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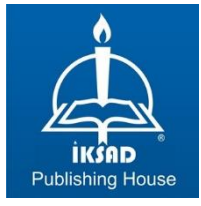

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CONTENTS

PREFACE

Contemporary medicine is developing and evolving constantly. Various teams and authors research and write by utilizing the former studies. Each study carries the flag further. In this context, we are pleased to offer our contribution to our colleagues all around the world who do their best day and night.

This book is the fruit of the long and challenging studies of its authors. It contains 16 topics which all highlight significant contemporary medical issues and provide insight. I kindly advise reading them and I am sure they will be quite helpful in both practical medicine and for the future studies of our colleagues.

I would like to express my sincere gratitude to all the authors for their high-quality contributions. Also, I would like to thank the family of İKSAD Publishing for their kind support. Without them it would not be possible to create and publish this book.

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CHAPTER 1

A DANGER BEHIND THE SCENES OF COVID-19: CHILDHOOD OBESITY

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INTRODUCTION

December 2019 was the start of a new era, especially for the new generation, bringing along a new way of living and lifestyle, much different from what we had experienced before. A disease named coronavirus disease 2019 (COVID-19) spread rapidly throughout the world, and we came across with the term ‘’pandemic’’ that would keep us concerned for the following two years (Cespedes & Souza, 2020); (González, 2020). Although it mostly affected adults in terms of disease severity and hospitalization & mortality rates in children and adolescents were lower (Dong, Mo, Hu, Qi, Jiang, & Jiang, 2020); (Garg, 2020); there was an unforeseen danger awaiting children behind the scenes: that was obesity. Studies after the pandemic show that body mass index (BMI) has significantly increased among children during the COVID-19 lockdown. This problem is possibly going to have an important impact on children’s health (Surekha, Karanati, Venkatesan, Sreelekha, & Kumar, 2021). We have to be more conscious of the problem and take measures earlier and more strictly next time in case of another wave of COVID-19 or another possible pandemic to prevent the complications.

Epidemiology and effects on health

Obesity is already a big problem of the westernized world (Hales, Fryar, Carroll, Freedman & Ogden, 2018), and the prevalence keeps on increasing in both European and Asian countries (Ng et al., 2014); (Sun, Ma, Han, Pan, & Xu, 2014). Although the rates can vary by age, ethnicity, social factors and location, it affects over 337 million children globally (NCD-RisC, 2017); (Ogden, Fryar, Hales, Carroll, Aoki, & Freedman, 2018). Obesity comes along with its risk factors including dysglycemia, metabolic syndrome, elevated blood pressure and hypertension which are threats for cardiovascular health (Lavie, Sanchis-Gomar, Henry, & Lippi, 2020); (Lavie, Laddu, Arena, Ortega, Alpert, & Kushner, 2018). Many cardiovascular diseases like coronary heart disease, heart failure, atrial fibrillation and peripheral arterial disease are found to be increased in presence of increased adiposity (Lavie, Laddu, Arena, Ortega, Alpert, & Kushner, 2018); (Elagizi, Kachur, & Lavie, 2018); (Neeland, Yokoo, Leinhard, & Lavie, 2020); (Lavie, Pandey, Lau, Alpert, & Sanders, 2017); (Carbone, Lavie, Elagizi, Arena, & Ventura, 2020). In addition to these factors; chronic lung disease, asthma and inflammation also increase in case of obesity (Lavie, Laddu, Arena, Ortega, Alpert, & Kushner, 2018); (Elagizi, Kachur, & Lavie, 2018).

The novel coronavirus disease (COVID-19) caused by SARS-CoV-2, firstly reported in Wuhan, China; spread rapidly throughout the world, more quickly than expected and was declared as a pandemic in March 2020 (Cucinotta & Vanelli, 2020). It had many impacts on social life, health, psychology, nutrition and economy. World Health Organization (WHO) reveals that over 522 million people have been infected with COVID-19 and over 6 million people died of COVID-19 ("WHO Coronavirus Dashboard," 2022). However, when excess mortality is calculated; that means deaths related with COVID-19 directly and indirectly because of overburdened health systems due to pandemic, this number exceeds 14 million.

The disease had a milder course and better prognosis in children. Pneumonia, hospitalization, need for ventilation and death rates were extremely lower when compared to adults (Ludvigsson, 2020). However, there was one group of children that were more prone to severe illness. These were obese children. In studies it was shown that hospitalization and intensive care unit (ICU) admission because of COVID-19 was higher among obese children and the need of mechanical ventilation increased parallel to BMI (Shekerdemian, Mahmood, Wolfe, Riggs, Ross, & McKiernan, 2020); (Zachariah, Johnson, Halabi, Ahn, Sen, & Fischer, 2020); (Simonnet, Chetboun, Poissy, Raverdy, Noulette, & Duhamel, 2020). In addition, clinical management of obese patients (like intubation, positioning,..) is generally more difficult and together with COVID-19, the ICUs were overloaded with this type of patients. Obesity is now recognized as an independent major risk factor for worse prognosis in patients with COVID-19.

Etiology

Obesity among children was already like a pandemic before COVID-19 had appeared. After start of COVID-19 pandemic, it has gained acceleration. What were the causes behind the increased rate of obesity, and could it be prevented?

The COVID-19 pandemic was so unexpected and stressful that it traumatized both children and adults. As COVID-19 spread worldwide, most adults started work online and schools were closed as a part of social distancing policies in most of the countries (Van Lancker & Parolin, 2020). This action brought many complications related with increased stress and increased weight gain among children. A metanalysis by Chang et al showed that body weight, BMI and prevalence of overweight and obesity had

increased significantly during lockdown among school age children and adolescents (Chang, et al., 2021).

There are multiple aspects that lead to increased weight gain during lockdown. First of all, physical activity (PA) was decreased intensely as children were stuck to their chairs in front of the screen during and between the lessons. PA plays a key role in mental and physical health and insufficient PA leads to reduced cardiorespiratory fitness and depression as well as weight gain (Lavie, Ozemek, Carbone, Katzmaryzk, & Blair, 2019). Children couldn't move much during the lesson because of the need to watch the screen closely. In addition to this, all the games in the breaks also turned out to be online. Moreover, they couldn't attend their routine sports activities, and some even couldn't get out of their houses for a certain period. This reduced opportunities of PA and sedentary behaviors increased the risk of weight gain and psychological stress (Qui, Shen, Zhao, Wang, Xie, & Xu, 2020).

The availability of healthy foods has also changed for most of the children. Previously, most children used to have the balanced diet program that school provided them, however after social distancing, they started eating more junk-food as most of the parents started to work from home and did not have enough time to cook all the meals. As a result, there was a big change in routine eating habits. A study from Poland showed that, children tend to eat salty foods, meat, dairy and snacks more during the pandemic and consumed less vegetables and fruits. This problem was more prominent especially in overweight children (Sidor & Rzymski, 2020). This means that already overweight children are more likely to become obese. In another study from India among 2000 children, there was a significant increase in BMI during lockdown and the overall obesity and overweight had also increased. The increase was more prominent in adolescent group (13-15 years) and male predominancy was seen (Surekha, Karanati, Venkatesan, Sreelekha, & Kumar, 2021). It was associated with decreased physical activity, sedentary life and increased screen time. In a study by Anderson et al, it is stated that each additional hour spent in front of screen increases obesity prevalence by 2% (Anderson & Butcher, 2006). In another study, Kapil et al reported that the time spent in front of TV correlates with consumption of salty snacks, sweets and sweetened beverages (Kapil & Bhadoria, 2014). There are other studies that show correlation between screen time, snacking and BMI (Marsh, Ni Mhurchu, & Maddison, 2013); (Tripathi & Mishra, 2020).

E-learning was beneficial for educational purposes, children had the chance to follow-up their lessons and be in contact with their friends and

teachers. However, online platforms started to be used more extensively by teenagers to play videogames and chat with each-other except school hours. This was one of the reasons of increased screen time. Although online gaming may be beneficial for socializing and stress reduction in some aspects; protracted periods of isolation and limited face-to-face interaction may have negative impacts on mental health, sleep patterns and physical health (King, Delfabbro, Billieux, & Potenza, 2020). Besides, choice of games is also important as there are many videogames promoting violence and other bad habits. In a study by Nagata et al, it was reported that increased screen time during pandemic had increased the risk of anxiety, depression and inattention (Nagata, Abdel Magid, & Pettee Gabriel, 2020). Psychological disorders like anxiety and depression are also risk factors for obesity (Chao, Wadden, & Berkowitz, 2019).

During the COVID-19 pandemic; appetite changes, stress eating and frequent snacking is also reported independent of increased screen time. Psychological distress is linked with greater food intake; especially increased intake of high fat, energy dense and palatable snacks (Epel, Lapidus, McEwen, & Brownell, 2001). In response to lock-down, stockpiling of foods has been increased and homes became snack food-rich environments that promote overconsumption (Nicola et al., 2020). Intake of fried food and sweet snacks were found to be increased. These changes in dietary habits lead to higher calory intake and possibly played a role in increased risk of obesity among children and adolescents.

COVID-19 pandemic also affected adults both physically and mentally. Some of them lost their job and most had to change their work routine (Aymerich-Franch, 2020). Besides economic problems, social problems were also remarkable. Parents were deprived of their support system that helped them to take care of their children, eg: other family members, friends, daycare and kindergartens. A study showed that parents were under more pressure than people with no children (Neubauer, Schmidt, Kramer, & Schmiedek, 2020). Especially mother's burden and stress increased. They had to work from home, do childcare and also help with schoolwork (Di Giorgio, Di Riso, Mioni, & Cellini, 2020). There are studies showing that parenting stress, psychological and financial stress can affect parent's food parenting practices (Gouveia, Canavarro, & Moreira, 2019); (Close, Mitchell, Brennan, & Hayes, 2009); (Gross, Mendelsohn, Fierman, Racine, Messito, & 2012. In a study by Baskind et al, parental stress was found to be associated with childhood weight gain and obesity (Baskind, Taveras, Gerber, Fiectner, Horan, &

Sharifi, 2019). Stressed parents are more likely to use food or snacks to manage problems related to children's behavior or emotions. However, there has to be more studies on relationship between stress and snack parenting practices.

Moreover, the whole food production and distribution system including agricultural production, transportation, and sale of nutritious, fresh, and affordable foods was also disrupted by the social distancing measures and families were forced to rely on nutrient-poor alternatives (Fore, Dongyu, Beasley, & Ghebreyesus, 2020); (IPES-Food, 2021). More shelf-stable, less expensive, ultra-processed, and high calory foods were bought instead of healthy fresh food (Fore, Dongyu, Beasley, & Ghebreyesus, 2020). Likewise, children who grow-up in low-income families are more likely to become obese related to the fact that high-calory junk foods are cheaper and easier to access and this problem became more severe during COVID-19 pandemic (Tester, Rosas, & Leung, 2020).

Precautions

Global movement behavior guidelines suggest minimum 180 minutes of moderate -vigorous intensity physical activity daily for preschool and minimum 60 minutes for school children ("WHO: Physical activity and young people, " 2020). Children mostly spend this time during active travel to school, physical education lessons, organized sports activities and playing in playgrounds and parks. The sedentary time is accumulated at home. However, during pandemic, after lockdown, these healthy movement behaviors all stopped and replaced by increased screen time and sedentary living. Guan H et al in their panel made recommendations for promoting healthy movement behavior in children's daily routine (Guan et al., 2020). They advised that parents and caregivers should incorporate physical activity into children's daily routine. Extended periods of sitting should be broken up every 30-60 minutes by physical activities like standing or stretching. Family should be involved in physical activities adhering to social distancing regulations. Educators and teachers should also incorporate healthy movement practices and messages into daily home-school routines and lessons. They should limit prolonged sitting and encourage changes in posture like stretching or moving. Governments and media should also engage influential people in promoting healthy movement behaviors. Children should be more active in speaking up for themselves about how to maintain a healthy life.

When all the factors related with weight gain during pandemic are considered, the first thing that should be done is to maintain the routine daily physical activity. Even during social distancing, aerobic activities like brisk-walking or bicycling can be performed with the family. Besides, bone-strengthening activities like jumping rope can be carried out at least 3 days a week according to physical activity guidelines ("WHO: Physical activity and young people, " 2020). Children can even participate in sports like tennis which don't require close contact.

According to screen-time recommendations of AAP&WHO, recreational screen-time should be limited to less than 2 hours ("AAP-WHO: Screentime- Recommendation-Chart-Final, " 2021). Screen time more than 2 hours is a sedentary behavior that takes away valuable time for physical activity and augments the risk of overweight and obesity in children and adolescents (Fang, Mu, Liu, & He, 2019). Besides, there must be controlled and balanced approaches to online gaming to support physical and psychosocial well-being.

High-calory, shelf-stable fast food and snack consumption should be reduced. Best way to avoid them is to stop food-piling at homes because when you buy them, they are in easy-reach for children and are consumed very fast. Healthy and low-calory snacks should be provided. In a study by Borgatti et al, limiting types of unhealthy food, making low-calorie and healthy food choices and controlling the activity of eating when bored helped preventing weight gain during COVID-19 pandemic (Borgatti et al., 2021).

We must also make sure that children get adequate sleep. Current guidelines suggest that children aged 5-13 years should have 9-11 hours and children aged 14-17 years should have 8-10 hours of uninterrupted sleep at night daily (Tremblay, Carson, Chaput, Connor Gorber, Dinh, & Duggan, 2016). That will contribute to maintain the healthy daily activity and decrease the sedentary behavior during daytime.

Government should also take action to provide affordable healthy food for all people. Cost of healthy food should be kept at a certain range that low-income families can also consume less calory dense and more nutritious food with their budget. Advertisements promoting consumption of junk foods and unhealthy beverages should be decreased and healthy foods must be promoted.

Lastly, psychological support system is very important for both children and parents as depression, stress and anxiety are all factors that can lead to over-eating.

CONCLUSION

During the last two years, COVID-19 pandemic worsened the burden of obesity which has many risks for health. The pandemic was an unexpected and stressful situation, and we were late to take measures against unhealthy way of living and weight gain. Maintenance of daily physical activity, limiting screen time and avoiding high calory fast food and snacks are the key points in prevention of obesity. Being more conscious about the problem will help us to act more rapidly in case of another COVID-19 wave or pandemic.

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CHAPTER 2

CLASSIFICATION OF AUTISM SPECTRUM DISORDER IN ADULT INDIVIDUALS BY MACHINE LEARNING METHODS

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INTRODUCTION

The first articles about autism date back to the 18th century. Jan Itard and Jon Hâlsam examined some children in the 1700s and stated that these children behaved as different individuals from the others. In fact, the term autistic is thought to have been used for the first time in 1911 by the Swiss scientist Bleuler (Frith, 1989).

Leo Kanner was the first to define Autism in 1943. Leo Kanner described common behavioral disorders in 11 children; Inspired by the Greek word “autos” meaning “self” as a term describing these children living in their own world, he named this clinical manifestation “early infancy autism” (Kanner, 1943).

At first, it was thought that autism was caused by the attitude of parents or the fear of establishing social relationships, but for the last 20 years, autism has been thought to have a neurobiological etiology, not related to the child's upbringing or past life (Bodur and Soysal, 2004).

