jr, J. E. D., & Gilman, M. Z. (1993). A common nuclear signal transduction pathway activated by growth factor and cytokine receptors. *Science*, *261*(5129), 1739–1744.

Sala, D., Cunningham, T. J., Stec, M. J., Etxaniz, U., Nicoletti, C., Dall'Agnese, A., … Sacco, A. (2019). The Stat3-Fam3a axis promotes muscle stem cell myogenic lineage progression by inducing mitochondrial respiration. *Nature Communications*, *10*(1), 1796.

Schust, J., Sperl, B., Hollis, A., Mayer, T. U., & Berg, T. (2006). Stattic: a small-molecule inhibitor of STAT3 activation and dimerization. *Chemistry & Biology*, *13*(11), 1235–1242.

Siddiquee, K. A. Z., Gunning, P. T., Glenn, M., Katt, W. P., Zhang, S., Schroeck, C., … Turkson, J. (2007). An oxazole-based small-molecule Stat3 inhibitor modulates Stat3 stability and processing and induces antitumor cell effects. *ACS Chemical Biology*, *2*(12), 787–798.

Siddiquee, K., Zhang, S., Guida, W. C., Blaskovich, M. A., Greedy, B., Lawrence, H. R., … Turkson, J. (2007). Selective chemical probe inhibitor of Stat3, identified through structure-based virtual screening, induces antitumor activity. *Proceedings of the National Academy of Sciences*, *104*(18), 7391–7396.

Simononic, P., Lee-Loy, A., Barder, D., Tremblay, M., & McGlade, C. (2002). The T cell protein tyrosine phosphatase is a negative regulator of Janus family kinases 1 and 3. *Current Biology*, *12*, 446–453.

Song, H., Wang, R., Wang, S., & Lin, J. (2005). A low-molecular-weight compound discovered through virtual database screening inhibits Stat3 function in breast cancer cells. *Proceedings of the National Academy of Sciences*, *102*(13), 4700–4705.

Stein, T., Salomonis, N., & Gusterson, B. A. (2007). Mammary Gland Involution as a Multi-step Process. *Journal of Mammary Gland Biology and Neoplasia*, *12*, 25–35.

Sullivan, N. ., Sasser, A. ., Axel, A. ., Vesuna, F., Raman, V., Ramirez, N., … Hall, B. . (2009). Interleukin-6 induces an epithelial–mesenchymal transition phenotype in human breast cancer cells. *Oncogene*, *28*, 2940–2947.

Takeda, K., Noguchi, K., Shi, W., Tanaka, T., Matsumoto, M., Yoshida, N., … Akira, S. (1997). Targeted disruption of the mouse Stat3 gene leads to

early embryonic lethality. *Proceedings of the National Academy of Sciences*, *94*(8), 3801–3804.

Tawara, K., Scott, H., Emathinger, J., Ide, A., Fox, R., Greiner, D., … Jorcyk, C. (2019). Co-expression of VEGF and IL-6 family cytokines is associated with decreased survival in HER2 negative breast cancer patients: subtype-specific IL-6 family cytokine-mediated VEGF secretion. *Translational Oncology*, *12*(2), 245–255.

Tawara, K., Scott, H., Emathinger, J., Wolf, C., LaJoie, D., Hedeen, D., … Jorcyk, C. (2019). HIGH expression of OSM and IL-6 are associated with decreased breast cancer survival: synergistic induction of IL-6 secretion by OSM and IL-1beta. *Oncotarget*, *10*, 2068–2085.

Thilakasiria, P. S., Dmelloa, R. S., Nerob, T. L., Parkerb, M. W., Ernsta, M., & Chand, A. L. (2021). Repurposing of drugs as STAT3 inhibitors for cancer therapy. *Seminars in Cancer Biology*, *68*, 31–46.

Tolomeo, M., & Cascio, A. (2021). The Multifaced Role of STAT3 in Cancer and Its Implication for Anticancer Therapy. *International Journal of Molecular Sciences*, *22*(2), 603.

Turkson, J., & Jove, R. (2000). STAT proteins: novel molecular targets for cancer drug discovery. *Oncogene*, *19*(2000), 6613–6626.

Tzeng, Y.-D. T., Liu, P.-F., Li, J.-Y., Liu, L.-F., Kuo, S.-Y., Hsieh, C.-W., … Shu, C.-W. (2018). Kinome-wide siRNA screening identifies Src-enhanced resistance of chemotherapeutic drugs in triple-negative breast cancer cells. *Frontiers in Pharmacology*, *9*, 1285.

Valeta-Magara, A., Gadi, A., Volta, V., Walters, B., Arju, R., Giashuddin, S., … J, R. S. (2019). No Inflammatory Breast Cancer Promotes Development of M2 Tumor-Associated Macrophages and Cancer Mesenchymal Cells through a Complex Chemokine Network. *Cancer Research*, *79*(13), 3360–3371.

Verhoeven, Y., Tilborghs, S., Jacobs, J., Waele, J. De, Quatannens, D., Deben, C., … Dam, P. A. van. (2020). The potential and controversy of targeting STAT family members in cancer. *Seminars in Cancer Biology*, *60*(2020), 41–56.

Walker, S.R, Nelson, E. ., Zou, L., Chaudhury, M., Signoretti, S., Richardson, A., & Frank, D. . (2009). Reciprocal effects of STAT5 and STAT3 in breast cancer. *Molecular Cancer Research*, *7*(6), 966–976.

Walker, Sarah R., Xiang, M., & Frank, D. A. (2014). Distinct roles of STAT3 and STAT5 in the pathogenesis and targeted therapy of breast cancer. *Molecular and Cellular Endocrinology*, *382*(1), 616–621.

Wang, T., Fahrmann, J. F. H. L., Li, Y.-J., Tripathi, S. C., Yue, C., Zhang, C., … Yu, H. (2018). JAK/STAT3-regulated fatty acid β-oxidation is critical for

breast cancer stem cell self-renewal and chemoresistance. *Cell Metabolism*, *27*(1), 136–150.

Weaver, A., & Silva, C. (2007). Signal transducer and activator of transcription 5b: a new target of breast tumor kinase/protein tyrosine kinase 6. *Breast Cancer Research*, *9*(6), R79.

Wegrzyn, J., Potla, R., Chwae, Y.-J., Sepurı, N. B. V., Zhang, Q., Koeck, T., … Larner, A. C. (2009). Function of mitochondrial Stat3 in cellular respiration. *Science*, *323*, 793–797.

Xin, P., Xua, X., Denga, C., Liua, S., Wang, Y., Zhou, X., … Sun, S. (2020). The role of JAK/STAT signaling pathway and its inhibitors in diseases. *International Immunopharmacology*, *80*(2020), 106210.

Xiong, A., Yang, Z., Shen, Y., Zhou, J., & Shen, Q. (2014). Transcription Factor STAT3 as a Novel Molecular Target for Cancer Prevention. *Cancers*, *6*, 926–957.

Yadav, A., Kumar, B., Datta, J., Teknos, T. ., & Kumar, P. (2011). IL-6 promotes head and neck tumor metastasis by inducing epithelial-mesenchymal transition via the JAK-STAT3-SNAIL signaling pathway. *Molecular Cancer Research*, *9*, 1658–1667.

Yue, P., & Turkson, J. (2009). Targeting STAT3 in cancer: how successful are we? *Expert Opinion on Investigational Drugs*, *18*(1), 45–56.

Zhang, Q., Raje, V., Yakovlev, V. A., Yacoub, A., Szczepanek, K., Meier, J., … Larner, A. C. (2013). Mitochondrial localized Stat3 promotes breast cancer growth via phosphorylation of serine 727. *Journal of Biological Chemistry*, *288*(43), 31280–31288.

Zhang, X., Yue, P., Page, B. D. G., Li, T., Zhao, W., Namanja, A. T., … Turkson, J. (2012). Orally bioavailable small-molecule inhibitor of transcription factor Stat3 regresses human breast and lung cancer xenografts. *Proceedings of the National Academy of Sciences*, *109*(24), 9623–9628.

Zhao, W., Jaganathan, S., & Turkson, J. (2010). A cell-permeable Stat3 SH2 domain mimetic inhibits Stat3 activation and induces antitumor cell effects in vitro. *Journal of Biological Chemistry, 285*(46), 35855–35865.

Zhao, Y., Zeng, C., Tarasova, N. ., Chasovskikh, S., Dritschilo, A., & Timofeeva, O. . (2013). A new role for STAT3 as a regulator of chromatin topology. *Transcription*, *4*, 227–231.

