MULTIDISCIPLINARY APPROACH IN MEDICAL SCIENCE V

EDITOR: ASSOC. PROF. DR. Ş. CEM YÜCETAŞ

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PREFACE

Dear readers and valuable scientists,

As a result of intense labor and effort, we are honored to present to you our book, which covers a wide range of valuable topics in the field of medicine and health.

Developments in health and health services give us hope every day. The efforts and diligent work of scientists, especially against the types of cancer that afflict humanity, are admirable. Great efforts are made to improve the quality of human life.

I greet you with respect, hoping to bring added value to humanity every day.

Doç. Dr. Ş. Cem YÜCETAŞ

Edıtor

CHAPTER 1

NOVEL APPROACHES IN CANCER IMMUNOTHERAPY

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INTRODUCTION

"Immunotherapy" encompasses various treatments aimed at stimulating the immune system to target and eradicate cancer cells. Effective cancer immunotherapy triggers a systemic, precise, and well-tolerated anti-tumor response, addressing the limitations often encountered with traditional treatment methods.

The review article provides an extensive overview of strategies targeting the immune system in adaptive T-cell cancer therapy, encompassing immunosuppression within the tumor microenvironment, cancer vaccines, and therapeutic cell interventions. It also explores advancements in combination therapies and molecular approaches, highlighting the significant impact of nanomaterials on enhancing cancer immunotherapy. Significant advancements have recently been made in the development of cancer vaccines, with a particular focus on vaccines containing purified antigenic epitopes.

However, the limited cytotoxic response induced by subunit vaccines poses challenges for their widespread application in cancer treatment. To overcome this challenge, nanoparticle-based vaccines can be utilized to enhance antigen presentation by phagocytic activity. Nanomaterials, with their multiple enhancing functions for concurrent suppression and immunization in the tumor microenvironment, have shown remarkable synergistic effects in combating tumorigenesis in preclinical models.

In this context, the article presents a hopeful perspective by addressing the constraints of existing therapeutic modalities. It aims to offer fresh insights into treatment approaches, aiming for impactful patient care with minimal adverse effects shortly.

1. Advancing Cancer Treatment: The Role of Adoptive Cell Therapy and Therapeutic Vaccines in Immunotherapy

Cancer ranks among the primary causes of mortality globally, with projections indicating a surge of over 70% in the coming two decades (Jemal et al., 2010). Conventional treatments like chemotherapy and radiotherapy often lack specificity and long-term efficacy.

Hence, there arises a necessity for a methodology capable of targeting cancer cells with precision while also offering sustained improvements by

mitigating mortality associated with treatment-related complications. With the proliferation of cancer, immune cells evade immune surveillance. Tumor expansion is mostly associated with cells harboring mutations in genes encoding proteins involved in biochemical control and cell growth(Weinberg R. The Biology of Cancer. 2nd ed. New York & 2013.).

ACT holds a distinguished position in the realm of cancer immunotherapy, boasting a rich history that spans several decades and setting it apart from other immunotherapeutic approaches (Greenberg et al., 1981).

This approach involves transferring tumor-specific lymphocytes, akin to therapeutic cancer vaccines aimed at bolstering immune responses against tumors. Esteemed institutions like the National Cancer Institute in the United States, alongside other research centers, have showcased the appeal of therapeutic vaccines for both clinical and preclinical trials owing to their ease of administration and minimal toxicity. Moreover, the effectiveness of vaccines can be augmented through combination regimens involving modifiers of the MHC, cytokines, and co-stimulatory molecules (Schlom, 2012).

2. Challenges and Advances in T-Cell-Based Immunotherapy: Exploring Tumor-Specific Targets and Engineering Strategies

Cancer vaccines induce tumor regression in less than 4% of patients undergoing treatment with Sipuleucel-T by the organization. Despite the approval of a therapeutic vaccine for prostate cancer aimed at reducing tumor burden, there has been no discernible difference in median survival rates over the four months of observation. Additionally, did not exhibit tumor progression nor sustained long-term responses during treatment (Klebanoff et al., 2011).

3. Immunosuppressive Mechanisms in Tumors and Advances in Monoclonal Antibody and CAR T Cell Therapies

While immunotherapy involving the transfer of tumor-infiltrating lymphocytes (TILs) stands as a promising treatment for metastatic melanoma patients, it comes with inherent limitations. Notably, not all patients qualify for clinical trials, and eligibility is confined to those with a favorable performance status capable of tolerating autologous TILs therapy alongside adoptive cell therapy (ACT). Consequently, IL-2-assisted therapy has

demonstrated sustained responses in melanoma, offering comparable efficacy irrespective of prior treatment history(Rosenberg et al., 2011). It has been shown that tumors may sometimes evade T-cell therapy through various mechanisms(Khong & Restifo, 2002).

Therefore, animal and human studies have focused on specialized targets of transferred cells. They have also investigated the microenvironmental nature and targeting mechanisms (Zhang et al., 2012) and the differentiation stages of involved cells. Engineered cells can be utilized to destroy cancers of various tissues. Certain types of tumors, including pediatric tumors, testicular germ cell tumors, and specific leukemias, exhibit a low mutational burden, with their cancer phenotypes primarily stemming from epigenetic abnormalities (Zhang et al., 2012).

It is noteworthy that melanomas are not the only tumors with highly mutated genomes; indeed, some primary lung tumors are also highly mutated, experiencing intense environmental mutagenesis induced by factors like ultraviolet light in melanomas, or by tobacco smoke in lung cancers. It appears that mutations are targeted. Many ACT approaches in lung cancer can be tailored independently. TIL expansion from melanomas, although engineered T cells may be found, can recognize various tumor cell types by engineering T cells to target tumor surface antigens and can be unleashed. Genetic engineering on tumor cells has expanded approaches to T cytokines and chemokines from these cells expressing specific receptors (Brentjens et al., 2011).
Tumor cells can secrete cytokines that exert potent immunosuppressive effects. Among these factors, TGF-β stands out as one of the most praised, capable of inhibiting differentiation (Abbasi et al., 2012; Rabinovich GA).

TGF-β plays a significant role in aiding tumor cells to evade the immune system. Plasma TGF-β levels exhibit a weak correlation with various malignancies. A fully human-specific monoclonal antibody, GC1008, demonstrates a high affinity for all three isoforms of TGF-β. Extensive studies are underway regarding this antibody's efficacy in patients with malignant melanoma. The production of TGF-β leads to the accumulation of regulatory T cells (Tregs) and FOXP3+, CD25+, and CD4+ cells (Petrausch et al., 2009). Clinical trials utilizing monoclonal antibodies targeting T-reg have demonstrated efficacy, inhibiting tumor formation and metastasis in breast cancer mouse models. Zenapax (Daclizumab), which targets CD25 on T reg

cells, is well tolerated and effectively depletes these cells in malignant breast cancer patients. Additionally, peptide vaccines targeting reverse transcriptase telomerase and anti-apoptotic CD proteins show promise when combined with these antibodies. However, CD25-targeted treatment may enhance T-cell proliferation potential post-activation. Further research on CD25 expression at tumor sites is necessary to understand the role of these cells, particularly in the absence of T-reg cells (Hong et al., 2010).

The lymphocytes address concerns about tolerance to tumor antigens and effectively induce frequency of responses. Host lymphocyte depletion facilitates the acquisition of adoptive T cells and the successful engraftment of ex vivo transferred TILs (Rosenberg et al., 2008) in patients with melanoma has provided remarkable immune responses, some of which are completely durable. With advances in methods and their engineering, coupled with retroviral vectors, the cultivation of CAR T cells or TCR engineered with domain receptors for stimulatory pathways, opportunities for adoptive therapy have been provided (June, 2007).

4. CTLA-4 Blockade and Neoantigen-Specific T Cell Responses in Cancer Immunotherapy

Ipilimumab, a monoclonal antibody targeting CTLA-4, has been extensively investigated by various research groups for many years, particularly in understanding T lymphocyte function and its association with CTLA. Blocking antibodies against T cells induces negative regulation, thereby controlling CTLA cell function. The fundamental importance of CTLA-4 has been well demonstrated, as disruption of Ctla4 leads to severe lymphoproliferative disorders in young mice. Binding is crucial for suppressing T cell function by further modulating the response of CTLA-4 cells. T regulatory cells are important for the immune system(Chambers et al., 2001).

In theory, two factors should determine the importance of neoantigens and mutated self-antigens, which are effective therapies in cancer immunotherapy: First, the frequency at which blockade occurs in TIL infiltrates and occurs.

Secondly, the efficacy of T cell responses against both antigen classes is crucial. Recent studies examining T cell specificity and response quality in murine models with carcinogen-induced cancer grafts have revealed

that blockade of immune infiltrates can alter the response to tumor-specific neoantigens by TILs, akin to common therapeutic modifications. This alteration is evident in the expression of CTLA genes in tumor-bearing mice treated with CD8+ antibodies, as well as in the magnitude of this response observed with PD-1 and/or combination therapy (Gubin et al., 2014).

Because these neoantigens have been identified in this model as cancer rejection antigens, these data provide convincing evidence that blockade at least in this setting is a part of the specific activity of neoantigen-specific T cells. However, in human melanoma, in locations where the tumor has been in contact with the immune system for many years, the situation is more complex in terms of T cell function.

As outlined earlier, neoantigen cell activity is prevalent in melanoma. Hence, a study has indicated the possibility of enhancement (van Rooij et al., 2013). Evidence supporting the role of neoantigens in modulating the magnitude of T cell responses to tumor cells has been established through observations linking increased mutation rates to higher infiltration of CD8+ T cells at the tumor site. Analysis of RNA sequencing data from tumors with varying mutation burdens has revealed a robust correlation, particularly in patients undergoing PD-1 inhibitor therapy, notably in non-small cell lung cancer patients with elevated mutation burdens, demonstrating clinical response. More specific neoantigen T data direct from studies on human tumor control cells have been quantitatively obtained from clinical studies that require T cell transduction or injection of a specific cell population. Therefore, although data on T cell receptor (TCR) sequencing supports the theory that recognition of neoantigens helps common immunotherapy. A direct comparison of the anti-tumor activity of specific neoantigens for further addressing these T cells and self-antigens derived from DC-derived cells has been obtained from patient data(Rizvi et al., 2015).

5. Dendritic Cell-Derived Exosomes: Immunomodulatory Roles and Limitations in Tumor Immunotherapy

Due to their role as immune molecules, Dendritic cell (Homesley et al.)-derived exosomes (Dexo) have played a significant role in stimulating T-cell responses. It was first reported in 1996 that Dexo induces CD8 antigen-specific responses, restricted to class II MHC molecules derived from B cells, for Zitvogel and colleagues. In animal models, T-cell responses to Dexo have been shown to root tumors and vaccination with Dexo vesicles. These

observations indicate their immunomodulatory bioactivity and anti-tumor effects, expressing MHC molecules derived from DCs(Zitvogel et al., 1998). Dexo directly stimulates T cell proliferation in vitro by interactions with T cells. The interaction is regulated by CD54/LFA-1 and MHC/TCR interactions on Dexo cells, inducing CD80/CD28 cell stimulation. However, it has been reported that the absent presence can facilitate host DC responses to pMHC peptide exchanges between DCs and CD4 T cells until the functional exchange is demonstrated in vitro(Thery et al., 2002). Nevertheless, most cytotoxic T lymphocytes (CTLs) only provide prophylactic immunization and no therapeutic effects have been observed with Dexo vaccines on established tumors. The tumor microenvironment may become immunosuppressive, causing immunogenic tumors to rapidly expand Tr CD4 cell responses with delayed Tr cell responses(Curiel et al., 2004; Ghiringhelli et al., 2004).

6. Enhancing Anti-Tumor Immunity: Strategies to Modulate Tumor Microenvironment and Improve Immune Modulator Delivery

In contrast, the release of these immune response Tr cells induces anti-tumor immunity and leads to tumor eradication. Tr cells, which are protected from the activity of Cyclophosphamide (CY), are capable of restoring immunological tolerance and facilitating acquired cancer immunotherapy. The anti-tumor effects of CPM are significantly enhanced in animals treated with Dexo, possibly due to the restoration of immune tolerance and the suppression of CD4 Tr cell activity. CPM may enhance specific antigen responses before Dexo application and may restore secondary immune responses associated with Dexo-specific suppression of CD4 Tr cell activity, resulting in a synergistic anti-tumor effect with Dexo(Steidl et al., 2010).

The microenvironment in the majority of solid tumors exhibits traits of immune evasion and pre-tumor inflammation, facilitated by the concurrent actions of cancer cells and TILs, including tumor-associated macrophages, Tregs, and MDSCs(Steidl et al., 2010).

MDSCs employ various mechanisms to counteract different tumor parenchyma effectively. These cells tend to locally inhibit cytotoxic effector function, thereby promoting tumor growth and metastasis while reinforcing the immunosuppressive milieu. Tumor immune priming, similar to molecular or cellular immunosuppression, may sometimes induce adequate anti-tumor immunity. This can synergistically impact the specialized cancer tumor

microenvironment (TME) when combined with approaches that enhance T cell production or expansion. Immunotherapies aimed at restoring immune suppression mimic types of immunogenic cancers found in a significant portion of the population, which were previously suppressed by TME-derived anti-tumor T cells (Le et al., 2001; Mellman et al., 2011). To facilitate the transfer of immune modulators like cytokines and antagonistic factors targeting cell surface receptors, it's crucial to design carriers that minimize extracellular interaction with phagocytic cells. For instance, the release of these modulators within the tumor microenvironment (TME) could be modified by coating certain carriers with bioinert polymers or incorporating anti-phagocytic signals such as PEGylation or PEG derivatives on their surface (Rodriguez et al., 2013).

Moreover, for delivering immune modulators like specific ligands or pathways targeting pattern recognition receptors (PRRs) involved in immune regulation, the carrier should be designed for efficient entry into the intracellular compartment. For instance, enhancing endolysosomal uptake can be achieved by adjusting particle surface properties or physical characteristics by surface modification with ligands targeting receptors associated with endocytosis (Zaimy et al., 2017).

Nanomaterials have been engineered to deliver immune modulators to the tumor microenvironment, aiming to target different mechanisms of immune suppression within the TME. One promising target is STAT3, recognized as a crucial regulator of immune dysregulation. In particular, STAT3 expression in infiltrating tumor cells promotes inflammation by Th-2 cells while inhibiting responses associated with Th-1 cells and the proliferation of T-regulatory (T-reg) cells (Wang L).

It is suggested that carriers of regulatory antigens, such as TNF and IL-6, be locally stimulated, inhibited, and regulated. For this purpose, carriers should be designed in a way that minimizes the side effects of interaction with phagocytic cells and prevents the risks of extracellular toxicity. For example, absorption can be reduced by changing the physical properties of carriers such as dimensions, aspect ratios, and stiffness. Additionally, patterns are designed to specifically deliver immune regulators such as ligands or RNA molecules to ensure their proper entry into cells. For instance, targeting endosomal delivery by regulating the surface properties of carriers can accelerate their uptake and endocytosis (Barreto et al., 2011).

7. Carrier Proteins and Cross-Presentation: Enhancing Antigen-Specific Immune Responses in Vaccine Development

The use of carrier proteins in vaccine formulations has been shown to enhance the immunogenicity of antigens by promoting their presentation on the surface of Subunit carrier proteins, which by APCs such as DCs via their Toll-like receptor (TLR) binding sites. Consequently, the activation of specific immune responses, including Th-1 cell-mediated immunity and CD4 T helper cell polarization, is facilitated by the interaction of APCs with the antigen-presenting region of the carrier protein, leading to enhanced CTL responses(Baumgaertner et al., 2012).

Antigen cross-presentation involves specialized APCs internalizing antigens originating from extracellular sources. These antigens are then presented on MHC class I molecules, facilitating their recognition by CD8+ T cells. This process ultimately triggers the induction of antigen-specific CTL responses (Li M).

Cross-presentation involves DCs uptaking exogenous antigens and processing them in specialized compartments to generate peptides suitable for loading onto MHC class I molecules. Particulate CpG adjuvants, such as E2 particles, have been demonstrated to BMDCs upon exposure to free CpG. E2 particles, delivering both CpG and antigenic peptides, facilitate efficient antigen uptake and processing into peptides suitable for presentation on MHC class I molecules. This process enhances the cross-priming of CD8+ T cells by these antigen-presenting cells (Stano et al., 2013).

8. Optimizing Antigen Cross-Presentation: Advanced Delivery Strategies for Enhanced Cancer Vaccine Efficacy

In cancer vaccines, the cross-presentation of tumor antigens by APCs is crucial for generating effective antitumor immune responses. To achieve this, strategies aimed at enhancing antigen cross-presentation involve the targeted delivery of antigens and adjuvants to specific cellular compartments, such as endosomes and lysosomes, where antigen processing and MHC class I loading occur. Various approaches, including nanoparticles and liposomes, have been explored to improve the targeting and delivery of antigens and adjuvants to these compartments, thereby enhancing antigen cross-presentation and subsequent CTL responses(Zaimy et al., 2016).

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CHAPTER 2

A DEVELOPING INDICATOR OF ACCESS TO HEALTH SERVICES IN TÜRKİYE OVER TIME: HISTORICAL ANALYSIS OF THE NUMBER OF PEOPLE PER HEALTH PERSONNEL

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1. INTRODUCTION

The right to life of all living beings on earth forms the basis of all other rights. The possibility of people who have lost their health and who have not solved their health problems as well as their economic problems to use their other rights is very low, even non-existent. In a society where the right to a healthy life is not guaranteed, it is meaningless to talk about the existence of other rights and to guarantee them. The guarantee of the right to life is the most fundamental reason for the emergence of human societies. In this case, the guarantee of the right to be healthy, along with the right to life, is ab initio adsum, that is, it has existed since the beginning. This basic justification directly forms the basis for the policies of states in the field of health (Karafazlı, 2013).

According to WHO, the main article that comes first among the basic principles related to health is 'accessibility'. In this regard, accessibility to health services constitutes an important part of the right to health. Accordingly, health institutions, goods and services should be accessible to everyone. Accessibility is possible with the existence of four interconnected features: non-discrimination, physical accessibility, economic accessibility and accessibility to information (De Vito et al., 2016).

The main purpose of health services is to protect, improve and ensure the continuity of individual and community health in terms of physical, mental and social aspects (Kayral, 2013; Patil, 2018; Purwanto et al., 2018). In this respect, health services provided throughout the country are extremely important. However, in order to achieve this basic purpose of health services, in addition to the quantity and quality of health services, access to such services and equity in access are also very important (Marşap et al., 2010).

The main criterion that shows the welfare or development level of a country is the access of citizens to health services. One way to measure this situation is the number of patients per health personnel working in the country. This calculated number reveals important situations related to the health system of the country. These include how the health system works, whether it is able to meet the needs, and the capacity of the health system (World Health Organization [WHO], 2006). Historically, changes in this indicator are very valuable in understanding the development of health policies and the distribution of resources in different regions. However, if country governments develop strategic plans in this sense, it will create

positive changes for individuals living in society (Yavan and Gazeloğlu, 2022).

The history of health services in Türkiye can be broken down into two main periods. The first period is the one before the Republic, which covers the Seljuk and Ottoman eras. The second period is the one after the Republic. The health system, which goes back to the Seljuk Empire, has a pretty sophisticated structure for the time. From the Seljuk Empire to the early days of the Ottoman Empire, health services were provided through foundations. However, the monarchical structure was reflected in the later periods of the Ottoman Empire. Health services were provided more to these two structures (Çavdar and Karcı, 2014) because they were focused on the palace and the military. The general public could also get services from freelance physicians for a fee. These services were available in major cities until the end of the 19th century. They expanded to reach more people by the end of the 19th century. As the 19th century came to a close, health services started to be seen as a basic responsibility of the state. This led to a wider rollout of these services across society, with the palace and the army becoming the main beneficiaries. On top of that, the legal framework was put in place to spread the services even further through different organisations (Fişek, 1983). The first modern hospital in Türkiye, Gülhane Military Hospital, opened in 1898. The medical education provided here under the leadership of German professors was really important for Turkish medicine. This education, which was led by Rieder, helped move from theoretical knowledge to practical training and showed that some medical knowledge, which had been known in the West for a long time, was lacking in Türkiye. Rieder and the physicians he trained played an important role in eliminating these deficiencies (Başustaoğlu, 2016).

Access to health services was frequently discussed as one of the important problems of the Turkish health system before the Health Transformation Program (HTP) (Tatar, 2007; Ökem and Çakar, 2015). Based on this, in order to ensure the effectiveness of health strategies, institutions apply continuous evaluation and feedback mechanisms, allowing individuals to create new systems in this regard over time, based on their opinions about the health system (Gazeloğlu and Özgören Ünlü, 2024). In addition, especially before the HTP, a significant portion of the society lacked health insurance and there was an unbalanced distribution between regions in terms of human resources and technical equipment (Ağartan, 2012; Yılmaz, 2013).

In the mid-20th century, like many other countries, Türkiye was facing a health services access issue in rural areas. The main reason was that there weren't enough health workers and the infrastructure was lacking. This general situation worldwide has been highlighted in many academic studies (Rosen, 1993). In the early 1900s, the government's focus was on preventing infectious diseases in the country, stopping them from spreading, and getting them to disadvantaged regions (Bryce et al., 2003).

Today, these steps taken by Türkiye in the field of health services are of great importance in terms of understanding how the number of people per health personnel has changed in the historical process and the effects of these changes on public health. In this context, the extent to which imbalances in the employment and distribution of health personnel affect public health and equality of access to health services should be carefully examined. This study aims to contribute to the academic knowledge in this field by revealing the structural transformations in Türkiye's health sector, the historical development of the number of people per health personnel and the reflections of these changes on health services.

2. METHOD

In this study, cluster analysis, which is a statistical method, was used to determine the similarities between the measured variables and to create natural groups that occur spontaneously. Cluster analysis helps to make the data more understandable and easier to interpret by grouping the observation values in multi-dimensional data sets to make the data more meaningful (Everitt et al., 2011). In this study, Hierarchical Clustering method, a type of clustering method, was used.