In the 2013 classification system of the American Psychiatric Association, the distinction between autistic disorder Rett syndrome, Asperger's syndrome, childhood disintegrative disorder and pervasive developmental disorder not otherwise specified has been removed and created a specific Autism Spectrum Disorder (ASD) category characterized by: (Fadıloglu, 2019).

• Persistent deficiencies in social communication and social interaction in many contexts.

• Restricted, repetitive patterns of behavior, interests, and activities in current or past clinical history.

• Significant impairments in social, occupational, or other important areas of functioning.

• Presence from early childhood (but the picture may not be fully revealed until societal expectations exceed the child's limited capacity).

• Not better explained by any general developmental delay or intellectual disability.

Although autism was a rare disorder seen in only 1 of every 10000 children until the mid-1970s, when 23 studies conducted between 1966-1998 were examined, it was detected in approximately 15 of every 10000 individuals (Fombonne, 1999). When the recent epidemiological data are examined, it is thought that this perception is wrong, in fact, it is seen in 66 of every 10000 individuals (Hill, Zuckerman and Eric, 2014).

In a study evaluating 55,266 schoolchildren aged 7-12 years in South Korea, the prevalence was found to be 2.64%, and it was found to be 3.74% among boys and 1.47% among girls (Kim et al., 2011). All recent studies with various media and methods show that the prevalence of ASD is over 1% (Kim et al., 2011). All recent studies with various media and methods show that the prevalence of ASD is over 1% (Mukaddes, 2014). According to the Autism and Developmental Disorders Follow-up Network (ADMM) data of the Centers for Disease Control and Prevention (CDC) from many studies in Asia, Europe and North America, the prevalence of ASD was 1/150 in 2006, 1/69 in 2012 and 1 in 2014. reported as /59 (CDCP,2018). It is also known that ASD is seen in all races and societies. The fact that the exact cause and the geography of its location are not known has led scientists to direct studies on this subject. While some of the studies are carried out on the diagnosis and treatment of the disease with medical methods, some of them are progressing to help specialists working in the medical field by classifying this disease by using machine learning methods in computerized environments. In addition, the increasing incidence of ASD in recent times has led to the development of action plans for this issue in many countries. The first step of these action plans is early diagnosis. As in every disorder, early diagnosis is of great importance in OBS. It will make a great contribution to helping experts in this field in early diagnosis and correct diagnosis, which are among the aims of this study.

Table 1 contains studies on some machine learning algorithms related to ASD. In the table, information such as in which computer programs the algorithms used in the studies were made and the correct classification rates of the algorithms are given.

Table 1: Studies on Classification of ASD Disorder with Machine Learning Algorithms (Thabtah, 2019)

Years	**Feature Selection**	**Machine Learning Algorithms Used**	**Computer Program Used**	**Data Properties**	**Best Algorithm**	**Accuracy rate %**
2012	no	SVM, LG, Tree, Probability and its types	Weka	29 variables, 612 people with ASD, 15 people without ASD	ADTree	99.70 %

2012	no	SVM, LG, Tree, Probability and its types	Weka	93 variables, people are ASD 891, 75 people are not OSB	ADTree	100 %
2014	no	Naive Bayes, SOM, Neural Fuzzy, LVQ Nueral Network, Kmeans, Fuzzy C Mean	Developed	16 variables and 100 people	SOM ve Naïve Bayes	100 %
2015	Yes	SVM, LR, DT, Probability and its types	R, Weka	28 variables 3885 people with ASD, 655 people without ASD	SVM LR	98.27 % 97.66 %
2016	Yes	SVM, LR, DT	Scikitlearn	65 variables, 2775 people with ASD, 150 people not with ASD	SVM	No
2016	Yes	SVM	LibSVM	65 variables, 1264 people ASD, 462 people not ASD	SVM	No

2. Method

2.1. Classification

The concept of classification can be basically defined as the distribution of data among the classes defined in a data set according to some rules. There are many classification methods in the literature. The important thing here is to determine the correct classification algorithm according to the data set and the success rate of the algorithm used is high.

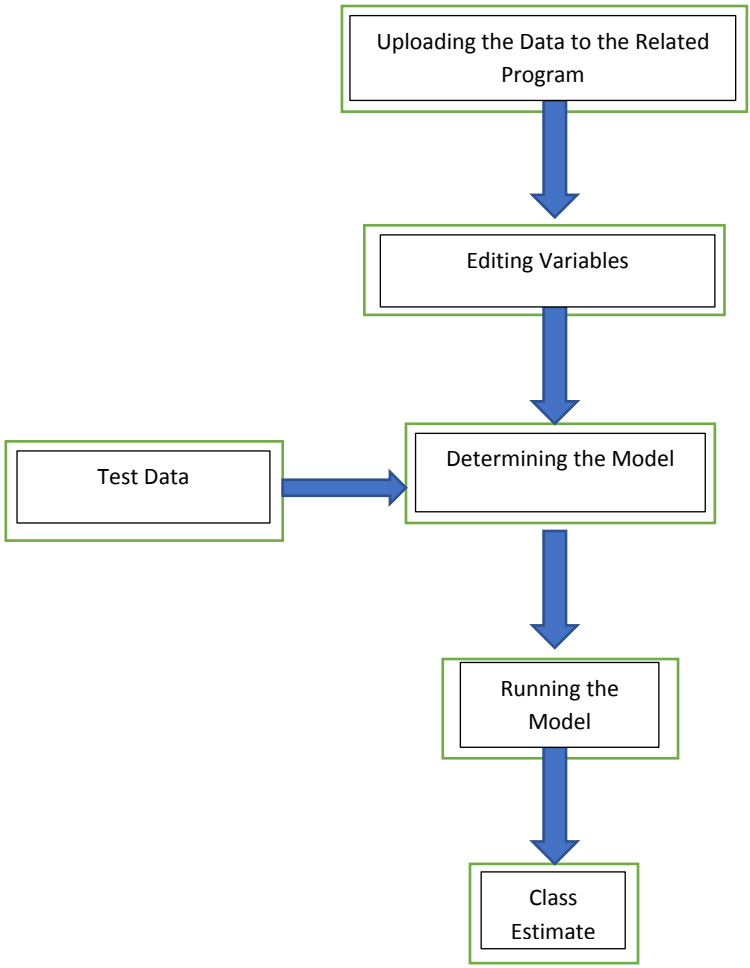

Figure 1: Flow Chart of Machine Learning

Figure 1 shows a basic flowchart of how the machine learning algorithm works. When the flow diagram is examined, first of all, the data set must be loaded into the relevant computer program connected to the user. Then, if the variables need to be transformed in accordance with the purpose of the study, necessary transformations are made. In the next steps, the model structure is created and the data classes are created by training the data through the model. Afterwards, the necessary tests are performed on the model used, and the correct classification rates are revealed.

2.2. Classification Algorithms and Validity Analysis

2.2.1. ZeroR Method

This method is the simplest and easiest classification method under the frequency-based classification framework. The operating logic of the method is as follows: first it determines the number of classes of the data loaded into the system. The class with the highest number of classes is assigned as the class of the new incoming data.

ZeroR Classification Algorithm;

*Upload data

*Determine the number of different classes

, C_3,…

*Count total class numbers

.

.

.

- . Line them up from the big to the small
- t
- New data class =

2.2.2. OneR Method

It can be said that this method is the second basic classification method among the classification methods based on the frequency. This method basically shares a lot of similarities with ZeroR logic. The main difference from the ZeroR classification method is that the class type is determined for each variable and the correct class numbers are assigned. Then, the variable that makes the correct classification is put to the beginning and the variables are sorted from the big value to small. Choosing between variables with the same accuracy value is totally left to the researcher.

OneR classification algorithm;

- One branch for each of the attribute's values
- Each branch assigns most frequent class

- Error rate: proportion of instances that don't belong to the majority class of their corresponding branch
- Choose attribute with lowest error rate

2.2.3. NaiveBayes Method

The Bayesian classification technique is a method that is able to calculate the likelihood of a new data that may fit into any of the existing classes using the existing, already categorized data. Algorithms and classification techniques based on the Bayesian rule are known by this name. Bayes' theorem can be expressed as:

$$\boldsymbol{P}(\boldsymbol{C_1}|\, x_i) = \frac{\boldsymbol{P}(\boldsymbol{x_i}|\, C_1)P(C_1)}{\boldsymbol{P}(\boldsymbol{x_i}|\, C_1)P(h_1) + \boldsymbol{P}(\boldsymbol{x_i}|\, C_2)P(C_2)} \quad (1)$$

It is assumed here that there are two separate hypotheses C_1 and C_2 in other words, two distinct classes.

$\boldsymbol{P}(\mathrm{C_1}|\, \mathrm{x_i})$ states the possibility that it is in $\mathrm{C_1}$ class of $\mathrm{x_i}$. $P(x_i)$ is the frequency / number of occurrences of $x_i$value in the database. Additionally, $P(\boldsymbol{C_1})$ and $P(\boldsymbol{C_2})$ are the frequency of C_1 and C_2 classes in the database.

If it is assumed that there are m hypotheses-classes, then the rule is as follows:

$$P(x_i) = \sum_{j=1}^{m} \boldsymbol{P}(\boldsymbol{x_i}|\, C_j)P(C_j) \quad (2)$$

In this case, the possibility that $\mathrm{x_i}$ is in class $\mathrm{C_1}$ is calculated by the following equation 3:

$$\mathrm{P(C_1|\, x_i)} = \frac{\mathrm{P(x_i|\, C_1)P(C_1)}}{\mathrm{P(x_i)}} \quad (3)$$

The Bayesian algorithm first calculates the value of $\mathrm{P(C_j)}$ in the learning set given to it and the frequency of each class in the given learning cluster. Next, $\mathrm{x_i}s$ are counted and found as $\mathrm{P(x_i)}$ $value$. Similarly, in each class, the frequency of occurrence of each value of $\mathrm{x_i}$ is obtained by counting $\mathrm{x_i}s$ in$\mathrm{P(x_i|\, C_j)}$, $\mathrm{C_j}s$ (Silahtaroglu, 2013).

Bayes classification algorithm;

- Get dataset : D
- Get Class : $C_i = 1,2,3, \dots n.$
- R : record to be classified

START

Do for each C

Calculate

$$P(C_1 | x_i) = \frac{P(x_i | C_1)P(C_1)}{P(x_i)} \quad (4)$$

Loop

Assign R to the C, which has the highest value

STOP

2.2.4. Decision Tree Method

The decision tree technique is one of the most intuitive and popular data mining methods, especially as it provides explicit rules for classification and copes well with heterogeneous data, missing data and non-linear effects. For applications concerned with database marketing, the only major competitor of the decision tree at present is logistic regression, which is preferred for risk prediction, owing to its greater robustness. It should be noted that decision trees are on the boundary between predictive and descriptive methods, since they create their classification by segmenting the population to which they are applied: thus they belong to the category of supervised divisive hierarchical methods (Tuffery, 2011).

Decision trees are one of the most popular classification and prediction methods used in data mining. As mathematical inference techniques are hard to be understood by users, decision trees are developed by machine learning researchers with the purpose of providing ease of human use and interpretation (Aksoy, 2013).

Compared to other classification methods, decision trees are a commonly used method of classification due to the fact that they are easier to configure and understand. The structure of the decision tree resembles a flow diagram. Each attribute (variable) is represented by a loop. Branches and leaves are elements of a tree structure. The last structure is called "leaf", the

top structure is called "root" and the structures between them are called as "branch" (Atılgan, 2011).

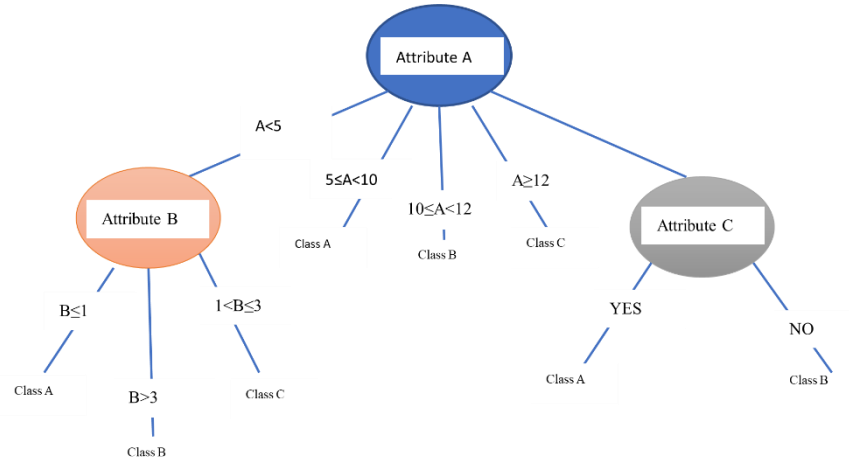

Figure 2: An example of a decision tree

Figure 2 displays an example of a decision tree with 3 variables and 3 separate classes.

The most important point in constructing decision trees is to determine which variable is the first loop, that is, the root loop (Dirican, 2001).

Steps to follow when constructing a decision tree;

- Get dataset : D
- Get Class : C
- Count No.of Fields : f
- START : Calculate general state entropy for class C

$$H(D) = \sum (p_i \log \left(\frac{1}{p_i}\right)) \tag{5}$$

Do for each f

Calculate Separation Information

$$H(\frac{|D_1|}{|D|}, \dots., \frac{|D_s|}{|D|}) \tag{6}$$

Calculate Gain Rate

$$Gain\ Rate\ (D,S) = Gain\left(\frac{D}{S}\right) Separation\ Information\ (D,S)$$

Loop
Assign Variable Nodes with the Smallest Gain Rate.
If Stopping Criterion =
Else

Go to START

2.2.5. Receiver Operating Characteristic (ROC), TP, FP and Cross Validation

The ROC analysis is used in the determination of the ability to distinguishing power of the test, comparison of various test techniques and in the determination of the appropriate positive threshold.

The area calculated by ROC analysis is one of the most important analysis methods used to evaluate the performance of classification algorithms.

(7)

Here indicates the sum of all positive samples while and give the number of positive and negative ones respectively.

The ROC curve method can be used within the following aspects (Takıcı, 2018).

- In the classification power of the established model
- Comparison of model performances
- In determining the equal value
- Quality tracking of model results
- Implementer(s) development follow-up
- Comparing different practitioner(s)

ROC analysis is a type of analysis used to evaluate the results of classification algorithms (Altman and Bland, 1994).

TP rate, can be defined as the classification of a true condition as true in the test result. It is also known in the literature as Sensitivity. Sensitivity is the proportion of true positives that are correctly identified by the test (Sanjay, 2018).

(3)

FP Rate, is deciding that it is correct after testing a condition that is incorrect in reality. It is also known in the literature as specificity. Specificity is the proportion of true negatives that are correctly identified by the test (Sanjay, 2018).

$$Specificity = TN/(FP + TN) \quad (4)$$

Cross validation is an algorithm that is used a lot in training and testing data in many fields such as machine learning algorithms, deep learning, artificial neural networks. On the basis of this algorithm, the data set is divided into equal parts as many as k given. While one of these parts is used for testing, the rest is used for training the data set. This loop is done as many as k. However, the dividing groups in each iteration must be different from the other iterations. In this way, the system is prevented from memorizing the data set. Figure 3 shows an example of cross-validation where k is 4.