Chapter 11

A General Overview of Epilepsy: its Classification, and Management

Yavuz Yücel[1]

Nor Osman Sidow[2]

Abstract

Epilepsy is one of the most common neurological disorders, with about 50 new cases per 100,000 people each year. It affects roughly 1% of the population and one-third of patients have refractory seizures. About 75% of patients present in childhood, reflecting the growing susceptibility of the developing brain to seizures. The classification of seizures is determined by their onset and can be divided into four types: focal, generalized, unknown or unclassifiable. The latest classification elaborates the basic categorization system previously described, extending the "motor" and "non-motor" groupings to include all three types of seizures (focal, generalized, and unknown).

Epilepsy is a difficult diagnosis with no simple, attainable gold standard. The key to diagnosis is a comprehensive history and reliable eyewitness account. Because no single symptom or sign is specific to epilepsy, determining whether a seizure has occurred is based on a combination of signs and symptoms. The primary treatment for diagnosed patients is administration of anti-seizure medications. The aim is to protect the individual from adverse effects that could potentially endanger the individual's standard of living and to terminate seizures immediately.

Epilepsy is a treatable condition; 80% of patients remain seizure-free and almost 50% remain seizure-free even after treatment is stopped. With more than 20 drugs used in treatment, it is possible to obtain effective treatment at rates close to 70% of diagnosed individuals. With this review we wrote, we aimed to summarize this very broad subject with recent articles.

1 Asst.Prof.Dr., Department of Neurology, Dicle University Faculty of Medicine, Diyarbakir, Türkiye, yyucel@dicle.edu.tr, ORCID ID: 0000-0003-4205-3138

2 Dr., Department of Neurology, Mogadishu Somalia Türkiye Training and Research Hospital, ziidoow113@gmail.com, ORCID ID:0000-0002-6039-5054

https://doi.org/10.58830/ozgur.pub128.c584

Background and definitions

A "seizure" is a paroxysmal change in neurologic function induced by the brain's excessive, hypersynchronous discharge of neurons[1]. The term "epileptic seizure" refers to a seizure induced by aberrant neuronal firing as opposed to a non-epileptic event, such as a photogenic seizure[2]. The disorder known as "epilepsy" is characterized by recurring, unprovoked seizures[3]. A seizure caused by reversibl insult (eg. Fever, hypoglycemia) does not qualify as epilepsy because it is a temporary secondary diseases rather than a chronic condition[4].

Epilepsy is one of the most prevalent neurologic disorders, with roughly 50 new cases per 100.000 people each year. Epilepsy affects roughly 1% of the population, and one-third of the patients have refractory epilepsy (seizures that are not controlled by two or more appropriately chose antiepileptic medications or other therapy).[5]

Approximately 75% of epilepsy originated in childhood, reflecting the developing brain's increased susceptibility to seiiuzres[6]. The term "epilepsy syndrome" refers to a combination of clinical characteristics that occur together on a continuous basis, such as comparable seizure types(s), age of onset, and response to antiepileptic drug (AEDs). The broad phase "seizure disorder" should be avoided[7].

Wirrell et al. outline in detail the methods used by the International League Against Epileptic (ILAE) Nosology and Definitions Taskforce (2017-2021) in defining epileptic syndromes and classifying them by age of onset[8]. An epilepsy syndrome is characterized by a distinct set of clinical and electroencephalographic (EEG) symptoms, which are frequently accompanied by particular etiological findings (structural, genetic, metabolic, immunological, and infections). A syndrome diagnosis in a person with epilepsy typically has prognostic and treatment consequences, syndromes frequently have age-dependent manifestations and a variety of unique comorbidities, a conditions has a "variable age" of onset if it can begin in both 18-year-old and 19-year-old (i.e., in both juvenile and adult patients)[9].

The ILAE defines epilepsy as any of the following conditions: (1) the occurrence of at least two unprovoked (or reflex) seizures that are more than 24 hours apart; (2) the occurrence of one unprovoked (or reflex) seizure with a possibility of subsequent seizures comparable to the general recurrence risk (at least 60%) after two unprovoked seizures that occur within the next ten yea4rs; and (3) the diagn0osi of an epilepsy syndorm[10].

The beginning of seizures might be focal (in one hemisphere of the brain). Generalized (in both hemisphere at the same time), or unknown. Active epilepsy is defined as the use of antiepileptic drugs on a regular basis or when the most recent seizure happened during the last 5 years. Status epilepticus (SE) is an epileptic episode that lasts long enough or is repeated at shout enough intervals to cause an epileptic condition. Depending on the kind and duration of the seizures, SE might have long-term repercussions such as neuronal injury or death, as well as changes in the neuronal networks. A new SE diagnostic categorization was recently developed and it will be seen in the last parts of this chapter[11].

Sudden Unexpected Death in Epilepsy (SUDEP) is a phenomenon characterized by sudden, unforeseen, and non-traumatic death in individuals with epilepsy, whether observed or not, and with or without evidence of a seizure. This definition excludes documented status epilepticus and requires that postmortem examination reveals no toxicological or anatomical cause of death[12].

Epidemiology and pathophysiology

The prevalence of epilepsy varies greatly across nations, based on the geographical distribution of risk and etiologic variables, the number of seizures upon diagnosis, and whether active epilepsy (active prevalence) or cases in remission (lifetime prevalence) is include. According to the Fiest et al. the total lifetime prevalence of epilepsy was 7.60 per 1,000 population (95%) confidence interval [CI] 6.17-9.38) and was greater in LMIC 98.75 per 1,000; the median point prevalence of active epilepsy was 6.68 (95% CI 5.45-8.10), while in HIC, it was 5.49 (4.16-7.26)[1314].

According to the sex, men have somewhat higher incidence and prevalence of epilepsy than women. The difference might be because the most common risk factors are more or less common in different places, or because women in some place hide the disease for social and cultural reasons[15].

Epilepsy is more common in the youngest and oldest age groups, with estimates of 86 per 100,000 per year in a well-defined population in the first year of life (figure 1), dropping to about 23-31 per 100,000 in people aged 30-59 years, and then going up to 180 per 100,000 in the over 85 age group. Epilepsy is most common in children in their first of life. By the end of age 10, the number of children with epilepsy drops to the same level as adults[16].

The most common type of seizure in both children and adults is a focal seizure. About 36% of all people who have seizures have a focal decreased

awareness seizure this is the most common type of focal seizure. In most LMIC, however, generalized tonic-colonic seizures are by far the most common type. The rate of SE has been found to range from 6.8 to 41 per 100,000 people per year, with peaks in children younger than 1 year and older people[17].

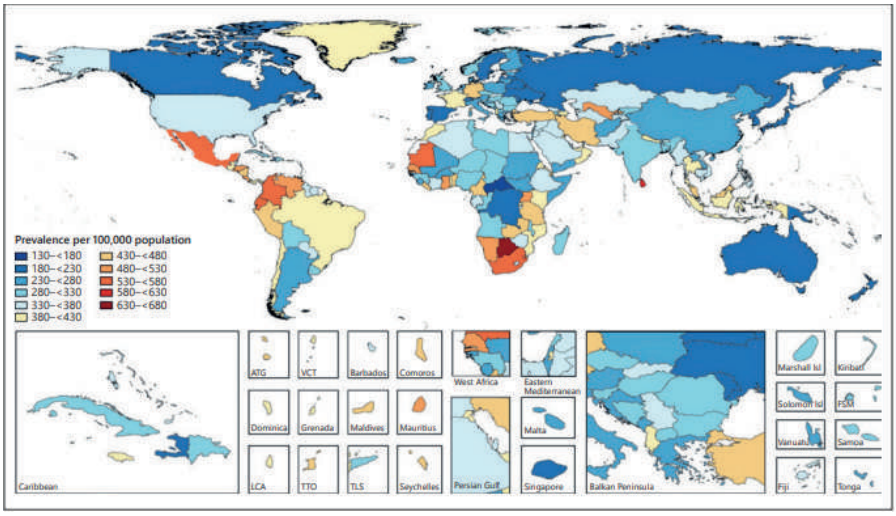


Figure 1: Age related prevalence per 100,000 of idiopathic epilepsy by country, 2016 form global burden of diseases 2016

Seizures happen when neurons in a part of the brain or the whole brain fire at the same time n a way that is not normal. This can happen when networks are not put together right or when there is a structural, infections, or metabolic problem. Most seizures in children are caused by genetics, injuries that happen before or during birth, or problems with how the brain develops. Common causes of seizures in people who do not have genetic predisposition of epilepsy include encephalitis/meningitis, traumatic brain injury, and brain tumors (figure 2). Epilepsy in older people is generally caused by neurodegenerative disorders, head injuries, or brain tumors. Because the causes of epilepsy are different for different age groups, the frequency of epilepsy is bimodal, with generic and developmental causes peaking in childhood and accumulated brain damage (such as from trauma or tumors) peaking in the elderly. It is important to know that the cause of about half of all seizures is unknown[18].