2.1. Cluster Methods

K-means algorithm assigns n observations in the data set to k clusters, ensuring that each observation is included in the cluster center closest to it. The algorithm works to minimize the Euclidean distance between each cluster center and the observations. This distance is the distance between two data points x and y.

$$d(x,y) = \sqrt{\sum_{i=1}^{p}(x_i - y_i)^2} \quad (1)$$

Equality 2 is used to minimize the total squared error of the K-means algorithm.

$$\arg min_s \sum_{j=1}^{k} \sum_{x \in S_j} \left\| x - \mu_j \right\|^2 \quad (2)$$

In Equation 2, each observation in cluster S_j represents the sum of the squared distances between x and the cluster center μ_j (Lloyd, 1982).

Hierarchical Clustering creates clusters based on similarities between observations and merges observations or existing clusters at each step. In hierarchical cluster analysis, clusters are merged according to the minimum distance between observations using the Nearest Neighbor (Single Linkage) method. The Nearest Neighbor method calculates the distance between two clusters

$$d(A, B) = \min \{d(x, y): x \in A, y \in B\}$$

where the groups are determined using the shortest distance between clusters A and B (Johnson, 1967).

2.2. Determining the Number of Clusters

One of the most important factors in accuracy of cluster analysis is the determination of the number of clusters. In the study, the Elbow Method and the Silhouette Coefficient were used to determine the optimal number of clusters. The Elbow Method allows determining the appropriate number of clusters by calculating the total within-cluster variance for each number of clusters. This method tries to minimize the total within-cluster sum of squares W_k for each number of clusters k:

$$W_k = \sum_{j=1}^{k} \sum_{x \in S_j} \left\| x - \mu_j \right\|^2 \quad (3)$$

Silhouette Coefficient evaluates clustering performance on a per-observation basis. The silhouette coefficient compares the proximity of an observation to its own cluster with the distance to its nearest neighbor cluster to evaluate whether the observation is assigned to the correct cluster. The silhouette coefficient s(i) for each observation is calculated as follows:

$$s(i) = \frac{\min \{d(i,j): i \neq j\}}{\max \{d_k: k=1,\ldots,k\}} \quad (4)$$

where d(i,j) represents the distance between clusters and dk represents the maximum distance within the cluster (Dunn, 1973).

3. FINDINGS

The study's data set was obtained from the official website of the Turkish Statistical Institute (International Statistical Institute, The dataset obtained from the source includes the number of physicians, dentists, nurses, midwives, pharmacists and other healthcare personnel in Türkiye from 2000 to 2021. You can access both definitions and other explanatory information regarding the dataset from the TÜİK official website.

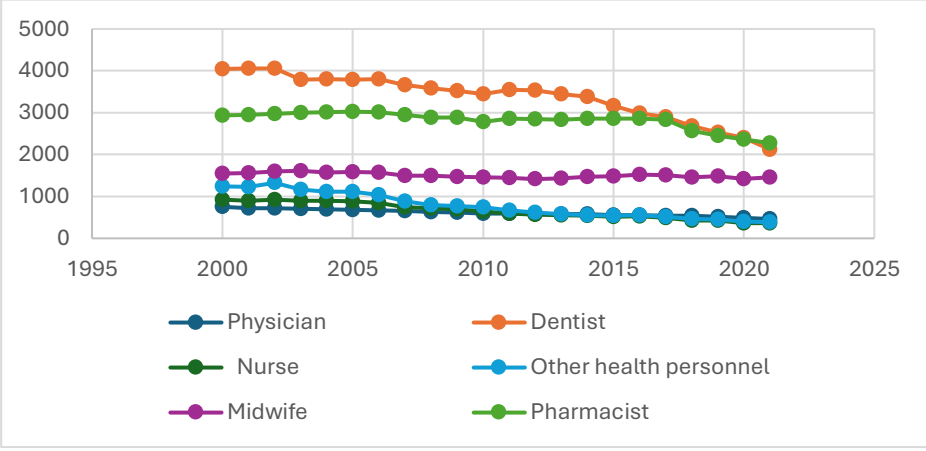

Figure 1: Graph of the number of healthcare personnel between 2000-2021

When Figure 1 is examined in detail, it is seen that the number of people per health personnel has decreased over time. This means that the number of health personnel in Türkiye has increased in proportion to the population. This shows that over the years, each citizen has increased their ability to reach a health personnel. In addition, it is seen that there are various fluctuations in some years. It can be said that migration, education programs, retirement system and rates are effective in these fluctuations. Considering such factors while creating health policies will minimize fluctuations and enable people to reach health personnel.

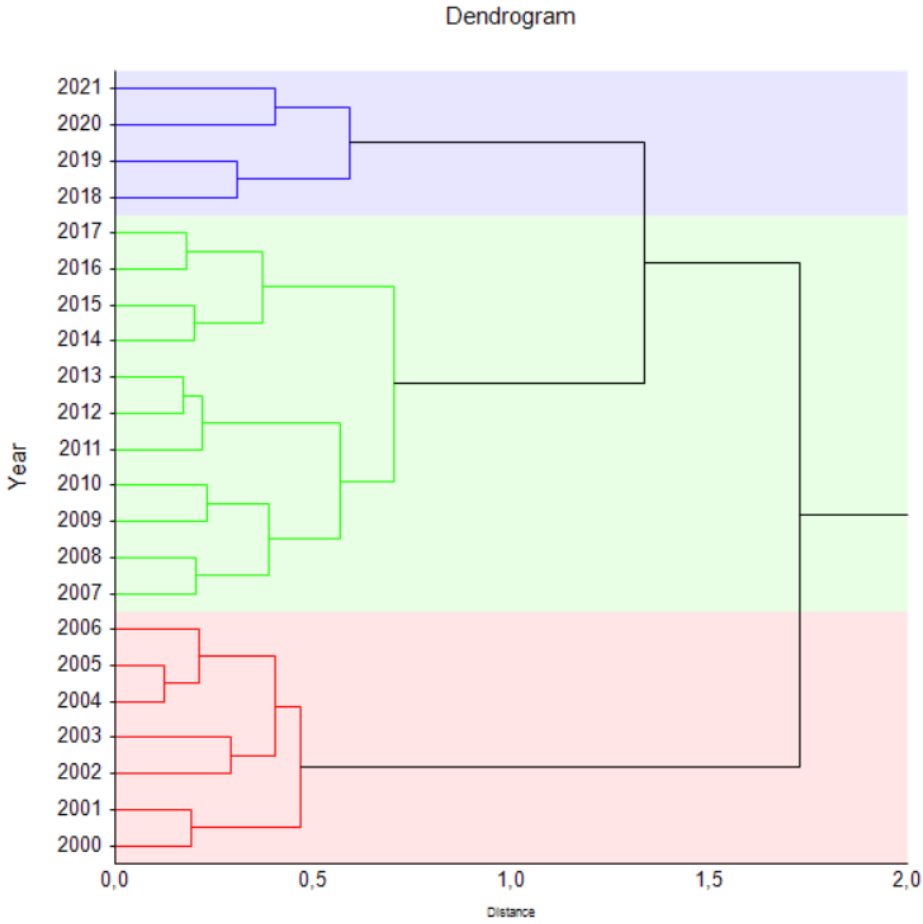

Figure 2: Hierarchical Cluster Analysis Dendrogram

Figure 2 shows the dendrogram created by hierarchical cluster analysis of the number of people per healthcare personnel in Türkiye between 2000-2021. According to these results, 3 clusters were formed. The first cluster, the region shown in red, covers the years 2000-2006. The green region covers the years 2007-2017. It is seen as the years when the number of healthcare personnel per capita decreased significantly. These years are known as the period when practices such as family medicine and general health insurance were implemented. The increase in healthcare personnel can be interpreted as people reaching doctors more easily. The third cluster, the blue region, covers the remaining years 2018-2021. In other words, the number of patients per personnel in these years is similar and is distinguished from other clusters.

4. DISCUSSION

Decreasing Number of People per Healthcare Personnel: Data shows that the number of people per healthcare personnel in Türkiye has decreased over the years, meaning that healthcare personnel have increased faster than the population. This trend indicates that citizens' access to healthcare services has become easier over the years. In particular, the increase in the number of doctors, nurses and other healthcare personnel may have enabled the population to benefit more from healthcare services.

Impact of Health Reforms: Health reforms initiated in the 2000s have led to radical changes in health services. With these reforms, there has been a significant decrease in the number of people per health worker. In particular, practices such as family medicine and universal health insurance have increased access to health services, and this is clearly evident in the graphs.

The Impact of the Pandemic: The fact that the years 2018-2021 are seen as a separate group in the dendrogram can be evaluated as an indicator of the impact of the pandemic period on the healthcare system. With the COVID-19 pandemic, the need for healthcare personnel has increased even more and it has been observed that this situation is reflected in the number of people per healthcare personnel. Rapid changes were made in the healthcare sector during the pandemic and efforts were made to increase the number of healthcare workers in these years.

Rural and Urban Differences: Although this study used general country data, it is important to consider the differences between rural and urban areas. For example, while the need for midwives may be greater in rural areas, there may be a greater need for nurses and doctors in cities. As a general trend, the increase in the number of health care professionals nurses and other health personnel may have helped to reduce the rural-urban difference.

Planning for Healthcare Personnel for the Future: Although the results show that significant improvements have been made in the field of health, considering the increasing population and the complexity of health services, more health personnel may be needed in the future. The increase in the elderly population and new health problems indicate the need for a continuous increase in personnel in the health sector.

5. RESULT

This study shows that changes in the number of people per healthcare personnel have been affected by different health policies and social events over the years. It has been concluded that factors such as healthcare reforms and pandemics have had significant effects on access to healthcare services. According to the data obtained, although access to healthcare services has increased over time in Türkiye, more healthcare personnel need to be trained and the current healthcare infrastructure needs to be strengthened to meet future needs. In this context, demographic changes and new needs in the healthcare field should be taken into account when planning healthcare personnel.

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CHAPTER 3

CAR-T CELLS IN CANCER

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INTRODUCTION

CAR-T is an innovative approach in immunotherapy for treating tumors.CAR is a synthetic receptor consisting of an antigen recognition domain and a T-cell signaling region, and It can be reprogrammed to detect and remove cells that display specific target antigens.(June et al., 2018). Clinical trials have shown that CAR-T cell therapy perfectly targeting CD19, a marker found on B cells, is highly effective in treating several blood cancers, including B-cell lymphoma and acute lymphoblastic leukemia (Neelapu et al., 2017, Parket al.,2018,Wang et al., 2019). FDA approved the preparation of CAR-T cells for refractory large lymphoma, followed by the market introduction of CAR-T products for various hematological tumors. In 2021, the JWCAR029 (Lowenberg et al.,2017)received marketing approval in China for treating large B-cell lymphoma that recurs or is refractory after two or more systemic therapies, becoming the first approved drug in this field. Simultaneously, scientists are investigating novel CAR design strategies aimed at expanding the applicability of CAR-T cell therapy to a broader spectrum of cancer types(Ying et al.,2021). Research has shown that CAR-T cell therapy can also effectively treat challenging cancers like lung and breast cancer(Tian et al.,2020). While CAR-T has shown success in treating blood cancers, it encounters significant obstacles when applied to solid tumors, mainly because of difficulties in accessing and infiltrating the tumor site(Porter et al., 2015). In theory, the ability of modified T cells to localize to tumor sites is limited, and numerous immunosuppressive cells exist within the tumor microenvironment (TME) (Lindoet al., 2020).

Numerous inhibitory factors can weaken the cytotoxic effectiveness of CAR-T during their migration, posing challenges in improving their efficiency and speed in targeting tumors—an area of active research.

1. Identification of Tumor Surface

CAR-T is a new strategy used in tumor treatment, cells are isolated and extracted from human blood, genetically engineered for their anti-tumor activity, and then expanded and reinfused into the patient. The CARs integrate the (scFv) with the transmembrane domain and the intracellular signaling component of the CD3ζ molecule from the T-cell receptor (Sadelain et al., 2013, Kochenderfer et al., 2009). When CAR-T cells bind to their target antigen, the extracellular domain transmits both activation and costimulatory signals to the T cells. This dual signaling enables to directly identify and

eliminate tumor cells, while also secreting cytokines that recruit additional immune cells to the tumor microenvironment. The incorporation of bispecific T-cell engagers, such as CD3, enhances the precision of CAR-T, improving their efficiency in targeting tumors and reducing the likelihood of off-target effects.

2. Choosing Tumor-Selective Surface Antigens for CAR-T Development

The selection of tumor-specific surface markers is fundamental to the efficient design of CAR-T cells. It is necessary to accurately identify antigens that are mainly found on tumor cells. Initially, the identification of these specific antigens relied heavily on databases and literature reviews. However, advancements in gene sequencing technologies and bioinformatics have transformed this process. Now, researchers conduct comparative analyses of tumor and normal tissues through whole-exome sequencing (WES) and RNA sequencing, allowing for the precise identification of regions with tumor-specific mutations. This novel strategy improves the precision of antigen selection, setting the stage for more targeted and effective CAR-T therapies (Zhang et al., 2022). Subsequently, dominant sequences exhibiting strong affinity, as identified through HLA typing and specific antigen-TCR interactions, can be selected.

Ultimately, the gene fragment that demonstrates the most effective stimulation of T-cell activation in laboratory settings is chosen. Clinical validation trials targeting specific antigen regions are currently ongoing, including NCT04749641 for glioma, NCT03715985 for melanoma, and NCT03558945 for pancreatic cancer. These trials aim to assess the safety of the selected antigen-targeting CAR-T cell therapies in treating these malignancies (Challita-Eid et al., 2016). Moreover, PVRL4, an emerging target in epithelial cancers, has been identified using the suppressive subtractive hybridization technique. Recent advancements in differential expression analyses, combined with suppressive subtractive hybridization and continuous gene expression studies, enable researchers to isolate genes with varied expression levels, thereby uncovering gene segments that are closely associated with malignancy. This progress enhances our understanding of tumor biology and opens new avenues for targeted therapies (Sasheva et al., 2017).

3. The Role of Injection Approaches in CAR-T Infiltration

Researchers have investigated the impact of different administration routes on the effectiveness of CAR-T. Targeting atypical rhabdomyoma, B7-H3 cells were delivered using various methods: directly into the tumor (intratumoral, IT), into the cerebrospinal fluid via the ventricle (intracerebroventricular, ICV), and through the bloodstream (intravenous, IV). The study aimed to assess how each delivery method influenced the overall success of the tumor treatment (Theruvath et al., 2020). The results indicated that intracerebroventricular (ICV) injection had a markedly superior effect compared to the other methods, successfully eliminating all established tumors (Theruvath et al., 2020). Simultaneously, it was noted that delivering CAR-T via intratumoral and intracerebroventricular routes did not cause an elevation in systemic inflammatory cytokines. Conversely, intravenous (IV) administration led to increased levels of systemic cytokines. This rise is probably attributable to the activation of monocytes in the bloodstream by CAR-T administered intravenously (Majzner et al., 2022, Brudno et al., 2019, Sheth et al., 2021).

This can lead to severe tissue necrosis, underscoring the intricate relationship between CAR-T cell transport and systemic inflammatory cytokine levels. The method of administration is essential in influencing the immune response during CAR-T therapy. To enhance treatment effectiveness, researchers need to comprehend the pathways of CAR-T cell migration to the tumor site determined by the chosen administration route. When administered intravenously, CAR-T cells navigate through the bloodstream and, upon detecting specific receptors on endothelial cells, they extravasate into the tumor microenvironment. Gaining insights into these pathways can facilitate the optimization of delivery techniques, ultimately improving therapeutic outcomes.

CAR-T cells can actively locate tumor remnants within tissues. Upon identifying the tumor mass, they can further amplify their activity, undergo activation, and ultimately breach the tumor membrane to infiltrate the tumor. However, when it comes to accessing local tumor tissues in the central nervous system, CAR-T cells encounter an additional hurdle: The blood-brain barrier presents a unique challenge for CAR-T cell therapy. Subsequently, they react to secondary signals, mainly from (ICAM-1) and (VCAM-1), facilitating their movement across this barrier (Kanda et al., 1998). Once

CAR-T successfully reaches the cerebrospinal fluid, they are guided to the tumor site by chemokines. In this environment, they aim to penetrate the tumor's protective capsule and must navigate the immunosuppressive factors that characterize the hostile tumor microenvironment. This enables them to unleash their tumor-killing potential while also mobilizing more immune cells to support the eradication of the tumor (Theruvath et al., 2020). Recent findings suggest that CAR-T cells may significantly lose their numbers while navigating through the circulatory system. Administering these cells via intratumoral injection helps to retain a higher count by reducing the chances of migration and systemic toxicity. Additionally, specific tumors situated in particular areas can be accessed through alternative delivery methods. For instance, leveraging the cerebrospinal fluid for central nervous tumors or focusing on lung cancers. CAR-T faces various challenges across all administration methods, including The extracellular matrix surrounding tumor tissue, which serves as an initial barrier for CAR-T infiltration. Research has shown that CAR-T cells modified to express heparanase can degrade key components of heparan sulfate chains within the matrix. This ability enables them to effectively penetrate the tumor's surface while maintaining their therapeutic action. (Neelapu et al., 2017, Caruana et al., 2015). The irregular vascular structure and elevated interstitial fluid pressure within tumors can hinder the anti-tumor activity of CAR-T. An experiment examining photothermal ablation indicated that near-infrared radiation generates enough heat to effectively kill tumor cells, thus boosting the tumor-eradicating potential of CAR-T (Chen et al., 2019). Another cutting-edge approach in nanoengineering involves CAR-T hybridization, which enhances the internal conditions of tumor tissues while preserving the fundamental properties of CAR-T. This strategy also facilitates fluorescence tracking and reconstruction of the tumor microenvironment, ultimately improving the functional effectiveness of CAR-T (Chen et al., 2021).

4. Enhancing CAR T Cell Targeting Through Chemokine Expression Modulation

Chemokines serve as navigational signals, steering CAR T toward the tumor area and facilitating accurate targeting (Mollica Poeta et al., 2019). Different tumor cells can secrete various chemokines, which interact with chemokine receptors on CAR T, thereby attracting them toward the tumor (Brentjens et al., 2013).

Incorporating CCR2b via vectors has been found to greatly enhance the infiltration of mesothelioma-targeted CAR T cells into the tumor microenvironment. This advancement enhances their effectiveness against tumors and supports the treatment of established mesothelin-expressing tumors (Moon et al., 2011).

Tumor cells, especially those related to IL-8, can be identified in mouse models. This IL-8 binds to the CXCR2 receptor, activating downstream signaling proteins and multiple pathways. The CXCR2 receptor is vital for important biological functions, such as the movement and degranulation of neutrophils and lymphocytes (Liu et al., 2020). These biological effects are vital for mediating inflammatory responses and tumor cell proliferation. Thus, CXCR2 are key players in creating a malignant tumor. CAR T that co-express CXCR1 and CXCR2 while targeting αvβ6 integrins demonstrate enhanced migration toward IL-8, retain their cytotoxic capabilities, and substantially improves therapeutic outcomes in xenograft models of ovarian cancers (Whilding et al., 2019).

5. Enhancing Vascular Endothelial Receptor Expression

Vascular endothelial receptors are essential for directing CAR T in the bloodstream. CAR T, these receptors guide the cells to specific sites, such as the bone marrow, especially when CAR T cells exhibit tetra-saccharide sialic acid-Lewis X (sLeX) glycosylation (Mondal et al., 2019). Increasing the autonomous sLeX expression/E-selectin binding capacity of CAR T cells enables them to bind to E-selectin receptors on vascular endothelium, facilitating their transport to the bone marrow (Sanchez-Martinez et al., 2021).

6. TME

While various strategies are available to direct CAR T toward tumor locations, the (TME) poses significant challenges. Factors such as the secretion of immunosuppressive cytokines like (Tregs) and myeloid-derived, PD-L1 expression on tumor cell surfaces, and an overall immunosuppressive metabolic milieu can greatly hinder the accumulation of CAR T at the tumor site. To address these obstacles, possible approaches include (1) reducing immunosuppressive elements by utilizing transgenic methods to express IL-15 directly in cytotoxic cells (Hurton et al., 2016); (2) Improving metabolic conditions for T cells in the tumor microenvironment—such as by providing essential amino acids like arginine and glutamine at the right time—can be

critical for maximizing T cell functionality; (3) Utilizing preconditioning methods to promote CAR T cell colonization. Research indicates that preconditioning with Ox and Cy boosts CAR T cell infiltration into tumors and enhances the effectiveness of immune checkpoint inhibitors (Martin-Otal et al., 2022). Oxaliplatin promotes immunogenic tumor cell death, contributing to improved accumulation of CAR T cells within the tumor. Additionally, it reduces STAT6-mediated immunosuppression, enhancing the responsiveness of cytotoxic T cells and promoting the recruitment of CAR T to the tumor site. However, it's crucial to note that using a combination of preconditioning agents is vital to fully optimize this therapeutic strategy (Nian et al., 2021, Davies et al., 2021).

Furthermore, (TLS) has become an important focus in the field of immunology. TLS consists of endothelial networks and T-cell zones, serving as sites where diverse immune cells congregate. They support the maturation and differentiation of T and B cells, boosting anti-tumor responses through efficient antigen presentation. The presence of TLS has demonstrated significant predictive value for cancer prognosis. Recent studies indicate that TLS can be identified in various solid tumors, including hepatocellular carcinoma (Calderaro et al., 2019, Meylan et al., 2021). These studies have established that tertiary lymphoid structures (TLS) facilitate the infiltration of CAR T. TLS boosts CAR T cell infiltration by (1) offering an abundance of immune cells for antigen presentation and a stable endothelial network, thereby enhancing CAR T cell survival (2) Facilitating the ongoing influx of naive immune cells is crucial for sustaining immune responses, which can substantially improve the effectiveness of CAR T (Meylan et al., 2021). Strategies to promote TLS formation include normalizing vasculature in melanoma microenvironments and employing STING agonists (Chelvanambi et al., 2021). Moreover, utilizing a scaffold made from diverse biomaterials can promote TLS formation by enabling immune cell infiltration.