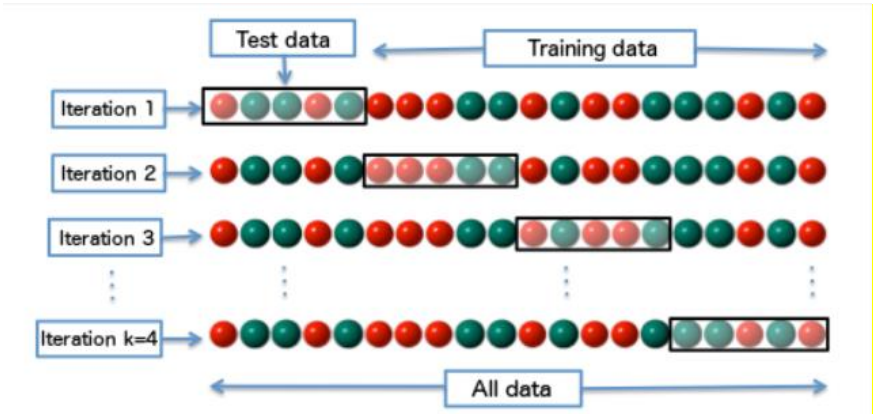

Figure 3: Cross Validation Model for k=4 (Sanjay, 2018)

Findings

The data used in the study consists of 21 variables in total. While 20 of these variables consist of variables related to the determination of ASD, 1 of them indicates whether ASD exists or not, that is, its class.

These data collected from adults are 704. Researchers who want to get more information about the data can refer to (UCI, 2017).

Table 2: Results of Classification Algorithms

Model	Correct Classification Rate (%)	TP	FP	ROC
OneR	100	1.00	0.00	1.000
ZeroR	73.15	1.00	0.00	0.495
Naïve Bayes	97.017	0.963	0.037	0.999
Karar Ağacı	100	1.00	0.00	1.000

According to Table 2, the results of OneR, ZeroR, Naive Bayes and Decision tree algorithms under the heading of frequency tables are given. As a result of the analysis, OneR and Decision Tree machine learning algorithms have the highest correct classification rate with 100% success rate. The lowest classification algorithm is ZeroR algorithm with 73.15% correct classification rate. Naive Bayes, on the other hand, ranks 3rd in terms of classification success rates with 97,017%.

TP is the determination of the test result as not disturbed when in reality it is a disturbed variable. According to this analysis, Naive Bayes algorithm was the lowest with a rate of 0.963 among the mentioned machine learning algorithms. The ratio of other algorithms is 1. The FP ratio, on the other hand, should be considered the opposite of the situation in the TP ratio. The said ratios are given in Table 2 in detail.

ROC analysis, on the other hand, is an analysis used to determine the discriminative power of a test, to compare various techniques, and to determine the appropriate threshold value. According to the results of this analysis, the ROC area was calculated as 1 in OneR and Decision Tree, 0.495 in ZeroR and 0.99 in Naive Bayes. The closer this value is to 1, the better the fit of the variables used in the detection of the disease.

Table 3: Classification Table for OneR Algorithm

a	b	
515	0	a=No
0	189	b=Yes

In the classification made according to the OneR algorithm, while 189 of 704 people in total were ASD, the remaining 515 people did not have the

disease in question. According to the results of the algorithm, all of the patients who are sick and those who are not are correctly classified. The relevant results are given in Table 3.

Table 4: Classification Table for ZeroR Algorithm

a	**b**	
515	0	a=Ho
189	0	b=Yes

Table 4 shows the classification results of the Zero algorithm. According to these results, while all 515 people who are not normally sick were classified correctly, 189 people who were sick were included in the class of not sick.

Table 5: Classification Table for Naive Bayes Algorithm

a	**b**	
496	19	a=No
2	187	b=Yes

Table 5 shows the classification results of the Naïve Bayes algorithm. According to Table 4, 496 of 515 people who were not sick were categorized in the no disease class, while the remaining 19 people were identified as sick even though they were not sick. Similarly, 187 of 189 people diagnosed as sick were included in the patient category according to the aforementioned algorithm, while 2 people were classified as not sick even though they were sick.

Table 6: Classification Table for Decision Tree Algorithm

a	**b**	
515	0	a=No
0	189	b=Yes

In the classification made according to the decision tree algorithm, while 189 of 704 people in total were ASD, the remaining 515 people did not have the disease in question. According to the results of the algorithm, all of the patients who are sick and those who are not are correctly classified. The relevant results are given in Table 6.

CONCLUSION

According to the results of the analysis, the highest accuracy rate among the frequency-based classification algorithms in the classification of ASD was OneR and Decision tree algorithms. OneR, ZeroR and Decision tree algorithms have achieved high success in diagnosing people who are actually sick. People who do research on this subject can use these algorithms. However, OneR and ZeroR algorithms are the most basic of machine learning algorithms and they do calculations without much logic, which may not always be good for the results of the study. However, the Decision Tree algorithm is recommended for researchers to use frequency-based classification algorithms for ASD detection in adults.

Finally, it is known that there are many algorithms used in classification problems. It is planned to compare the results by using other algorithms in the scientific literature in the future.

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CHAPTER 3

STATISTICAL INVESTIGATION OF DNA DAMAGE IN OBESE PEOPLE

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INTRODUCTION

Studies have shown that obesity alters the repair mechanism of DNA strand breaks caused by genotoxic chemicals. In addition, the increase in oxidative stress and inflammation that occur with obesity can increase genotoxic damage and prevent the function of DNA repair mechanisms, resulting in the transformation of a cell with accumulated DNA damage into a cancerous cell. Studies over the past few years have shown that obesity can affect genome stability. Also, epidemiological studies linking obesity with increased risk of cancer is steadily increasing (Satayesh et al., 2018). High Body Mass Index (BMI) was knew to change the repair of double-strand breaks induced by toxic chemicals (Włodarczyk.et al., 2019). Increased BMI, a broad range of DNA lesions two times higher DNA damage in cells than in normal-weight subjects have been showed (Sancar, 1995; Bukhari et al., 2011). Studies demonstrated that animals fed a high-fat diet increased mitochondrial DNA damage (Yuzefovych et al., 2013; Pazmandi et al., 2014). It is known that excessive increase in BMI is a source of increase in reactive oxygen species and cytokines, which are characteristic for inflammation. The specified process leads to damage to the genetic material, leading to the formation of cancer. The incidence of this condition is increased in overweight or obese people (Lee & Chan, 2015). The Alkaline Single Cell Gel Electrophoresis is one of the methods used to evaluate DNA damage in human cells for monitoring genotoxicity (Møller et al., 2020; Azqueta et al., 2020]. Micronucleus (MN) assay in buccal exfoliated cells is a minimally invasive method for monitoring genotoxic effects in humans (Thomas et al., 2009). In the past ten years, there have been a large number of research analyzing the possible relation among obesity and DNA damage (Setayesh et al., 2018; Włodarczyk et al., 2019; Egusquiza & Blumberg, 2020). However, there is no information on such studies in obese subjects from Turkey. We aim to fill this topic with our throughputs.

Materials and methods

Study Design

Our study was planned as a cross-sectional analysis of Turkish adults. Also, it was carried out from November to April 2020 with underweight, normal, and obese participants (n=79). A 25-question survey was administered to participants to save information on several lifestyle parameters. In addition, study groups sex, age, level of education, height and

body weight information was recorded. BMI was calculated and groups were classified according to BMI results.

Alkaline Single-Cell Gel Electrophoresis

Genotoxic damage in peripheral blood samples of our study subjects was comparatively tested by the Comet assay (Azqueta et al., 2020). For measure of DNA damage, the comet assay parameters mean that tail intensity (DNA% in comet tail), tail length (expressed in μm), and tail moment were calculated by the Comet Assay III image analysis system.

Micronucleus Assay

MN assay was used to detect possible genotoxic effects. For this purpose, the mouth of the subjects was rinsed thoroughly with water and a buccal epithelial smear was taken gently from both cheeks with a tongue depressor above the slides. Two slides were made for each subject. The slides were delivered to the laboratory and processed according to standardized procedures (Thomas et al., 2009). Briefly, the slides were fixed with 80% methanol for 10 min and then stained using a Feulgen reaction. The slides were then counterstained with fast green. MN, micronucleated cell (MNed), and nuclear bud (NB) frequency were scored in 2,000 cells and other nuclear anomalies; e.g. binuclear (BN), condense chromatine, karyolitic, pyknotic, and karyolitic were scored in 1,000 cells for each subject, and taken photography with x1000 magnification. All parameters were recorded in 1,000 cells (‰).

Statistical Analysis

Genotoxicity tests results were analyzed using the statistical program Statistical Package for the Social Sciences (SPSS) measured to determine relations between the obese group and control group in the study population. Frequency and percentage analyses were used for analyzing participants' demographic characteristics and mean scores from the measurement tools. A p-value less than 0.05 was considered statistically significant.

Results

The present study was planned from November to April 2020. Finally, 37 females (M_{age}=28.94±7.36) and 42 males (M_{age}=35.69±10.77) who were 18 and older in Turkey were included. Baseline characteristics and BMI results of 79 participants 37 females and 42 males were shown in Table 1, which shows 23.1% of women and 76.9% men were obese.

Comet assay results (tail moment, tail intensity, tail length) in underweight, normal, and obese subjects was shown in Table 2. Comet assay results show that the relationship between groups and DNA damage is statistically significant ($p>0.05$). According to the comet assay tail moment results, the BMI of participants and DNA damage was statistically non-significant ($p=0.382$). Comet assay tail intensity results, BMI of participants, and DNA damage were statistically non-significant ($p=0.406$). In addition, comet assay tail length results, BMI of participants, and DNA damage were statistically non-significant ($p=0.573$).

Table 1. General characteristics of study group

Variables	**Age (Years)**	**Height (cm)**	**Weight (kg)**	**BMI (kg/m^2)**
Underweight (n=19)	27.89±7.67	167.84±8.40	57.78±8.05	19.18±0.83
Female (n=12)	26.50±5.77	162.50±5.00	53.08±3.84	19.02±0.82
Male (n=7)	30.28±10.12	177.00±3.31	65.85±6.89	19.46±0.84
Normal (n=47)	31.70±9.66	171.34±8.90	66.35±10.29	22.48±1.96
Female (n=22)	28.22±6.19	167.45±6.62	62.07±8.98	22.04±2.07
Male (n=25)	34.76±11.15	174.76±9.35	70.12±10.04	22.86±1.82
Obese (n=13)	42.30±6.98	171.84±11.29	95.61±11.82	32.57±2.94
Female (n=3)	44.00±3.00	162.00±10.39	89.66±4.04	34.35±3.46
Male (n=10)	41.80±7.85	174.80±10.20	97.40±12.93	32.03±2.74

The association between the tail moment, tail intensity, tail length results of underweight, normal weight, and obese subjects was not statistically significant ($p>0.05$). Table 2 was shown the comparison of comet assay results of underweight, normal, obese individuals. The relationship between obesity and BMI of the study groups was statistically non-significant ($p>0.05$).

Table 2. Comet assay results of the study groups

	Mean ±SD	**Min.**	**Max.**	***p-value*** †
Tail Moment	1.39±1.31	0.15	10.14	
Underweight	1.16±0.47	0.47	2.14	0.382
Normal	1.34±1.22	0.41	8.99	
Obese	1.96±1.78	0.41	7.66	

Tail Intensity (%)	5.31±2.09	1.44	9.52	
Underweight	4.85±1.82	1.97	9.07	
Normal	5.16±2.04	1.80	9.04	0.406
Obese	6.04±2.59	1.44	9.21	
Tail Length (μm)	29.02±4.63	18.02	43.07	
Underweight	29.10±4.37	22.86	38.39	
Normal	28.34±5.37	18.02	43.07	0.573
Obese	29.08±4.17	24.07	35.83	

**p>0.05 (there is no statistically significant difference compared with those of underweight, normal, obese subjects with 95% confidence interval [95% CI].*

Table 3 was shown MN (‰) and MNed cell (‰) frequency in underweight, normal, obese study groups. According to Table 3 MN (‰) frequency in underweight, normal, obese study groups 0.63±0.76, 0.95±1.64, and 2.07±2.49 respectively. Evaluated MN assay results in study groups there was no statistically significant difference (p=0.094) with a 95% confidence interval [95% CI].

Table 3. MN (‰) and MNed cell (‰) frequency in study groups

	Mean±SD	**Min.**	**Max.**	***p*-value†**
MN	**1.26±2.06**	**0.00**	**10.00**	
Underweight	0.63±0.76	0.00	2.00	
Normal	0.95±1.64	0.00	8.00	0.094
Obese	2.07±2.49	0.00	8.00	
MNed cell frequency	**0.89 ±1.37**	**0.00**	**7.00**	
Underweight	0.63±0.76	0.00	2.00	
Normal	0.70±1.12	0.00	6.00	0.225
Obese	1.46±1.80	0.00	7.00	

*p>0.05 (there is no statistically significant difference compared with those of underweight, normal, obese subjects with 95% confidence interval [95% CI].

Buccal micronucleus cytome assay results in underweight, normal, obese study groups were shown in Table 4. In Buccal MN cytome assay the frequency of nuclear anomalies (pyknotic, karyorrhectic, karyolytic, condensed chromatin, binucleated, basal, nuclear bud) in buccal epithelial cells of individuals in study groups was evaluated.

According to buccal MN cytome assay results, there was no statistically significant difference compared with underweight, normal, obese groups ($p>0.05$). We evaluated the relation between the participant's BMI and possible genotoxic damage. The most valuable result of this study is that there were no statistical differences found between BMI and DNA damage ($p>0.05$).