Etiologies of Epilepsy by Age

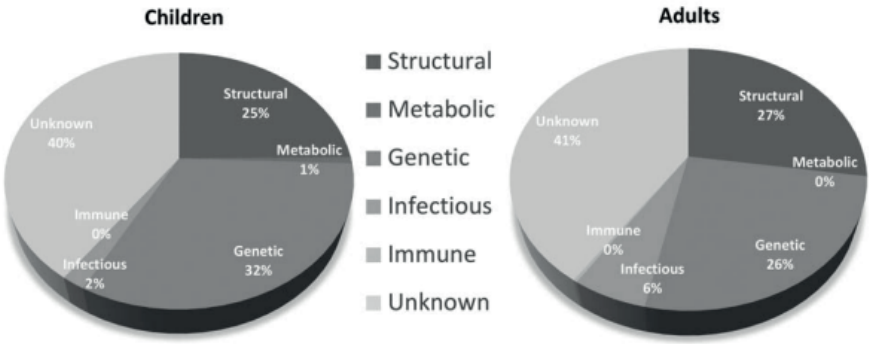


Figure 2: Etiologies of epilepsy according to the age by ILAE

The condition known as epilepsy is distinguished by an atypical synchronization of firing among clusters of neurons, which arises form an in-equilibrium in the neurotransmission on excitatory and inhibitory signals, historically, epilepsy research has been primarily focused on the neurological aspects of the conditon. In the past twenty years, there has been a significant increase in research regarding the function of glial cells in regulating and altering neuronal activity. This has resulted in compelling evidence supporting the involvement of glial cells in the development of epilepsy[19].

Classification of seizure and epilepsy

Basic classification

The categorization of seizures is determined by their initiation and can be classified into four types: focal, generalized, unknown, or unclassifiable, as presented in Table 1. The term "focal" can be considered as a synonym for the previously used term "partial." The term "generalized" has remained unaltered. A seizure of generalized onset is characterized by the simultaneous activation of both hemispheres, which may exhibit asymmetry, at the onset of the seizure, as determined by behavioral and electroencephalographic observations. The term "unknown onset" pertains to a scenario wherein the onset remains unidentified, while other indications are recognized. Further elaboration is provided below. The category of "Unclassified" persists, although its usage may diminish with the inclusion of novel seizure types and the "unknown onset" classification. There exist a limited number of occurrences that are unequivocally seizures, yet they cannot be categorized[20].

Table 1: International League Against Epilepsy 2017 basic classification of seizures.

Focal seizure	**Generalized onset**	**Unknown**
Aware Impaired awareness Motor onset Non-motor onst Focal to bilateral tonic-clonic	Motor • Tonic-clonic • Other motor Non-motor absence	Motor • Tonic-clonic • Other motor Non-motor absence Unclassified

Expanded classification

The extended categorization system elaborates on the fundamental categorization system delineated earlier, by extending the "motor" and "non-motor" groupings to encompass all three types of seizures (focal, generalized, and unknown), as presented in tables 2 and 3.

Table 2: ILAE 2017 expanded classification of seizures

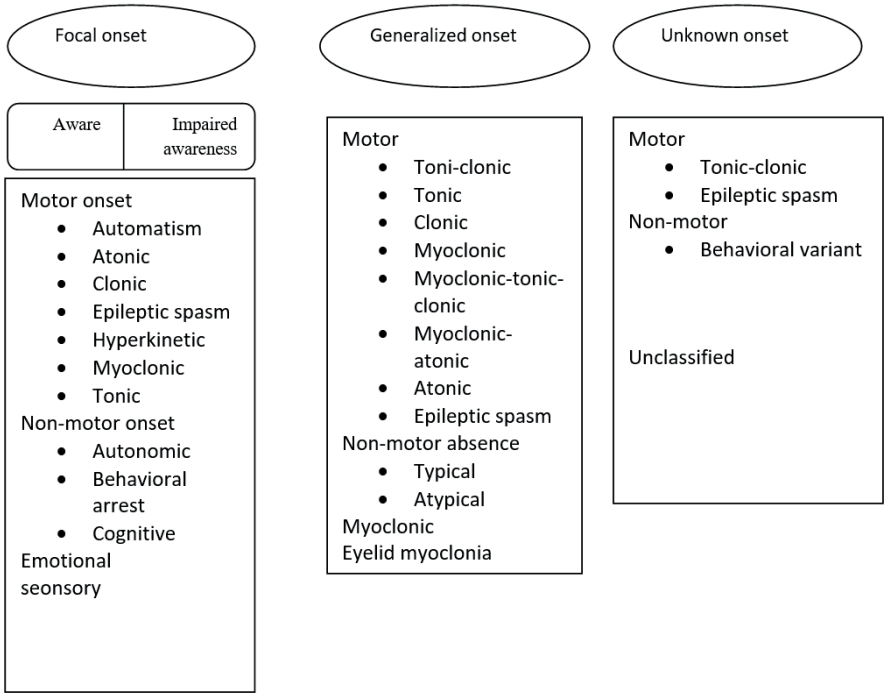
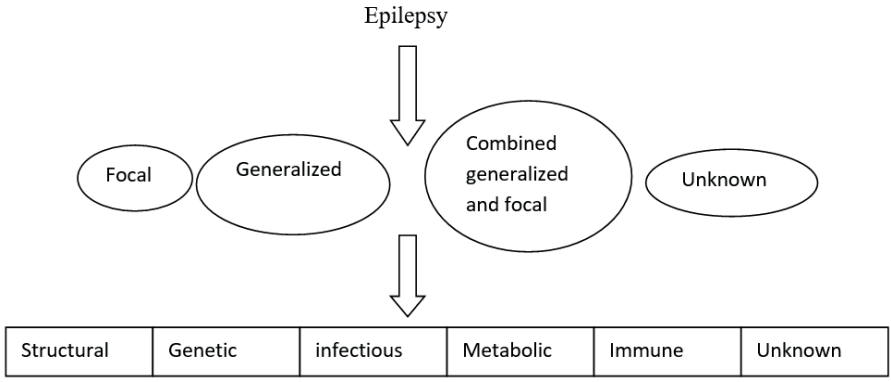


Table 3: The 2017 ILAE classifications of the Epilepsies



Epilepsy syndrome

Etiology

The revised categorization of epilepsy offers a tripartite diagnostic structure that underscores the significance of etiological factors throughout all phases. The initial step involves the delineation of the seizure classification. The second category pertains to epilepsy classification, encompassing focal, generalized, combined focal and generalized, and epilepsy of unknown etiology. The third aspect pertains to the diagnosis of the epilepsy syndrome. The classification of etiological categories, which holds significant implications for management, encompasses structural, genetic, infectious, metabolic, immune, and unknown factors[21].

Diagnostic evaluation

Epilepsy is a difficult diagnosis that lacks a simple, obtainable gold standard. The key to diagnosis is a thorough history and a credible eyewitness narrative, because no single symptom or sign is distinctive to epilepsy, the determination of whether a seizure has occurred or not is based on a combination of symptoms and signs. Epilepsy is polymorphic with numerous presentations and a wide range of mimics, which adds to its complexity. Always rule out non-epileptic paroxysmal occurrences because epilepsy misdiagnoses are frequent and can have negative effects. The most frequent syncope presentation is a brief loss of consciousness, and the most significant epileptic mimics are those caused by psychogenic or functions factors. All people who may be having seizure should be given electrocardiogram, especially if they also have a brief loss of consciousness. Epilepsy is not defined by an abnormal electroencephalogram (EEG), but

inter-ictal epileptiform discharges may support a clinical diagnosis. Finding the likely type of epilepsy (focal or generalized), diagnosing and epileptic syndrome, and determining the likelihood of recurrence are all made easier by an abnormal EEG. Long-term video-EEG monitoring may be able to provide a conclusive diagnosis in patients who still have diagnostic challenges following clinical evaluation and standard EEG, particularly if the episode frequency is high[22].

The history and neurological examination serve as the fundamental basis for the diagnosis of seizures and epilepsy, while laboratory testing is utilized as supplementary measures. The historical features that are significant include the clinical setting in which the seizure took place, which encompasses premonitory indications, as well as the seizure's specific characteristics, such as phenomenology, responsiveness, focal features, and the post-ictal state. Subsequent inquiry is directed towards ascertaining the presence of an epilepsy syndrome, which serves to delineate the nature and extent of the assessment, as well as to establish the appropriate course of treatment and prognosis.