7. Boosting Cytokine Release and Sustaining Immune Memory

Local CAR T cell infiltration on its own may not be adequate to guarantee total tumor destruction during the elimination phase. As a result, CAR T need to produce an increased quantity of cytokines to attract additional immune cells, including dendritic cells and T cells, to the tumor microenvironment. Notable cytokines that have been effectively expressed

include CCL21, IL-7, CCL19, CXCL11and CCR2b (Adachi et al., 2018, Pang et al., 2021).

While the short-term effects of drugs can lead to considerable tumor reduction, their transient nature in the human body often results in the exhaustion of CAR T. Merely achieving short-term tumor clearance is not the ultimate objective. To address this, some researchers are investigating strategies to enhance CAR T cells with intrinsic immunity by examining the mechanisms underlying their exhaustion (Pietrobon et al., 2021). At present, the capacity for immune memory can be enhanced using immune stimulants such as RN7SL1 and STING. These agents stimulate endogenous RNA, promoting the proliferation and differentiation of CAR T into effector memory cells. This enhances the drug's effectiveness in the body and helps maintain anti-tumor efficacy over time (Johnson et al., 2021, Xu et al., 2021).

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CHAPTER 4

APPROACH TO IRON DEFICIENCY ANEMIA

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INTRODUCTION

Anemia is when the hemoglobin concentration is below average for a given age and gender. The World Health Organization (WHO) defines anemia as a hemoglobin level below 12.0 g/dL in women and below 13.0 g/dL in men (Cappellini, and Motta, 2015). Hemoglobin concentrations exhibit variability influenced by gender, ethnicity, and physiological states. The definition of the lower threshold for normal hemoglobin levels has been revised to incorporate considerations like ethnicity, gender, and age. Anemia typically results from a combination of causes and should not be considered an isolated condition. Accurate classification and diagnosis of anemia require an integrated evaluation of hematological parameters, underlying pathological processes, and the patient's clinical history. While numerous factors contribute to anemia, iron deficiency is the most common cause. Iron is one of the most abundant elements on Earth, and iron deficiency is one of the most prevalent nutritional deficiencies worldwide, affecting approximately 4 to 6 billion people (Stelle, et al. 2019). Iron deficiency anemia (IDA) is a prevalent form of anemia globally, defined by insufficient iron levels, which result in decreased hemoglobin concentration. While IDA can affect individuals across all age groups, it is widespread among women of reproductive age, infants, and young children due to their higher iron needs. Iron is a key component in producing hemoglobin, a protein responsible for oxygen transport in the bloodstream.

Iron deficiency disrupts oxygen delivery to various tissues, leading to symptoms such as fatigue, pallor, and dyspnea. The causes of iron deficiency are diverse and may include insufficient dietary intake, impaired absorption, chronic blood loss, and increased iron requirements during periods of growth or pregnancy. Timely diagnosis and appropriate treatment are essential to avoid potential long-term health issues.

1. IRON METABOLISM

1.1. Distribution of Iron in the Body

Iron plays a crucial role as a micronutrient in the human body, fulfilling various functions and being distributed in a balanced manner across tissues (Gkouvatsos, et al. 2012).

- **Hemoglobin (65-70%):** The majority of the body's iron is concentrated in red blood cells, which play an essential role in oxygen transport.
- **Ferritin and Hemosiderin (15-30%):** These are the storage forms of iron located in the liver, spleen, and bone marrow. Ferritin is water-soluble, while Hemosiderin is less soluble.
- **Myoglobin (5-10%):** This protein, found in muscle cells, is involved in storing and utilizing oxygen.
- **Enzymes (1-5%):** Iron-containing enzymes, such as cytochromes, catalase, and peroxidase, are critical for energy production and cellular defense mechanisms.
- **Plasma Iron (<1%):** Iron in the bloodstream is transported via a protein called transferrin**.**

1.2. Iron in Foods and Its Absorption:

Iron in foods is typically found in ferric hydroxide, ferric protein, and heme-protein complexes. The amount of iron and its absorption rate in foods vary depending on the source. Liver and meat products are rich in iron, whereas vegetables, eggs, milk, and dairy products contain lower amounts. A typical diet provides about 10-15 mg of iron, of which 5-10% is absorbed. In cases of iron deficiency or during pregnancy, the absorption rate can increase to 20-30% (Yiannikourides, et al. 2019). Some of the organic iron from the diet is absorbed in its heme form, while a portion is converted to inorganic iron in the intestines and excreted in the feces. Iron absorption primarily occurs in the duodenum. Heme iron, which is found in animal-based foods, particularly meat and fish, has a high bioavailability due to its efficient absorption. Heme iron is absorbed through specific receptors in duodenal enterocytes, and free iron is released after digestion of the heme molecule. Non-heme iron, found in plant-based foods, is influenced by various factors that affect its absorption. Vitamin C enhances absorption, while substances like phytates, tannins, and calcium may inhibit it. Non-heme iron is first reduced to its ferrous (Fe^{2+}) form by the ferri-reductase enzyme and then absorbed into enterocytes via the Divalent Metal Transporter 1 (DMT1) protein (Yiannikourides, et al. 2019).

1.3. Iron Transport into Cells and Its Utilization:

1.3.1. DMT1 (Divalent Metal Transporter 1):

Non-heme iron is absorbed into enterocytes through the DMT1 transporter. Heme iron, on the other hand, enters cells through heme transporters. Once inside the cell, free iron can bind to storage proteins such as ferritin or Hemosiderin (Nemeth, and Ganz, 2021).

1.3.2. Iron Binding:

Iron within the cell can be bound to ferritin for storage. This helps the body maintain iron homeostasis.

1.3.3. Iron Transport:

Iron passes from the intestine's enterocyte cells into the bloodstream through the ferroportin transporter protein. Ferroportin facilitates the export of iron, and its activity is regulated by the hormone hepcidin (Nemeth, and Ganz, 2021).

1.4. Iron Transport in the Body:

Iron in the serum is transported by a carrier protein called “transferrin”. Transferrin enables the delivery of iron to cells, allowing it to be used in various body parts. The transferrin receptors on the cell surface recognize the transferrin-bound iron and facilitate its uptake into the cell. This mechanism is crucial in regulating iron metabolism and helps prevent iron deficiency.

1.5. Factors Affecting Iron Absorption:

1.5.1. Factors that Enhance Iron Absorption (Piskin, et al. 2022):

- **Vitamin C:** Vitamin C is a powerful nutrient that significantly enhances the absorption of non-heme iron. It promotes the reduction of iron to its ferrous state, thereby improving its bioavailability for absorption in the body.
- **Acidic Environment:** Stomach acid is essential for iron absorption. Iron is more easily reduced in an acidic environment, becoming suitable for absorption.
- **Ferrous (Fe^{2+}):** Iron Utilization: Ferric (Fe^{3+}) iron must be reduced to its ferrous (Fe^{2+}) form to be absorbed through the intestines.

- **Heme Iron**: Heme iron, predominantly present in animal-derived foods such as meat, poultry, and fish, is absorbed more efficiently in the intestines than non-heme iron.

1.5.2. Factors that Decrease Iron Absorption (Piskin, et al. 2022):

- Phytates and Tannins: Phytates (especially in legumes) and tannins (found in tea) can inhibit the absorption of non-heme iron.
- Calcium: Calcium can interfere with iron absorption, particularly when consumed in large amounts, reducing iron uptake efficiency.
- Phosphates and Oxalates: Oxalates, found in foods such as spinach, rhubarb, and certain nuts, and phosphates can decrease iron absorption.
- Fiber: Foods high in fiber can limit iron absorption in the intestines. Excessive fiber intake, in particular, can negatively impact the absorption process.
- Low Stomach Acid (Achlorhydria): Stomach acid is required for the reduction of iron from its ferric (Fe^{3+}) form to ferrous (Fe^{2+}) form. Absorption is reduced in conditions of acid deficiency (e.g., in individuals using proton pump inhibitors).
- Antacids and Proton Pump Inhibitors (PPIs): Antacids that neutralize stomach acid and proton pump inhibitors (PPIs) that suppress acid production can hinder the reduction of iron and subsequently reduce its absorption (Hamano, et al. 2020).
- Intestinal Diseases: Conditions like celiac disease, Crohn's disease, inflammatory bowel disorders, and a history of small intestine surgeries can adversely impact iron absorption.

1.6. Utilization of Iron in Cells:

Most iron transported in circulation bound to transferrin reaches erythroid precursor cells, which are used for hemoglobin synthesis. This process primarily occurs in the bone marrow. The remaining 10-15% of iron is utilized in other biological functions, including synthesizing myoglobin in muscle tissue, forming enzymes, and assembling cytochromes. Additionally, iron is stored in liver parenchymal cells and macrophages of the reticuloendothelial system (Yiannikourides, and Latunde-Dada, 2019). A significant portion of the iron in macrophages of the reticuloendothelial system is derived from hemoglobin following the breakdown of dead

erythrocytes. This process is crucial for maintaining the sustainability of the body's iron cycle.

1.7. Iron Storage:

Iron must be stored appropriately to prevent excessive iron accumulation in the body. To fulfill this function, iron is stored by binding to proteins such as ferritin and Hemosiderin. The stored iron is released into the bloodstream and transported to needy tissues when required. This regulation is vital in maintaining iron balance and preventing toxic effects.

1.8. Iron Excretion:

Iron excretion in the body is quite limited and typically occurs through physiological processes. During the renewal of enterocytes, iron bound to ferritin in these cells is also excreted from the body. Additionally, sweat, bile, and urine can lose small amounts of iron. However, blood loss can lead to considerable iron depletion, particularly in significant amounts. The average daily iron loss in a healthy adult male is approximately 1.0 mg. In premenopausal women, this amount can increase to around 1.5 mg. Especially during each menstrual cycle, an average blood loss of 30-40 ml leads to the loss of approximately 15 mg of iron. Consequently, women are at a higher risk of iron deficiency (Hunt, et al. 2009).

2. Iron Deficiency Anemia

Iron deficiency anemia (IDA) is the most common nutritional deficiency and the primary cause of anemia worldwide. This condition particularly affects children living in developing countries and women of reproductive age (Means, 2013).

2.1. Clinical Features of IDA

During the process of iron deficiency, the body's iron stores gradually decrease before anemia develops, eventually becoming depleted. This primarily occurs through the consumption of iron reserves such as ferritin and Hemosiderin (Gkouvatsos, et al. 2012). When there is a disruption in iron balance in the body, these reserves are initially utilized. However, if iron intake is insufficient or if iron loss accelerates, the stored iron is exhausted, leading to a significant decrease in serum ferritin levels. At this stage, anemia has not yet developed, but laboratory tests can reveal a reduction in the body's

iron reserves. If this process progresses further, the synthesis of hemoglobin, which affects the tissues' oxygen-carrying capacity, is negatively impacted, ultimately leading to the development of iron deficiency anemia (Percy, et al. 2017). The depletion of iron stores before the onset of anemia is critical for early diagnosis and intervention. During this phase, accurate diagnosis and timely treatment are essential in preventing the clinical consequences of iron deficiency. Iron deficiency anemia usually develops gradually, with initial symptoms being mild and becoming more severe as the condition progresses. Clinical manifestations vary depending on the severity and duration of the iron deficiency (Stelle, et al. 2019).

General Symptoms:

- **Fatigue and Weakness:** Commonly seen due to lack of energy.
- **Pallor:** Occurs in the skin and mucous membranes due to reduced oxygen-carrying hemoglobin.
- **Dizziness and Syncope** Develop due to insufficient oxygen supply to the brain.
- **Palpitations:** The heart works harder to compensate for the lack of oxygen.
- **Shortness of Breath:** Becomes more pronounced during physical exertion as the body's oxygen demand increases.
- **Headaches and Difficulty Concentrating:** The brain is particularly sensitive to oxygen deficiency.

Specific Symptoms:

- **Koilonychia:** Iron deficiency can lead to significant changes in the structure of nails. Initially, this is seen as the loss of the natural convex shape of the nails. Over time, it progresses into a condition called "spoon nails" (koilonychia), characterized by inwardly concave nails. This nail deformity is considered a distinctive sign in individuals with iron deficiency.
- **Angular Stomatitis:** Sensitivity and sores at the corners of the mouth.
- **Atrophic Glossitis:** Burning sensation on the tongue, with a shiny and smooth appearance.
- **Pica:** The craving for non-nutritive substances like dirt, ice, or paper.
- **Hair Loss:** Thinning and shedding of hair.

- **Dysphagia:** In some cases, iron deficiency can cause mucosal protrusions in the esophagus, particularly in the area just below the cricoid cartilage. This condition, known as dysphagia, can be part of the Patterson-Kelly or Plummer-Vinson syndrome.

Systemic Effects:

- **Weakened Immunity:** Iron deficiency can increase susceptibility to infections.
- **Muscle Weakness:** Due to impaired energy metabolism, muscle strength may decline.

2.2. Causes of IDA

Iron deficiency anemia is the most prevalent form of anemia globally and can arise from various etiological factors. These causes can be classified into four primary categories: inadequate iron intake, increased iron demand, iron loss, and impaired iron absorption (Killip, et al. 2007).

2.2.1. Insufficient Iron Intake:

Insufficient dietary intake of iron is a common cause of iron deficiency anemia.

- Nutritional Deficiency: In developing countries, the consumption of iron-poor foods is a common issue.
- Vegetarian/Vegan Diets: Plant-based iron's bioavailability is lower than animal-derived sources.

2.2.2. Increased Iron Requirement:

Certain physiological conditions can increase the body’s iron requirement:

- **Pregnancy and Lactation:** Increased iron is required to meet the demands of both the mother and the fetus.
- **Growth Phases:** During childhood and adolescence, the body's rapid growth leads to a higher iron requirement.
- **Erythropoietin Therapy:** Iron utilization increases as erythropoiesis (red blood cell production) is stimulated.

2.2.3. Iron Loss:

Excessive loss of iron can lead to the rapid depletion of iron stores. (Killip, et al. 2007).

- **Gastrointestinal Bleeding:** Conditions such as peptic ulcers, esophageal varices, aspirin and other NSAID use, partial gastrectomy, stomach cancer, cecal cancer, colorectal cancer, rectal cancer, hookworms, angiodysplasia, hemorrhoids, and diverticulosis can lead to chronic blood loss (Nielsen, et al. 2016).
- **Menstrual Bleeding:** Heavy and prolonged menstrual periods in women can increase iron loss (Percy, et al. 2017).
- **Trauma or Surgery:** Acute bleeding resulting from trauma or surgery can also lead to rapid iron loss.

2.2.4. Iron Absorption Disorders

Inadequate absorption of iron from the intestines is also a significant factor in iron deficiency (Alkdede, et al. 2020):

- **Celiac Disease:** Damage to the intestinal mucosa in celiac disease reduces iron absorption.
- **Gastrointestinal Surgery:** Gastrectomy, or the removal of parts of the intestines, reduces the absorptive surface area, leading to decreased iron absorption.
- **Inflammatory Bowel Diseases:** Chronic inflammation in conditions like Crohn's disease and ulcerative colitis can impair iron absorption.

2.3. Laboratory Diagnosis in IDA

Laboratory tests play a crucial role in diagnosing iron deficiency anemia, helping to support clinical findings and assess the severity of the deficiency. Basic parameters, such as hemoglobin and hematocrit levels, indicate the presence of anemia, while specific tests that evaluate iron metabolism confirm the diagnosis and assist in understanding the underlying cause (Cappellini, et al. 2020).

2.3.1. Complete Blood Count (CBC)

- **Anemia:** The severity of anemia can vary from person to person. Sometimes, anemia may be discovered incidentally while

evaluating a patient for another condition. During this process, anemia may present mildly without obvious symptoms. Hematocrit levels are typically low and correlate with hemoglobin levels. In terms of erythrocyte indices, the Mean Corpuscular Volume (MCV) is reduced (<80 fL), indicating microcytic anemia. The Mean Corpuscular Hemoglobin (MCH) is also decreased, reflecting hypochromia. Additionally, the Red Cell Distribution Width (RDW) is increased (>15%), indicating heterogeneity in cell size (Killip, et al. 2007).

- **Leukocytes:** The leukocyte count is generally within the normal range. However, in chronic iron deficiency anemia, the granulocyte count may slightly decrease.
- **Platelets:** Reactive thrombocytosis is commonly observed in iron deficiency anemia (Maryala, and Vaddiparti, A. (2021). In adults, when thrombocytopenia is present, it should be considered a potential sign of an early response to iron therapy or an indication of an underlying condition (such as iron deficiency anemia resulting from bleeding associated with immune thrombocytopenia).

2.3.2. Serum Iron Parameters:

Serum iron levels are decreased (<30 mcg/dL). Total Iron Binding Capacity (TIBC) is elevated (>400 mcg/dL), indicating that there are more available binding sites for iron. Transferrin Saturation is reduced (<15%), indicating a decrease in iron transport capacity (Bouri, and Martin, 2018).

2.3.3. Ferritin Level:

A low serum ferritin level (<15 ng/mL) indicates depletion of iron stores and is the earliest sign of iron deficiency anemia (Bouri, and Martin, 2018).

2.3.4. Peripheral Smear:

Erythrocytes appear microcytic (small) and hypochromic (pale). Anisocytosis (variation in red blood cell size) and poikilocytosis (abnormal shapes) may be observed (Bouri, and Martin, 2018).

2.3.5. Bone Marrow Examination:

A bone marrow examination may be required to evaluate iron stores (such as Hemosiderin) in rare instances. However, serum ferritin levels generally provide adequate information for assessment (Bouri, and Martin, 2018).

2.3.6. Further Investigations:

- **Gastrointestinal Bleeding Investigation:** A fecal occult blood test may be performed as a screening tool. If gastrointestinal diseases, bleeding, or cancer are suspected, endoscopy and colonoscopy should be performed to screen the entire gastrointestinal system (Killip, et al. 2007).
- **Evaluation of Menstrual Irregularities and Bleeding:** Patients with prolonged and excessive menstrual bleeding should be evaluated for gynecological cancers by an obstetrics and gynecology specialist. Additionally, patients with significant bleeding should be assessed for underlying bleeding disorders (Killip, et al. 2007; Percy, et al. 2017).

2.4. Treatment of IDA

The treatment of IDA aims to correct the underlying cause of the deficiency and replenish iron stores. The treatment plan is tailored to the patient's characteristics, the severity of the anemia, and the underlying cause (Alkdede, et al. 2020).

2.4.1. Dietary Modifications:

A key component of treating iron deficiency is adopting a diet high in iron content:

- **Animal-Based Foods:** Red meat, chicken, and fish, which are sources of heme iron, should be prioritized. This type of iron is more readily absorbed in the intestines.
- **Plant-Based Foods:** Foods containing non-heme iron, such as spinach, legumes, and whole grains, can be consumed. However, the absorption of this type of iron is lower.

- **Vitamin C Intake**: Consuming foods rich in Vitamin C, such as oranges, lemons, and tomatoes, enhances iron absorption.
- **Avoiding Foods that Inhibit Iron Absorption:** Foods such as tea, coffee, and those high in calcium should be avoided, as they reduce iron absorption.

2.4.2. Pharmacological Treatment

Oral or parenteral iron supplements are used to correct the deficiency.

- **Oral Iron Supplements:**

Ferrous sulfate, ferrous fumarate, or ferrous gluconate are commonly preferred. A daily dose of 100-200 mg of elemental iron (typically administered in 2-3 doses) is recommended (Nielsen, OH., et al, 2016). Taking the supplement on an empty stomach enhances absorption, but it can be taken with food if gastrointestinal side effects occur. The absorption is further supported when taken with vitamin C. The most common side effects include constipation, nausea, abdominal pain, and black-colored stool. Even after hemoglobin levels normalize, iron therapy typically lasts 3-6 months to replenish iron stores.

- **Parenteral Iron Supplements:**

Parenteral iron therapy is preferred when oral iron treatment is poorly tolerated or ineffective. It is indicated in cases of severe anemia, malabsorption syndromes (such as celiac disease and Crohn's disease), and chronic blood loss. The most commonly used intravenous iron preparations include iron sucrose, dextran, and carboxymaltose. There is a risk of allergic reactions, so caution is necessary during administration. Other potential side effects include anaphylaxis, shock, hypotension, headache, dizziness, urticaria, nausea, and vomiting (Asma, et al. 2009; Nielsen, OH., et al, 2016).

2.4.3. Treatment of Underlying Causes

If the primary cause of iron deficiency is not addressed, anemia may recur. Therefore, the underlying cause must be treated**:**

- **Correcting the Source of Bleeding:**

If gastrointestinal bleeding is present, it should be managed with endoscopic or surgical methods. In cases of heavy menstrual bleeding, hormonal therapy or other gynecological interventions may be required.

• Managing Malabsorption Disorders:
Conditions like celiac disease should be treated appropriately.

2.5. Treatment Monitoring

• Laboratory Monitoring:

Hemoglobin and hematocrit levels are monitored to assess the response to treatment (an increase is usually expected within 2-4 weeks). Serum ferritin levels are tracked to determine if iron stores are replenished.

• Treatment Success:

After treatment, the goal is for hemoglobin levels to return to normal and for ferritin levels to be ≥50 ng/mL.

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CHAPTER 5

GLOBAL HEALTH TOURISM MARKET: RECENT DEVELOPMENTS

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INTRODUCTION

According to the World Health Organization (WHO), health is a comprehensive state of physical, mental, and social well-being, extending beyond the mere absence of illness or infirmity. Health is a fundamental human right, and governments have made commitments to safeguard and advance it, most recently within the context of the Sustainable Development Goals (SDGs). A healthy population drives economic growth and is a critical component of both national and global security.

Health has been a primary motivator for travel since the beginning of human history. As tourism has become a crucial part of modern life, health tourism has grown steadily in popularity. Medical tourism, a specific type of health tourism, generates significant foreign currency for countries due to its high spending per tourist. The past 25 years have witnessed new trends in healthcare consumption and delivery, driven by factors such as increased global mobility, medical advancements, financial investments, and regulatory changes. A major development in the expanding healthcare industry is medical tourism, where patients travel internationally for medical treatment. This involves individuals seeking healthcare outside their home countries, often as part of a tourism package that includes a range of medical services and other offerings (Ates & Sunar, 2024).