Table 4. The frequency of nuclear anomalies in buccal epithelial cells in study groups

Nuclear Anomalies ‰	**Mean±SD**	**Min.**	**Max.**	***p*-value**
Pyknotic	16.90±21.75	0.00	122.00	
Underweight	24.42±34.70	2.00	122.00	
Normal	19.02±22.53	0.00	101.00	0.154
Obese	9.92±9.44	0.00	29.00	
Karyorrhectic	21.16±20.10	1.00	92.00	
Underweight	17.94±15.91	1.00	59.00	
Normal	19.78±19.87	1.00	70.00	0.399
Obese	17.46±16.39	2.00	56.00	
Karyolytic	44.34±44.66	0.00	222.00	
Underweight	36.42±36.23	0.00	106.00	
Normal	37.72±48.14	0.00	222.00	0.267
Obese	46.66±31.53	0.00	97.00	
Condensed chromatin	18.59±14.48	0.00	59.00	
Underweight	20.63±14.11	1.00	59.00	0.143
Normal	20.72±15.43	0.00	56.00	

Obese	10.69±8.45	2.00	32.00	
Binucleated	5.60±9.72	0.00	60.00	
Underweight	4.16±7.41	0.00	29.00	
Normal	7.70±12.85	0.00	60.00	0.275
Obese	5.38±8.08	0.00	26.00	
Basal	2.64±3.88	0.00	29.00	
Underweight	3.53±7.83	0.00	29.00	
Normal	2.36±2.04	0.00	7.00	0.672
Obese	3.45±4.27	0.00	14.00	
Nucleer Bud	0.90±1.33	0.00	7.00	
Underweight	1.42±1.98	0.00	7.00	
Normal	0.68±0.95	0.00	4.00	0.238
Obese	0.84±1.21	0.00	4.00	

**p>0.05 (there is no statistically significant difference compared with those of underweight, normal, obese subjects with 95% confidence interval [95% CI].*

Discussions

Last decade, there have been limited analyses of the possible relationships between DNA damage and overweight or obese outcomes (Teixeira & Pestana, 2020; Gandhi & Kaur, 2012). The present study using alkaline single cell gel electrophoresis was showed increasing in DNA damage in obese subjects in contrast to controls. Moreover, the number of subjects in our study is comparable with those in other studies that also have shown significant effects (Zaki et al., 2019; Dupont et al., 2013). Tomasello et al. (2011) show that all increase in DNA damage both in preobese-obese, compared with control groups. In addition, DNA is involved in oxidative stress related to metabolic abnormalities occurring in obesity. Also, Włodarczyk et al. (2018) compare genotoxic damage non-obese and obese subjects (n=114) by comet assay. They found that genotoxic damage was approximately two times higher in the obese than in the control group (p <0.001). Gandhi and Kaur (2012) evaluate that DNA damage in obese subjects. They observed genotoxic damage was significantly (p<0.001)

elevated in obese subjects with a mean damage index of 47.34±0.79, damage frequency of 77.77±1.12, and DNA migration of 29.14±0.93 μm compared to related results in controls. Zaki et al. (2019) investigate DNA damage in obese women (n=172). They evaluate DNA damage by comet assay and found high DNA damage in obese women. Włodarczyk et al. comet assay results indicate that levels of % DNA in the tail were significantly higher in obese than in controls. Their results showed that obesity has a significant effect on the levels of genotoxic damage. Usman & Volpi (2018) measured DNA damage by micronucleus assay in buccal epithelial cells. Research indicates that was positively related to obesity and the total frequency of nuclear anomalies found in MN assay. Santovito & Gendusa (2020) found a significant correlation between MN, nucleoplasmic bridges, nuclear buds frequencies, age, and body mass index were scored in 1,000 cells per subject. However, Satayesh et al. and Milić et al. study results indicate the knowledge concerning the impact of increased body weight and genotoxic damage is poor (Setayesh et al., 2018; Milić et al., 2013). Present study, the first comprehensive study regarding the impact of obesity on DNA compared with normal-weight subjects in Turkey. According to the results of both genotoxicity experiments (Comet assay and MN), there is no statistically significant relationship between DNA damage and obesity.

CONCLUSION

This study evaluated the possible genotoxic effects of obesity. DNA damage may play a role in the etiology of cancers. Recent studies support that obesity can impact genome stability. prominent relation obesity and a raised risk of genotoxic damage. Obesity causes genotoxic damage, which is causally related to several disorders including cancer. In the future, should plan new studies to investigate the relationship between obesity and DNA damage to determine the precise mechanism.

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Conflict of Interest

No conflict of interest was declared by the authors.

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CHAPTER 4

COVID-19 PATHOPHYSIOLOGY IN HIGH-RISK PATIENTS WITH COMORBID DISEASES

"Narrative Review:"

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INTRODUCTION

A silenced outbreak has now become a worldwide pandemic. In December 2019, there was an outbreak of an acute atypical respiratory syndrome in Wuhan, Hubei Province, China, at a local seafood market (known as "wet market"). It was later discovered that a novel coronavirus was responsible for such an outbreak. The novel coronavirus SARS-CoV-2 which caused acute respiratory distress syndrome (ARDS). Due to its rapid transmission, increased fatality that caused public and health-care-related casualties worldwide, the World Health Organization (WHO) sounded an alarm of international warning, declaring the epidemic to be a 'Public Health Emergency of International Concern' on 31st January 2020. The origin of SARS-CoV-2's transmission was initially thought to be zoonotic (associated with the seafood market). Later it was recognized that human-to-human transmission played a major role in the subsequent pandemic.

COVID-19 primarily affects the respiratory system; however, it can also involve other organ systems. Symptoms surprisingly range from minimal, to significant hypoxia, and eventually death. These patients show significantly high blood levels of cytokines, chemokines, erythrocyte sedimentation rates and D-dimer. Concerning the respiratory system, higher leukocyte numbers and abnormal respiratory findings are observed since the symptoms arise from both the upper and lower respiratory tracts. The main findings of COVID-19 infection are severe pneumonia, combined with the incidence of ground-glass opacities, and acute cardiac injury. There are many different hypotheses out there regarding each high-risk comorbidity and its role in COVID-19 severity. The purpose of this paper is to do a literature review on the possible pathophysiology of COVID-19 progression in those comorbid patients, who are considered at high-risk of COVID-19 severity. Furthermore, we discuss the impact of COVID-19 around the world.

Methods

An electronic literature search was done using PubMed, Google Scholar, Research Gate, ScienceDirect and Mendeley. The search was focused, but not limited to articles published from December 2019 to

August 2021. Search through references of retrieved articles were also done. Articles were selected based on the keywords related to comorbidities, such as “Comorbidity”, “Obesity”, “Diabetes”. “DM”, “Thyroid”, “Cardiovascular diseases”, “CVD”, “Hypertension”, “HTN”, “Respiratory diseases”, “COPD”, “Liver injury” and “Kidney injury”, “AKI”. Additionally, keywords related to COVID-19 such as “COVID-19”, “SARS-CoV-2", “vaccine” “demographics”, “ethnicity”, “healthcare”, “outbreak”, “epidemiology”, “variants”, were also searched. The search was dynamic with keywords added on subject to relevancy. Articles were then reviewed and selected on applicability to our topic.

Discussion

I. Covid-19 & related co-morbidities:

A) Chronic obstructive pulmonary diseases (copd) and asthma

COPD is one of the most prevalent respiratory conditions found in public. It comprises the respiratory tract in patients by increasing mucus production like in chronic bronchitis, alveolar destruction like in emphysema, hypersensitive bronchoconstriction like in asthma, and bronchial dilation like in bronchiectasis. In COVID-19 however, it is suggested that the acute lung injury is caused when the viral spike protein of SARS-COV-2 find an entry in alveolar cells by binding to the abundant angiotensin converting enzyme 2 (ACE-2) extracellular domains present on them, with the help of transmembrane serine protease2 (TMPRSS2) protein (Gómez-Zorita S,2021).

Pulmonary fibrosis is one of the main findings in severe COVID-19 patients. Researchers have found progressive fibrotic tissue in alveolar spaces in the first week, followed by interstitial and air spaces in the second week and finally dense septal and alveolar fibrosis by week 3 [2]. In a hypothesis suggested by these researchers, SARS-CoV-2 infection induces transforming growth factor beta (TGFb) which promotes fibrosis and suppresses ACE2 which in turn has a negative regulation of pulmonary fibrosis. They argue that myofibroblasts in the lungs produce extracellular matrix (ECM) and modify the lung structure and function when they undergo transdifferentiation into pulmonary

lipofibroblasts (LiF) that contain lipid droplets and a high level of perilipin 2. According to them, since they are closely situated to type 2 alveolar epithelial cells and 2% of these cells have ACE-2 receptors, there may be a phenomenon of ectopic fat deposition leading to pulmonary fibrosis-like fat deposition from adipose tissue in non-alcoholic fatty liver disease (NAFLD).

There are also other microscopic changes seen in COPD patients. In a set of autopsy results reported by researchers in China, COVID-19 patients with COPD were found to have edema, proteinaceous exudate patchy inflammatory cellular infiltrations, multinucleated giant cells, and focal reactive hyperplasia of pneumocytes [3]. In yet another research, patients were found to have elevated ACE-2 expression in their alveolar epithelial and bronchial cells [4]. Additionally, ACE- 2 expression was observed to be higher in nasal epithelium than in bronchial epithelium and they were also found to be high in COPD and current smokers in comparison to non-COPD patients and former/never smokers [5].

Scope of disease progression and mortality in COPD patients depends on multiple factors. According to Song et al., old males with COPD were more likely to progress into a severe form of COVID-19 in comparison to non-COPD patients and non-asthmatic patients or even asthmatic patients. They have also found a decreased neutrophil to lymphocyte ratio in patients with COPD and suggested that it is an independent risk factor for mortality in them. Additionally, COPD with dyspnea was found to be the strongest predictive factor for ICU admissions [6-7] and has a 5.9- fold higher risk of severity progression [8]. 15 – 20% of COVID-19 patients develop hypoxemia which may require mechanical ventilation [9]. However, COPD patients are more likely to receive mechanical ventilation than non-COPD patients due to their preceding condition [4]. COVID-19 patients with pre-existing COPD are four times likely to die [10]. They also get delayed care because these patients refuse to show up to the hospital due to the fear of the infection, leading to "excess mortality" [5].

Of note, CVD was found to be the prime comorbidity in COPD and asthmatic patients. Additionally, in COPD, anemia was recognized

as an independent predictor of hospitalization and mortality [11].

Interestingly, COVID-19 progresses differently in patients with asthma. Asthmatic patients are least likely to develop a severe form of COVID-19 infections and ACE-2 expressions were found to be decreased in these patients in comparison to healthy controls [4]. Moreover, ACE-2 expression in airway epithelium cells is lower in inhaled corticosteroids (ICS) users in comparison to non-ICS users [5]. Hence, it may be suggested that asthma had protective factors against COVID-19 infections. Further research must be done in this direction.

B) Cardiovascular diseases system and hypertension

ACE-2 receptors are found in abundance on the cardiac muscle. Therefore, the involvement of the cardiovascular system is highly predictive. Pre-existing CVD can increase fatality by 10.5% in COVID-19 patients [12].

According to Nishiga et al, ACE-2 expression is higher in the heart and arteries than in the lungs, predominantly seen in the pericytes of the heart. The symptoms that patients with cardiac disorders exhibit are myocardial infarction, arrhythmias, acute coronary syndrome, and thromboembolisms [13].

Higher adipose tissue ratio in the body contributes to the manifestation of cytokine storm seen in COVID-19 patients due to its propensity of viral reserve after infection. One hypothesis suggested by El-Sayed Moustafa et al., is the involvement of the cardiometabolic system [14]. Since adipose has been observed to have the highest ACE-2 expression among all sites in the body, they argue about the possible relationship between adipose tissue, microvascular endothelial cells, inflammation, age dependency, and ACE-2 expression in tissues. They propose that a higher proportion of microvascular endothelial cells lead to higher ACE-2 expression and therefore evidently increases the risk of the thrombolytic event, whereas lower ACE-2 expression was associated with higher total triglyceride levels and higher macrophage infiltration.

Hypertensive patients are increasingly observed to suffer from the COVID-19 infections. According to Schiffrin et al., however, frequency

of hypertension in COVID-19 patients is unrelated to COVID-19 infections since this condition is exceedingly found in the elderly population worldwide [15]. They also suggested that although the risk factor for uncontrolled hypertension is unknown, blood pressure control must be a precautionary step in hypertensive patients. Unlike previously proposed, they suggest that angiotensin converting enzyme inhibitors (ACEi) and angiotensin receptor blockers (ARBs) do not seem to increase susceptibility to the virus rather have anti-inflammatory effects. It is suggested that taking ACEs and ARBs increase circulating ACE2 and acts as a decoy by preventing SARS-CoV-2 from entering cells [16]. It has been observed by Yang et al., that females and patients with comorbidities have relatively higher achievement rates for target blood pressure [17]. Monotherapy was observed to be more effective in comparison to combination and blood pressure did not fluctuate based on the age theory [18].

Patients with hematogenic imbalance further complicate the disease progression. According to a linear mixed model study done in Wuhan [19], comparing survivors and non-survivors, thrombocytopenia, increased neutrophil to lymphocyte ratio, longer prothrombin time >16s, and

>2mg/L D-dimer are independently associated with increased mortality. Hence, they deduced that the higher the intensity of coagulopathy, severe the outcome of COVID-19. They also proposed that injury to the endothelial cells might have led to the thrombotic microangiopathic, and microcirculatory impairment, and this proposal is consistent with autopsy reports that found clots and the endothelial apoptosis during postmortem of COVID-19 patients.

Of note, coagulopathy in COVID-19 and disseminated intravascular coagulation (DIC) is different in the fact that extreme hypercoagulopathy state with very high D-dimer levels is rarely seen in DIC [19]. DIC in COVID-19 patients is indicated to have a 71.4% mortality rate and 0.6% survival rate [20].

Another factor responsible for COVID-19 progression in patients with hematogenic imbalance is oxidative stress. A high concentration of free heme released under oxidative conditions. According to Tracz et al.,

free heme that can denature proteins, activate apoptotic caspases that further lead to a complete cessation of O2 in the organelle [21]. Heme oxygenase 1 (HO1) is observed to get upregulated in stress organs which channel as a protective mechanism for heme related injuries. Hence, activating the HO1 pathway in targeting COVID-19 may have a preventing role for inflammatory induced coagulopathy [22]

C) Diabetes and thyroid

Type 2 diabetes (T2D) has already been a global pandemic and for it to be combined by the COVID-19 pandemic led to detrimental outcomes. Previously too, during the SARS epidemic, type 2 diabetic patients had a poor prognosis [23].

Diabetic patients have a higher tendency to develop complications when infected by COVID–19. According to a study conducted by Zhu et al., not only hyperglycemia but hypoglycemia can also lead to complications in prognosis and increase mortality [23]. They observed that T2D patients with uncontrolled hyperglycemia reported having higher dyspnea, systolic blood pressure neutrophil, leukocyte elevation ratio, and higher incidence of lymphopenia. They also seem to activate the renin-angiotensin system in tissue that increases the propensity of COVID-19 infections in these patients. Moreover, they suggested that an increase in blood glucose in turn leads to an increase in glucose in the airway epithelial secretion that reduces their defensive ability. Both hyperglycemia and insulin resistance lead to the synthesis of advanced glycation end products (AGEs) and pro-inflammatory cytokines, oxidative stress, in addition to stimulating the production of adhesion molecules that mediate tissue inflammation [24]. Hypoglycemia has been shown to increase monocytes and platelet reactivity, contributing to higher cardiovascular mortality in patients with diabetes [24]. These patients required more intensive in-hospital treatment in comparison to non-diabetic patients, yet T2D caused mortality and all-cause mortality (comorbidities) was higher compared to well-controlled T2D patients [23].

There is also an altered inflammatory response in diabetic patients. According to Varghese et al., increased blood glucose leads to

heightened inflammatory response and with a combination of

SARS-CoV-2 infection could progress to worsened prognosis [25]. Their patients are found to have elevated serum levels of interleukins (ILs) mainly IL 6, IL2, IL10, interferon (INF) gamma, T cells with cluster of differentiation (CD) 4+, and decreased levels of CD8+ T cells, thereby possibly producing cytokine storm. Dysregulated renin-angiotensin-aldosterone-system (RAAS) were found by them to interfere with phosphoinositide-3-kinase/protein kinase B (PI3K/AKT), mitogen-activated protein kinase (MAPK), and nuclear factor kappa light chain enhancer of activated b cells (NF-kB) pathway to decrease glucose transporter 4 (GLUT-4) translocation and cause inflammation following vascular damage.