The neurological exam evaluates localized indications that may implicate or locate cerebral disease. Increased tone on one side of the body, for example, could suggest pathology in the contralateral hemisphere, such us cortical dysplasia. A general physical examination is also necessary to assess whether or not the patient has an underlying ailment. Atypical skin marks, for example cough suggest a neurocutanous illness characterized by epilepsy, such as tuberous sclerosis or neurofibromatosis[23].

Electroencephalogram (EEG): an EEG is a recording of the electrical activity of the brain. It is capable of detecting aberrant electrical activity such as focal spikes or waves (which are compatible with focal epilepsy) or diffuse bilateral spike waves (which are consistent with generalized epilepsy). Because the prevalance of epileptiform abnormalities changes in the distinct states of consciousness, routine EEG should cover wakefulness, drowsiness, and sleep. Hyperventilation and photic stimulation are EEG activation procedures used to increase the yield of epileptic activity. The clinical information is used to make and epilepsy diagnosis and the EEG should be considered confirming rather than diagnostic. The conventional wisdom is to "treat the patient, not the EEG." Absence epilepsy is an exception to this rule, as brief generalized bursts of spike-wave activity, even if not linked with evident clinical alterations, indicate a significant likelihood of unreported absence seizure recurrences[24].

Metabolic evaluation: seizures are frequently associated with other anomalies in metabolic diseases, such as developmental delay, unexpected vomiting, or unconsciousness. A metabolic study is required in neonatal convulsions, including a screening of serum amino acids and urine organic acids, as well as blood lactate to rule out mitochondrial dysfunctions. Cerebrospinal fluid can examine for glucose transporter abnormalities (LUT1 deficient syndrome) in addition to its more usual usage to evaluate CNS illness.

Immunology: The identification of encephalopathies and epilepsies that were previously unknown has been made possible by the discovery of neuronal antibodies. The prevalence of autoimmune epilepsy is currently uncertain; however, it seems to affect a considerable proportion of patients with focal epilepsy. The most commonly observed etiologies of encephalitis are antibodies directed against glutamic acid decarboxylase (GAD)-65, LGI1, CASPR2, and NMDA receptors. This is particularly true when the initial assessment fails to reveal an underlying cause and the patient presents with symptoms or indications of limbic encephalitis. Diagnostic indicators include cognitive decline, personality changes, autonomic seizures, dyskinesia, comorbid autoimmune conditions, and mesial temporal MRI alterations that may potentially progress to mesial temporal sclerosis.

Imaging: The predominant modality for imaging is magnetic resonance imaging (MRI), which discloses epileptogenic lesions in nearly 20% of recently diagnosed epilepsy patients and over 50% of those with drug-resistant focal epilepsy. In contrast to individuals without an MRI lesion, those with an MRI lesion exhibit a greater likelihood of recurrence following a first seizure or persistent seizure activity after treatment. The MRI protocol necessitates the incorporation of volumetric T1-weighted imaging in three dimensions with a minimum slice thickness of 1mm. Additionally, the protocol must include axial and coronal T2-weighted and fluid attenuated inversion recovery sequences, which should encompass hippocampal angulation. Furthermore, the protocol should comprise axial hemosiderin or calcification-sensitive T2 sequences or susceptibility-weighted sequences.

Epileptic syndromes

The contemporary classification system has incorporated the epilepsy syndrome as a novel component. The term refers to a constellation of symptoms that typically co-occur, encompassing various types of seizures, electroencephalogram (EEG) readings, and imaging results. Epilepsy syndrome is influenced by various factors such as age of onset, remission,

triggers, diurnal variation, intellectual and psychiatric dysfunction, EEG findings, imaging studies, familial history, and genetic predisposition. The International League Against Epilepsy (ILAE) has not officially categorized a comprehensive list of epileptic syndromes. Nevertheless, recognized and established syndromes have been delineated and a selection of them is examined herein. For a comprehensive inventory of epilepsy syndromes, it is recommended to consult the website of the International League Against Epilepsy (ILAE). In the past, the term "benign" was utilized to classify certain epilepsy syndromes. However, this terminology is no longer favored as it suggests that the epilepsy has negligible effects on the patient. Presently, it is acknowledged that all types of epilepsy can have social implications and may be linked to comorbidities such as psychiatric and learning disorders. Presently, the phrase "self-limiting" is employed.

Idiopathic or Genetic Generalized Epilepsy Syndromes:

The category of idiopathic generalized epilepsies encompasses various types of seizures, including childhood absence epilepsy, juvenile absence epilepsy, juvenile myoclonic epilepsy, and generalized tonic-clonic seizures occurring in isolation (as presented in table 4). The utilization of the term idiopathic in the classification of epilepsy has been a subject of controversy, with some advocating for its removal. The term "idiopathic" was originally coined to connote a self-originating or genetically determined condition. There exists a concern regarding the use of the term "genetic" as it may connote inheritance, while a significant proportion of epilepsy patients exhibit de novo mutation or complex genetic syndromes that may manifest with or without environmental exposure. The ILAE task force has resolved to maintain the usage of the term "idiopathic generalized epilepsy" to denote the epilepsies mentioned earlier, owing to the preference of a significant number of stakeholders in the epilepsy domain to retain its usage. In cases where a clinician has identified a specific genetic cause, the term "genetic generalized epilepsy" may be employed to characterize the epilepsy syndrome. Patients diagnosed with idiopathic generalized epilepsies exhibit typical electrographic background and generalized spike-wave patterns in their EEGs.

Childhood absence epilepsy is a condition that is known to be self-limiting and has a higher incidence rate in neurologically normal females as compared to males. Onset of the condition is commonly observed to manifest between the ages of 4 and 10, while remission is typically observed to occur during the period of adolescence. Patients exhibit episodes of absence seizures and occasionally experience tonic-clonic generalized seizures. The premature

manifestation of generalized tonic-clonic seizures is linked to a poorer prognosis.[27].

Juvenile absence epilepsy manifests during the period of adolescence and early adulthood, with the most favorable age of onset ranging from 10 to 13 years. Both genders are equally affected by absence seizures, although these occur less frequently in childhood than absence epilepsy. At the outset of the presentation, there is a manifestation of generalized tonic-clonic seizures, and albeit infrequent, myoclonic seizures may also transpire. In contrast to infantile absence epilepsy, the aforementioned syndrome is not characterized by a self-limiting course.

One of the most prevalent epilepsy syndromes is juvenile myoclonic epilepsy, the onset period spans from before the age of 10 through the middle of the 20s, and occasionally later. Women are more likely than men to develop juvenile myoclonic epilepsy[28]. Every patient experiences myoclonic seizures, which are frequently followed by generalized tonic-cloni seizures. Rarely do absence seizures happen, most patients don't go into spontaneous remission and need to take antiepileptic drugs for the rest of their lives.

The hallmark of piously with generalized tonic-clonic seizures only is characterized by generalized tonic-clonic seizures that occur within an age range spanning from childhood to mid-adulthood, with a peak onset typically observed during the second decade of life. The term "generalized tonic-clonic seizures at awakening" was initially used, but was later revised upon the recognition that seizures could occur at any point during the day. Generalized tonic-clonic seizures in epilepsy, as well as juvenile absence epilepsy and juvenile myoclonic epilepsy, are not inherently self-limiting conditions. As a result, individuals with these forms of epilepsy often require lifelong treatment with antiepileptic medication.[29].