Over the past several decades, the demographic composition of OECD countries has undergone a notable shift, with the proportion of the population aged 65 and older has resulted in a significant rise from less than 9% in 1960 to 18% in 2021. Prior to the COVID-19 pandemic in 2019, OECD countries allocated an average of 8.8% of their gross domestic product (GDP) to healthcare expenditures. This figure remained relatively stable compared to previous years, dating back to 2013. However, by 2021, healthcare spending had increased significantly, reaching 9.7% of GDP (OECD, 2024).

The Medical Tourism Association reports that over 14 million individuals travel abroad for medical treatment annually. The increasing number of patients seeking regular checkups is a significant driver of the global medical tourism market. Regions such as Dubai, Singapore, and Thailand are particularly attractive due to their lower treatment costs. Singapore, for example, receives 500,000 medical tourists per year, with Indonesia accounting for half of this number. The anticipated growth in the number of medical tourists and the ongoing demand for cosmetic and medical

procedures are expected to further fuel the expansion of the global medical tourism market. The affordability of medical procedures and surgeries is another key factor contributing to the market's growth (www.presedencereaserch.com, 2024).

BODY

With populations rapidly aging, healthcare systems face a growing need for long-term care (LTC) services to support daily activities of older adults. This necessitates a multi-pronged approach. First, promoting healthy aging through preventive measures is crucial. Second, improving the quality and accessibility of LTC options is essential. Finally, balancing social adequacy with financial sustainability is critical for long-term system viability. To navigate these challenges, effective assessment of health system performance becomes paramount. Evaluating care quality, identifying resource allocation needs, and measuring progress towards key objectives allows policymakers to optimize healthcare delivery. Additionally, building health system resilience is vital. The ability to absorb and adapt to unforeseen events like pandemics requires robust systems that prioritize resilience as a core objective. Furthermore, addressing health workforce shortages necessitates increased training initiatives, improved working conditions for staff retention, and innovative solutions leveraging technology to meet the growing demand for care (OECD, 2024)

Medical tourism involves traveling to another country for healthcare services, such as medication, surgery, or general medical treatments, often because of lower costs and shorter wait times (Enderwick & Nagar, 2011, p. 329). Medical tourists often choose to seek healthcare in foreign countries due to high treatment costs, lengthy wait times, the unavailability of certain treatments within their home countries, and other factors (Brady, 2007, p. 1098). Individuals who seek medical care in a foreign country can be categorized as consumers of medical tourism services. This type of tourism encompasses both the medical procedures and any associated leisure activities, such as sightseeing or relaxation, often undertaken before or after treatment (Mohsen et al., 2018).

The rising cost of healthcare is a major concern globally, affecting its quality, accessibility, and affordability. The COVID-19 pandemic exacerbated these issues, increasing costs in areas like staffing while also highlighting the need for affordability and access. Inflation is driving up the prices of drugs,

medical supplies, and other healthcare resources, further straining budgets. The pandemic also led to a backlog of healthcare needs, putting pressure on funding and forcing prioritization of care. In response, healthcare providers are exploring more affordable and efficient delivery models, including those utilizing technology and innovation. Rising healthcare costs are influenced by both higher labor costs and widespread inflation. In the U.S., the highest inflation in four decades has exacerbated healthcare price increases, which historically outpace overall inflation. Health insurance premiums surged by 28% in 2022, far exceeding the inflation rate, raising concerns about affordability for many consumers (Deloitte, 2024).

In academic literature, the term "health tourism" is often employed interchangeably with "medical tourism" or "destination tourism" (Hall, 2011; Pesonen, 2011). The modern era has witnessed the rise of medical tourism as a dynamic market segment. The growth of this sector is attributable to the proliferation of high-quality medical care (Ryndach et al. 2024). The attractiveness of a country or region as a medical tourism destination is primarily influenced by its tourist appeal and the efficacy of the medical services provided by healthcare organizations (Lade et al. 2020).

Individuals aspire to achieve a holistic state of well-being, encompassing physical, mental, and spiritual dimensions (Chen et al., 2013). The convergence of health and tourism sectors, catering to both healthy and ill travelers, offers broader economic benefits at both global and local levels (Hofer et al., 2012). Positive health behaviors exhibited by tourists can contribute to enhanced mental health and overall well-being (Dale et al., 2014).

The intricate relationship between health and tourism encompasses a wide range of direct and indirect impacts, encompassing the tourism workforce, safety and security, sanitation and hygiene practices, and socioeconomic factors. The diverse health benefits and risks associated with tourism are influenced by various factors, including tourism types, travel modes, individual traveler characteristics, behavior, the tourism workforce, host-community conditions, the quality of healthcare systems, public health measures, and existing safety and security protocols (WHO, 2022).

Health tourism encompasses three distinct subsectors: medical, wellness, and spa tourism. Medical tourism involves individuals traveling specifically to receive medical treatment for existing conditions. Wellness

tourism focuses on preventative and restorative practices aimed at maintaining or improving overall health and well-being. Spa tourism centers on holistic approaches to healing, relaxation, and beautification, with services encompassing both prevention and curative treatments. Medical tourism addresses illness on one end, while wellness tourism promotes proactive health management on the other. Spa tourism occupies a middle ground, aiming to prevent illness and sustain overall health. General tourism plays a complementary role by providing accommodation and amenities that cater to individuals with healthcare needs, disabilities, or existing health conditions (Mainil et al. 2017).

The global medical tourism industry experienced a significant expansion, increasing by 17% between 2018 and 2019. In 2019, the market value was estimated to be between $65 billion and $80 billion USD (Globalmedik, 2020). Projected growth rates from 2019 to 2025 suggest a sustained increase of over 12% annually. Furthermore, approximately 45 million medical tourism trips were undertaken in 2019 (Knoema, 2020).

Table. 1 Medical Tourism Market Size and Forecast 2024 to 2034.

Year	Market Size ($Billion)
2023	$29.43
2024	$35.78
2025	$43.51
2026	$52.91
2027	$64.34
2028	$78.24
2029	$95.34
2030	$115.69
2031	$140.68
2032	$171.06
2033	$206.01
2034	$252.94

Source: www.presedenceresearch.com (2024).

The global medical tourism market, valued at USD 35.78 billion in 2024, is projected to experience substantial growth, reaching an estimated USD 252.94 billion by 2034, with a compound annual growth rate of 21.6% over this decade. The North American region dominated the global medical tourism market in 2023, accounting for 26% of the total market share. Among the geographic regions, the Asia-Pacific region is projected to exhibit the most rapid growth rate from 2024 to 2034. When considering treatment types, the cancer treatment segment constituted the largest revenue share in 2023. The cardiovascular treatment segment is anticipated to experience significant growth during the forecast period (www.presedencereaserch.com, 2024).

The United States represents the largest global consumer of healthcare services. Deloitte Consulting (2008) projected a significant increase in outbound medical tourism among Americans, with estimates indicating that 750,000 individuals sought healthcare abroad in 2007, and this number was anticipated to grow by 100% to reach six million outpatients by 2010. On the supply side, both developed and developing countries are actively competing to attract medical tourists, with over 35 nations serving more than one million such patients annually. In 2008, the United States also received a substantial influx of international patients, with over 400,000 non-US residents seeking healthcare and contributing approximately $5 billion to the US healthcare economy (Cattaneo, 2009).

Health tourism comprises approximately 5% of general tourism within the EU28, contributing roughly 0.3% to the EU's overall economy. In comparison to general tourism, health tourism exhibits a significantly higher domestic component. Expanding the share of health tourism within the EU has the potential to mitigate tourism seasonality, enhance sustainability and labor quality, and contribute to reducing healthcare costs by preventive measures and decreased pharmaceutical consumption (Mainil et al. 2017).

Health tourism encompasses two main types: wellness tourism and medical tourism. Wellness tourism focuses on improving and balancing various aspects of human life, including physical, mental, emotional, occupational, intellectual, and spiritual well-being. Wellness tourists primarily engage in activities like fitness, healthy eating, relaxation, and healing treatments to promote a healthy lifestyle. Medical tourism involves utilizing evidence-based medical resources and services, both invasive and non-invasive, for diagnosis, treatment, cure, prevention, and rehabilitation. The

UNWTO General Assembly in 2017 adopted recommendations for defining health tourism, medical tourism, and wellness tourism. People are spending more time on leisure activities, including healthcare and prevention, due to increased free time and disposable income. This supports healthy lifestyle motivations. Medical tourism is often driven by overburdened healthcare systems and public health insurance. Long-term care expenditures have risen as populations age, requiring more healthcare and social support. Urbanization is a significant public health challenge. Over half of the world's population now lives in cities, and this is expected to increase to two-thirds by 2050. Urban living is linked to health conditions and chronic diseases, leading to a growing demand for healthier travel, natural alternatives, and escapism. Historically, medical tourism has involved individuals traveling from lower-income countries to higher-income countries with better medical facilities and professionals. However, this trend is now shifting, with centers of medical excellence emerging and attracting patients regionally. Many countries participate in medical tourism as both importers and exporters of healthcare services. The primary countries of origin for medical tourists are in North America and Western Europe, while exporting countries are located across all continents. Countries and hospitals often specialize in particular medical procedures (World Tourism Organization and European Travel Commission, 2018).

While there are different definitions of medical tourism, it's generally considered a type of health tourism, alongside wellness tourism. The key difference lies in the fact that medical tourism involves addressing a medical issue through diagnosis and treatment, making it a reactive form of health tourism. In contrast, wellness tourism is proactive, focusing on preventing or maintaining health through alternative methods without requiring specialized clinics, medical professionals, or invasive procedures (Fetscherin & Stephano, 2016).

As international travel, transportation, and cooperation become easier, the availability of higher-quality or lower-cost healthcare in different countries attracts patients. This has significantly increased the importance of health tourism. The combination of affordable, high-quality healthcare with a beach or cultural holiday is appealing to patients with various budget constraints. Health tourism can be defined as individuals traveling to another country for curative, preventative, rehabilitative, or health-promoting services.

The health tourism sector includes four concepts: medical, thermal health, elderly, and disability tourism (Ozad & Celebi, 2024).

The increasing strain on healthcare systems, rising expenditures, and insurance premiums in many countries have led consumers to seek more affordable care. This has contributed to the growth of medical tourism. Medical service providers have recognized the potential of this market, offering opportunities for access to quality care, safety, privacy, lower-cost procedures, and procedures not available domestically. Countries have developed specialties in various fields, such as cosmetic surgery, dentistry, orthopedics, cardiovascular procedures, reproductive health, and nanotechnology, to attract specific patient groups and gain a competitive advantage. For example, Hungary is known for its dentistry expertise, South Korea is a popular destination for cosmetic surgery and cardiology, and Mexico is expanding its offerings beyond cosmetic procedures to include orthopedics (WTTC, 2019).

Medical tourism has become a lucrative industry for countries and private institutions, offering opportunities for international healthcare trade, foreign exchange generation, addressing personnel shortages, and improving healthcare system efficiency. Thailand, Singapore, India, and Malaysia are leading medical tourism destinations due to their affordable costs, high-quality JCI-accredited care, minimal wait times, access to specialized surgeons, comprehensive post-surgery support, and attractive tourist features (Collins et al., 2022, p. 488).

Within the EU, health tourism contributed to 233.7 million domestic guest nights and 16.7 million international guest nights, totaling 250.4 million overnight stays. The average domestic trip duration was 4.1 nights, whereas international travelers typically stayed for 8.5 nights. When comparing international health tourism arrivals to general tourism across the EU28, the average revenue per trip exhibits a minimal difference, with health tourism generating €791 per trip compared to €783 for general tourism. However, significant variations exist among individual countries. Finland, for instance, experiences revenues per trip that are 39% lower than the average for all international arrivals, while Italy demonstrates a 28% higher revenue per trip for health tourism compared to the overall health tourism average (Mainil et al. 2017).

The average healthcare costs per person have increased in most countries since 2020. The United States has the highest healthcare spending per individual, rising to over $12,500 in 2022, which is about 17% of its GDP. This is significantly more than other countries like Belgium, Denmark, and Finland, which spend around 2% of their GDP on healthcare. U.S. healthcare costs are projected to increase by another 36% to over $17,000 per person by 2027 (The Economist, 2023). Hospital costs per patient in the U.S. rose by 22.5% since the pandemic, with labor costs accounting for nearly 25% of this increase. A significant number of healthcare workers left their jobs during the pandemic, leading to widespread staffing shortages and increased workload for those remaining (Leo & Satija, 2023).

The high cost of cosmetic surgery in the United States, with average prices between $4,500 and $12,500, has driven many individuals to pursue cosmetic procedures overseas. The prospect of receiving equivalent care at a 50% reduction in cost is a major incentive for medical tourism, contributing to the growth of the global market. Asian nations have emerged as prominent destinations for medical tourism, primarily due to their lower treatment costs. India, ranked tenth globally in the Medical Tourism Index, is a particularly popular choice. The cost of bypass surgery in India, at approximately $10,000, is significantly lower than the corresponding cost of $113,000 in the United States. This substantial cost differential is a driving force behind the expanding medical tourism market in the Asia-Pacific region. The North American region maintains a dominant position in the global medical tourism market as of 2023. The growth of this market is primarily driven by the increasing number of international patients seeking treatment for orthopedic and cardiological conditions in the United States. A study conducted by the World Travel and Tourism Council revealed that the United States received inbound medical tourism expenditures of $3.93 billion in 2019, further contributing to the expansion of the North American medical tourism market. Europe also holds a significant market share in the global medical tourism industry. The development of advanced medical facilities and infrastructure in countries such as France, the United Kingdom, and Spain, coupled with the rising popularity of medical tourism, has been driving the growth of this sector in Europe. However, the COVID-19 pandemic had a substantial negative impact on the European medical tourism market (www.peresedenceresearch.com, 2024).

The demand for medical tourism services is characterized by volatility, influenced by economic conditions, external factors, and evolving consumer preferences (Lunt et al., 2011). In Germany, a notable example of this volatility can be observed in the fluctuating number of foreign medical tourists, with a 4.4% increase in 2014 followed by a period of stagnation in 2015 (1.4%). This instability was primarily attributed to the dynamic performance of the Russian and Arabic markets, which experienced rapid growth and decline in response to favorable or unfavorable national economic conditions (Juszczak, 2017). Similar to general tourism, health tourism is a labor-intensive industry that necessitates a diverse skill set encompassing tourism, hospitality, healthcare, healing modalities, fitness, sports, and spirituality (Dvorak, Saari, & Tuominen, 2014).

Popular destinations like Thailand, Mexico, India, Türkiye, Malaysia, Costa Rica, and Singapore attract patients seeking significant cost savings of 40-80%. Over 90% of medical tourists report high satisfaction with their care. The global medical tourism market is projected to reach $35.9 billion by 2032, with Asia-Pacific leading the market at 75%. This industry contributes over $100 billion annually to the global economy. Sharma predicts continued steady growth in the medical tourism market (Media Market, 2023).

Turkey's leadership in health tourism stems from its early adoption of the industry, providing extensive experience. The country's new strategy involves offering package deals to medical tourists, including hotel, transportation, sightseeing, and hospital expenses, ensuring no additional costs. Turkey's proximity, easy visa process, affordability, and accessibility are factors contributing to its popularity as a medical tourism destination (Gündüz, Gündüz, & Yavuz, 2019).

CONCLUSION

Over the past several decades, the demographic composition of OECD countries has undergone a notable shift, with the proportion of the population aged 65 and older experiencing a twofold increase on average. This demographic transition has resulted in a significant rise from less than 9% in 1960 to 18% in 2021. However, the extent of this demographic change varies considerably across member countries, with Japan and Italy reporting notably higher percentages of their populations aged 65 and older at 28.9% and 23.6%, respectively. In contrast, Mexico and Colombia have lower proportions of their populations in this age group, at 7.9% and 8.8%,

respectively. This ongoing trend is projected to persist, with a further increase in the proportion of the population aged 65 and over anticipated in the future (OECD, 2024).

Health tourism is one of the fastest-growing forms of tourism. Analyzing the supply and demand in the health tourism market highlights important issues, including the diversity of service providers and the lack of reliable, comparable data and evidence-based information. This makes it difficult to benchmark performance at the country, destination, or individual facility level (World Tourism Organization and European Travel Commission, 2018).

The global medical tourism market is experiencing significant growth due to the increasing prevalence of chronic diseases and the availability of advanced medical technologies. Government support and initiatives from both source and host countries are expected to further drive market expansion. Patients are increasingly seeking medical tourism as a means to access affordable and timely treatment, particularly in cases where domestic healthcare options are limited or expensive. The globalization of healthcare, characterized by the free movement of patients, healthcare professionals, technologies, and capital, has played a pivotal role in the growth of the medical tourism industry. Rising awareness of medical tourism and the decreasing cost of advanced medical treatments are additional factors contributing to the market's expansion. Government investments in healthcare infrastructure, favorable policies, and improvements in transportation connectivity are also instrumental in promoting medical tourism development (www.presedence.com, 2024).

Many governments worldwide are recognizing the economic benefits of medical tourism and are supporting its development. Effective policies for medical tourism should align with the country's overall tourism strategy and be integrated into the broader travel and tourism sector. National and regional governments play a crucial role in developing, managing, and prioritizing such policies. As destinations aim to grow their medical tourism sub-sectors, governments should consider the following elements in their strategies: Establishing clear regulations and quality standards for healthcare providers and facilities. Creating visa policies that make it easier for medical tourists to visit. Addressing shortages of skilled healthcare professionals and providing training to improve their skills. Developing targeted marketing campaigns to

attract medical tourists. Offering financial incentives to encourage investment in the medical tourism sector (WTTC, 2019).

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CHAPTER 6

THE SUCCESS OF TITANIUM-BASED ABUTMENTS (TI-BASE) USED IN IMPLANT-SUPPORTED PROSTHETIC RESTORATIONS

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INTRODUCTION

Dental implants are a widely used treatment method to restore lost aesthetics and function in cases of single or multiple tooth loss for various reasons. Dental implants have revolutionized restorative dentistry by providing a durable and aesthetic solution for tooth replacement (Block, M. S. ,2018., Gupta, R., Gupta, N., & Weber, K. K., 2017). Dental implants are artificial roots made of titanium materials and are securely and stably integrated into the jawbone. The success of dental implants depends on several factors. In general, the success of implant-supported fixed prostheses is multifaceted, including the implant-abutment connection, the choice of crown material, the type of abutment-crown connection, patient-specific factors, maintenance routines, design elements, and psychosocial influences (Takahashi, T., Kihara, M., et al., 2022). From a prosthetic perspective, it also depends on abutment selection, the intermediate component connecting the prosthesis restoration to the implant (Elias, C. N., 2011, Raikar, S. et al., 2017). Abutments are divided into two types: basic prefabricated and custom design. The most used prefabricated abutments are made of titanium. They are produced with platforms with different angles and gingival heights at reasonable costs and with easy production. This cost-effectiveness makes them a prudent choice for many dental implant procedures. However, these abutments are only suitable for restorations to be cemented. In cases where the emergence profile needs to be customised, limited interocclusal distance and difficulty in removing excess cement, custom abutments are required. Custom abutments can be made of metal alloys, titanium or Zirconia. Custom abutments can be manufactured using Computer-aided design (CAD) and Computer-aided manufacturing (CAM) technologies or universal casting long abutments (UCLA) using metal alloys. Even when custom abutments made of metal alloys are placed subgingival, a metallic colour can be observed, or an abnormal blue-green colour can be observed in the gingival mucosa. The thin gingival biotype in the anterior region further aggravates this problem. In line with these demands in the field of esthetics, custom zirconia abutments have been introduced to the industry as an alternative. However, studies have shown that custom zirconia abutments fail more frequently than titanium abutments, implant-abutment connections or transmucosal components. Frictional wear between titanium and zirconia has made long-term success uncertain (Moreno ALM., Dos Santos DM., 2022., Moreno ALM., Dos Santos DM, 2022.,). Furthermore, the restoration connection to the implant platform

can be achieved with a three-component or two-component system. In a three-component system, the implant consists of three separate components: the implant, the abutment, and the crown. In a two-component system, the abutment and crown are combined as a single unit and connected to the implant (Misch, C. E., 2004, Sailer, I. et al., 2022). This type of restoration, where the abutment and crown function as a single unit and are cemented together, is called a Titanium Base (Ti-base) abutment and ceramic crown restoration, which is secured to the implant with a screw. Ti-base abutments were developed to combine the benefits of a titanium-to-titanium connection with the aesthetics of a tooth-coloured abutment. Ti-base abutments can be preferred in cases where stock abutments cannot solve implant angulation issues or where high aesthetic expectations exist. In addition, these abutments are frequently preferred due to their advantages, such as reflecting the soft tissue anatomical contour with satisfactory aesthetic properties, providing an optimal emergence profile, the absence of ceramic material at the implant-abutment connection level, cementation control under laboratory conditions and improved biomechanical properties (Moreno ALM., Dos Santos DM, 2022). In recent years, Ti-Base abutments have been widely used to enhance the success of implant-supported restorations (Al-Thobity, A. M., 2021, Elsayed, S., & Elbanna, K., 2021, Smith A, Johnson B., 2015). This article aims to evaluate the success of 'Ti-Base abutments, examine their advantages and disadvantages, and examine the existing studies on the properties, construction techniques and clinical applications of restorations prepared with Ti-base abutments.

1. METHOD

This article is designed as a review article supported by a literature review in various scientific databases. A search was carried out on the databases PubMed / MEDLINE, Web of Science, Google Scholar, and Scopus to find articles published between 2017 and 2024 on Ti-base abutments and aimed at review purposes. Clinical trials randomized controlled trials, prospective and retrospective cohort studies, systematic reviews and meta-analyses of "Ti-Base abutments" were primarily examined.

2. RESULTS AND DISCUSSION

The use of 'Ti-Base abutments' can enhance the success of dental implants in various aspects. These abutments possess several advantages, including good tissue compatibility, improvement of gingival contour, the

ability to provide a more natural aesthetic closer to natural teeth, and more accessible application of restorations. Moreover, the mechanical stability and tissue compatibility offered by 'Ti-Base abutments' have been observed to reduce complications and contribute to long-term success. However, these abutments also have disadvantages, such as cost considerations and limited indications for certain specific cases (Al-Thobity, A. M., 2021, Calderon, U., et. al., 2022, KILIÇARSLAN, M. et.al., 2022, KESKİN, D. E., 2022, Edelhoff, D., et.al., 2019).