There are also other mechanisms for increased thrombotic events in infected diabetic patients. Both insulin resistance and T2D are associated with endothelial dysfunction, and enhanced platelet aggregation and activation, both of which can also favor the development of a hyper-coagulable prothrombotic state [24].

Hyperglycemia may also be involved directly in the viral replication in the body. According to a study conducted by L. S et al., elevated glucose is found to directly increase and sustain SARS- CoV-2 replication by increasing ACE-2 expression in pancreatic cells and glycolysis that produce mitochondrial reactive species activating hypoxia-inducible factor 1 alpha respectively [26]. Increased reactive species, they suggest, can lead to dysregulation of RAAS causing insulin resistance, hyperglycemia, and endothelial damage that further leads to thrombotic manifestations as mentioned earlier.

Cytokine storms seen in COVID-19 patients is not a new phenomenon. It is observed that patients who have an imbalance in thyroid functions, have disruption in hypothalamic-pituitary-thyroid (HPT) axis. In a study conducted by Lorenzo et al., ACE-2 expression and TMPRSS expression are abundant in thyroid cells, even more so than the alveolar cells [27]. They found that SARS- CoV-2 induces cytokine response directly by affecting thyroid hormones in hypothyroidism, thyrotoxicosis, non-thyroidal illness syndrome, graves' disease, and thyroid cancer, and indirectly by affecting the HPT axis.

D) Obesity

Obese individuals are under constant metabolic stress. With the addition of COVID-19 infection, obese patients have a 1.41 times higher risk of experiencing disease progression, increasing the risk of worse prognosis, but not mortality, by three times [28]. It also leads to hyper-inflammation- like cytokine release syndrome (CRS) [29]. According to Tsankov et al., obesity causes low-grade inflammation that might lead to T and B cell exhaustion, further leading to the delayed capacity of producing interferons and allowing more time for viral replication, which in turn gives rise to fulminant cytokine production [30]. They observed that interleukins, especially IL6, are elevated in obese individuals and the viral trigger may increase the propensity of such individuals to develop cytokine storms. Obese individuals have impaired innate and adaptive immunity due to persistent inflammation and this leads to a weakened response to diseases [31].

There is a close relationship between COVID-19 and body mass index (BMI). According to a study conducted by Gao et al., independent of T2D, there is a linear relationship between an increase in BMI above 23kg/m2 and hospital admission along with an increase in the risk of death

in people with a BMI 28kg/m2 [32]. The youngest age group (20-39) was associated more with the risk of death and the association weakened and became insignificant as the age progressed above 80 [28,32]. It may be because the patients on the higher age spectrum might have already been treated by ACEIs and ARBs making them less likely to get affected by the said process [33].

Nevertheless, a paradox has come into existence where obese patients with pneumonia have a decreased possibility of mortality, which may be because obesity helps them during the deficit caloric intake during the intensive care period that led to sepsis and ventilator-induced lung injury and thus decreasing the mortality risk [29].

Interestingly, the risk of hospital admission with a unit increase of BMI was lower in T2D, CVD, HTN, and other comorbid patients [32]. There could be multiple reasons for this phenomenon. Patients with these comorbidities are high-risk groups and can be admitted earlier and

treated more aggressively resulting in better outcomes [34]. Additionally, the greater abundance of ACE-2 in adipose tissue has shown to have a protective effect in patients due to its role in increasing the anti- inflammatory markers in the body [1].

Adiponectin is an anorexigenic peptide that helps in fatty acid oxidation and glucose uptake. Circulating adiponectin is inversely proportional to adipose mass in the body and can be used for therapy [2]. Circulating levels can be increased by certain medications such as thiazolidinediones and can be researched further.

E) Acute liver injury

Patients infected with COVID-19 are shown to exhibit symptoms related to liver injuries. According to A. Sharma et al., elevated aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels have been shown to lead to a poor prognosis, and 1/3 of the patients who had elevated AST and ALT had longer hospital stays [35]. Furthermore, they observed cirrhosis to be associated with higher hospitalizations and mortality in COVID-19 patients. In a study conducted by Marjot et al., Patients with highly elevated ALT levels also had high levels of D-dimers and IL6 [36]. They proposed that there is a direct effect of SARS-COV-2 on cholangiocytes and hepatocytes which has 57.9% and 2.6% ACE-2 expressions, respectively. However, they observed very few hepatocytes that co-expressed ACE-2 and TMPRSS2. They found moderate macrovesicular steatosis and mild lobular portal activity in postmortem autopsy along with an increase in mitotic cells with eosinophilic bodies and ballooning hepatocytes indicating virus-induced apoptosis and liver injury. However, due to multifactorial reasons, it might not show a direct temporal relationship.

Bleeding is common in liver diseases which manifests due to the defect in the production of coagulant proteins. According to Caldwell et al., portal hypertension, endothelial dysfunction, bacterial infection, and renal failure play a major role in bleeding diathesis and the prevalence of decreased fibrinogen is only seen in advanced cirrhosis patients with acute liver failure in comparison to patients with stable disease [37]. According to their studies done in vivo, excessive amounts of nitric

oxide and prostacyclin along with von-Willebrand factor (vWF) in plasma promotes defective platelet activation and adhesions in patients with cirrhosis. This phenomenon further explains the thrombotic manifestations in COVID-19 patients. Other liver abnormalities in

COVID-19 patients could be maybe ischemic hepatitis, congestion related to cardiomyopathy, and transaminase release due to the breakdown of skeletal and cardiac muscles [38].

There is said to be a relationship between NAFLD, BMI and COVID-19. NAFLD is one of the leading conditions that cause liver failure and transplantation. Currently, there is an inconsistency in the relation of NAFLD and COVID-19 because of the confounding effects of virus-induced steatosis which results in difficulty in separating the effects of NAFLD and other comorbidities [36]. NAFLD proportionally increases with BMI and waist circumference similar to the Metabolic Syndrome (MetSyn) [39]. Hence, both central (abdominal) obesity and NAFLD must be considered as potential risk factors for COVID-19 infection.

F) Acute kidney injury

Acute tubular injury is the most common injury found in acute kidney injury (AKI) COVID-19 patients [40]. According to Izzedine &Jhaveri, 20-40 % of ICU admitted COVID-19 patients are affected by acute kidney injury and the main renal symptoms in those patients are reduced glomerular filtration rate (GFR), prolonged hematuria, proteinuria, and cytokine storm that causes hypo-perfusion tubular injuries [20]. They proposed that the kidney injury may have taken place due to a direct cytopathic effect through CD68+ interstitial macrophages and a tubular deposition of complement C5b-9 that led to kidney infarctions. They observed that patients were found to have erythrocyte aggregation and obstruction were also found in the glomerular lumen and peritubular capillaries along with glomerular ischemia and endothelial cell injury along with fibrin and thrombin within glomerular capillary loops indicating coagulation activity in COVID-19 patients. Additionally, they suggested that the cause of proteinuria is seen because of the imbalance of RAAS activation due to

SARS-CoV-2 attachment on membrane-bound ACE-2 leading to down-regulation and dysregulation of angiotensin 1-7, that may prevent internalization of ACE-2 via lysosomes and increase the interaction between spike protein and ACE-2.Gabarre et al., suggest that the already existing comorbidities such as hyalinosis arterioles(diabetes) arteriosclerosis (hypertension), and radiographic contrast media used for investigations might also play a role in the development of AKI [16].

One of the main manifestations seen in patients admitted to ICU is hypokalemia. Chen et al., found that 93% of severe ICU admissions were hypokalemic and suggested that it was because of the degradation of ACE2 receptors [41]. Hence, they proposed that the end of potassium ion loss could be a good prognostic factor and sensitive biomarker reflecting the RAAS system in COVID-19 progression.

Interestingly, according to certain autopsy reports, patients experience multiple organ failure with selective tropism for kidneys even if they are not critically ill and do not have a history of kidney diseases [42]. This may be explained by the heart-kidney axis and the lung-kidney relationship. Conditions such as cardio-renal syndrome (CRS) cardiomyopathy and acute viral myocarditis may lead to renal venous congestion, renal hypo-perfusion, and hypotension, and furthermore injured renal tubular epithelium upregulates IL6 which is seen to be associated with an increase in permeability of alveolar capillary permeability [43].

Genetically, ACE1 polymorphism in intron 16 with the deletion or insertion (D/I) leads to an increase or decrease of a D allele, Low D allele has been associated with increased expression of ACE2 and has been found in Europeans, Asians, and Africans [20]. Although some patients are reported to have collapsing glomerulopathy due to heavy proteinuria [44]. APOL1 in the black population seems to predispose them to collapse glomerulopathy [16].

II. Impact of covid-19 on the comorbid population

The impact of the pandemic is varied around the world. Highly populated areas such as urbanized and highly urbanized states in India are mostly resided by the working class and are more likely to get

infected [44]. At the beginning of the pandemic, low temperatures were said to be the reason for the low spread of the infection even in highly populated areas. This could be because people tended to stay at home which made practicing social distancing easier [45]. People also volunteered and successfully followed in-house guidelines at the beginning of the pandemic leading to a lower prevalence of infection. However, later during the months of quarantine, they failed to properly practice social distancing in public or had in-house gatherings without proper ventilation and this has continuously sprung back incidences. Nonetheless, this does not explain whether people who did not follow the guidelines ended up getting infected or not [46].

The odds of COVID-19 infection were four-fold higher in African Americans than non-African Americans [47]. This may be because comorbidities associated with a higher risk of severe COVID-19-related morbidity are more prevalent in African American and Hispanic than non- African American and non-Hispanic populations [47]. However, later infants and children were seen to be getting affected as well. The attack rate dramatically declined for all age groups except children (<20 years) [49]. The probable reasons for children to get infected may be due to improper social distancing and indoor familial clustering. The mortality rate in those testing positive for COVID-19 was higher in White British patients (25.4%) than those of South Asian origin (18.1%) but this was not statistically significant [48].

Periodic announcements of variants of interests (VOI) and variants of concerns (VOC) sent the public in spirals. The more recent variant named Delta is found to be more lethal in comparison to its prior counterparts. It changes the phenotype of the virus due to multiple genetic mutations and in a combination with other variants. People are more likely to die especially the comorbid patients, than equivalent infected patients with previously circulating variants [50-51]. Even so, there are only small differences between the vaccination efficiency against the Alpha and Delta variants. Any vaccine is 87.5% effective with the Alpha variant and 79.6% effective with the Delta variant [52]. It is also advised to take annual flu shots since Influenza virus vaccines could help differentiate between influenza-like and COVID-19

symptoms and reduce COVID-19 infection in comorbid patients by reducing the probability of getting the flu [9].

According to the phylogenetic analysis of the coronavirus, among the three central variants, type A, B and C, type B is the most common type found in East Asia and interestingly does not spread to other countries before getting mutating, hence indicating the resistance of this variant outside this region [53]. Additionally, several RNA viruses tend to mutate inside the host. It has been reported that COVID-19 intra-host variants are supported with genetic differentiation and can lead to bottleneck phenomenon reducing the viral population at some point in time during the pandemic [54].

Outcomes of the outbreak are dependent on several factors such as exposure, individual health consequences, social vulnerability, and pandemic control measures. Health workers are the front liners most exposed to the infection. At least 115,000 health workers are said to have died due to COVID-19. Children too are at risk since child healthcare was seen to be reduced up to 60% by the initiation of lockdowns [55]. Exposure is also significant in overcrowded localities and localities with unfavorable environmental conditions. Minority ethnic groups due to their lack of proper nutritional status are also being affected adversely. They may also be at high risk of developing long covid (long COVID) which is a term described for the continuing effects of COVID-19 infection beyond initial illness. Additionally, they face the unpleasant reality of insecure working conditions, employment contracts, and insurance insufficiency [56]. Ironically, the negative impact on the public debt due to an increase in public expenditures by the government have led to negative moods among customers that may cause them to spend even less in the market, affecting the economic situation at a large [57]. Proposedly, some countries may never be able to reduce the public debt at a level before the pandemic.

CONCLUSION

The possible pathophysiology of COVID-19 progression in patients with respiratory conditions, cardiovascular conditions, endocrine conditions, obesity, liver injury and kidney injury were

explored in this paper. We observed that adipose tissue plays an important role in many comorbidities and may predispose patients suffering from these conditions to COVID-19. Myofibroblasts in lungs undergo transdifferentiation into lipofibroblasts that cause ectopic fat deposition leading to pulmonary fibrosis. Similarly, since adipose tissue contributes to the highest ACE-2 expression in the body, in comparison to other tissues, it may have a relationship with microvascular endothelial cells that increases the risk of coagulopathy events. Cytokine storm is a known phenomenon in COVID-19 patients. Since adipose tissues have the propensity to have a viral reserve. They may be responsible for cytokine storm in obese patients as these patients are in chronic low-grade inflammation state. Additionally, it is also responsible for the phenomenon in diabetic patients who already have highly elevated inflammatory markers along with hyperglycemia. Viral induced microvesicular steatosis leading to apoptosis and liver injury found in autopsied bodies could indicate a similar correlation of fat deposition and liver diseases. Renal coagulation activity seen in infected patients may reflect vascular pathologies stemming from diabetic and hypertensive pathology, a relation of which with the adipose tissue has been mentioned already. Interestingly, however, the greater abundance of ACE-2 in adipose tissue has also shown to have a protective effect in co-morbid patients due to its role in increasing the anti- inflammatory markers in the body decreasing their need for hospital admission.

The impact of the pandemic on different demographics, ethnicities, and economy were also discussed. The highly populated, African-American populations in vulnerable working and/or

living conditions are at the highest risk of COVID-19 infection. Any vaccination given to the public has a higher effective percentage than no vaccination and it can prevent severe progression and hospitalization in comorbid and non-comorbid populations alike.

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CHAPTER 5

SURGICAL MANAGEMENT OF ABDOMINAL TRAUMA

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INTRODUCTION

Abdominal trauma is one of the most common causes of morbidity and mortality worldwide. There traumas can be blunt, mostly resulted from motor vehicle accidents, or penetrating, caused by gunshot or stab wounds. The most commonly involved organs in abdominal trauma cases include the stomach, liver, spleen, small intestines, duodenum, pancreas, colon and rectum. Laparotomy is the standard method used in surgical management of abdominal trauma, while laparoscopy may be used occasionally. Two critical steps of the management are the evaluation of the patient's stability and damage control. Surgical procedure is then performed depending on the extent of the involvement and severity of injury. This chapter provides basic information on surgical management of abdominal trauma involving various organs.

1. Abdominal Trauma

In patients under 35 years old, trauma is the leading cause of death worldwide (Soreide, 2009). It leads to significant burden and challenge on health care providers. In 2013, approximately 671 billion dollars have been spent on trauma victims in the USA (Florence, Simon, Haegerich, Luo and Zhou, 2015). Abdominal traumas account for 9-14.9% of all trauma cases (Lefering and Nienaber, 2015). Abdominal trauma is divided into two subtypes as penetrating and blunt traumas. Motor vehicle accidents are responsible for approximately 75% of blunt abdominal traumas, while gunshot and stab wounds are the main mechanism of injury in penetrating traumas.