Tabel 4: Idiopathic or Genetic Epilepsy Syndromes

Epilepsy Syndrome	Seizure types	Age of onset	Self-limiting	EEG findings
Childhood Absence Epilepsy	Absence generalized tonic-clonic seizure (rare)	4 to 10 years	Yes	Normal background, occipital intermittent rhythmic delta activity, 3-3.5Hz generalized spike wave discharges
Juvenile Absence Epilepsy	Absence generalized tonic-clonic seizure, myoclonic (rare)	adolescence to early adulthood	No	Normal background, polyspikes may be present, -3.5Hz generalized spike wave discharges
juvenile Myoclonic Absence	myoclonic, generalized tonic-clonic, absence (rare	10 years to mid-20s	No	Normal background, -3.5Hz generalized spike wave discharges, > 4Hz generalized spike wave discharges, high amplitude polyspike wave discharges with myoclonic seizures, photoparoxysmal response in up to 40% of patients
Epilepsy with generalized tonic-clonic seizures alone	generalized tonic-clonic	childhood to mid-adulthood	No	Normal background, generalized, spike or polyspike wave discharges,

Focal Epilepsy Syndromes

Focal epilepsy disorders such as childhood epilepsy with centro-temporal spikes and Panayiotopoulos syndrome have been extensively documented. Formerly, the condition known as childhood epilepsy was referred to as benign epilepsy with centro-temporal spikes (as presented in Table 5). Childhood epilepsy with centro-temporal spikes is a type of epilepsy that is self-limited. It typically presents during the school years and is characterized by short focal motor hemifacial seizures and nocturnal focal motor seizures that eventually develop into bilateral tonic-clonic seizures. The occurrence of centro-temporal spikes during sleep is in accordance with the electroencephalogram (EEG) background. Panayiotopoulos syndrome is a type of self-limited epilepsy that is distinguished by focal autonomic seizures that are often prolonged, as well as focal occipital high-amplitude sleep-

activated spikes on the electroencephalogram (EEG). Possible autonomic symptoms include vomiting, pallor, mydriasis, as well as symptoms related to the digestive, respiratory, and thermoregulatory systems. Additionally, incontinence and hypersalivation may also be observed.

Reflex Epilepsy Syndromes:

Reflex epilepsy syndromes refer to a type of epilepsy in which the occurrence of seizures is triggered by a specific stimulus. Whilst generalized tonic-clonic seizures are the most commonly occurring type of seizure, it is important to note that other types of generalized seizures may also manifest. Focal seizures may occasionally present as reflex epilepsy. The most commonly occurring reflex epilepsy syndromes are those that are triggered by photosensitivity. Two additional types of epilepsy that fall under the category of reflex epilepsy are reading epilepsy and startle epilepsy[30].

Table 5: Examples of epilepsy syndromes according to age of onset:

Neonatal

- Benign familial neonatal epilepsy (BFNE)

Infancy

- West syndrome
- Dravet syndrome

Childhood

- Generalized epilepsy with febrile seizures plus (GEFS+)
- Childhood absence epilepsy
- Lennox-Gastaut syndrome
- Landau-Kleffner syndrome

Adolescence and adulthood

- Juvenile myoclonus epilepsy

Disorders that mimic epilepsy

According to their clinical presentation or medical history, many paroxysmal behaviors resemble epileptic seizures. Here, a few of the more typical illnesses are mentioned, it is critical to distinguish between epileptic and non-epileptic behaviors since some non-epileptic phenomenon can be treated with drugs other than EDs and others only need reassurance or avoidance of the situationsthattrigger the spell[31].

Non-epileptic Seizures (NES): NES are episodic changes in motor function or behavior that bear resemblance to epileptic seizures, but do not exhibit any corresponding electroencephalogram (EEG) activity. These events are commonly referred to as psychogenic seizures or pseudo-seizures, as they differ from epileptic seizures. The condition known as NES has the potential to cause significant impairment and is often associated with underlying psychopathological conditions. The manifestation of the medical condition, namely seizure, is a genuine occurrence. However, as abnormal neural discharges are not involved, the primary objective of therapy is to target the fundamental psychological issues. This statement provides reassurance to both the patient and their family.

Breath-Holding Spells (BHS): BHS are not voluntary responses but rather involuntary reflexes, despite their name. BHS are at their peak in preschoolers and are often gone by the time children reach the school age. There are two subtypes of BHS: cyanotic and pallid. Cyanotic BHS is also referred to as cyanotic infantile syncope, while pallid BHS is often referred to as reflex anoxic seizures. Anger and irritation are two of the most prominent triggers for cyanotic BHS, which is the more prevalent form. Thee kid will stop breathing (in expiration), get cyanotic, and lose consciousness while they are weeping, whichisthedefining characteristics of this conditon. A pallid BHS is more prone to become agitated in response to a frightening experience or an unpleasant stimulation (such as a minor form of trauma). After a gasp, the patient will experience loss of consciousness, as well as pallor, bradycardia, diaphoresis, and limpness. Neither a type of BHS is associated with and increased predisposition to epilepsy, although seizure activity can occur at the end of a BHS.

*Syncope: H*istory is generally enough to tell the difference between syncope (fainting) and an epileptic seizure. Lightheadedness, blurred vision, pallor, nausea, and diaphoresis are all possible pre-attack warning indicators (presyncopal symptoms). Loss of consciousness and a gradual descent to the ground follow these wringing sings, in contrast to the sudden collapse seen in myoclonic and atonic seizures. When compared to the extended postictal state that follows and epileptic seizure, consciousness returns relatively quickly. Syncope is caused by a temporary drop in blood flow to the brain. This can be caused by an irregular heartbeat (arrhythmia) a condition called orthostasis or Valsalva, or a vasovagal response (fear, pain, or mental upset).

Parasomnias: are problems with sleep that can look like seizures. Night terrors are a common parasomnia that happens to kids between the ages of 18 months and 8 years. In early (non-REM) sleep, the child wakes up

screaming, sweating, and moving his or her arms and legs out of sync. The children then goes back to sleep and doesn't remember what happened. Night terrors are often passed down from generation to generation. The case history is use to make the diagnosis; video-EEG is rarely needed. Nightmares which happen during REM sleep, and nighttime epileptic seizures that start in the frontal lobe are the main things that make a diagnosis difficulty.

General approach of epilepsy management

The primary mode of treatment for individuals diagnosed with epilepsy involves the administration of anti-seizure medications. The objective is to promptly terminate seizures while avoiding any adverse effects that may potentially compromise the individual's standard of living. By utilizing a pharmacological arsenal comprising over 20 medications, it is possible to achieve effective treatment in up to 70% of individuals who have received a recent diagnosis of epilepsy[32]. Pharmaceutical agents employed in the management of epilepsy function by impeding neuronal depolarization through the blockade of sodium or calcium channels, augmenting the activity of potassium channels, inhibiting glutamate-mediated neuronal excitation, or enhancing the inhibitory effects of gamma-aminobutyric acid (GABA) on neuronal activity. The efficacy of medications varies depending on the underlying etiology as they operate through distinct mechanisms. Patients with idiopathic conditions are more likely to experience improvement, particularly if their developmental and neurological assessments yield normal results[33]. The selection of a particular seizure medication by a neurologist is contingent upon various factors such as the nature of the seizure, the age of the patient, and the coexistence of other medical ailments. Furthermore, the potential adverse effects of the intervention should be taken into consideration. Oftentimes, a medication with a wide range of efficacy is employed due to the possibility of an insufficient description of a seizure provided by a witness. Levetiracetam has become increasingly favored as a primary treatment option in recent times owing to its effectiveness, convenient dosage adjustment, and established adverse effects profile. In the past, carbamazepine was the favored therapeutic option for focal seizures, whereas valproic acid was the preferred treatment for generalized seizures.

Typically, it is advisable to administer an initial modest dosage of medication in order to mitigate the likelihood of adverse effects. In the event of a need for escalation, incremental dosages may be administered at predetermined intervals. The aim is to effectively control seizures while utilizing the minimum feasible dosage. A trial that is deemed appropriate entails a duration of two months during which a therapeutic dosage is

administered and well-tolerated.[34]. The possibility of increased toxicity resulting from drug interactions is a potential concern in combination therapy. Nevertheless, specific combinations, such as lamotrigine and valproic acid, have demonstrated notable efficacy in treating generalized seizures (refer to table 6). The central nervous system (CNS) adverse effects are a common occurrence among anticonvulsant drugs, owing to their respective mechanisms of action. As an illustration, drowsiness is a prevalent unfavorable outcome of almost all antiepileptic drugs (refer to table 7). Certain medical practitioners may consider the possibility of discontinuing a particular medication in the event that seizures have not manifested for a minimum duration of two years. In cases where medication proves to be ineffective in managing seizures, alternative options such as dietary therapy (ketogenic diet), epilepsy surgery (lesionectomy, hemispherotomy), and palliative epilepsy surgery (stimulation therapy,callosotomy) may be considered[35]. Modifying one's lifestyle is an essential element of the management of epilepsy. Improving epilepsy outcome can be achieved through the optimization of sleep, enhancement of medication adherence, and minimization of stress. Ultimately, it is imperative that individuals in good health advocate for those who are experiencing digestive wellness. Individuals diagnosed with epilepsy encounter considerable obstacles to achieving a typical way of life as a result of the negative attitudes and prejudicial treatment they encounter.