2.1. Ti-Base Abutment Design and Design of Restorations Using Ti-Base Abutment

"Ti-base abutments are made from grade 5 titanium, an alloy of titanium, aluminium, and vanadium. Grade 5 titanium combines excellent mechanical properties and biocompatibility with high strength, corrosion resistance, and lightness. Dental implants are also manufactured from grade 5 titanium, making them ideal for ensuring long-term durability and reliability. The fact that both the implant and the ti-base abutment are made from the same material results in two materials with similar elastic behaviour being mechanised. Thus, the absence of a ceramic or non-titanium alloy material between the implant and the Ti-base abutment increases the success of the connection. Ti-base abutments come in two designs based on the connection area to the implant: 1) hexagonal Ti-base and 2) non-hexagonal Ti-base. The hexagonal ti-base features an apical conical connection with hexagonal positioning grooves on the implant contact surface to allow for positioning of the restoration. In contrast, the non-hexagonal Ti-base has an apical conical connection without positioning grooves. The conical connection piece is the same in both designs. Although the hexagonal Ti-base grooves help transmit forces to the implant, the primary load transfer is provided by the conical connection formed by the contact plane between the abutment and the implant (ERSÖZ, E., ÖZER, N. E., et.al.,2024, Al-Thobity, A. M., 2021, Spitznagel, F. A., Bonfante, E. A. et.al., 2022, Martínez-Grau, J., Robles, D., Pérez, R. A. et.al., 2024) (Fig 1).

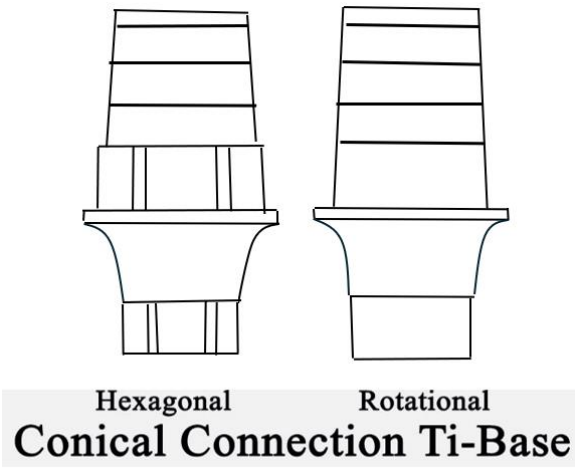

Fig 1: Ti-Base Abutment Design

2.2. Restoration Designs Using Ti-Base Abutment

As mentioned above, the connection designs of the Ti-base abutment to the implant are hexagonal-nonhexagonal(rotational). There are two design types for implant-supported restoration using Ti-base abutments (Fig 2). The first design form is the crown restoration designed on the Ti-base abutment, which is directly cemented. Then the hybrid abutment crown, which becomes a single piece, is the connection form screwed to the implant. The second design is a hybrid abutment superstructure designed on the Ti-base abutment, cemented, and these two are screwed onto the implant. It comprises a separate full ceramic crown form cemented on this structure (Al-Thobity et al., 2021, Spitznagel, F. A., Bonfante, E. A. et.al., 2022.)

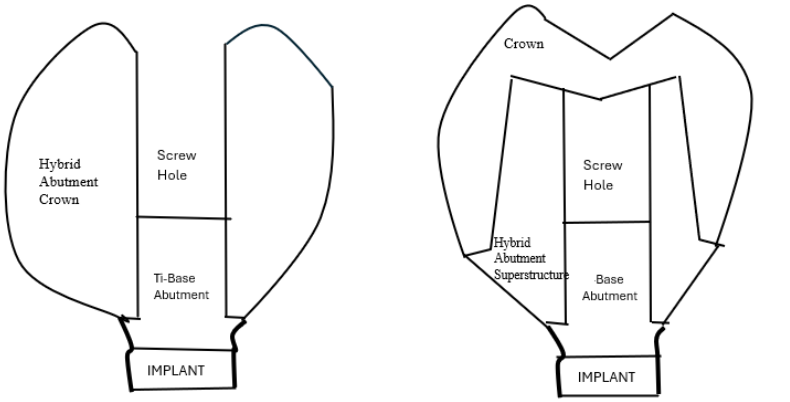

Figure 2: Restoration Designs Using Ti-Base Abutment

The meticulous process of the first design is a significant advantage. It allows for the extraoral removal of excess cement, ensuring a precise fit for the Ti-base abutment and crown restoration before they are screwed onto the implant, providing reassurance in the design's quality. Nouh et al. assessed the fracture resistance of these two designs using zirconia and lithium disilicate restorative materials. They found that both designs exhibited high fracture resistance, with the abutment bonded to the titanium base with a separate zirconia crown having the highest (3,730 N), followed closely by the one-piece zirconia abutment and crown bonded to the titanium base (3,400 N), instilling confidence in the durability of the designs.

2.3. Advantages of Ti-Base Abutments:

Ti-Base abutments offer several advantages that contribute to their success in dental implant restorations:

1. Their design allows for a stable and secure connection between the implant and the prosthetic restoration, promoting implant stability and longevity.
2. Titanium is the material of choice for Ti-Base abutments, as it ensures excellent biocompatibility, reduces the risk of adverse reactions and supports healthy peri-implant tissues.
3. The adaptability of Ti-Base abutments simplifies the restorative process, enabling clinicians to achieve optimal esthetic outcomes.

Ti-base abutments contain the mechanical durability of titanium material and the aesthetic properties of ceramic material.

Since the surface in contact with the implant is titanium, implant neckwear and abutment fractures caused by Zr abutments are prevented.

On the other hand, since the oral part of the abutment is made of ceramic material, gingival discolouration, grey colour reflection and aesthetic limitations seen in titanium abutments are prevented.

Therefore, Ti-base abutments offer higher resistance to chewing forces and aesthetic results.

In such abutments, the possibility of residual cement in conventional cement-retaining restorations is avoided, as the cementation of the restoration to the titanium substructure is performed on the model. Therefore, one of the most significant advantages of ti-base abutments is eliminating soft and hard tissue discomfort around the implant due to residual cement (Al-Thobity, A. M., 2021, Yilmaz, B., et.al., 2015, Turkoglu, P., Kose, A., & Sen, D., 2019, Ladino, L. G., Sanabria, A., & Cruz, M., 2020).

2.4. Important Factors Affecting the Success and Survival of Prosthetic Restorations Prepared with Ti-Base Abutments

Titanium-base (height): Ti-base abutment height is a determining factor in cementation performance. Higher abutments provide better retention forces for restorations. In particular, ti-bases with straight screw access channels of 4 mm height provide better retention forces (Karasan et al., 2024, Ntovas, P., Ladia, O., Pachiou, A. et al., 2024).

Titanium-base (geometry): It was found that the Ti-Base abutment geometry had significantly increased cement retention when it had anti-rotation properties (Karasan et al., 2024, Ntovas, P., Ladia, O., Pachiou, A. et al., 2024).

Titanium-base (surface treatment): Air-Abrasion: When 50 μm Al2O3 is applied to the Ti-base surface during the air abrasion process, the surface roughness increases. This contributes to the expansion of the bonding surface and is an effective method of increasing the retention strength of restorations.

Chemical Etching: Hydrofluoric acid can be especially effective in chemical etching the Ti-base surface. This process improves the microstructure of the Ti-base surface and increases the bonding strength.

Cleaning Procedures: Ultrasonic cleaning is not just a recommendation but a crucial step in reducing the contamination of the Ti-base surface. Cleanings performed before the restoration are vital, as they significantly increase the bonding performance, providing reassurance about the effectiveness of these procedures.

Application of a primer: Primers containing MDP (methacrylate-phosphate diester) increase the surface's bonding capacity and help prevent bonding errors. These primers provide strong adhesion, especially between the ti-base and restoration surfaces.

Effects on Success: Increased Bond Strength: Surface treatments are critical in providing strong adhesion between the Ti-base and the restoration. Air abrasion increases the surface roughness, allowing better-holding forces to be obtained.

Low Fracture Risk: Applied surface treatments help to reduce fracture and bond problems by improving the interaction with the restoration material. These effects are especially evident in restorations made with materials such as zirconia and lithium disilicate.

Surface modifications, particularly those related to Ti-base height, play a significant role in improving bonding performance. Increasing the height and applying appropriate surface treatments significantly improves bonding performance, instilling confidence in the effectiveness of these techniques.

Combining these methods contributes to the longevity and durability of restorations made with Ti-base abutments. Surface treatments are critical to increasing bond success.

Restoration (surface treatment): Surface treatments applied to the inner surface of crowns bonded to Ti-base abutments:

1. Surface Abrasion: Air abrasion, a commonly favoured technique, has shown promising results in enhancing the adhesion strength of zirconia restorations bonded to Ti-base abutments. In a study by Zahoui et al. (2020),

the adhesion strength of zirconia restorations bonded to 4 mm high Ti-bases air-abraded with silica-coated alumina particles significantly increased. This suggests a potential for surface abrasion to be a beneficial technique. However, it's worth noting that no significant increase in adhesion strength was found when applied to 2 mm high Ti-bases (Zahoui et al., 2020).

2. Primer Applications: The role of primer applications in enhancing the adhesion strength of restorations Increases the bond strength of restoration and ti-base abutment. In a study by Maltzahn et al. (2016), it was reported that the application of phosphate-based ceramic primer significantly increased the adhesion strength of air-abraded single zirconia crowns bonded to 3.5 mm high ti-bases (Maltzahn et al., 2016). Similarly, Chiam et al. (2023) demonstrated that bonding systems that included primer application provided higher adhesion than bondings made without primer application (Chiam et al., 2023).

3. Silane Applications: Silane application can increase adhesion, especially in temporary restorations. A study by Filokyprou et al. (2023) found that applying MDP-containing silane to 4 mm high air-abraded ti-bases before cementation increased the adhesion strength. However, it was reported that silane application and air abrasion in restorations produced from 3D printers did not provide a significant advantage (Filokyprou et al., 2023).

4. Restorative Material Selection: The material from which the crowns are fabricated is crucial to retention success. Research by Oddbratt et al. (2021) has shown that the effect of air abrasion on temporary restorations made of PMMA depends on the resin adhesive used. Additionally, studies by Pitta et al. (2020) found that PMMA restorations abraded with silica-coated alumina and treated with MMA (methyl methacrylate)-based fluid provided higher retention than restorations that were not air abraded or were treated without MMA-based fluid (Pitta, Bijelic-Donova et al., 2020b; Pitta, Zarauz et al., 2020a).

5. Long-Term Success: Studies on the effect of surface treatments on long-term stability on Ti-base abutments have shown promising results. Primer and surface treatments have been found to maintain implant stability in the long term. In particular, the studies of Al-Thobity (2021) and Korsch et al. (2018) concluded that appropriate surface treatments not only increase peri-implant tissue health but also significantly contribute to the long-term success of implant-supported restorations (Al-Thobity, 2021; Korsch et al., 2018).

Restoration (material): The crown materials used with Ti-Base abutments are a factor that especially affects success rates and durability. The restoration materials used:

Zirconia Crowns: However, the fracture rate of zirconia abutments may vary significantly depending on the height and surface processing techniques, underscoring the necessity of proper surface treatment. The bonding success of Zirconia crowns is also perfectly satisfactory.

Lithium Disilicate Crowns:: Lithium disilicate (IPS e.max) crowns offer high bonding strengths when used with Ti-Base abutments. This material is also advantageous in aesthetics and has generally been observed to provide better retention strengths than other crown materials. Lithium disilicate also exhibits good performance in terms of fracture resistance.

Polymer-infiltrated ceramic network: When used with Ti-Base, polymer-infected ceramic mesh (PICN) material provides good results, especially with 4-7 mm high ti-bases. This material reduces the risk of bonding failure and increases durability.

Materials that Increase Success: High zirconia and lithium disilicate materials increase success rates by reducing bonding and fracture problems. Studies have shown that both materials provide high bonding strength and compatibility with Ti-Base abutments after appropriate pre-bonding processes, such as surface cleaning, conditioning, and priming.

Connection Problems: Zirconia and lithium disilicate crowns stand out as materials with fewer connection problems. These problems, which include fit, stability, and adaptation, can be mitigated by applying appropriate surface treatments and connection protocols, thereby increasing the success of restorations made with such materials.

As a result, zirconia and lithium disilicate crown materials offer high success rates and low fracture risks when used with Ti-Base abutments, reassuring the audience about their effectiveness. Both materials are preferred in clinical applications and are among the critical factors that increase success.

Luting agents: When the studies are examined, it is emphasized that different cementation systems and some methods give more successful results.

Cementation Systems: Dual-Curing Resin Cements: Dual-curing systems such as Panavia V5 and RelyX Ultimate have been reported to provide high bond strengths, especially in the cementation of zirconia coatings. Such systems are also compatible with various restoration materials.

Self-Adhesive Resin Cements: Self-adhesive resins such as Panavia SA and RelyX Unicem show promising potential, giving more effective results, especially with short ti-bases. However, their performance on long or air-abraded ti-bases may vary, suggesting a need for further exploration and optimization.

The height of the ti-base and the method of surface treatment play a crucial role in the success of cementation. For ti-bases with a height of less than 3.5 mm, the impact of surface modifications is limited, a factor directly linked to the choice of cementation system.

Conclusively, the studies underscore the effectiveness of dual-curing resin cement and air-abrasion on the Ti-base abutment surface among the various cementation systems.

2.5. Clinical Performance and Long-Term Success:

Numerous studies have investigated the factors contributing to the success of Ti-Base abutments. Biomechanical stability, achieved through a precise fit and firm connection between the abutment and implant, has been identified as a crucial factor. The design of Ti-Base abutments, such as their ability to optimise load distribution and preserve soft tissue contours, has also been shown to influence success rates. Furthermore, studies have highlighted the importance of properly selecting and customising Ti-Base abutments based on the specific clinical case (Korsch, M., et.al., 2018, Pamato, S., et.al., 2022).

Several clinical studies have evaluated the performance of Ti-Base abutments in terms of their success rates and long-term outcomes. These studies have reported favourable results, demonstrating high implant survival rates and low rates of complications associated with Ti-Base abutments. Long-term follow-up examinations have shown stable peri-implant tissues, minimal bone loss, and excellent esthetic integration, supporting the long-term success of Ti-Base abutments in implant restorations (Al-Thobity, A. M., 2021, Korsch, M., et al., 2018, Pamato, S., et al., 2022, Wittneben, J. G., Millen, C., & Brägger, U., 2014).

In the meta-analysis conducted by Linkevicius et al. (2018), it was emphasized that surface treatments such as primer and adhesive applications increased the strength of the connection between the Ti-base abutment and the restoration. They stated that phosphate-based primer applications, especially, increased the adhesion strength between the abutment and the crown in the long term, providing practical knowledge that can empower dental professionals in their restorative work.

Long-term studies conducted between 2015 and the present have provided valuable insights into the clinical performance of Ti-Base abutments. For example, a study by Tajti, P. et al. (2023) reported a success rate of 97.5% over a 5-year follow-up period for restorations using Ti-Base abutments. Another study by Cosyn et al. (2019) demonstrated that Ti-Base abutments maintained the implant bone level and minimized bone loss over a 10-year follow-up period. These findings emphasize Ti-Base abutments' long-term stability and success in implant-supported restorations.

2.6. Esthetics and Patient Satisfaction:

Prosthetic restorations on Ti-base abutments aim to provide high patient satisfaction in aesthetics, biomechanical stability and longevity. Achieving optimal aesthetics is a critical aspect of implant-supported restorations, and Ti-Base abutments play a significant role in this regard (Al-Thobity, A. M., 2021, Mostafavi, A. S., Mojtahedi, H., & Javanmard, A., 2021) . Studies have shown that Ti-Base abutments facilitate proper seating of porcelain restorations and help preserve the integrity of the soft tissue barrier (Wittneben et al., 2014, Cabrera, J. E., 2017). The natural emergence profile created by Ti-Base abutments enhances esthetic outcomes and patient satisfaction (Joda et al., 2016, Al-Thobity, A. M., 2021). Ti-base abutments are crucial in ensuring high patient satisfaction, particularly with their natural gingival emergence profile and aesthetic results. A meta-analysis by Al-Thobity (2021) found that zirconia and lithium disilicate crowns, when prepared on ti-base abutments, significantly enhance aesthetic satisfaction, especially in anterior restorations. The unique contribution of zirconia restorations to the natural appearance by improving soft tissue harmony is a convincing aspect that positively influences patient satisfaction (Al-Thobity, 2021). Functional success and chewing comfort are fundamental elements of patient satisfaction. In the meta-analysis of Korsch et al. (2018), it was stated that zirconia and lithium disilicate restorations made on Ti-base abutments

gave positive results, especially regarding chewing comfort and functional stability. The study concluded that Ti-base abutments provided long-term chewing comfort by increasing biomechanical stability in implant-supported restorations, and therefore, patient satisfaction was high (Korsch et al., 2018).

2.7. Fracture Resistance of Prosthetic Restorations Prepared with Ti-Base Abutments.

Fracture resistance of prosthetic restorations prepared with Ti-base abutments. Different restorative materials are used on the Ti-base abutment. Factors such as different material types, surface treatments, and bonding protocols affect the fracture resistance of implant-supported crowns restored with Ti-base abutments. These are ceramic, Zirconia, and PEEK. In a recent study, restorations made of Zr, lithium disilicate and PEEK (polyetheretherketone) on a Ti-base abutment were cemented and subjected to the fracture test after thermal cycling in vitro. Studies have shown that the fracture resistance of ti-base abutments is higher than that of traditional Zirconia abutments. These abutments are also indicated for use in posterior areas with intense chewing forces. According to the study results, hybrid abutments made of Ti-base Zr material are suitable for clinical use, and hybrid abutments made of lithium disilicate and PEEK have low fracture resistance. According to the results, the reason for the low fracture resistance in lithium disilicate hybrid abutments is that; In addition to the mechanical properties of the ceramic material, the screw entry hole in the occlusal makes the material more fragile. Another study emphasized that the fracture resistance and chipping of ti-base crowns were higher when the fracture resistance of lithium disilicate porcelain crowns prepared as on-implant cement and ti-base was investigated after the fatigue test (Al-Thobity, A. M., 2021, Mostafavi, A. S., Mojtahedi, H., & Javanmard, A., 2021, Adolfi, D, et al., 2020, Al-Zordk, W., Elmisery, A., & Ghazy, M., 2020). PEEK material, known for its flexibility and biocompatibility, was the subject of a study by Adolfi et al. (2020). The study found that PEEK restorations, when placed on Ti-base abutments, exhibited lower fracture resistance than zirconia. However, they demonstrated sufficient durability even in high-chewing force areas, suggesting that PEEK could be a viable option in specific cases.

The study by Zahoui et al. (2020) showed that air abrasion increased the fracture resistance of zirconia restorations. This study found that air abrasion, especially silica-coated alumina particles, provided a stronger bond

between the restoration and the Ti-base abutment and increased fracture resistance (Zahoui et al., 2020). Maltzahn et al. (2019) reported that applying a phosphate-based primer increased the adhesion strength of zirconia restorations placed on Ti-base abutments and increased the fracture resistance. The study revealed that the primer application effectively increased long-term durability (Maltzahn et al., 2019). Al-Zordk et al. (2020) examined the fatigue resistance of zirconia restorations applied on Ti-base abutments and found that their resistance to intense chewing forces increased. The study showed that zirconia restorations provided long-term durability and achieved high results in fracture resistance (Al-Zordk et al., 2020). Pitta et al. (2020) investigated how micro movements affected the fracture resistance on Ti-base abutments and stated that these movements could increase fracture risk, especially in high-stress regions. Minimizing micro-movements is critical in increasing the long-term stability of restorations (Pitta et al., 2020). Mostafavi et al. (2021) studied the effect of materials used in posterior restorations on fracture resistance. This study reported that zirconia and PEEK materials placed on Ti-base abutments increased the strength in the posterior regions, but PEEK was not as resistant to masticatory forces as zirconia (Mostafavi et al., 2021).

2.8. Biological Success of Prosthetic Restorations with Ti-Base Abutments

Implant-support connection: This is significant for the long-term success and stability of the prosthesis. The incompatibility between these components is an issue that needs to be considered because it causes mechanical problems, such as screw loosening and damage to the internal screw threads and biological complications due to microorganism colonization inside the implant. Due to these biological complications, inflammation occurs in the peri-implant tissues, followed by pain, marginal bone loss, and disruption of osseointegration, which may lead to the worst-case scenario. One of the biggest causes of biological complications is residual cement around the biological tissue and implant (Al-Thobity, A. M., 2021, Mostafavi, A. S., Mojtahedi, H., & Javanmard, A., 2021, Pamato, S. et al., 2020, Linkevicius, T. et al., 2018). With Ti-base Abutments, cementation of the crowns is done outside the mouth, so no cement is left. No significant difference was found in the 1-year clinical follow-up study in which the amount of bone loss and soft tissue health around the implant of Zirconia crowns prepared with Ti-base abutments of different lengths were investigated. While bone loss in short ti-base abutments is 0.6 ± 0.51 mm, this

amount is 0.45 ± 0.59 mm in long ti-base abutments (Linkevicius, T. et al. 2022). In the meta-analysis by Wittneben et al. (2014), low bone loss, stable peri-implant tissue health, and low screw loosening rates were reported in restorations using Ti-base abutments. These low complications improve patient satisfaction (Wittneben et al., 2014).