Stomach, small intestine, duodenum and colon injuries are common in penetrating abdominal trauma, while these injuries are relatively rare in blunt trauma (Durso et al., 2020). The most commonly injured site is small intestines in both types of trauma (Ntundu et al., 2019). Abdominal trauma cases should be carefully triaged for appropriate intervention, because nearly 25% of these cases require surgical management (Ülkü, 2018).

1.1. Blunt and Penetrating Abdominal Traumas

Most cases of abdominal trauma are reported to be blunt with reported rate of 90% (Özpek, Yücel, Atak, Baş and Alimoğlu, 2015). The most important causes of blunt trauma include motor vehicle accidents (62%) and fall from height (27%) (Pimentel et al., 2015). Mortality rate of blunt trauma has been reported as approximately 10% (Pimentel et al., 2015). The causes of

mortality mostly involves extra-abdominal injuries such as central nervous system or thorax that have been reported as 97% (Malkomes et al., 2019).

The main causes of penetrating abdominal trauma are gunshot and stab wounds. These events are seen more commonly in regions where crime or terrorism levels, or interpersonal conflicts are high (Brenner and Hicks, 2018). The mortality rate of these traumas has been reported between 2-13% (Ülkü, 2018). The causes of mortality in penetrating abdominal trauma are death at the scene due to septic complications or due to multiorgan failure in the postoperative period (Ülkü, 2018; Brenner and Hicks, 2018).

1.2. Evaluation of Stability in Patients with Abdominal Injury

One of the major challenge in the management of patients with abdominal trauma is to classify them as stable or unstable in order to determine the need for resuscitation or surgical intervention. Stability is evaluated considering several factors such as age of the patient, severity of injury and comorbidities (Ball, 2014). In abdominal trauma, hematological instability arises from major hemorrhage and thus, resuscitation should involve transfusion of blood and blood products (Benz and Balogh, 2017). Hemodynamic stability with vital signs should be assessed frequently.

In patients with major abdominal trauma, hemodynamic instability, impaired coagulation, metabolic acidosis from low tissue perfusion, infections and pulmonary complications contribute to instability, making the management of patients challenging and contributing to morbidity and mortality (Feliciano, 2017).

1.3. Laparotomy

Most abdominal trauma cases are managed non-operatively, although generally laparotomy remains mainstay of surgical management. The first laparotomy procedure was performed in the USA in a patient with abdominal gunshot wound in 1884 (Barrow, Anderson, Varley, Pichel, Peden, Saunders and Murray, 2013). Today, 30000 to 50000 laparotomy operations are performed in the United Kingdom annually (Hershberg, 2013).

1.4. Basic Surgical Approach

Preparation of the patient is made with a sterile cleansing solution applied on a large area of the abdomen. A midline abdominal incision is best for an effective exposure. Over the midline, an incision is made beginning from the xyphoid and extending to the pubis. The abdomen is explored systematically by quadrants in the case of blunt trauma. In a penetrating

trauma, the injured site should be focused on for exploration. Following exploration, the relevant definitive operations are performed to repair traumatic injuries (Baloch, Arif, Zaheer and Aziz, 2017).

1.5. Damage Control Laparotomy

In the case of patient presenting with multiple traumatic injuries and unstable vital signs, it is often not possible to repair traumatic injuries at the first stage. Instead, damage control laparotomy, which has been developed in early 1990s to salvage trauma patients from life threatening events, is attempted. The aim of damage control laparotomy is to identify injuries and stabilize the patient. The initial operation is performed in order to stop bleeding and prevent gastrointestinal spread. Every trauma patient does not require damage control operation. Selection criteria are shown in Figure 1 (Karateke et al., 2017). Damage control laparotomy may lead to several complications such as intra-abdominal abscess, abdominal compartment syndrome and sepsis.

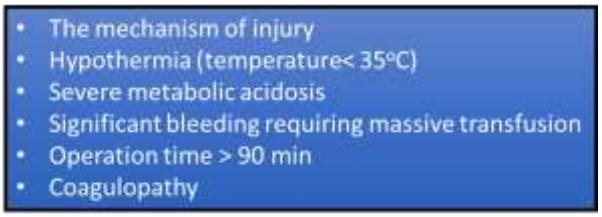

Figure 1. Selection criteria for damage control laparotomy

1.6. Laparoscopy

Laparoscopy is occasionally performed especially in penetrating abdominal traumas for exploration particularly the left upper quadrant. In addition, laparoscopy play a role in the diagnosis of intraperitoneal injuries following trauma (Pucher, Carter, Knight, Toh, Tucker and Mercer, 2018).

Laparoscopy is less invasive compared to laparotomy and results in less blood loss. However, there may be missed injuries with laparoscopy and examination of the intestines may be difficult especially if blood is present in the abdomen. When performed by experienced and specialized teams, laparoscopy is a useful method in order to decrease mortality and morbidity in abdominal trauma. Pucher et al. reported that performing laparoscopy instead of emergency laparotomy decreases mortality, morbidity, health related costs and hospitalization (Nicol, Hommes, Primrose, Navsaria and Krige, 2007).

2. Surgical Management of Abdominal Trauma

2.1. Surgical Management of Hepatic Injuries

The liver is the most frequently injured organ in blunt abdominal trauma. Liver injuries cause large amount of blood loss. Therefore, the patient may be hemodynamically unstable when transferred to the operating room. Communication between caregivers and planning of resuscitation are of paramount importance in these patients. The patient may have symptoms of hypothermia or coagulopathy.

The first step in operation for hepatic injury is to stop bleeding. The surgical and anesthesia teams should be ready to attempt this through resuscitation when the abdomen is opened. Hemorrhage can be effectively controlled with perihepatic packing (Yu, Li and Gong, 2016). Earlier use of packing has been associated with better survival rates. In more expensive hepatic injuries, large sutures may be placed to approximate a wound. Penetrating injuries can be approached through tamponade with a balloon or packing. If a part of the liver is severely injured, a segmental resection may be performed (Claridge and Hambley, 2015).

2.2. Surgical Management of Splenic Injury

Operative exploration should be performed in patients with splenic injury who exhibits hemodynamic instability. It has been reported that approximately 45% of patients with splenic injury require emergency surgery (Yoganandan, Pintar, Gennarelli and Maltese, 2000). During preparation for surgery it is important to be aware of the possibility of injury in structures surrounding the spleen. The most commonly injured structures concurrently with the spleen include stomach, small intestines, pancreas and diaphragm (Weledji, 2014). Placing a nasogastric tube will be helpful for better surgical exposure of the splenic area.

Primary repair of the spleen may be achieved by suturing a lacerated area. In order to stop hemorrhage from the injured parenchyma, electrocautery can be used. In addition, fibrin coagulation products can also be used for hemostasis. In hemodynamically unstable patients with severe injury, splenectomy is undertaken. First, the spleen is mobilized and splenic area is packed. Control of the vasculature during splenectomy is achieved by ligation (Rahnemai-Azar, Rahnemaiazar, Naghshizadian, Kurtz and Farkas, 2014).

2.3. Surgical Management of Gastric Injury

In the beginning of operation for gastric injury, first a nasogastric tube is placed to improve exposure. Surgical team should have high suspicion for

gastric injury in case of sanguineous fluid from the nasogastric tube (Liu, Dai, Ye, Zhao, Shi and Peng, 2018). Giving the patient a reverse Trendelenburg position will improve gastric and surrounding exposure to better assess the injury. If a diaphragmatic injury presents, there is a risk for contamination of the pleural space with gastric content (Coleman and Zarzaur, 2017). In this case, a thoracotomy may be needed to wash out the pleural space or a thoracostomy to drain gastric fluid.

Gastric injuries are treated according to their severity. Evacuation of a hematoma and primary closure of laceration are performed for the treatment of Grade I and II injuries (Fonseca, Schuster, Maung, Kaplan and Davis, 2013). Primary closure is also used in Grade III injuries, but the closure should be made with staples due to the size of the injury. A partial gastrectomy may be needed in Grade IV injuries. Whereas, in the case of Grade V injuries total gastrectomy may be needed with Roux-en-Y esophagojejunostomy. In the case of anterior gastric injury, the stomach should be carefully examined for a possible posterior wound (Yoganandan, Pintar, Gennarelli and Maltese, 2000).

2.4. Surgical Management of Small Bowel Injury

Definitive repair is performed with necessary interventions at the time of presentation in stable patients with small bowel injury. In hemodynamically unstable patients with acidosis and/or coagulopathy, surgery team should be ready to complete the procedure quickly. Placing a nasogastric tube will be helpful to decompress the small intestines (Weinberg and Croce, 2015). After the abdomen is opened, entire small bowel is examined for injury and bleeding vessels are clamped.

Grade I injuries are repaired by suturing the tears. In Grade II injuries of the small bowel, debridement and primary closure is performed (Ozsoy, Ersen, Ozsoy, Yilmaz and Arıkan, 2018). Resection is usually not recommended in these injuries. Grade III and IV injuries are repaired by surgical resection and anastomosis. Grade V injuries that lead to devascularization of a segment of the bowel is repaired by resection of the entire segment and anastomosis (Ozsoy, Ersen, Ozsoy, Yilmaz and Arıkan, 2018).

2.5. Surgical Management of Duodenal Injuries

The extent of the repair will be dependent on the nature and severity of duodenal injury. When beginning the operation, a nasogastric tube is placed

for decompression. After the abdomen is opened, the duodenum is exposed and inspected visually.

Grade I injury is managed non-operatively by nasogastric decompression and bowel rest. Grade I and II injuries with laceration are repaired with simple suture approximation (Yoganandan, Pintar, Gennarelli and Maltese, 2000). Grade III injury is usually repaired with duodenorraphy, Roux-en-Y duodenojejunostomy or an end-to-end duodenojejunostomy. Grade IV and V injuries require more complicated decision making and management. The repair include pancreatoduodenectomy, reimplantation of the ampulla and reimplantation of the common bile duct into the duodenum or primary anastomosis of the common bile duct (Mzoughi, et al, 2012).

2.6. Surgical Management of Pancreatic Injury

Pancreatic traumatic injury cause challenge to the surgeon due to anatomical location of the pancreas. Recent studies recommend to treat low-grade pancreatic injuries non-operatively (Bishop and Simo, 2022). In Grade I and II injuries, it is crucial to ensure that the main pancreatic duct is not disrupted. In these cases, magnetic retrograde cholangio-pancreatogram (MRCP) or endoscopic retrograde cholangio-pancreatogram (ERCP) is performed to examine ductal involvement. Placing a drain for hemostasis is usually sufficient in these injuries.

Distal injuries where the main pancreatic duct is disrupted (Grade III), are managed based on the amount of the tissue involved (Yoganandan, Pintar, Gennarelli and Maltese, 2000). If 50% of the pancreas will remain intact to maintain its function, a distal pancreatectomy can be performed (Girard et al, 2016). A pancreatic injury with more proximal involvement of the main duct (Grade IV) is surgically managed with resection of the involved section and suturing of the duct.

Pancreatic injuries with massive disruption of the head are especially challenging (Grade V). These patients are frequently unstable. Studies have shown that a damage control approach followed by definitive operation after resuscitative attempts is the best way for the management of these injuries (Mitchao, Lewis, Strickland, Benjamin, Wong and Demetriades, 2022).

2.7. Surgical Management of Colon Injury

Non-destructive colon injuries are managed with primary repair without need for diversion. Destructive colon injuries are managed with colon resection and anastomosis in patients who are not in shock state (Lasinski, Gil, Kothari, Anstadt and Gonzalez, 2018). The location of injury will

determine the anatomical boundaries of the resection. Anastomosis with diversion has been shown to be effective in patients with comorbidity or a high need for transfusion (Campbell, Alderson, Smith and Warttig, 2015). Abdominal washout is performed in all patients before abdominal closure.

2.8. Surgical Management of Rectal Injury

Surgical management of rectal injury is based on anatomic location. Rectal injuries are divided into two subtypes as intraperitoneal and extraperitoneal rectal injuries. Intraperitoneal rectal injuries that involve lateral or anterior two-third upper part of the rectum are managed in a similar way to colon injuries because of their similarity of the left colon (Yoganandan, Pintar, Gennarelli and Maltese, 2000). Extraperitoneal rectal injuries can be occasionally repaired primarily. The best management of these injuries is proximal diversion, which provides injury to heal without fecal contamination.

2.9. Surgical Management of Abdominal Vascular Injuries

Patients presenting with abdominal vascular injuries are often unstable and may have lost a large amount of blood before brought to the operating room. Therefore, the time between presentation and operation is one of the most important determinant of outcomes. It is of paramount importance that surgical, anesthesia and other teams should work together in order to minimize this time. The necessary blood products should be readily available and an auto-transfusion device should be prepared for using intraoperatively. Warming considerations are critical because of the risk of hypothermia induced coagulopathy. Body cavities should be irrigated with warm saline to increase body temperature. After the abdomen is opened through a large midline incision, a rapid visual exploration should be carried out. Any bleeding should be controlled by packing the solid organs or clamping the bleeding vessels and repair procedure should be performed only after all injuries are identified.

CONCLUSION

The abdomen is often injured during trauma, making it a critical region for surgical interventions. Some low-grade abdominal injuries can be managed non-operatively, while the others require surgical management. Laparotomy is the mainstay of operative care. Surgical intervention depends on the nature, extent and severity of injury. The most commonly injured organ is the liver. The other injured organs include the spleen, stomach, duodenum,

pancreas, colon, and rectum. Surgical management of abdominal trauma focuses on injury identification and bleeding control. Surgical management may range from primary repair to resection and anastomosis. Vascular injuries in an abdominal trauma have a high rate of mortality.

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CHAPTER 6

OCCLUSION IN IMPLANT-SUPPORTED PROSTHESES

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INTRODUCTION

In this study, implant-supported prostheses will be examined using the classification made by Hobo et al. (1989) classified the prosthesis using the dentitions of the patients as:

a) Implant prostheses applied to total edentulous patients; implant-supported full and implant-supported overdenture dentures.

b) Implant prostheses applied in partially edentulous cases.

Classical total dentures are functionally much worse compared to natural teeth, no matter how carefully and successfully they are applied. On the other hand, upper prostheses applied using implants bring the patient's muscular and dental functions closer to the functions provided by the natural tooth.

There are three implant-supported prosthesis options in total edentulous patients. These are fixed, removable, and hybrid prostheses.

Implant-supported overdenture prostheses

In order to increase retention and stability, prostheses made on a certain number of implant support with support from soft tissues are called overdenture prostheses.

Overdenture prostheses on implants are very useful in restoring tissues in the post-resorption area. Adequate soft tissue supports, good phonation compared to conventional full dentures, less cost compared to fixed prostheses on implants, ease of application, allowing the patient to use the prostheses used in the past during osteointegration, the low number of implants placed, and being removable in terms of cleaning (Sandallı, 2000; Hobo et al. 1989; Schroeder et al. 1991) can be listed as other advantages of overdenture prostheses. In addition, in these prostheses, different types of connector options such as O-ring, bar, locater, magnetic attachments, and ball attachment can be used.