Table 6: Range of efficacy and chose of Antiepileptic Drugs

Antiepileptic Drug	Focal seizures	Generalized tonic-clonic seizures	generalized absence seizures	generalized myoclonic seizures	Lennox-Gastaut syndrome/Infantile spasm/ Dravetsyndorme
Phenobarbital	Class 1 trial	Suggested, but not proven in Class 1 trails	Not effective	Class 1V evidence	
Phenytoin	Class 1 trial	Suggested, but not proven in Class 1 trails	Not effective	Not effective	
Carbamazepine	Class 1 trial	Suggested, but not proven in Class 1 trails	Not effective	Not effective	
Oxcarbazepine	Class 1 trial	Unknown	Not effective	Not effective	
Eslicarbazepine acetate	Class 1 trial	Unknown	Not effective	Not effective	
Valproate	Class 1 trial	Suggested, but not proven in Class 1 trails	Class 1 trial	Suggested, but not proven in Class 1 trails	Suggested, but not proven in Class 1 trails
Ethosuzimide	Not effective	Not effective	Class 1 trial	Not effective	Class 1 trial Lennox-Gastaut syndrome
Clobazam	Suggested, but not proven in Class 1 trails	Suggested, but not proven in Class 1 trails	Suggested, but not proven in Class 1 trails	Suggested, but not proven in Class 1 trails	Class 1 trial Lennox-Gastaut syndrome
Felbamate	Class 1 trial	Suggested, but not proven in Class 1 trails	Unknown	Unknown	
Gabapentin	Class 1 trial	Not effective	Not effective	Not effective	
Pregabalin	Class 1 trial	Not effective	Not effective	Not effective	
Lamotrigine	Class 1 trial	Class 1 trial	Suggested, but not proven in Class 1 trials	Variable	Class 1 trial Lennox-Gastaut syndrome
Topiramate	Class 1 trial	Class 1 trial	Not effective in Class 1 trial	Unknown	Class 1 trial Lennox-Gastaut syndrome
Tiagabine	Class 1 trial	Not effective	Not effective	Not effective	
Levetiracetam	Class 1 trial	Class 1 trial	Suggested, but not proven in Class 1 trials	Class 1 trial	
Brivaracetam	Class 1 trial	Unknown	Unknown	Unknown	

Zonisamide	Class 1 trial	Suggested, but not proven in Class 1 trails	Suggested, but not proven in Class 1 trials	Suggested, but not proven in Class 1 trials	
Lacosamide	Class 1 trial	Unknown	Not effective	Not effective	
Vigabatrin	Class 1 trial	Not effective	Not effective	Not effective	Class 1 trial Infantile Spasm
Rufinamide	Class 1 trial, but not FDA approved	Suggested, but not proven in Class 1 trails	Unknown	Unknown	Class 1 trial Lennox-Gastaut syndrome
Perampanel	Class 1 trial	Class 1 trial	Unknown	Class IV evidence	
Cannabidiol	Class IV evidence	Unknown	Unknown	Unknown	Class 1 trial Lennox-Gastaut syndrome/ Infantile Spasm

Table 7: Pharmacokinetic aspects of Antiepileptic Drugs

Antiepileptic drug	Oral bioavailability	Protein binding[a]	Metabolism	Half-life[b]	Drug interaction
Phenobarbital	Good	Low	>70%	Long	High
Phenytoin	Variable	High	Extensive not linear	Intermediate (long with toxicity)	High
Carbamazepine	Good	Intermediate	Extensive	Intermediate	High
Oxcarbazepine	Good	Low	Extensive	Short	Moderate
Eslicarbazepine acetate	Good	Low	~40%	Intermediate	Moderate
Valproate	Good	High	Extensive	Intermediate	High
Ethosuzimide	Good	Low	Extensive	Long	Moderate
Clobazam	Good	High	Extensive	Intermediate	High
Felbamate	Good	Low	~50%	Intermediate	High
Gabapentin	Low	Low	None	Short	No/minimal
Pregabalin	Good	Low	None	Short	No/minimal
Lamotrigine	Good	intermediate	Extensive	Intermediate	Moderate
Topiramate	Good	Low	~30%	Intermediate	No/minimal
Tiagabine	Good	High	Extensive	Short	High
Levetiracetam	Good	Low	~30% non-hepatic	Short	No/minimal
Brivaracetam	Good	Low	Extensive	Short	Moderate
Zonisamide	Good	Low	~65%	Short	Moderate
Lacosamide	Good	Low	~60%	Intermediate	No/minimal
Vigabatrin	Good	Low	None	Intermediate	No/minimal
Rufinamide	Good	intermediate	Extensive	Short	Moderate
Perampanel	Good	High	Extensive	Long	Moderate
Cannabidiol	Low	High	Extensive	Long	HIgh

[a] Low: <50%; intermediate: 50% to 85%; high: >85%.

[b] Short: <10 hours; intermediate: 10 to 30 hours; long: >30 hours.

Status Epilepticus

Status epilepticus (SE) is considered to be one of the most critical neurological emergencies. It is characterized by prolonged or recurrent seizures that do not allow the individual to fully return to their baseline state. Based on estimates, there exists a range of 10-40 cases and a corresponding mortality rate per 100,000 individuals, approximately 20% of whom experience a return to previous levels of functionality at a rate of only one-third.[36].

In 2015, the ILAE Task Force on Classification of Status Epilepticus emphasized the significance of timing in the assessment of seizures. The International League Against Epilepsy (ILAE) introduced two operational dimensions that pertain to the duration of a seizure. These dimensions are defined by the period of epileptic activity that may lead to permanent brain damage with long-term consequences, and the duration beyond which a seizure should be identified as unusually prolonged. According to findings from studies conducted on both animals and humans, it has been projected that the anticipated time intervals for convulsive tonic-clonic status epilepticus (SE) are 5 minutes and 30 minutes, correspondingly. The available data on non-convulsive status epilepticus (NCSE) is limited as most studies have primarily focused on convulsive forms. However, the International League Against Epilepsy (ILAE) has recommended time points of 10 minutes (t1) and 60 minutes (t2) for focal status epilepticus with impaired consciousness[37].

The extant guidelines and protocols for the management of status epilepticus (SE) prescribe specific timing recommendations for each line of therapy. These recommendations stipulate that first-line treatment should be administered within the initial 10 minutes of seizure activity, second-line treatment within the initial 20 minutes, and third-line treatment should be considered if SE persists despite the administration of at least two anti-seizure medications (ASM) with optimal dosing within the initial 60 minutes. Several studies and meta-analyses have concurred that there exist systematic delays in the treatment of SE when compared to the recommended guidelines (refer to Table 8).

Current guidelines recommend several pharmacological treatments for SE. initial administration of benzodiazepines recommend occurs in the earliest prehospital phases of seizures, in the early phase of an established SE, anticonvulsant medications are administered intravenously (IV) after hospital admission (table 9a and 9b). If IV ASM administration fails to control refractory seizures, general anesthesia is administered, such as by

IV infusion of midazolam, propofol, or ketamine. Continuous infusion of anesthetics is administered until the seizure subsidies and is continued for 12-24 hours after the last seizure. If SE persists or recurs despite the use of anesthetic for at least 24 hours, it is defined as super-refractory SE (SRSE)[38].

Table 8: General approach of Status epilepticus management

	General Measures	Medications
Immediate Management (0-5 minutes)	➢ Note the time, call for help ➢ Secure airway (semi-prone position, nasopharyngeal airway), give oxygen ➢ IV cannulation, finger glucose, blood gas, LFT, RFT, electrolytes, AED levels, CRP ➢ History: past medications, overdose and drug addiction	➢ Observe prehospital treatment ➢ Give thymine if suspected of Alcohol excess ➢ Dextrose infusion if hypoglycemic ➢ Benzodiazepines for IV preparations
Early Status Epilepticus (5-20 minutes)	➢ Monitoring vital signs ➢ Cardiac monitoring ➢ ICU possibility	give benzodiazepines if no response repeat after 5 minutes
Established Status Epilepticus (20-40 minutes)	➢ ICU review ➢ chest x ray ➢ CT head ➢ Consider intubation	Give 2nd line AEDs ➢ Phenytoin/ Fosphenytoin ➢ Sodium valproate ➢ Levetiracetam
Refractory Status Epilepticus (>30 mins)	➢ Intubate and admit ICU ➢ continuous EEG monitoring	Anesthetic agents like (Thiopental, propofol, midazolam)

Table 9: Doses of first-line and second-line ASM for SE

Table 9a: Benzodiazepines as first-line in SE

Intravenous	Non-intravenous
Lorazepam 0.1mg/kg (max 4mg)	Midazolam IM/IN/buccal 10mg 5mg in elderly or <40kg
Diazepam 0.15-2mg/kg (max 10mg)	Diazepam 10mg rectal 5mg in elderly or <40kg
Clonazepam 0.015mg/kg (max 1mg)	Lorazepam intranasal 0.1mg/kg or <40kg