2.9. Misfit and Torque Loss of Ti-base Abutment Prosthetic Restorations

Incompatibility can cause stresses at the implant-bone interface and, in advanced cases, cause biological and mechanical complications such as loss of torque and screw loosening, breakage of abutment screw, marginal bone loss around the implant neck, and loss of implant osseointegration. Studies have reported that a gap of 150 µm is a clinically acceptable mismatch value (Al-Thobity, A. M., 2021, Mostafavi, A. S., Mojtahedi, H., & Javanmard, A., 2021). In the study of Ramalho et al., they compared the compatibility of the systems with 2 and 3 stages connected to the implant platform. As a result, it was emphasized that the internal fit and marginal fit of the crowns prepared for ti-base abutments were statistically better than the fit on the implant platform. In a recent study, the amount of torque loss after bonding ti-base zirconia, lithium disilicate and polyetheretherketone to restorations was evaluated, and it was found that the superstructure material did not have a significant effect on the amount of torque loss (Adolfi, D. et al., 2020, Al-Zordk, W., Elmisery, A., & Ghazy, M., 2020, Linkevicius, T. et al., 2018). Incompatibility, a gap or deficiency in the implant-abutment connection, typically induces stress at the bone-implant interface. Structural incompatibility (misfit) and torque loss in abutments can compromise implant-prosthesis integrity, leading to biomechanical issues and clinical complications. Linkevicius et al. (2018) established the acceptable incompatibility value at 150 µm in Ti-base abutments, warning that higher incompatibilities could detrimentally impact peri-implant tissue health. Research indicates that structural incompatibilities can trigger marginal bone loss around the implant, torque loss, and screw loosening (Linkevicius et al., 2018). Al-Thobity (2021) found that torque loss is often linked to structural incompatibility in Ti-base abutment restorations. The study noted that torque loss is more prevalent in posterior regions, particularly those subject to intense chewing forces, and that torque power should be regularly monitored to prevent screw loosening. The study underscored the crucial role of surface treatments in reducing torque loss, instilling confidence in their effectiveness

(Al-Thobity, 2021). Filokyprou et al. (2023) reported that the application of primer containing MDP strengthened the connection between the Ti-base abutment and the restoration, reducing the mismatch rate. Furthermore, the primer application effectively prevented torque loss and contributed to long-term clinical success (Filokyprou et al., 2023). The study by Korsch et al. (2018) advocates for the use of surface treatments to reduce implant-abutment mismatch. The study found that mismatch and torque loss decreased in long-term patient follow-up. Ti-base abutments offer long-term patient satisfaction with high stability and low complication rates (Korsch et al., 2018). Studies on the effect of different restorative materials on mismatch and torque loss show that material selection can significantly affect mechanical stability. In a study conducted by Adolfi et al. (2020), the performance of materials such as PEEK (Polyetheretherketone) and zirconia on Ti-base abutments was evaluated. Zirconia restorations provided higher fracture resistance and stability than PEEK, while reduced torque loss was observed. Therefore, restorative material is crucial, especially for long-term stability (Adolfi et al., 2020).

2.10. Limitations and Future Research Directions:

While Ti-Base abutments have demonstrated favourable outcomes, certain limitations and potential complications should be considered. One limitation is the relatively higher cost than conventional abutments, which may influence treatment decisions. Furthermore, the complexity of the design and the need for specialised laboratory procedures may require additional technical expertise and resources. Additionally, the risk of peri-implant inflammation or soft tissue complications, although minimal, should be considered (Al-Thobity, A. M., 2021; Mostafavi, A. S.; Mojtahedi, H., & Javanmard, A., 2021, Edelhoff, D., at al., 2019). Future research should focus on optimising Ti-Base abutments, further understanding long-term outcomes and minimising potential complications. Comparative studies with different implant systems and investigations into the effects of new materials and surface treatments are also warranted.

CONCLUSION

This scientific review article provides a comprehensive assessment of the success of Ti-Base abutments and Ti-Base abutment implant-supported prosthetic restorations based on studies conducted from 2017 to the present. Upon reviewing the included studies, in vitro studies have shown that

titanium-based abutments exhibit high fracture resistance, particularly suitable retention values with resin cement, and good marginal and internal fit. Although the clinical evaluation of monolithic zirconia crowns prepared with titanium-based abutments is limited, they have been reported to demonstrate comparable performance to stock abutments, especially in short-term evaluations in the anterior and premolar regions and meet aesthetic expectations better. The evidence indicates that Ti-Base abutments contribute to improved clinical performance, aesthetics, and long-term stability of implant-supported restorations. However, further research is needed to optimise Ti-Base abutments, enhance our understanding of long-term outcomes, and minimise potential complications.

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CHAPTER 7

CLINICAL IMPLICATIONS AND INTERPROFESSIONAL COLLABORATION OF TAXANE-RELATED LYMPHEDEMA IN BREAST CANCER PATIENTS

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INTRODUCTION

Breast cancer and lymphedema

Breast cancer remains one of the most common and prevalent cancers worldwide, representing a significant health burden for women. As per World Health Organisation statistics, over 2.3 million new breast cancer cases were reported in 2020. The disease impacts women of all ages, races, and ethnicities, but certain factors can increase risk, including age, family history, and certain genetic mutations, such as BRCA1 and BRCA2 (WHO, 2020; Bray et al. 2018). While various treatment options exist, the choice is influenced by the cancer's characteristics and the patient's health status. Treatment options for breast cancer are dependent on the type and stage of the cancer, as well as the patient's overall health. Common treatment strategies include surgery (like a lumpectomy or mastectomy), radiation therapy, chemotherapy, such as, taxanes, hormonal therapy, targeted therapy, and immunotherapy. Often, a combination of these treatments is employed [Burguin et al., 2021].

The lymphatic system comprises a network of vessels, similar to the blood circulatory system, but its role is to drain excess fluid (lymph) from tissues and return it to the bloodstream. The lymphatic system plays a crucial role in fluid homeostasis and immunity by transporting lymph fluid from tissues back into circulation. This system also contains immune cells that help fight infections and diseases. When this system is damaged or obstructed, it can lead to the accumulation of lymph fluid in the interstitial tissues, resulting in lymphedema [Johnson et al. 2021; Brix et al. 2021].

Lymphedema is a chronic condition characterised by localised fluid retention and swelling of the limbs due to impairment and a disruption in the normal drainage function of the lymphatic system 5. There are two main types of lymphedema: primary and secondary. Primary lymphedema is a rare condition, accounting for 25–30% of all lymphedema patients, usually due to genetic abnormalities affecting the development of the lymphatic system. Secondary lymphedema is more common, accounting for one case in 1000 individuals, with an average age of patients at diagnosis of between 50 and 58 years. It occurs when the lymphatic system is damaged or blocked, commonly due to surgery, radiation, infection, or cancer. In breast cancer patients, it can develop following surgical removal of lymph nodes in the underarm area or radiation therapy [Brix et al. 2021; Telinius et al., 2018; Grada et al. 2017;

Warren et al. 2014]. Breast cancer related lymphedema is the most common form of secondary lymphedema. The risk of developing lymphedema after breast cancer treatment was reported to be about 15–20% [Mortimer et al. 2014]. A systematic review and meta-analysis of BCRL cases shows an overall incidence of 15.5% after cancer treatment [Cormier et al., 2010].

Symptoms of lymphedema can range from mild to severe and include swelling in the arm, hand, breast, or torso on the side of the body where the lymph nodes were removed or damaged. The skin may become hard or thick, and there may be discomfort or aching. The complications of lymphedema can be serious if left untreated. Chronic inflammation and fibrosis can lead to changes in the skin and underlying tissues, potentially resulting in decreased function in the affected limb. Furthermore, patients with lymphedema are at an increased risk of developing infections in the affected limb, such as cellulitis or lymphangitis, which can cause serious illness and require hospitalisation [Ebaugh et al., 2011; Clark et al., 2005; Fu et al., 2014; Shih et al. 2009]. On the other hand, physical symptoms like swelling, heaviness, and discomfort can limit functional mobility and impact daily activities. Moreover, psychosocial impacts due to altered body image, ongoing discomfort, and anxiety about the chronic nature of the condition can significantly affect a patient's emotional well-being and can significantly impact the patient's quality of life [Fu et al., 2013, Chachaj et al., 2010].

Taxane and breast cancer treatment

Taxanes represent a class of drugs widely used in the field of oncology and are a key component of chemotherapy regimens at various stages of breast cancer treatment. They can be used in the neoadjuvant setting, aiming to shrink the tumour prior to surgery, or in the adjuvant setting post-surgery to eliminate any remaining cancer cells. The two most known and utilised taxanes are paclitaxel and docetaxel. They function by interfering with cell division, more specifically by stabilising the microtubule network crucial for mitosis. By stabilising these structures, taxanes prevent their disassembly, which halts the cell in the metaphase of cell division, leading to apoptosis, or cell death. This mechanism is especially effective against cancer cells, which have a rapid rate of division compared to most normal cells [Willson et al., 2019; Gradishar et al., 2012]. In terms of administration, taxanes are typically delivered intravenously. The dosing frequency and treatment duration are variable, generally depending on the individual

patient's health status, the stage of the disease, and whether the taxane is being administered alone or in combination with other anticancer agents. Clinicians often have to balance the potential therapeutic benefits with possible side effects, such as neuropathy, myelosuppression, and the potential for lymphedema [Zaheed et al., 2019; McLaughlin et al., 2020].

Taxane-related lymphedema in breast cancer

While taxanes impact on cancer cells is beneficial, these antineoplastic drugs can have a damaging effect on other cells in the body, including those that make up the lymphatic system. Taxane-related lymphedema is a significant concern for patients undergoing breast cancer treatment. Taxane chemotherapy might contribute to lymphedema through several mechanisms. It is postulated that taxanes can cause injury to the endothelial cells lining the lymphatic vessels which increase the permeability of lymph vessels via autophagy in lymphatic endothelial cells and gap junction disruption, which results in loss of the gap junction protein and VE-cadherin. Some evidence suggests that taxanes may cause fibrosis or scarring in the lymphatic vessels, disrupting the normal flow of lymph fluid. Additionally, taxanes could potentially induce inflammation or oxidative stress, which might also contribute to lymphatic dysfunction [Wong et al., 2020; Pal et al., 2022]. In *vitro* studies also found that taxanes attenuate tumour lymph-angiogenesis, attenuate proliferation and inhibition migration and tubule formation of lymphatic endothelial cells [Zamora et al., 2019; Harris et al., 2018].

It is evident that the development of taxane-related lymphedema in breast cancer patients is a multifactorial issue, encompassing both treatment-related and patient-related factors. These factors intertwine to create a complex risk profile for each individual patient. Understanding these factors can help healthcare professionals predict which patients are at higher risk and take the necessary measures to mitigate this risk. The incidence of taxane-related lymphedema in breast cancer patients varies, depending on several factors, such as the specific taxane used, the dosage, and the individual patient's characteristics. Furthermore, patients undergoing combination therapy, which includes surgical removal of lymph nodes or radiation, may have a compounded risk of developing lymphedema. These factors can be categorised into treatment-related and patient-related factors [McLaughlin et al., 2020]. Treatment-related factors are primarily associated with the intensity

and extent of breast cancer treatment. Patients undergoing more aggressive treatment regimens, including higher doses of taxanes, have been reported to be at higher risk for developing lymphedema. Additionally, the risk is compounded in patients who have undergone axillary lymph node dissection or radiation therapy, both of which can disrupt the normal function of the lymphatic system. Patient-related factors also play a significant role. Factors such as obesity, older age, and a sedentary lifestyle have been associated with an increased risk of lymphedema. In addition, patients with a pre-existing condition that affects the lymphatic or vascular system may be more susceptible to developing lymphedema following taxane treatment. Genetic factors may also play a role in the development of taxane-related lymphedema. Some studies suggest that certain genetic variations may make some individuals more susceptible to lymphatic damage following chemotherapy. However, more research is needed in this area to fully understand the genetic implications [Hayes et al., 2008; Gillespie et al., 2018; Nguyen et al., 2017; Ugur et al., 2013].

Earlier clinical studies have identified a link between the use of taxanes and the development of lymphedema in breast cancer patients. A study published by Hayes et al. [Hayes et al., 2008] found that breast cancer patients treated with taxanes were significantly more likely to develop lymphedema. This risk was further amplified in patients who also underwent axillary lymph node dissection, highlighting the cumulative effect of these therapies on the lymphatic system. Another systematic review and meta-analysis study by Qin et al. [Qin et al.i 2011] found that among breast cancer survivors, those who had received taxane-based chemotherapy showed higher rates of lymphedema. A summary of clinical findings from earlier studies regarding the impact of taxane-related lymphedema in breast cancer patients is presented in Table 1 [Ohsumi et al., 2012; Lee et al., 2014; Cariati et al., 2015; Swaroop et al., 2015; Kilbreath et al., 2016; Zhu et al., 2011].

Table 1: Summary of findings for the included clinical studies

Author	Type of Study	Duration (Post Treatment)	Study Objectives	Summary of Findings	Main Outcomes
Hayes et al.	Cohort	6 and 18 months	Prevalence and incidence of lymphedema	40% of the sample had long-term lymphedema	Earlier detection and benefits of physical activity to prevent and mitigate lymphedema symptoms
Qin et al.	Meta-analysis	-----	Drug-related toxicities of taxanes	Significant increased rate of oedema in the taxane-based treatment arm (OR=6.61, 95% CI 2.14–20.49)	Drug-related toxicities should be balanced
Ohsumi et al.	Randomized controlled trial	21 weeks	Time course of taxane-induced edema	Edema scores worsened up to 1-2 months after chemotherapy. Body weights increased remarkably	Patients receiving taxane for >4 cycles significantly had edema
Lee et al.	Longitudinal	6 months	Describe the incidence of lymphedema in women receiving taxane-based chemotherapy	Elevated ECF ratios after 6 months of taxane-based chemotherapy 32% meeting the criteria for lymphedema	Increased incidence of lymphedema following taxane-based chemotherapy
Cariati et al.	Retrospective analysis	2 years	Identify risk factors for BCRL	33.5% of the sample developed BCRL	Adjuvant taxanes play a key role in the development of BCRL after surgery
Swaroop et al.	Prospective cohort	7 years	Determination of adjuvant taxane-based chemotherapy increased risk of lymphedema	5.27 % of the sample developed lymphedema 2 years post treatment with taxanes	Docetaxel causes mild swelling of the upper extremity
Kilbreath et al.	Prospective cohort	18 months	Identify risk for lymphoedema	LE developed in 18.2% of the sample	Arm swelling in the first year poses a very

			(LE) based on axillary surgery	with ≥5 nodes	strong risk for presence of LE at 18-months
Nguyen et al.	Cohort	10 years	BCRL incidence	BCRL cumulative incidence = 9.1% [95% CI: 7.8-10.5%]	Higher BCRL rates in patients receiving taxane chemotherapy
Zhu et al.	Retrospective	4 years	Relationship between docetaxel-based chemotherapy and BCRL	Increased all-grade lymphedema incidence (32.09 %; p=0.011), Docetaxel an independent risk factor for all-grade lymphedema	Docetaxel significantly increased the risk of BCRL

BCRL: Breast cancer-related lymphoedema, CI: Confidence interval

Management

Current therapeutic approaches to minimize lymphedema risk: effectiveness and limitations

Taxane-related lymphedema is a complex condition that requires multifaceted treatment approaches. Existing therapies vary in their effectiveness and have their own set of limitations. Understanding these factors is crucial for managing lymphedema effectively. Current therapies for managing taxane-related lymphedema have varying degrees of effectiveness and associated limitations. Complex decongestive therapy (CDT) is the standard of care for managing lymphedema. It is a two-phase treatment that involves an intensive phase of manual lymph drainage, compression therapy, skin care, and exercise, followed by a maintenance phase. Compression therapy, a component of CDT, involves wearing a fitted garment to prevent fluid accumulation. While effective, these garments can be uncomfortable, and adherence can be a challenge. Although CDT has been shown to be effective in reducing limb volume and improving quality of life, it requires significant time and effort from patients, and access to trained therapists may be limited in some areas. In addition, the cost of compression garments, which need to be replaced regularly, can be a burden for some patients [Ramachandran et al., 2022; Schaverien et al., 2018; Marotta et al., 2023].

Surgical interventions, such as lymph venous anastomosis or vascularized lymph node transfer, are increasingly being used for patients with severe, refractory lymphedema. While these procedures can be effective, they are invasive, have risks associated with surgery, and require specialised surgical expertise [de Sire et al., 2022]. There are currently no FDA-approved medications used for the treatment or prevention of lymphedema. However, few drugs are used to try to alleviate lymphedema, such as diuretics, which have been used to manage lymphedema symptoms. However, their effectiveness in long-term lymphedema management is limited, and they have potential side effects, including electrolyte imbalances. Other drugs, such as benzopyrones, have shown some promise but require further research [Harris et al., 2001].

While therapies like CDT and compression garments have proven beneficial, they can be time-consuming, uncomfortable, and costly. Surgical interventions, while potentially effective for severe cases, carry inherent risks, and pharmacological treatments offer some relief but can have side effects.

New pharmacological approaches are being investigated. One of the most promising agents is ubenimex, a drug traditionally used for leukaemia treatment. Preclinical studies suggest that ubenimex may improve lymphedema by enhancing lymphangiogenesis and suppressing inflammation. However, clinical trials are still ongoing to confirm these effects in human subjects.

Advances in surgical techniques are also promising [Ogino et al., 2022]. Lymphatic microsurgical preventive healing approach (LYMPHA), for instance, is a prophylactic surgical technique performed at the time of axillary lymph node dissection. It involves creating a lymphatic-venous bypass to reduce the risk of lymphedema. Early clinical trials have shown promising results, but more research is needed to validate their effectiveness and long-term outcomes [Campisi et al., 2024]. In the realm of therapeutic devices, pneumatic compression devices are gaining attention. These devices, which deliver controlled compression to the affected limb, have been shown to reduce limb volume and improve symptoms in some patients with lymphedema. More advanced models can mimic the body's natural lymphatic pumping action, potentially improving their effectiveness [Muluk et al., 2013; Zaleska et al., 2014]. Bioimpedance spectroscopy (BIS) is another promising development. BIS is a non-invasive device that can detect changes in tissue composition and fluid levels, allowing for earlier detection of lymphedema. Early detection may allow for interventions that could prevent or slow the progression of lymphedema [Shah et al., 2016].

Potential role of pharmacogenomics to minimize lymphedema risk

The latest research on emerging therapies for lymphedema offers promising options for improved patient care. From novel pharmacological approaches and surgical techniques to innovative therapeutic devices and diagnostic tools, these advancements can potentially revolutionise the management of taxane-related lymphedema. However, it's important to note that many of these therapies are still in the research phase, and further studies are necessary to confirm their effectiveness, understand their limitations, and ensure their safety for widespread use. As such, a personalised, patient-centred approach is crucial, factoring in the effectiveness and limitations of each treatment option considering the patient's unique circumstances. Therefore, pharmacogenomics may play a crucial role in personalising breast cancer treatment to reduce the risk of lymphedema, particularly in patients

undergoing taxane therapy [Al-Taie et al. 2022; Angelini et al., 2017]. For instance, variations in the gene CYP3A4, which encodes a major drug-metabolising enzyme, can alter the metabolism of taxanes. Taxanes are substrates of the adenosine triphosphate-binding cassette B1 (ABCB1 gene), a P-glycoprotein (P-gp) membrane-bound efflux pump that plays an important role in biliary absorption and intestinal excretion [van Zuylen et al., 2000]. Single-nucleotide polymorphisms (SNPs) in the ABCB1 and CYP P450 (CYP) genes may be linked to interindividual variance. SNPs in the ABCB1 gene, specifically, are paired with phenotypic differences in P-gp, which play the most critical role in drug clearance. The ABCB1 C1236T polymorphism in exon 12 is linked to lower docetaxel clearance [Bosch et al., 2006; Gréen et al., 2006]. Identifying these genetic variations could allow for individualised dosing or the selection of alternative chemotherapy agents to reduce the risk of taxane-induced lymphedema.

Moreover, certain genetic factors might predispose individuals to lymphedema. Understanding these genetic predispositions could help identify patients at high risk of lymphedema, enabling early interventions to mitigate this risk. Several genes have been identified that are involved in the development and maintenance of the lymphatic system, such as FOXC2 and SOX18. Genetic variants in these genes might increase susceptibility to lymphedema following breast cancer treatment [Newman et al., 2012]. On the other hand, personalised medicine can also extend to the management of lymphedema. For instance, genetic factors can influence the response to certain medications used to manage lymphedema symptoms. Pharmacogenomic testing could inform the selection of these drugs, leading to more effective symptom management [Guan et al., 2012].

Patient-centric care

1. Multidisciplinary approach in management of taxane-related lymphedema

The management of lymphedema in breast cancer patients, particularly related to taxane therapy, requires a multidisciplinary approach. Such an approach involves several healthcare professionals working together, each contributing their unique expertise to provide comprehensive patient care. The multidisciplinary approach to lymphedema management underscores the complexity of this condition and the need for comprehensive,

coordinated care. This collaborative approach provides patients with the highest level of care, addressing both the physical symptoms of lymphedema and the associated emotional challenges. As a result, this can lead to improved patient outcomes and a better quality of life. Oncologists are integral to coordinating the overall treatment plan, as they play a critical role in adjusting the chemotherapy regimen based on the patient's response and side effects. They may also refer patients to lymphedema therapists or vascular surgeons, for specialised care as needed. Dietitians can contribute to the team by providing guidance on weight management and nutritional strategies to reduce inflammation, both of which can help manage lymphedema. Meanwhile, physical therapists play a vital role in the management of lymphedema. They can instruct patients on specific exercises designed to promote lymph drainage and reduce swelling. They can also assist with the fitting and use of compression garments, which can help manage lymphedema symptoms. Psychologists or counsellors can provide emotional support, as the diagnosis and treatment of lymphedema can take a psychological toll on patients [Gillespie et al., 2018; Vafa et al., 2020; Megens et al., 1998; Fu et al., 2013].

By collaborating closely with other healthcare professionals, clinical pharmacists can ensure that the patient's lymphedema is effectively managed as part of their overall cancer treatment plan. As the clinical pharmacists are a key member of the multidisciplinary team, they can provide collaborative care and play a pivotal role in managing cancer patients through evaluating the medication regimens for potential drug interactions or contraindications, providing patient education and counselling on the proper use of medications and adverse effects, including the risk of lymphedema. This involves an in-depth understanding of various treatment options and their impacts on the lymphatic system, as well as tailoring the medication regimen to each patient's unique needs and circumstances [Walko et al., 2011; AlTaie et al., 2020; McKee et al., 2011].