The disadvantages of implant-supported overdenture prostheses are that the chewing activities are less than fixed prostheses on implants, the constant wearing and removal of removable dentures disturb the patients, and the prosthesis covers more space in the mouth (Hobo et al., 1989).

Implant-Supported Prostheses Used In Partially Edentulous Patients

Implant-fixed prostheses can be used in cases where there are parts missing teeth. It is indicated in conjunction with removable dentures to avoid

preparing other teeth in case of a single missing tooth or when the patient does not have sufficient abutment tooth support for a fixed prosthesis. (Sandallı 2000; Hobo et al. 1989; Schroeder et al. 1991).

In Kennedy Class I And II Cases

In these cases with edentulous ends, a single or multi-member fixed prosthesis can be applied by applying enough implants to the edentulous area to make chewing effectively.

Implants are frequently used in Kennedy I and II cases. In these cases, the implants should be applied to the posterior region, where the function will be restored in the missing region, but these regions contain anatomical formations such as mandibular canal, maxillary sinus, etc. In addition, increased bone resorption complicates implant applications in this region. Although manufacturers have developed mini-implants for these cases, it is impossible to implant posteriorly in some cases. In such cases, cantilever prostheses supported by more anterior implants can be planned (Hobo et al., 1989; Schroeder et al., 1991).

In the application of cantilever prostheses, basic biomechanical rules such as keeping the cantilever length shorter than the distance between the implants should be considered. This type of prosthesis should be prevented from creating a destructive force on the implants. When Cantilever prostheses are prepared in accordance with biomechanical rules, they provide service to patients for many years. However, in some cases, the above-mentioned anatomical formations and bone insufficiency do not even allow cantilever prostheses. In this case, removable partial dentures may have to be planned for the patients.

In Kennedy Class III Cases

In Kennedy's class III cases, the missing tooth may be in one tooth, or it may involve many teeth. In such cases, implant-supported applications can be planned.

Fixed prostheses supported by implants and teeth can be mentioned in these cases. It should also be considered that in cases where conventional fixed partial dentures are insufficient, an implant-supported prosthesis application with support from two or three implants will yield more successful results than a prosthetic application with support from the teeth with a single implant (Hobo et al., 1989; Schroeder et al., 1991).

In Kennedy class III cases, since there are natural teeth mesial and distal to the edentulous area, the implant must have a certain crown size, but the size of the implant in the bone is limited due to anatomical factors. This poses a biomechanical problem. This becomes a problem, especially when a short implant is used, and the crown length must be long due to the adjacent teeth. To prevent this, different procedures may have to be applied, such as using natural teeth as a support, placing more implants, and applying longer implants with further surgery (Hobo et al., 1989). If there is a single missing tooth in Kennedy class III cases, problems may occur due to the rotational forces on the restoration in the horizontal plane while the implant is applied in these patients. These problems can be solved by using systems with spacer, support options that prevent rotation and providing occlusion that does not allow rotational forces to occur. In cases where it cannot be resolved, conventional treatment methods should be preferred (Hobo et al. 1989; Kosinski 2000).

In Kennedy Class IV Cases

In such cases, the area where there is a missing tooth causes aesthetics to become more important than many other factors because the missing tooth is such that it crosses the midline. Placing the implants at the points where the teeth should be is very important from an aesthetic point of view. If the bone structure is suitable, 2 or 6 implants are placed, and implant-tooth supported plans are not made (Hobo et al. 1989). If a cantilever is to be made, it should be symmetrical on both sides, and this cantilever should touch the posterior teeth exactly. However, the ideal is the application of treatments that do not use cantilevers.

Occlusion types and ideal occlusion in implant-supported prosthesis

The natural occlusion of the patient should be examined to ensure ideal occlusion in implant-supported prostheses. An occlusion with optimum aesthetic and chewing efficiency and compatibility with the stomatognathic system is the ideal occlusion. According to Hobo and Guichet, the occlusion type is the ideal occlusion in which vertical forces are distributed appropriately, the teeth are in maximum intercuspation, the condyles are in a centric position, and horizontal movements are not prevented (Hobo et al. 1989).

1.1. Bilateral Balanced Occlusion

This type of occlusion is the most commonly used occlusion type in implant-supported prostheses as well as in total prostheses. In this occlusion, all teeth are in centric position, and at the same time, they are in contact with the maximum tubercle. During lateral movements, the occlusal forces come equally to the teeth and the temporomandibular joint (Ismail and Yacoub 2000). In this occlusion, it is thought that the force occurs in the horizontal direction, not vertically, during the chewing movement. Lateral forces damage the teeth periodontally. In order to reduce the effect of these forces, the contact surface should be wide during closing and movements.

Balanced occlusion is basically based on three theories. These are Bonwill's Three-Point Theory of Equilibrium, Manson's Circular Theory, and Spee's Spee Curve Theory.

The main factor in the occurrence of occlusal abrasion problems is excessive contact. Although Goldstein revealed in his work that the abrasion will decrease when the occlusion surfaces are arranged, since all teeth are in contact in all tooth movements, even if abrasion can be reduced, it cannot be prevented. Generally, balanced occlusion is not seen in mouths where there is no problem in the periodontium. If it is seen, there is attrition in the teeth. It is known that it is difficult to achieve fully balanced occlusion. Goldstein et al. (1962) argued that in this type of occlusion, the contact points could be created with a very sensitive and fully adjustable articulator, but the contact cannot be maintained when extreme movements are made.

1.2. Group Functional Occlusion

The foundations of this occlusion were laid by Schuyer in 1929. He thought that excessive exposure of the canine tooth to the incoming forces during lateral movements damaged the tooth and described an occlusion on the working side of the vestibular surface of the mandibular tooth tubercles, while there was no contact on the non-working side. In addition, this type of occlusion is a frequently observed occlusion in natural teeth. In this occlusion, in lateral movements, the forces should be distributed equally to all teeth, there should be no conflicts between the teeth while coming to the closure and during the lateral movements, and a suitable closing relationship should be provided.

Group function occlusion is not difficult to create as in bilateral balanced occlusion, and destructive forces do not occur in this occlusion type

as in bilateral balanced occlusion. For these reasons, it is thought to be the ideal occlusion type for short-bodied prostheses (Karaca 2014).

1.3. Canine-Protected Occlusion

D'amico (1961) defined canine-protected occlusion and stated that eccentric movements of the mandible occur under the guidance of the canine. He defined it as an occlusion with no tooth contact during the period until the mandible movement is completed under the guidance of the canine while the teeth are in maximum intercuspation. The key to occlusion is the canine tooth. The reason for this is that this tooth has a long root length, has compact bone around it, is the tooth farthest from the temporomandibular joint in the arch, and is the tooth that is least exposed to induced stresses (D'Amico 1961).

Advantages of canine-protected occlusion can be listed as:

a. It enables the lower and upper components to be clamped and to be in the maximum tubercle fossa relationship and supports the centric occlusion with too many teeth.
b. The forces acting on the tooth come parallel to their long axis.
c. It properly arranges the marginal and transversal ridges of the teeth, thus providing optimum chewing efficiency.
d. In a study on canine-sparing occlusion in humans and animals, it was observed that canine-sparing occlusion functions in all eccentric movements except protrusive movement

Despite all this information, D'Amico also reported that the canine tooth is exposed to quite a lot of lateral stress despite its anatomical advantages. In 1961, Lucia claimed that canine-sparing occlusion is contraindicated in patients with masticatory cycles that are too exposed to horizontal forces. Dawson argued that anterior guidance in the absence of a canine tooth should correct this missing tooth and named this type of occlusion as anterior group functional occlusion (Dawson et al. 1996).

Another disadvantage of this occlusion is that the amount of disocclusion in the posterior teeth is not clear between anterior and lateral movements in canine-sparing occlusion. Stellard (1961) argued that this disocclusion should be the smallest possible amount, while Thomas (1967) argued that this amount should be 1 mm. Later, Thomas's view was accepted

by other researchers, and this occlusion was defined as "organic occlusion" (Yogesh et al. 2016).

According to Thomas's study in 1967, the characteristics of organic occlusion were defined as follows: There is tooth-to-tooth contact in the posterior teeth, and they are in the relationship of the tubercle fossa, each tubercle touches the contact tooth at three points, while there is a 25-micron disocclusion between the anterior teeth. In protrusive movements, the palatal surface of the anterior incisors and the vestibuloincisal surface of the lower anterior incisors guide the occlusion. In lateral movements, however, the palatal inclination of the maxillary canine and the mesiobuccally inclination of the mandibular canine and premolars direct the occlusion (Çalıkocaoğlu 1998).

1.4. Lingualized Occlusion

Lingualized occlusion was described by Howard Payne in 1941. It is an occlusion in which the lingual tubercles of the maxillary teeth on the working or non-working side fit into the central fossa of the lower teeth. In order to achieve this occlusion, a different tooth shape must be used. The incisors are not replaced, while the tubercles of the upper molars have an inclination of 33 degrees. In the lower molars, the central fossae are enlarged. While the teeth are aligned, an inclination is created between the anterior and posterior teeth, and during the protrusive movements of the mandible, balance is achieved in the closure. In addition, a medial-distal slope is provided, which creates balance in mandibular lateral movements. There are many benefits of lingualized occlusion: It provides stabilization in parafunctional movements and reduces the vertical and lateral forces on the jaw bones. It is aesthetic and provides balanced occlusion during lateral movements. It can be applied easily. Chewing efficiency is high (Sonugelen et al. 1997).

1.5. Ideal Occlusion

It is a type of occlusion that provides effective chewing, is compatible with the surrounding bone and muscle tissues, does not cause physiological problems, and is aesthetically pleasing. According to Hobo (1978) and Guichet (1970), the ideal occlusion is one where the teeth are in maximum intercuspation, and the condyles are in a centric position. It should reduce the stress created by vertical forces and be able to carry horizontal forces and perform horizontal movements during the intercuspal position when the mandible is centric (Çalıkocaoğlu 1998).

According to Dawson (1985), ideal occlusion should reduce the stresses caused by vertical loads, maximum intercuspation should be provided while in the concentric position, and it should be able to perform horizontal movements and carry horizontal loads in this position, stabilize the contacts in the teeth when the condyle is in the centric position, and contact between all posterior teeth between protrusive movements. It is a type of occlusion that ensures that the patient is cut off and there is no contact with the non-working side. (Sohn 2011)

Occlusion on implant-supported prostheses

1.6. Ideal Occlusion in Implant-Supported Prostheses

In 1983, Leihom conducted the first research on the appropriate occlusion form for implant-supported prostheses. He said that in this type of prosthesis, the forces are not evenly distributed on the alveolar bone, so the type of fully balanced occlusion would be appropriate in implant-supported prostheses in order to avoid bone resorption. However, although this occlusion distributes the forces evenly, it puts a lot of load on the posterior teeth in particular. This type of occlusion has devastating effects in implant-top prostheses since the posterior regions are more unstable than the anterior regions (Schroeder et al. 1991).

In 1986, Jemt advocated that occlusion in implant-supported prostheses should be guided by anterior teeth in lateral movements, disocclusion in posterior teeth, and all teeth should be in contact with the tubercle fossa in closing. This type of occlusion is especially useful in full-mouth fixed prostheses. Because in this type of prosthesis, the posterior region is not resistant to lateral forces (Çalıkocaoğlu 1998).

As a result of the research, it has been understood that the ideal occlusion in prostheses on implants is as in natural teeth. In the following sections, the types of occlusions that should be applied in implant-supported prostheses are mentioned.

3.2. Occlusion In All Arch Fixed Prostheses

For full-mouth fixed dentures, group-functional occlusion should be applied if there are natural teeth on the opposite jaw, and bilaterally balanced occlusion should be applied if there is a total prosthesis. In fixed prostheses on full implants, canine-sparing occlusion should be applied. It has been suggested that mild anterior guidance should be provided even if there is an implant-supported prosthesis against the natural tooth (Vanlıoğlu et al., 2011).

The occlusal plates of the teeth in the lower jaw should be prepared in such a way that they are straight and taper towards the gingival. Buccolingual dimensions should be reduced so that a more aesthetic appearance is formed. The lingual tubercles of the upper teeth should be straightened. In order to reduce the forces that are not parallel to the long axis of the teeth, a crossbite can be created if the patient is not disturbed. If the upper lingual tubercles are not straightened and left pointed, this tubercle may break. Buccal tubercles may not be flattened but should be prepared in a shorter and rounded form. It is also necessary that the buccal tubercles do not come into contact during lateral movements. If the inclination of the tubercles increases by 10 degrees, there is an increase in the torque of 30 degrees in the implant. In the protrusive movement of the mandible, there should be no contact with the posterior teeth. In order for the prosthesis to be successful in the long term, an occlusal surface must be created to reduce the forces that do not come parallel to the long axis. These forces should be distributed in the anterior region by using as many implants as possible (Karaca 2014).

If there are areas where wings are applied, there should be no contact during lateral movements. During the centric relationship, 1-1.5 mm of freedom should be provided in the occlusal contacts so that premature contacts in function are prevented. Contact should be made on the anterior working side to avoid overloading the posterior region. If there are wings in full mouth fixed dentures, if a small amount of infraocclusion is provided on the teeth in this region, the incoming overloads will be reduced. In addition, the shortening of the wing length increases the success of the prosthesis. It has been reported that more successful results are achieved when a wing shorter than 15 mm in the mandible and shorter than 12 mm in the maxilla is applied. In full-mouth implant-supported fixed prosthesis applications, occlusion should be carefully evaluated, and forces should be distributed evenly (Vanlıoğlu et al., 2011).

3.3. Occlusion In Overdenture Prostheses

Bilateral balanced occlusion type should be used in overdenture prostheses. The teeth in the anterior region are supported by overdenture attachments and in the posterior region by mucosa and soft tissues, and changes can be made by creating disocclusion in the molar region. In recent studies, the use of bilaterally balanced lingualized occlusion type for overdenture prostheses has also come to the fore.

If there is excessive alveolar bone loss in the crest, monoplane occlusion should be used.

There is a consensus that maximum stability is achieved when bilateral balanced occlusion is used in overdenture prostheses, but there are not many studies comparing it with other occlusion types (Kim et al. 2005; Kesercioğlu et al. 1992).

3.4. Occlusion In Case Of Partial Edentulism

There are two approaches to free-ending cases:

1- Fixed prostheses made using implants in the edentulous area without support from the patient's own teeth,

2- Fixed prostheses made by placing an implant in the edentulous area and taking support from the patient's own tooth.

If there are teeth in the anterior region, canine-sparing occlusion should be used. During the lateral and protrusive movements of the mandible, canine-guided disocclusion should be present in the posterior teeth. If the canine is absent or damaged, but premolars are present, group function occlusion should be used. In this way, the forces coming during the function are not only loaded on the implant but are shared between the teeth and the implant (Acar and İnan 2001).

Minsley and Koth (1991) revealed that the first occlusion rule to be applied in edentulous class I and II partial cases is to provide simultaneous bilateral posterior contacts during the correct relationship of the maxilla and mandible. In Class II cases, edentulous areas are well suited for fixed denture applications because the occlusion is determined by the natural teeth already in the mouth. The occlusion of the prosthesis on the implant applied in the edentulous area should be done with a 30 μm gap. (Kesercioğlu et al. 1992; Acar and İnan 2001)

In Class III and IV cases, if the fixed implant-supported prosthesis includes the canine tooth, canine-sparing occlusion or group function occlusion should be used (Unger and Crabtree 1991).