Table 9b: Second line of ASMs in SE

Drug	Dose; Rate, Maximum	Suggestion	Caution
Phenytoin/ Fosphenytion	20mg/kg 50g/min (2000mg)	Already taking phenytoin. Suspected poor adherence	• significant hypotension • bradycardia, heart block • Porphyria • generalized Epilepsy • Overdose of recreation drugs or antidepressants
Valproate	30mg/kg 10mg/kg/min (3000mg)	Already taking Valproate. Suspected poor adherence generalized epilepsy	• women of childbearing age • pre-existing of liver disease or pancreatitis • known metabolic disorder • caution in acute stroke or brain injury
Levetiracetam	60mg/kg 6mg/kg/min (4500mg)	Already taking Levetiracetam, Suspected poor adherence	• acute or brain injury • known mood/behavioral disorder • renal impairment

Women and epilepsy

The occurrence of seizures and the administration of antiepileptic medications have the potential to interfere with hormone regulation, thereby posing a threat to the sexual and reproductive well-being of women diagnosed with epilepsy. Women diagnosed with epilepsy encounter social stigmatization in several developing nations. According to a study conducted by Komolafe et al. (2012), the economic status of WWE in Nigeria is comparatively lower than that of non-epileptic women.[39]. According to Santosh et al. (2007), a significant proportion of individuals with epilepsy in India concealed their condition from their prospective spouses due to apprehension of social ostracism and the possibility of disrupted marriage negotiations. Specifically, over 50% of individuals with epilepsy who participated in the study reported hiding their condition prior to marriage[40]. Women with epilepsy typically experience a higher frequency of seizures during periods of hormonal fluctuations such as puberty, menstruation, pregnancy, and menopause. Catamenial epilepsy exacerbates seizures during menstruation in females with epilepsy. The prevalence of catamenial epilepsy among women with epilepsy (WWE) ranges from 33% to 50%. The works cited are those of Foldvary-Schaefer and Falcone (2003) and Morrell (1999).[41]. The

prevalence of menstruation issues in WWE was found to be 2.5 times higher than that of the general population. The occurrence of epilepsy in relation to the menstrual cycle, known as catamenial epilepsy, has been found to have a correlation with the varying levels of hormones, particularly estrogen and progesterone. It has been observed that progesterone exhibits anticonvulsant properties while estrogen has proconvulsant effects. The condition known as catamenial epilepsy exhibits three discernible patterns of heightened seizure frequency, namely C1 (perimenstrual pattern), C2 (perovulatory pattern), and C3 (luteal pattern). C1 takes place in the follicular phase, which spans from day 4 to day 10 of the menstrual cycle. C2 occurs during the ovulatory phase, which takes place from day 10 to day 14 of the cycle. Finally, C3 occurs in the luteal phase, which spans from day 17 to day 3 of the menstrual cycle. According to research conducted by Harden and Pennell (2013) and Reddy, the levels of progesterone exhibit a decline during phases C1 and C3, whereas estrogen levels experience an increase during phase C2[42]. Antiepileptic drugs (AEDs) have the potential to impact or bear resemblance to the menstrual cycle. Seizures have the potential to induce disturbances in neuroendocrine activity, resulting in menstrual irregularities in women with epilepsy, as well as serving as an adverse reaction of antiepileptic drugs. An increasing body of evidence suggests that the utilization of valproate among females is associated with a higher likelihood of encountering menstrual irregularities, such as polycystic ovarian syndrome (PCOS). According to Johnston and Crawford (2014), there exist bidirectional pharmacokinetic interactions between oral contraceptives and AEDs. There exist a number of antiepileptic drugs (AEDs) that are recognized for their ability to induce cytochrome activity, specifically within the CYP3A4 group. Such drugs include carbamazepine, oxcarbazepine, topiramate, phenobarbitone, and phenytoin[43]. The cytochromes play a role in the metabolic process of the primary constituents of the combined oral contraceptive pill, namely estrogen and progesterone. Hence, the effectiveness and efficacy of oral contraceptive pills (OCPs) could be reduced when co-administered with cytochrome-inducing antiepileptic drugs (AEDs).

According to Harden et al. (2009a), a significant proportion of women with epilepsy (WWE) do not experience any alteration in seizure activity during gestation compared to their pre-pregnancy baseline. This finding is noteworthy as WWE often express apprehension regarding the efficacy of seizure management during pregnancy. Given that AEDs are excreted in breast milk in minimal quantities, it is commonly accepted that breastfeeding while taking AEDs is a safe practice. According to the National Institute for Health and Care Excellence (2012), it is recommended to administer vitamin

K intravenously to neonates born to mothers undergoing antiepileptic drug (AED) therapy for women with epilepsy (WWE).[44]

Prognosis

From a demographic perspective, the majority of individuals with epilepsy exhibit a positive prognosis. The likelihood of recurrence following an initial seizure is subject to significant variability contingent upon whether the seizure was characterized by acute and symptomatic features or occurred spontaneously. In contrast to single unprovoked seizures, acute symptomatic seizures exhibit a comparatively lower recurrence rate of approximately 19% over a decade According to Beghi's (2003) findings, the aggregate likelihood of recurrence subsequent to an initial unprovoked seizure varies between 23 and 71%. Two factors that have a high likelihood of predicting return are the presence of a known cause and an atypical EEG pattern, characterized by epileptiform and/or slow activity[45].

Epilepsy is a treatable conditon, with up to 80% of people having long times without seizures and up to 50% of people still not having seizure after treatment stops. Patients with unexplained or cryptogenic first seizures have shown that there is a link between having seizures again and having them in the past. Only in LMIC, where most people with epilepsy don't get treatment (the treatment gab is between 70 and 94%), can be the outcome of untreated epilepsy be determined. The cause of epilepsy is the best prediction of whether or not seizures will happen again, in a well-defined US community, people with symptomatic epilepsy had a much lower chance of remission after 5 years than those with idiopathic epilepsy (42 vs. 30% at 15 years), and people with neurological dysfunction at birth had the lowest change of remission. Type of seizure and EEG epileptiform changes were also used to predict the outcome. Europe also had lower remission rates for people with symptomatic epilepsy[46]. The chance of dying from epilepsy is low, but when incidence and prevalence studies area compared, mortality rates are likely to be different. When epilepsy or seizures cause death, some of the most important causes are SUDEP, SE, accidental injury, and suicide.

References

Osawa, S. I., Iwasaki, M., Hosaka, R., Matsuzaka, Y., Tomita, H., Ishizuka, T., ...&Mushiake, H. (2013). Optogenetically induced seizure and the longitudinal hippocampal network dynamics. PloS one, 8(4), e60928.

Fisher, R. S., Boas, W. V. E., Blume, W., Elger, C., Genton, P., Lee, P., & Engel Jr, J. (2005). Epileptic seizures and epilepsy: definitions proposed by the International League Against Epilepsy (ILAE) and the International Bureau for Epilepsy (IBE). Epilepsia, 46(4), 470-472.

Falco-Walter, J. J., Scheffer, I. E., & Fisher, R. S. (2018). The new definition and classification of seizures and epilepsy. Epilepsy research, 139, 73-79.

Falco-Walter, J. (2020, December). Epilepsy—definition, classification, pathophysiology, and epidemiology.In Seminars in neurology (Vol. 40, No. 06, pp. 617-623).Thieme Medical Publishers, Inc..

Fisher, R. S., &Leppik, I. (2008). Debate: When does a seizure imply epilepsy?. Epilepsia, 49, 7-12.

Camfield, P., &Camfield, C. (2015). Incidence, prevalence and aetiology of seizures and epilepsy in children. Epileptic disorders, 17(2), 117-123.

Stafstrom, C. E., &Carmant, L. (2015). Seizures and epilepsy: an overview for neuroscientists. Cold Spring Harbor perspectives in medicine, 5(6), a022426.

Riney, K., Bogacz, A., Somerville, E., Hirsch, E., Nabbout, R., Scheffer, I. E., ...&Tinuper, P. (2021). ILAE classification and definition of epilepsy syndromes with onset at a variable age: position statement by the ILAE Task Force on Nosology and Definitions. Currently in Review by the ILAE. Available online at: https://www. ilae. org/guidelines/definition-and-classification/proposed-classificationand-definition-of-epilepsy-syndromes/proposed-classification-syndromeswith-onset-at-variable-ages (accessed October 26, 2021).

Patel, P., &Moshé, S. L. (2020). The evolution of the concepts of seizures and epilepsy: What's in a name?. Epilepsia Open, 5(1), 22-35.