One aspect of medication management is adjusting the dosage and administration schedule of taxane chemotherapy. It has been shown that certain dosing strategies, such as lower doses administered more frequently (also known as dose-dense or metronomic dosing), may be associated with a lower risk of lymphedema compared to traditional dosing schedules. Clinical pharmacists can also provide recommendations on alternative dosing strategies and monitor patients for any adverse effects [Al-Taie, & Sheta, 2024]. They can also explore alternative chemotherapy agents that may pose a

lower risk of lymphedema. For instance, as mentioned earlier, platinum-based chemotherapy drugs have not been strongly linked to lymphedema and may be a suitable alternative for some patients. Additionally, clinical pharmacists can recommend medications to manage the symptoms of lymphedema. Diuretics, for example, can help reduce swelling by removing excess fluid from the body. However, these must be used judiciously due to potential side effects, such as electrolyte imbalances. Pharmacists can also advise on over-the-counter options, such as topical creams to care for the skin and prevent infection.

2. Patient education about lymphedema post-treatment

Education is a fundamental role of the clinical pharmacist, and it's particularly important when it comes to informing breast cancer patients about the risk and signs of toxicities, including lymphedema post-treatment. This education can empower patients to be vigilant about changes in their bodies and to seek help promptly if lymphedema symptoms develop. This also includes recommending appropriate pharmacological and non-pharmacological treatments, monitoring treatment outcomes, and adjusting treatment plans as necessary [Al-Taie & Yilmaz, 2021]. Proper education can empower patients, leading to earlier detection and treatment of lymphedema and, ultimately, better patient outcomes. This includes explaining the potential impact of taxane chemotherapy on the lymphatic system and how it can lead to lymphedema. It's also essential to discuss other factors that can contribute to lymphedema, such as surgical removal of lymph nodes or radiation therapy. Patient education should start with a discussion about the risk of developing lymphedema following breast cancer treatment. Clinical pharmacists can also be a part in this area and can explain the signs and symptoms of lymphedema, the importance of early detection, and strategies for risk reduction.

3. Patient-centric care and shared decision-making

Patient-centric care and shared decision-making are vital in managing taxane-related lymphedema. Such an approach respects the patient's autonomy, fosters a collaborative relationship between the patient and healthcare provider, and ensures that the care plan aligns with the patient's needs and preferences. Shared decision-making is another fundamental aspect of patient-centric care. Patients should be active participants in making decisions about lymphedema preventive measures, and management strategies. This requires clear, open communication between the healthcare

provider and patient about the benefits, risks, and alternatives of each treatment option. In managing lymphedema, a patient-centric approach also considers the patient's quality of life. Lymphedema can cause significant physical discomfort and functional limitations, impacting daily activities and mental well-being. Therefore, treatment decisions should aim not only to manage lymphedema but also to minimise its impact on the patient's lifestyle and overall quality of life. As key members of the healthcare team, clinical pharmacists' role in managing taxane-related lymphedema in breast cancer patients is multifaceted and crucial, contributing significantly to patient care across multiple levels. Their involvement spans from patient counselling and education to active participation in treatment decision-making and research. Moreover, they can significantly contribute to delivering patient-centric care, through patient education, facilitating shared decision-making, and advocating for the patient's needs. By doing so, clinical pharmacists can help improve not only lymphedema management but also the overall care experience for patients undergoing breast cancer treatment.

Recommendations and clinical practice points

Gaps in current knowledge and research opportunities

1. The precise mechanism of how taxanes contribute to lymphedema remains unclear. More research is needed to understand the pathophysiology at a molecular level. This could lead to the development of preventative strategies or therapies to mitigate the risk of lymphedema in patients undergoing taxane therapy.
2. While certain risk factors for taxane-related lymphedema have been identified, the list is not exhaustive. Identifying more comprehensive and specific risk factors, such as genetic predispositions, would allow clinicians to predict more accurately which patients are at higher risk. This would enable personalised strategies for lymphedema prevention and early intervention.
3. Many emerging therapies for lymphedema are still in the early stages of research. Rigorous, large-scale clinical trials are needed to validate the efficacy and safety of these new treatments. The role of pharmacogenomics in personalising breast cancer treatment to minimise lymphedema risk also requires further exploration.
4. There is a lack of standardised criteria for diagnosing and staging lymphedema, leading to variations in reported incidence rates and challenges in comparing studies. Research efforts should focus on

developing and validating standardised diagnostic criteria and staging systems to improve consistency in research and clinical practise.

Future strategies for preventing taxane-related lymphedema

Preventing taxane-related lymphedema in breast cancer patients is a complex challenge that will require the implementation of future strategies rooted in growing understanding of the disease and advancements in breast cancer treatment. These strategies include:

- *Early risk assessment and education:* as our understanding of the risk factors for taxane-related lymphedema expands, we can better identify patients at increased risk before they begin chemotherapy. Providing these patients with education on signs of lymphedema and lifestyle modifications that can reduce risk may facilitate early detection and intervention, possibly preventing the progression of the condition.
- *Personalised medicine:* pharmacogenomics has the potential to revolutionise how we approach breast cancer treatment. Identifying genetic markers that could influence a patient's response to taxanes may allow us to tailor treatment plans that are both effective against the cancer and lower the risk of lymphedema.
- *Physical therapy:* incorporating exercise into the treatment plan may also be beneficial. Research suggests that guided exercise may improve lymphatic function and reduce the symptoms of lymphedema. This can be combined with other preventive strategies, such as compression garments, to manage the condition more effectively.
- *Regular monitoring and follow-up:* appointments with physical examinations and, potentially, bioimpedance spectroscopy measurements, can help detect lymphedema at its earliest stages, when interventions are most effective.

CONCLUSION

Reflecting on the presented evidence, taxane-related lymphedema is a substantial health issue in breast cancer patients that warrants serious

attention. The interplay of various factors, the compounded damage of the lymphatic system, and the availability of a variety of symptoms contribute to the complexity of this oncology disorder. Moreover, complications associated with lymphedema not only affect physical health but also have psychological implications and can considerably lower a patient's quality of life. It is crucial to develop tailored treatment plans considering each patient's unique characteristics and risk profile. Furthermore, with a deeper understanding of the impact of taxanes on the lymphatic system, the gaps in current knowledge across understanding the underlying mechanisms, identifying risk factors, validating emerging therapies, and standardising diagnostic and staging criteria present valuable opportunities for further research for early risk assessment and education, personalised medicine, physical therapy, and vigilant monitoring, thereby paving the way for advanced patient care and outcomes and better prevention and management strategies in the future. The integration of these strategies requires a concerted and coordinated effort by the entire healthcare team, including clinical pharmacists. The multidisciplinary team can play a significant role in identifying high-risk patients and managing taxane-related lymphedema. These insights are multifaceted and vital in bridging these gaps and implementing these strategies, providing an additional layer of support for breast cancer patients in managing this complex and challenging side effect through patient-centred care, providing patient education, and empowering them to recognise the early signs of lymphedema and seek treatment promptly, thereby optimising patient outcomes and potentially improving their quality of life and outcomes.

Ethics approval and consent to participate

Not applicable.

Author contributions

AA, and AA drafted the original manuscript, and tables; AA, and AA were involved in manuscript revision; AA established the concept. AA, AA, and MYB reviewed and revised the manuscript, and supervised. All the authors have read and approved the final version of the manuscript.

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Consent to publish

All the authors have given consent for the publication of this manuscript.

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CHAPTER 8

NEUTRON AND PROTON DENSITY

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INTRODUCTION

Neutron and proton density distributions in the nucleus are of fundamental importance in the understanding nuclear properties and the description of nuclear structure. The density is a direct measure of the size of the nucleus and plays an important role in the cross sections of nuclear reactions. The algebraic form of nuclear density is especially critical for analytical studies of nuclear scattering and reaction processes (Gambhir et al., 1989; Lalazissis et al., 1997; Pei et al., 2005; Chu et al., 2010). The proton distribution in the nucleus is determined by the charge distribution measured from electromagnetic interactions such as electron-nucleus scattering, and the physical meaning of the proton density distribution is expressed by the charge density distribution (Richter and Brown; 2003; Centelles et al., 2010).

Neutron distribution in the nucleus is insensitive to electromagnetic interactions and is not sufficient to obtain information on neutron distribution (Patterson and Peterson, 2003; Schmidt et al., 1999). Strong interactions such as protons, pions and alpha particles are required to study neutron distribution. However, hadronic studies show uncertainties in the reaction mechanisms due to incomplete knowledge of the nucleon-nucleon interaction mechanism, and the neutron densities obtained from these studies are model-dependent. This book chapter discusses the approaches usually used to calculate proton and neutron densities in the nucleus and presents some studies using these approaches.

NEUTRON AND PROTON DENSITY

Nuclear matter is ideally defined as a system in which infinitely large neutrons and protons interact only through the strong interaction; electromagnetic interactions are ignored. In this context, nuclear matter is considered in two forms: symmetric nuclear matter, where neutron and proton densities are equal, and asymmetric nuclear matter, characterised by the difference between neutron and proton densities (Alonso and Sammarruca, 2003). Early in modern physics, it was realised that the neutron and proton distributions of stable nuclei would not be equal. A nuclear model proposed by Johnson and Teller (1955) predicted that in nuclei with an excess of neutrons, neutrons would spread over a larger radius than protons.

The most important effect of the Coulomb potential is to increase the difference between neutron and proton numbers. The Coulomb potential

changes very slowly within the nucleus and this change tends to increase the density of protons compared to neutrons at the surface. Johnson and Teller (1953) also mentioned an effect that increases the number of neutrons at the surface. Johnson and Teller considered two important effects: When the number of neutrons exceeds the number of protons, neutrons on average have a higher kinetic energy than protons and spread more widely than protons. The Coulomb potential creates a barrier that prevents the passage of proton wave functions. For these arguments to be valid, protons and neutrons must have approximately equal nuclear potentials (Berg and Wilets, 1956).

In this context, the interaction of kaons with the nucleus has been studied (Burhop, 1967; Davis, et al., 1967; Cotanch, 1981). These studies analyzed the possible products of the interaction of K mesons with neutrons and protons in heavy and light nuclei and, considered as important evidence that heavy nuclei have a surface largely composed of neutrons and that neutrons are spread over a larger radius than protons in heavy nuclei. The proton distribution in the nucleus is expressed as the nuclear charge distribution and is used to describe nuclear structure. Due to the simple reaction mechanism of electromagnetic research, precise information about the charge density in the nucleus can be obtained. General information on charge densities is obtained from studies such as electron and muon scattering, nuclear scattering and reactions, meson atoms and isobaric energy shifts.

Nuclear charge densities are generally expressed using Fourier-Bessel constants, Fermi distribution, Gaussion distribution and Harmonic oscillator distribution (Vries, et al., 1987; Wong, 2004). The charge density, where the charge density radius R_c is expressed by the zeroth order spherical Bessel function $j_o(\xi)$, is expressed as follows:

$$\rho_{ch} = \begin{cases} \sum_k a_k j_o(k\pi r/R_c) & r \le R_c \\ 0 & r > R_c \end{cases}$$

Here the parameter a_k is the Fourier-Bessel constant and is taken as follows in relation to the charge density:

$$a_k = \frac{2k^2\pi^2}{R_c^3}\int_0^{R_c} \rho(r) j_o(k\pi r/R_c) r^2 dr$$

It is very difficult to express the density distribution in terms of Fourier-Bessel constants. Moreover, the density is almost constant outside the surface region ($R \approx r$). This feature is particularly pronounced in heavy nuclei, where the density decreases exponentially towards the surface. This distribution is usually represented by the two-parameter Fermi distribution.

$$\rho_{2pF}(r) = \frac{\rho_o}{1 + exp[(r-c)/z]}$$

Here, the parameter c is the distribution diameter at the point where the center density is halved, while the parameter z is the surface distribution parameter related to the thickness of the surface region. Both parameters were obtained from experimental studies using various atomic nuclei. The density distributions observed by studying different nuclei are better described by the three-parameter Fermi distribution, which is regularized by the parameter ω added to the two-parameter Fermi distribution. The three-parameter Fermi distribution is expressed as follows:

$$\rho_{3pF}(r) = \frac{\rho_o(1 + \omega(r/c)^2)}{1 + exp[(r-c)/z]}$$

Here, $z = t/4.4$ is taken as and if $\omega = 0$, the t parameter corresponds to the distance at which the surface thickness decreases from 90% to 10% (Bellicard and Oostrum, 1967). Another density distribution expression is the three-parameter Gaussian distribution,

$$\rho_{3pG}(r) = \frac{\rho_o(1 + \omega(r/c)^2)}{1 + exp[(r^2 - c^2)/z^2]}$$

and the Harmonic oscillator density distribution.

$$\rho_{HO}(r) = \rho_o[1 + z(r/c)^2]e^{-(r/c)^2}$$

Nuclear charge density is usually described in Gaussian and Fermi types. In general, the Gaussian distribution is suitable for describing the charge density of light nuclei, while the Fermi distribution is more effective in describing the charge density of heavy nuclei (Chu, et al., 2010).

Another approach developed to study the density profiles of nuclei at different temperatures is the Thomas Fermi approach (De, et al., 1996; 1998). The general expression for this approach is taken as follows:

$$\rho(r,T) = \frac{2}{h^3}\int n(r,p)dp = A_T^* J_{1/2}(n(r))$$

Here, $A_T^* = \frac{4\pi}{h^3}[2m^*(r)T]^{3/2}$ and The Fermi integral expression is taken as follows

$$J_K(\eta) = \int_0^\infty \frac{x^K}{1 + \exp(x - \eta)}dx$$

In the expression for density above, h is the Planck constant, T is the temperature, and m^* is the effective nucleon mass.

Reliable information about the proton distribution is usually obtained through electron-nucleus scattering, and experimental data are analysed using

charge parameters taken in accordance with proton density parameters (Patterson and Peterson, 2003). Information about the proton distribution can also be obtained by analysing the spectrum of muonic X-rays (Gils and Rebel, 1976; Lalazissis, et al., 1997). Although information on the proton density distribution can be obtained, the neutron density distribution is generally unknown and the information obtained is less and less precise (Pei, et al., 2005). Since neutrons are uncharged particles, measuring their spatial distribution is much more difficult than determining the spatial distribution of positively charged protons (Warda et al., 2010).

Many theoretical and experimental studies have been carried out to determine the neutron density. Since these studies use strongly interacting particles such as protons, alpha particles and pions, the analysis of scattering data varies from model to model and the results are affected by uncertainties in many-particle scattering theories (Ray and Hodgson, 1979). Hadronic studies, especially in the nuclear environment, carry uncertainties in reaction mechanisms due to incomplete information on nucleon-nucleon scattering amplitudes. Therefore, information on neutron densities is less reliable. To obtain precise information on the neutron density distribution, it is necessary to carefully select suitable probes and effective nucleon-nucleon interactions. In this context, the scattering of α particles from the nucleus due to their strong interaction with nuclear matter has become an important tool in nuclear structure research (Bernstein and Seidler , 1972; Tatischeff, et al., 1972). Gils and Rebel (1976) obtained information on neutron distribution by measuring the elastic scattering cross sections of 104 MeV α particles from $Pb^{204,206,208}$isotopes. Proton distributions were taken from electron scattering analyses. Neutron distributions are expressed in terms of Gaussian parameters modified according to the experimental cross sections. Whether the nuclei have a neutron surface is discussed both experimentally and theoretically.

Proton-nucleus elastic scattering at moderate energies is one of the most frequently used research methods to obtain information about the nuclear interior and surface. In these studies where the sum of Gaussian distribution was used, the reaction model for the neutron density distribution and the measurement results of the uncertainties in the experimental data were reported (Thomas, 1969; Egelhof, 2001; Piekarewicz and Weppner, 2006; Zenihiro, et al., 2010).

In the studies of the interaction of antiprotons with the nucleus, the neutron and proton density on the surface of the nucleus was investigated using two different methods, namely the examination of the antiproton-X-ray spectrum and the determination of the annihilation efficiencies of the products resulting from the nuclear antiproton reaction. (Baran, et al., 1996; Schmidt, et al., 1999; Trzcinska, et al., 2001; Klos, et al., 2007).

In these studies, using these two methods, the neutron and proton density on the surface of $Yb^{172\text{-}176}$ (Schmidt, et al., 1999) nuclei and the neutron density for Pb^{208} and Bi^{209} (Klos, et al., 2007) nuclei were investigated using the antiproton-nucleus optical potential. In this context, by considering the differences between the neutron and proton densities of medium and heavy nuclei, the square of the mean radius of the radial neutron distribution and the difference between the squares of the mean radii of neutrons and protons (Δr_{np}) were obtained and it was predicted that the surface of heavy isotopes was largely composed of neutrons.

In addition to the experimental determination of the neutron and proton density distribution in nuclei, the density distribution in superheavy nuclei with atomic numbers between 104 and 120 was investigated using the Skyrme-Hartree Fock model in a theoretical study based on the model (Pei, et al., 2005). In this study, using different Skyrme interactions, collapses in the density distribution at the center were observed due to the deformation effect.

In some theoretical studies (Gambhir and Patil, 1985; 1986; 1989; Gambhir, et al., 2001), simple semi-phenomenological expressions for neutron and proton densities have been obtained. These expressions are developed by considering the asymptotic behavior of the density and near-center properties. Using these expressions, predictions are made about the surface thicknesses and radii of the nuclear densities depending on the energy of separation of the last particle. As a result of quasi-phenomenological studies, it was observed that the proton center density decreases with atomic number Z and that there are significant increases in density in nuclei with magic proton number. In this context, it was determined that closed neutron shells caused an increase in proton density. When the neutron center density is approximately constant, proton closed shells are also found to increase the neutron density. It is found that the Coulomb interaction causes significant differences in the neutron-proton surface thicknesses, and in general the surface thickness and rms radius of neutrons are larger than protons.

The neutron and proton densities of the Pb^{208} nucleus have been studied due to the spherical structure of this nucleus and the fact that it has magic numbers for both neutrons and protons. Moreover, this nucleus is used as a target nucleus in controlled reactor systems where energy is generated by burning nuclear waste (Gokbulut et al., 2013).). In this study, the neutron and proton density distributions of the Pb^{208} nucleus were calculated using a new analytical expression involving the Fermi integral. The calculations using the Thomas-Fermi approach for the nucleon distribution of the Pb^{208}nucleus were compared with other theoretical and experimental studies and a new analytical expression was obtained that can be used for other nuclei. Furthermore, in another study using the Thomas-Fermi method, the neutron densities of even-even Sn isotopes (A=116-132) were calculated by a new analytical expression based on the Fermi integral (Eser, et al., 2016). In this study, the neutron densities of individual isotopes were analyzed and the nuclear density regions where the Thomas-Fermi approach can be used were determined. In another theoretical study (Koc et al., 2019), the Coulomb potential for spherical nuclei was calculated using the Fermi distribution function suitable for the charge distribution in the nuclei and it was concluded that the nuclear diffusion parameter should take different values for all spherical nuclei.

In another study, the proton and neutron density profiles of 760 nuclei in the $A = 16 - 304$ mass region were analyzed using the Skyrme energy density for the Sly4 parameter set (Seif and Mansour, 2015). In this study using the two-parameter Fermi distribution, the local proton (neutron) density was obtained as the sum of singlet particle- occupied states, and it was reported that neutron and proton densities generally increase linearly with the radii $N^{1/3}$and $Z^{1/3}$, respectively.

Recent investigations into the proton and neutron skins of nuclei provide significant insights into proton and neutron density research. In this context, a study (Mahzoon, et al., 2017) investigating the neutron skin thickness for the Ca^{48} isotope using nonlocal dispersive optical model analysis determined the charge and neutron matter distributions in $Ca^{48}$and predicted that the neutron matter distribution expands towards large radii and forms a neutron skin.

In another study conducted in this context (Thiel, et al., 2019), both statistical and systematic errors specific to the theoretical models used to relate the measured experimental observables to the neutron skin were

investigated. Additionally, Gaidarov et al. (2020) explored the relationship between nuclear charge radii and neutron skins using Skyrme interactions and the Hartree-Fock-Bogoliubov method, making predictions about the differences between the neutron skins of nuclei and the proton radii of their mirror nuclei.

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CHAPTER 9

SILVER AND GOLD NANOPARTICLES INTEGRATED WITH ESSENTIAL OILS: A NOVEL APPROACH FOR COMBATING ANTIBIOTIC RESISTANCE

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INTRODUCTION

Pathogenic microorganisms negatively impact public health worldwide, causing serious infections and, consequently, morbidity and mortality. The excessive and inappropriate utilization of antimicrobial drugs in therapeutic applications has led to the rapid global spread of multi-drug-resistant microorganisms through events such as mutations and horizontal gene transfer (CDC, 2020 a; Yakimov et al., 2021; WHO, 2021). The increase in resistant strains adversely affects treatment, leading to extended hospitalization periods and exacerbating the economic burden on countries by increasing outbreaks (Friedman et al., 2016). The presence of drug-resistance genes (DRGs) in the environment suggests a correlation between DRG transmission and human activities (Zhuang et al., 2021). The use of increased amounts of antibiotics in the therapy of multidrug-resistant organism (MDRO) diseases results in toxic side effects, necessitating the search for alternative treatments (Muzammil et al., 2018; Natan et al., 2017).

MNPs produced through diverse physical and chemical techniques, including precipitation, ultrasonication, ball milling, thermal dissolution, spray pyrolysis, thermal hydrolysis, and sol-gel methods, pose environmental risks attributed to the employment of hazardous solvents and the execution of procedures involving elevated temperatures and pressures. (Alphandéry, 2019a,b). Environmentally friendly plants and products (flavonoids, aldehydes, phenolic compounds, etc.) are served as reducing agents in the manufacturing process of MNPs such as silver and gold (Kanchi et al., 2018; Kratosova et al., 2019; Rozhin et al., 2021). Naturally synthesized MNPs offer benefits like small-scale production, economic efficiency, and easy accessibility (Mülhopt et al., 2018; Saravanan et al., 2008). Bionanotechnology contributes to the advancement of potential antimicrobial solutions by facilitating the interaction of medicinal plants and products with nanostructures through biological applications (Backx et al., 2022; Raja et al., 2022).