Fisher, R. S., Acevedo, C., Arzimanoglou, A., Bogacz, A., Cross, J. H., Elger, C. E., ... & Wiebe, S. (2014). ILAE official report: a practical clinical definition of epilepsy. Epilepsia, 55(4), 475-482.

Specchio, N., Wirrell, E. C., Scheffer, I. E., Nabbout, R., Riney, K., Samia, P., ...&Auvin, S. (2022). International League Against Epilepsy classification and definition of epilepsy syndromes with onset in childhood: Position paper by the ILAE Task Force on Nosology and Definitions. Epilepsia, 63(6), 1398-1442.

Nashef, L., Hindocha, N., &Makoff, A. (2007). Risk factors in sudden death in epilepsy (SUDEP): the quest for mechanisms. *Epilepsia*, *48*(5), 859-871.

Brodie, M. J., Barry, S. J. E., Bamagous, G. A., Norrie, J. D., & Kwan, P. (2012). Patterns of treatment response in newly diagnosed epilepsy. *Neurology*, *78*(20), 1548-1554.

Engel, J. (2005). *Epilepsy: global issues for the practicing neurologist* (Vol. 2). Demos Medical Publishing.

Kandar, H. K. M. C. C., Das, S. K., Ghosh, L., & Gupta, B. K. (2012). Epilepsy and its management: A review. *Journal of PharmaSciTech*, *1*(2), 20-26.

McHugh, J. C., &Delanty, N. (2008). Epidemiology and classification of epilepsy: gender comparisons. *International review of neurobiology*, *83*, 11-26.

Behr, C., Goltzene, M. A., Kosmalski, G., Hirsch, E., &Ryvlin, P. (2016). Epidemiology of epilepsy. *Revue neurologique*, *172*(1), 27-36.

Thurman, D. J., Beghi, E., Begley, C. E., Berg, A. T., Buchhalter, J. R., Ding, D., ... & ILAE Commission on Epidemiology. (2011). Standards for epidemiologic studies and surveillance of epilepsy. *Epilepsia*, *52*, 2-26

Patel, D. C., Tewari, B. P., Chaunsali, L., &Sontheimer, H. (2019). Neuron–glia interactions in the pathophysiology of epilepsy. *Nature Reviews Neuroscience*, *20*(5), 282-297.

Hirose, G. (2013). An overview of epilepsy: its history, classification, pathophysiology and management. *Brain and nerve= Shinkeikenkyu no shinpo*, *65*(5), 509-520.

Sharma, R., &Pachori, R. B. (2015). Classification of epileptic seizures in EEG signals based on phase space representation of intrinsic mode functions. *Expert Systems with Applications*, *42*(3), 1106-1117

Peker, M., Sen, B., &Delen, D. (2015). A novel method for automated diagnosis of epilepsy using complex-valued classifiers. *IEEE journal of biomedical and health informatics*, *20*(1), 108-118

Kutlubaev, M. A., Xu, Y., Hackett, M. L., & Stone, J. (2018). Dual diagnosis of epilepsy and psychogenic nonepileptic seizures: systematic review and meta-analysis of frequency, correlates, and outcomes. *Epilepsy & Behavior*, *89*, 70-78.

Malmgren, K., Reuber, M., & Appleton, R. (2012). Differential diagnosis of epilepsy. *Oxford textbook of epilepsy and epileptic seizures*, 81-94.

Schmitt, B. (2015). Sleep and epilepsy syndromes. *Neuropediatrics*, *46*(03), 171-180

Seneviratne, U., Cook, M. J., & D'Souza, W. J. (2017). Electroencephalography in the diagnosis of genetic generalized epilepsy syndromes. *Frontiers in neurology*, *8*, 499.

Zuberi, S. M., Wirrell, E., Yozawitz, E., Wilmshurst, J. M., Specchio, N., Riney, K., ...&Nabbout, R. (2022). ILAE classification and definition of epilepsy syndromes with onset in neonates and infants: Position statement

by the ILAE Task Force on Nosology and Definitions. *Epilepsia*, *63*(6), 1349-1397.

Hirsch, E., French, J., Scheffer, I. E., Bogacz, A., Alsaadi, T., Sperling, M. R., ...& Zhou, D. (2022). ILAE definition of the idiopathic generalized epilepsy syndromes: position statement by the ILAE task force on nosology and definitions. *Epilepsia*, *63*(6), 1475-1499.

van Baalen, A., Vezzani, A., Häusler, M., &Kluger, G. (2017). Febrile infection–related epilepsy syndrome: clinical review and hypotheses of epileptogenesis. *Neuropediatrics*, *48*(01), 005-018.

Camfield, P., &Camfield, C. (2015). Incidence, prevalence and aetiology of seizures and epilepsy in children. *Epileptic disorders*, *17*(2), 117-123.

Louis, E. K. S., &Cascino, G. D. (2016). Diagnosis of epilepsy and related episodic disorders. *CONTINUUM: Lifelong Learning in Neurology*, *22*(1), 15-37.

Perucca, P., Scheffer, I. E., & Kiley, M. (2018). The management of epilepsy in children and adults. *Medical Journal of Australia*, *208*(5), 226-233.

Brown, C. (2016). Pharmacological management of epilepsy. *Progress in Neurology and Psychiatry*, *20*(2), 27-34c

Volkov, I. V., &Volkova, O. K. (2020). Juvenile myoclonic epilepsy. Update. *Epilepsy and paroxysmal conditions*, *12*(1S), S41-S49

Nascimento, F. A., Friedman, D., Peters, J. M., Bensalem-Owen, M. K., Cendes, F., Rampp, S., ...&Beniczky, S. (2023). Focal epilepsies: update on diagnosis and classification. *Epileptic Disorders*

Trinka, E., &Leitinger, M. (2022). Management of status epilepticus, refractory status epilepticus, and super-refractory status epilepticus. *CONTINUUM: Lifelong Learning in Neurology*, *28*(2), 559-602.

Treiman, D. M. (2020). Status epilepticus.In *The medical treatment of epilepsy* (pp. 183-194).CRC Press.

Shorvon, S., & Sen, A. (2020). What is status epilepticus and what do we know about its epidemiology?. *Seizure*, *75*, 131-136.

Komolafe, M. A., Sunmonu, T. A., Afolabi, O. T., Komolafe, E. O., Fabusiwa, F. O., Groce, N., ... &Olaniyan, S. O. (2012). The social and economic impacts of epilepsy on women in Nigeria. *Epilepsy & Behavior*, *24*(1), 97-101.

Santosh, D., Kumar, T. S., Sarma, P. S., & Radhakrishnan, K. (2007). Women with onset of epilepsy prior to marriage: disclose or conceal?. *Epilepsia*, *48*(5), 1007-1010

Foldvary-Schaefer, N., & Falcone, T. (2003). Catamenial epilepsy: pathophysiology, diagnosis, and management. *Neurology*, *61*(6 suppl 2), S2-S15.

Harden, C. L., & Pennell, P. B. (2013). Neuroendocrine considerations in the treatment of men and women with epilepsy. *The Lancet Neurology*, *12*(1), 72-83.

Johnston, C. A., & Crawford, P. M. (2014). Anti-epileptic drugs and hormonal treatments. *Current treatment options in neurology*, *16*, 1-9.

Harden, C. L., Hopp, J., Ting, T. Y., Pennell, P. B., French, J. A., Allen Hauser, W., ... & Le Guen, C. (2009). Management issues for women with epilepsy—Focus on pregnancy (an evidence-based review): I. Obstetrical complications and change in seizure frequency: Report of the Quality Standards Subcommittee and Therapeutics and Technology Assessment Subcommittee of the American Academy of Neurology and the American Epilepsy Society. *Epilepsia*, *50*(5), 1229-1236.

Beghi, E., Gatti, G., Tonini, C., Ben-Menachem, E., Chadwick, D. W., Nikanorova, M., ... & BASE Study Group. (2003). Adjunctive therapy versus alternative monotherapy in patients with partial epilepsy failing on a single drug: a multicentre, randomised, pragmatic controlled trial. *Epilepsy research*, *57*(1), 1-13.

Shorvon, S. D., &Goodridge, D. M. (2013). Longitudinal cohort studies of the prognosis of epilepsy: contribution of the National General Practice Study of Epilepsy and other studies. *Brain*, *136*(11), 3497-3510.

Current Researches in Health Sciences-II

Editor:
Dr. Enes Karaman
Dr. Gözde Özge Önder

ÖZGÜR YAYINLARI

ISBN 978-975-447-651-4