EOs, secondary metabolites of plants, have been utilized in folk medicine due to their biological properties, including antimicrobial activity (Wińska et al., 2019). EOs contain a variety of bioactive components and exhibit antibacterial effects *in vitro* and *in vivo* by disrupting bacterial cell wall integrity, bacterial biofilm, ATP, and protein synthesis pathways (Orhan-Yanikan et al., 2019). However, their instability, susceptibility to oxidation

and degradation, volatility, low water solubility, and toxic effects at high concentrations limit their applications (Altun et al., 2023; Kavetsou et al., 2021; Tsitlakidou et al., 2023). Encapsulation of EOs into biopolymeric microparticles or nanoparticles is a viable alternative to preserve them without degradation (Volic et al., 2018). MNPs loaded with medicinal plants exhibit antibacterial, antifungal, and antibiofilm effects based on their morphological, physical attributes, and chemical compositions. Moreover, they can be utilized in developing effective methods against antimicrobial resistance (Dikshit et al., 2021; Kambale et al., 2020; Mohanta et al., 2020; Shkodenko et al., 2020; Singh et al., 2020). This study aims to assess the antibacterial effects of Ag and Au nanoparticles loaded with various EOs.

Essential Oils

EOs commonly used in phytotherapy are typically derived from the *Myrtaceae, Myristicaceae, Piperaceae, Rutaceae, Asteraceae,* and *Lamiaceae* families. EOs possess a diverse range of compounds that determine their biological activities, exhibiting both polar and non-polar characteristics (Macwan et al., 2016; Matos et al., 2019). Climatic conditions, genotypes, environmental factors, production methods, and physiological factors contribute to the composition of EOs. Volatile components produced by plants for protection against pathogens contain compounds such as aldehydes, alcohols, esters, ketones, oxides, phenol ethers, hydrocarbons, terpenes, and acids, which play a role in detecting their antimicrobial features (Chouhan et al., 2017; De Groot et al., 2016; Eslahi et al., 2017; Eze, 2016; Ramsey et al., 2020). Particularly, monoterpenes and sesquiterpenes are effective in various biological activities of EOs (Sharmeen et al., 2021).

EOs obtained through techniques such as steam, water, and dry distillation, as well as mechanical processing, exhibit antibacterial, antioxidant, antiseptic, anti-inflammatory, anesthetic, and anti-cancer properties. Recent studies have noted the bacteriostatic and bactericidal impacts of EOs such as fennel, mint, thyme, lavender, basil, rosemary, eucalyptus, manuka, and tea tree on bacteria (Ali et al., 2015; Bassanetti et al., 2017; Najafi-taher et al., 2018; Nazzaro et al., 2017; Reichling et al., 2009). EOs have broad usage in the pharmaceutical, food, and cosmetic fields (Sharmeen et al., 2021; Souto et al., 2020). External factors like light, temperature, oxidation, or hydrolysis impact the chemical stability of EOs, resulting in degradation. (Turek et al., 2013). Although inherently organic and

eco-friendly, the application of EOs in bacterial infection treatment faces limitations stemming from factors like limited solubility, evaporation, and the potential toxicity of solvents. Encapsulation with MNPs has been employed to boost the stability, and biological activities of EOs (Miguel et al., 2020).

Nanoparticles

Particles within the size range of 1 to 100 nanometers exhibit distinctive traits, characterized by their expansive and active surface areas dictating adsorption capabilities, reactivity, and antimicrobial properties. Factors such as color, shape, size, and aggregation level influence the bioactivities of nanoparticles (Chang et al., 2019; Hosseinzadeh et al., 2020; Penyala et al., 2008). The substantial surface area of nanoparticles leads to increased interactions with microorganisms, making them effective in bacterial inhibition at low concentrations (de Oliveira et al., 2020). Nanoparticle-based systems, such as nanobactericides, demonstrate antimicrobial activity by deactivating bacterial enzymes, decreasing membrane permeability, enhancing the expression of efflux pumps, and damaging drug-binding targets (Vassallo et al., 2020). Additionally, nanoparticles disrupt the integrity of microbial membranes, generate reactive oxygen species (ROS), and impede protein and RNA synthesis, exhibiting toxic effects due to different cytotoxic targets in physicochemical reactions (Fröhlich et al., 2013; Gold et al., 2018).

Nanoparticles can be produced using various methods, including physical, chemical, and biological approaches that incorporate plants, bacteria, fungi, and algae. (Ijaz et al., 2020; Iravani et al., 2014). On the other hand, chemical approaches involve environmental hazards as a result of employing organic solvents and harmful chemicals (Sulaiman et al., 2013). With antimicrobial activity, nanoparticles find numerous applications (Aref et al., 2020; Hasanin et al., 2022; Milanezi et al., 2019; Salem et al., 2022). Nanoparticles contribute to enhancing drug solubility, reducing toxicity, improving stability, and decreasing drug resistance, making them valuable in various applications (Medhi et al., 2023).

Metal Nanoparticles

Nanotechnology is a rapidly evolving field, and MNPs, with their distinct size and shape-dependent properties, find applications in various sectors such as medicine, pharmaceuticals, cosmetics, textiles, and food.

Furthermore, they have found applications in biotechnology, and more recently, *in vitro* plant cultures (Kamat et al., 2002; Navarro et al., 2008; Ocsoy et al., 2018). The most commonly used metals in nanoparticle synthesis are gold and silver. Silver nanoparticles (AgNPs) exhibit antimicrobial activities, while gold nanoparticles (AuNPs) easily penetrate cell walls allowing particles of 5-20 nm in size to transit, stimulate cell renewal, and demonstrate therapeutic effects (Choi et al., 2008; Hackenberg et al., 2011; Nair et al., 2010).

Factors such as bacterial types and strain diversity, density, culture conditions (temperature, pH), nanoparticle size, shape, and zeta potential, the type of discs can influence the inhibition zone diameter (IZD) and minimum inhibitory concentration (MIC), thereby altering antimicrobial activity (Balouiri et al., 2016; Eloff et al., 2019; Jorgensen et al., 2009; Shanmugakani et al., 2020). MNPs achieve their antimicrobial effects through different mechanisms such as disrupting the microorganism's cell wall structure, inhibiting DNA replication, protein and enzyme synthesis, increasing ROS production, and inhibiting biofilm formation (Baptista et al., 2018; Siddiqi et al., 2018; Singh et al., 2014). The mechanisms of action for MNPs depend on their origin and biological, physical, and chemical properties (Rudramurthy et al., 2016).

MNPs synthesized using chemical methods exhibit high reactivity due to the chemicals used, leading to negative impacts on the environment and human health. This has particularly restricted their use in clinical applications (Dhand et al., 2016). AgNPs and AuNPs exhibit antibacterial and biostability activities due to their electrical, magnetic, and thermal conductivity features (Qin et al., 2021). Additionally, owing to their biocidal effects, these nanoparticles have long been used as antimicrobial substances in agriculture and the health industry. Coated MNPs, in particular, are more effective on host cells compared to bare MNPs (Maduray et al., 2021; Palza et al., 2015). In recent years, AgNPs and AuNPs have been employed in oncology, photothermal therapy, biomolecule labeling, nanoscience-based diagnostics, pharmaceutical distribution, genetic treatment, immune chromatography analyses, and pathogen identification in clinical samples (Botha et al., 2019; Pasparakis et al., 2022).

AgNPs

Silver is known to interact with the negatively charged cell membranes of microorganisms, releasing ions, increasing oxidative stress, and reacting with phosphorus in DNA, exhibiting antimicrobial effects (Burdus et al., 2018; Phan et al., 2019). Silver is utilized as an antimicrobial agent in the form of nanomaterials or metal salts, used in water purification, chemistry, the food industry, agriculture, and biomedical applications (Anjali Das et al., 2020). Silver ions can be biologically synthesized into MNPs, enhancing their antibacterial effects and reducing toxicity (Kambale et al., 2020). Within the field of nanoscience, silver attracts interest due to its exceptional traits, such as its ability to conduct heat, maintain chemical stability, demonstrate catalytic abilities, and exhibit antibacterial properties. Nano-scale silver (nano-Ag) displays distinct mechanical, optical, and electrical characteristics owing to surface and quantum effects, which also impact its chemical reactivity. Recent studies highlight that biocatalytic processes can yield potent, biocompatible AgNPs with antifungal, antibacterial, and antioxidant properties. Nonetheless, the therapeutic effectiveness of synthesized AgNPs hinges on factors like size, shape, surface area-to-volume ratios, and surface modifier composition (Zakeri et al., 2021).

Through nanotechnology, AgNPs at the nanometer scale show better antibacterial activity against resistant bacteria in contrast to silver, owing to their elevated surface-to-volume ratios (Herman et al., 2014; Nowack et al., 2011; Rai et al., 2012). AgNPs are used in various commercial products, including medical devices, food supplements, animal feed, packaging materials, and kitchen utensils, due to their ability to exhibit good antimicrobial activity at low concentrations (Ameta et al., 2018; Deshmukh et al., 2019; Rafique et al., 2017). Additionally, silver nanomaterials have been reported as the most commonly used medical nanoparticles (Lee et al., 2019). AgNPs have been utilized for centuries as antimicrobial agents in the treatment of wounds, eye infections, and the preservation of food and water (Alexander et al., 2009; Chernousova et al., 2013). It has been reported that AgNPs exhibit lower toxicity towards hosts when compared to silver ions (deLima et al., 2012). The enhanced antimicrobial efficacy of AgNPs is attributed to their ability to generate free radicals (Duran et al., 2016). Bacteria do not have the metabolism to overcome the toxicity of AgNP, making them vulnerable to toxicity (Khina et al., 2021). AgNPs exhibit bactericidal mechanisms, including binding to cell surface components,

disrupting permeability, respiration, and energy synthesis, interfering with transcription by interacting with nucleic acids, and inhibiting protein synthesis. Additionally, they disrupt metabolic activity by generating ROS (Eghbalifam et al., 2005; Morones et al., 2005; Simbine et al., 2019; Tang et al., 2018).

AgNPs can be incorporated into hydrogels, cyclodextrins, and lipid-based formulations for regulated discharge and precise administration (Barbinta-Patrascu et al., 2013; Celebioglu et al., 2019; Gupta et al., 2020). Furthermore, the production of AgNP formulations is used in the development of diagnostic and detection platforms (Vishwakarma et al., 2018). Factors such as pH, temperature, environment, solvent type, preparation method, and $AgNO_3$ concentration are crucial in the production of AgNPs (Aragaw et al., 2022). Their effectiveness is determined by physical characteristics like shape, size, and coating (Fahmy et al., 2019). AgNPs are prepared using chemical methods, utilizing hydrazine hydrate and sodium citrate as agents for reduction, along with sodium dodecyl sulfate as a stabilizer in an $AgNO_3$ solution (Guzman et al., 2012). These methods can negatively impact applications due to the adsorption of toxic reducing and coating chemical agents on nanoparticles (Soni et al., 2021). Although AgNPs can be used after purification from chemicals, their broad application is restricted by the challenges and expenses associated with purification (Luo et al., 2015). To overcome these challenges, research has focused on green synthesis methods.

AuNPs

In recent years, advances in nanotechnology have increased interest in AuNPs for therapeutic applications (Vijayan et al., 2018). Gold has been used as a coating material in the pharmaceutical industry since the 16th century (Sane et al., 2013). AuNPs are preferred in biological studies for their specific structural features that enable the detection of biological molecules and the monitoring of surface events. The activities of these particles are determined based on characteristics such as size, shape, and dispersion (Huang et al., 2006; Khan et al., 2019). Normally, gold exists in two oxidation forms, Au^{+} and Au^{3+} both of which can be found in the colloidal form at the nanometer scale and can be easily reduced to Au^{0} (Colacio et al., 1996). AuNPs exhibit various color tones, ranging from red to blue, depending on their sizes, shapes, and quantities. Additionally, they possess features such as high stability, sensitivity, and consistency (Chang et al., 2019). Gold's resistance to

chemical reactions and biological compatibility make it preferable in biomedical applications. Moreover, the optical properties of AuNPs can be modified based on their sizes, geometries, and environmental dielectric mediums. The flexibility of AuNPs allows them to be effectively functionalized by various biomolecules, extending beyond the inherent properties of their metal cores (Oueslati et al., 2021). Due to these characteristics, AuNPs find applications in biomedical fields such as treatments, imaging, pharmaceutical transport, and immunochromatographic detection of pathogens in medicine, materials engineering, physics, and biology (Chung et al., 2022; Fan et al., 2022; Hammami et al., 2021; Rai et al., 2013). The antimicrobial and antibiofilm activities of AuNPs on pathogens have led to their extensive utilization in cosmetics, the environment, and agriculture (Anwar et al., 2021; Bahrulolum et al. 2021; Ben Haddada et al., 2020; Lahtinen et al., 2019). Applications such as photo-reduction, ultrasonic waves, microwave irradiation, chemical reduction, and thermal decomposition in organic solvents utilized for the functional synthesis of AuNPs in shape and size lead to adverse effects on health and the ecosystem (Kishore et al., 2022; Nasrollahzadeh et al., 2015). Due to the toxic effects of traditional synthesis methods, materials scientists prefer plant-based approaches for AuNP production. This has garnered increasing attention due to its enhanced chemical, physical, electrical-optical, and biological properties (Al-Radadi, 2018; Katas et al., 2018). AuNPs can be easily attached through surface modification with coating materials. This allows them to specifically bind to targets and be imaged. Studies have shown that chemically synthesized AuNPs exhibit antibacterial properties only at high concentrations (Amin et al., 2009; Chatterjee et al., 2011; Dasari et al., 2015; Zhang et al., 2015). Biogenic AuNPs are being investigated as antibacterial and chemotherapeutic agents due to their remarkable selectivity, bioactivity, and minimal toxicity (Abbasi et al., 2016).

Green Synthesis

Green synthesis is acknowledged as a biological method for obtaining environmentally friendly and cost-effective nanoparticles, devoid of the use of toxic chemicals (Emeka et al., 2017; Thomas et al., 2019). Intensive research has been directed towards natural substances such as EOs and plant extracts, known for their harmless, eco-friendly, antibacterial, and antioxidant properties for nanoparticle synthesis (Hosseinzadeh et al., 2020). Green chemistry plays a crucial role in preserving biodiversity (Thomas et al., 2019).

The synthesis of MNPs through plant-mediated processes is considered easy, rapid, environmentally friendly, and sustainable, making them easily adaptable to clinical applications and scalable for industrial use (Kambale et al., 2020; Vanlalveni et al., 2021). Moreover, secondary plant metabolites like flavonoids, tannins, terpenoids, and alkaloids act as both reducing and coating agents during the synthesis of MNPs. (Rónavári et al., 2021; Roy et al., 2019). The combination of plant components with MNPs has attracted considerable attention in the fields of phytotechnology, pharmaceuticals, clinical microbiology, and medicine (Ahmed et al., 2016; Dikshit et al., 2021). Green AgNPs, acting as effective coating agents, have demonstrated their efficacy in reducing skin infections and preventing bacterial adherence to medical apparatus such as catheters and prosthetics (Khan et al., 2018; Makarov et al., 2014; Makarov et al., 2018; Raji et al., 2019). Encapsulation techniques covering both physical and chemical processes have found applications in biomedicine and the food industry (Lis Arias et al., 2022).

Essential Oil-Loaded Nanomaterials

Through nanotechnological applications, EOs are integrated into various metal nanoparticles including gold, silver, platinum, iron, copper, chitosan, and zinc with sizes varying between 1 to 100 nm. These nanoparticles exhibit high reactivity, sensitivity, large surface area, stability, and antimicrobial activity. EO-loaded nanoparticles serve to protect the EOs against external factors like heat and UV, consequently extending their shelf life and therapeutic effectiveness. Furthermore, EO-loaded nanoparticles facilitate faster diffusion of EOs through cell membranes, contributing to a synergistic antimicrobial effect (Nair et al., 2022). Nanoencapsulation provides numerous advantages, including protection of EOs from external influences, increased solubility, reduced aroma intensity, prevention of negative interactions with other elements, heightened biological effectiveness, and precise delivery. EO-loaded nanovesicles demonstrate antioxidant, anti-inflammatory, and antibacterial effects (Albuquerque et al., 2022; Kumar et al., 2020). Combining certain EOs with nanoparticles holds promise for controlling multi-drug-resistant pathogens (Basavegowda et al., 2020; Chi et al., 2019; Nair et al., 2022).

Antibacterial Activity of EO-Loaded AgNPs

Nanoparticles loaded with EOs have demonstrated their effectiveness in skincare applications, exhibiting remarkable antibacterial properties against

various pathogens. For instance, AgNPs synthesized with oregano (*Origanum vulgare*) EO exhibited bactericidal effects against 17 strains with MIC values between 0.298 and 1.193 mg/mL (Scandorieiro et al., 2016). Thyme (*Thymus vulgaris*) EO-loaded AgNPs demonstrated stronger antibacterial efficacy against various bacteria compared to $AgNO_3$, proving to be synergistically effective (Aldosary et al., 2021). Additionally, thyme EO-reduced AgNPs were found effective against *Escherichia coli* (*E.coli*) and *Staphylococcus aureus* (*S.aureus*) (Melo et al., 2020). In a study replacing toxic chemicals with natural EOs from thyme, clove, rosemary, and *Poiretia latifolia* EO-loaded AgNPs showed antibacterial activity against *S.aureus* at various concentrations (Maciel et al., 2020). Cinnamon, cardamom, and clove EO-loaded AgNPs demonstrated potent antimicrobial activities, against *S.aureus* and *Klebsiella pneumoniae* (*K.pneumoniae*) (Pervaiz et al., 2023). A study synthesizing *Litsea cubeba* EO (Lceo)-AgNPs shows the stability-enhancing role of Lceo along with significant *in vitro* antibacterial effects against multi-drug-resistant *E.coli* and methicillin-resistant *Staphylococcus aureus* (MRSA) (Wang et al., 2022). *Myristica fragrans* EO, containing terpenes and phenylpropanoids, used as a reducing and stabilizing agent, demonstrated substantial antibacterial activity against *S.aureus* and *E.coli* (Vilas et al., 2014). Orange EO-coated AgNPs demonstrated antibacterial effectiveness against *Bacillus subtilis* (*B.subtilis*) and *E.coli* (Phan et al., 2022). Moreover, AgNPs synthesized with *Mentha spicata* EO (average size of 24 nm) were reported to be effective against *E.coli*, *Listeria monocytogenes* (*L.monocytogenes*), *Salmonella typhimurium* (*S.typhimurium*), *S.aureus*, and *Bacillus cereus* (*B.cereus*) (Moosavy et al., 2023).

Antibacterial activity of EO-loaded AuNPs

EO-loaded AuNPs with spherical shapes and sizes ranging from 15.6 to 28.4 nm were biosynthesized using *Nigella sativa* EO. They exhibited antibacterial activity against *S.aureus* (IZD: 16 mm) and *Vibrio harveyii* (IZD: 5 mm) and dose-dependently inhibited bacterial biofilms (Manju et al., 2016). In a study using the medicinal plant *Cymbopogon flexuosus* EO, crystal-sized AuNPs ranging between 10 and 32 nm were synthesized and proven effective against *S.aureus*, *E.coli*, and *Fusarium oxysporum* strains (Pathania et al., 2022). AuNPs prepared with *Curcuma pseudomontana* EO as the reducing agent, with approximately 20 nm sizes, demonstrated effective antibacterial properties against both gram-positive and gram-negative bacteria according to the report (Muniyappan et al., 2014). AuNPs prepared with

Lavandula angustifolia EO exhibited stronger antimicrobial effects against *Proteus mirabilis* with MIC of 8 µg/mL and minimal biofilm eradication concentration (MBEC: 16 µg/mL) compared to AuNPs alone (MIC and MBEC: 256 µg/mL) (Fadel et al., 2023). Moosavy et al. (2023) synthesized spherical Au nanoparticles measuring 19.61 nm using *Mentha spicata* EO, showing antibacterial effectiveness against *E.coli*, *L.monocytogenes*, *S.typhimurium*, *S.aureus*, and *B.cereus* with IZDs ranging from 8.0 to 10.33 mm at a concentration of 100 µg/mL. EO obtained from the leaves of *Coleus aromaticus* was used as a reductant in the synthesis of Au/Ag alloy nanoparticles, and they were reported to be bactericidal (IZD: 28 mm) against *E.coli* (Vilas et al., 2016).

CONCLUSIONS and FUTURE PERSPECTIVES

Bacteria developing resistance to conventional antibiotics poses a significant challenge to the treatment of bacterial diseases. EOs derived from plants, containing a plethora of biologically active components, have been utilized as antimicrobial agents in traditional medicine. However, factors such as volatility, low solubility, susceptibility to oxidation, instability, and solvent toxicity limit their applications. AgNPs and AuNPs, synthesized through physical and chemical methods, exhibit antimicrobial activity but pose environmental risks due to their toxicity. Environmentally friendly EOs have been utilized as agents for reduction in the production of MNPs. The incorporation of EOs into AgNPs or AuNPs imparts stability to the structures, reducing the toxic impact on the environment and production costs. Furthermore, EO-loaded AgNPs and AuNPs facilitate controlled release and have shown synergistic antibacterial effects against resistant bacterial strains. EO-AgNPs and EO-AuNPs hold potential for use as antimicrobial agents in sectors such as medicine, food, and agriculture. In conclusion, the use of EOs in the synthesis of MNPs not only addresses environmental concerns but also enhances the stability and efficacy of these nanoparticles. The controlled release and synergistic antibacterial effects of EO-loaded AgNPs and EO-loaded AuNPs make them promising candidates for combating antibiotic-resistant bacterial strains. This study highlights the potential applications of EO-AgNPs and EO-AuNPs as effective antibacterial agents.

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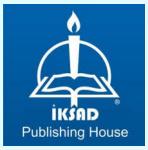

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