In cases with edentulousness in the Class IV anterior region, the following factors should be considered:

1- If a fixed prosthesis is to be applied, there should be no contact between the teeth,

2- In patients with a removable prosthesis in the anterior region, there should be passive or no contact in the anterior region prosthetic teeth. Occlusal contacts should only be made in anterior and lateral movements.

3- If monoplane occlusion is chosen, it is desirable to have contact with the teeth in the anterior region.

4- For bridges located in the anterior region, the moment of tipping in the distal direction should be less than the anterior-posterior width of the implant.

According to Skalak (2002) and Sullivan (1996), implant-tooth-supported restorations are dangerous as they will damage natural teeth. El Charkawey et al. (1997), Cohen and Orenstain (1994) argued that the implant-tooth connection should not be rigid.

3.5. Occlusion Applied In A Single Tooth Missing

Single-tooth implants applied in the anterior region are more successful than single-tooth implants placed in the posterior region. Because both the bone height is insufficient in the posterior region and the anatomical structures such as the mandibular canal, the implant cannot be applied bicortical. In addition, the occlusal loads in this region are higher than in the anterior region. The widest implant that can be placed in the area should be placed so that offset contacts can be eliminated. However, the screw of the implant may break or loosen due to occlusal forces (Acar and İnan 2001).

In order to avoid such situations and to minimize the damage to occur, three-point contact should be ensured in single tooth implants, the occlusal surface should be reduced to be aesthetically pleasing, and the forces on it should be adjusted to be parallel to the long axis of the implant (Kesercioğlu et al. 1992; Acar and İnan 2001). During centric occlusion, the implant-supported prosthesis should have an occlusal gap of 30 μm. Because while the natural teeth can compensate for the stress caused by moving in their sockets under extreme forces, the implant cannot move.

If this occlusal space distance cannot be achieved, heavy forces will damage the implant-supported prostheses. Patients cannot tell whether implant-supported prostheses have sufficient occlusal space because they do not have a periodontal membrane and do not have the proprioceptive mechanism to detect height. The occlusion type found in the natural teeth of the patient with a single missing tooth should also be used in the new restoration. However, the implant used in the absence of canine teeth is exposed to great stress due to the occlusal forces on it while providing the disocclusion of the posterior teeth. In order to distribute these forces evenly to the teeth in the mouth, the use of group function occlusion should be preferred. In the restoration to be applied, occlusion should be checked before

and after permanent cementation with 40 μm thick bite paper, premature contacts should be removed, and bilateral occlusal contact should be ensured (EurJ, 2015).

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CHAPTER 7
DRUG RESISTANCE IN CANCER: A TUMOR MICROENVIRONMENTAL PERSPECTIVE

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INTRODUCTION

Cancer is a lethal disease which results in uncontrolled division of cells due to genetic mutations. Traditional treatment options such as surgery, chemotherapy and radiotherapy are often used for cancer therapy. However, the emergence of drug resistance is still one of the most challenging factors in response to cancer treatment. Emerging evidence suggests that the tumor microenvironment (TME) is effective factor in the development of drug resistance. TME contains several cellular and non-cellular components each of which influence drug response to tumor cells. TME not only plays a significant role in tumorigenesis as well as therapeutic resistance. The tumor microenvironment can influence the susceptibility of the tumor cell to drug treatment by affecting the drug concentration. In this review, we focus on how the tumor microenvironment mediates drug resistance in cancer therapy.

1. Cancer

Cancer is one of the most serious diseases and biggest mortality cause in the world. Only in 2020, an estimated 19.3 million new cases were detected, and almost 10.0 million patients died of cancer globally (Sung et al., 2021). Cancer disease occurs when cells begin to divide uncontrollably. Cancer cells can originate from various tissues and organs. These growing tumor cells often spread to the other parts of the body, which can invade the surrounding tissue and metastasize to distant organs through the lymphatic system, or vascular system (Fidler, 1989; Rusciano & Burger, 1992). The formation of cancer may be due to several risk factors, however, it is established that most of cancer formation results from the accumulation of multiple mutations in DNA (Bièche & Lidereau, 1995; Devilee & Cornelisse, 1994). These mutations can alter the regulatory pathways of the cells and lead normal cells to become cancer.

The development of cancer cells is composed of several stages. The tumor development begins with tumor initiation, caused by the genetic alteration. This genetic alteration leads to the aggressive proliferation of cells. The increased proliferation leads to a group of cells dividing abnormally, which leads to a tumor. The proliferation of cells continues with some additional genetic mutations in tumor progression. Invasion is the initial stage of metastasis and requires cell adhesion and extracellular matrix (ECM) adhesion. Cancer cells can invade through the epithelial basement membrane (BM), ECM, and stromal cell layers. During the invasion, cells avoid both adaptive and innate host surveillance mechanisms of the organism (Leber

Felix & Efferth, 2009; Valastyan & Weinberg, 2011). At the end of the invasion, cancer cells gain new properties (Lu & Kang, 2007; Minn et al., 2005).

There are several treatment strategies such as chemotherapy, radiotherapy, immunotherapy and targeted therapy for cancer treatment (Szakács et al., 2006). However, the development of resistance to these treatments has been a critical obstacle affecting patient survival rate (Liu et al., 2018). Therefore, patients in advanced stages of cancer often show poor clinical outcomes with treatment (Miller et al., 2019). Tumor microenvironment is an important cause leading to several therapeutic resistances (Steinbichler et al., 2018). New viewpoints have proposed that tumor progression is a dynamic and complex process that interacts closely with the TME (Hanahan, 2014).

2. Tumor microenvironment

The tumor microenvironment (TME) that surrounds and interacts with tumor cells, is an extracellular environment and act as a crucial role in tumorigenesis. TME contains cellular components such as endothelial cells, fibroblasts, endothelial, immune, vascular and smooth muscle and inflammatory cells and non-cellular components such as ECM, cytokines, growth factors, and hormones (T. Wu & Dai, 2017). In 1889, the seed and soil hypothesis is stated that cancer progression depends on the crosstalk between tumor and their microenvironments (Paget, 1889). These components in the TME limits the ability of anti-cancer drugs to permeate and kill tumor cells. The complexity and heterogeneity of TME promotes excessive cell growth and effects the susceptibility of tumor cells to drug treatment, thereby developing drug resistance (Trédan et al., 2007). Moreover, recent studies have demonstrated that TME induces abnormal cell proliferation and malignancy. TME is characterized by hypoxia, vascular abnormalities, acidosis, low pH, poor vascular perfusion and altered metabolic states (Roma-Rodrigues et al., 2019; Vaupel, 2004). It has been found that transformed cancer cells interact with stromal cells and lead to tumor development and drug resistance. Uncontrolled tumor cell proliferation, hyperplasia and blockage of apoptotic cell death contribute to increased extracellular matrix (ECM) stiffness, acidosis and hypoxia in TME, resulting in drug resistance (Lin et al., 2019; Maman & Witz, 2018). Cancer-associated fibroblast (CAFs) stimulate tumor progression and drug resistance via secrete cytokines, chemokines, ECM remodeling factors and extracellular vesicles (Fu et al.,

2016). The stromal cells in TME create an environment for tumor cells to escape immune elimination (Hui & Chen, 2015). There are studies on combined drug therapies targeting TME to overcome resistance to drug treatment (Jo et al., 2018; P. Wu et al., 2021).

3.Targeting the tumor microenvironment for therapy

TME is considered as a potential target in recent studies to prevent cancer progression and the development of anticancer therapeutic resistance. Recent investigations have shown that combination drug treatments are effective in drug-resistant cancer cells (Jin & Jin, 2020; P. Wu et al., 2021). Therefore, it is thought that targeting the TME may have the potential to reverse resistance to therapeutics in cancer (Figure 1).

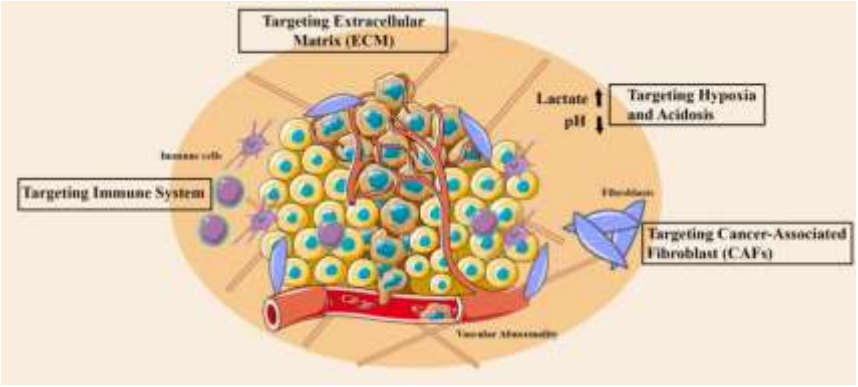

Figure 1: Strategies for targeting the TME in cancer therapy

3.1. Targeting Cancer-Associated Fibroblasts (CAFs)

CAFs are the most prominent stromal cells in the TME. CAFs induce tumor progression and provide signals for cancer cells to evade drug therapy (Kalluri & Zeisberg, 2006). Studies have shown that in the TME, a large amount of CAFs are related to poor prognosis in several cancer types including breast, lung, and pancreatic cancer (Orimo et al., 2005; Räsänen & Vaheri, 2010). Activated CAFs perform ECM remodeling through the synthesis and secretion of ECM and proteolytic enzymes. In addition, CAFs produce growth factors, interleukin 6 (IL-6), hepatocyte growth factor (HGF), fibroblast growth factor (FGF) and cytokines which acts as a role in tumorigenesis, angiogenesis and drug resistance (T. Wu & Dai, 2017). Activated CAFs produce proinflammatory mediators which stimulate neovascularization, tumor progression and angionesis through NFκB

signaling (Erez et al., 2013; Nagasaki et al., 2014). GPR77 and CD10 cell surface markers are expressed by CAFs responsible for the development of chemo-resistance in lung and breast cancer (Vaquero et al., 2018). Additionally, CAFs express fibroblast activation protein α (FAP) which is related to poor prognosis in many types of cancer including pancreatic, colon and ovarian, suggesting that promising target to overcome drug resistance for cancer treatment. CAFs induce the formation of desmoplasia which is related to progression and metastasis of several cancer types such as ovarian, oral, breast and pancreatic (Coletta & Salo, 2018; Ishii et al., 2019; Otranto et al., 2012; Yang et al., 2017). Although several studies have focused on the blockage of secretory factors, it is necessary to understand the relationship between relevant signaling pathways to overcome drug resistance (Son et al., 2017; Yu et al., 2015).

3.2. Targeting the Extracellular Matrix

The ECM is produced by cells in the TME and weaves a complex fiber network that not only serves as a structural support to cells but also act as a role in invasion, metastasis, angiogenesis and therapeutic resistance (Martino et al., 2014). The ECM is a three-dimensional structure and its composition is important for tumor prognosis. The ECM has several components, including elastin, collagen, glycoproteins, fibronectin, and proteoglycans which provide mechanical strength, hydration and pH homeostasis. Furthermore, the ECM acts as a depot for growth factors (Roma-Rodrigues et al., 2019). Cell adhesion-mediated drug resistance (CAM-DR) depends on the relation between ECM components and integrin. The altered ECM components may be associated with poor prognosis and a high recurrence rate (Sun, 2015, 2016).

Matrix metalloproteinases (MMPs), main enzymes of ECM, act as serine proteases to detach cancer cells from ECM. The upregulation of MMPs expression is associated with tumorigenesis, metastasis, poor prognosis and resistance to anticancer drugs. Additionally, transforming growth factor-β (TGF-β) promotes the interaction between ECM and tumor cells (Najafi et al., 2019).

While it is appropriate to target the ECM to overcome drug resistance, some researchers demonstrate that targeting the ECM has limited results in late-stage tumors including glioblastoma, prostate and melanoma which have different extracellular matrices (Eisele et al., 2014; Hirata & Sahai, 2017).

3.3. Targeting Hypoxia and Acidosis

Rapid tumor cell proliferation is associated with high oxygen demand, resulting in limited oxygen levels to cells called hypoxia. The partial pressure of oxygen within the TME is typically lower than in healthy tissues (Vaupel & Mayer, 2007). Under hypoxia conditions are stimulated many cellular responses coordinated by hypoxia-induced factor-1 (HIF-1) to prevent oxygen deficiency (Ziello et al., 2007). Hypoxia conditions induce cell proliferation, angiogenesis, metastasis and inhibits apoptosis by changing gene expression levels in tumor cells. Moreover, hypoxia negatively affects chemotherapy and radiotherapy by reducing the oxygen level for the generation of free radical (T. Wu & Dai, 2017).

In the TME, hypoxia and acidosis conditions significantly influence tumor response to treatment. Therefore, studies are focused on the manipulation of acidosis and hypoxia for the inhibition of cancer progression (Roma-Rodrigues et al., 2019). It has been considered that acidosis conditions protect cells from cancer therapy by altering pH and drug concentrations. A family of enzymes carbonic anhydrases promote the pH of cells and/or tissues in tumors, are important for altering acidosis conditions (Supuran, 2018).

3.4. Targeting the Immune System

Immune cells such as dendritic, macrophages, natural killer cells (NKs) and lymphocytes in TME produce cytokines, chemokines, proteolytic enzymes, and growth factors that modulate tumor progression or tumor suppression. In addition, there are many myeloid cells such as myeloid-derived suppressor cells (MDSCs), tumor-associated macrophages (TAMs) and tumor-associated neutrophils (TANs) which induce therapeutic resistance (Son et al., 2017).

Tumor-associated MDSCs act as a role in therapeutic resistance via blockage of immune cells' activation and polarization of myeloid cells (Gabrilovich et al., 2012; Ozao-Choy et al., 2009).

The immune system impacts the tumor response to several treatment strategies. For this reason, multiple strategies are being developed to target the immune system including block the macrophages recruitment to tumor sites, and differentiation of macrophages to myeloid cells TAMs, improve anti-tumor activation of immune cells (Roma-Rodrigues et al., 2019; Tsai et al., 2014). TAMs produce several proteins such as MMPs, plasmin, interleukin (IL)-8, FGF, and vascular endothelial growth factor (VEGF) that can induce

angiogenesis and TAMs-mediated therapeutic resistance can be stimulated by angiogenesis and immunosuppressive factors (Table 1) (Wang et al., 2015).

However, combining macrophage-targeted strategies with other traditional therapeutic approaches is thought to show more effective results. Besides the macrophage-targeted strategies, MDSC-targeted approaches are important for tumor response to drug treatments. These MDSC-targeted strategies include the blockage of MDSC expansion and immunosuppression potential and stimulation of MDSC differentiation into mature cells. Although targeting MDSCs alone has failed to improve cancer treatment, it may have the potential to elevate the efficacy of immunotherapies against cancer. In addition, it has been reported that immune checkpoint molecules in NKs are the potential immunotherapeutic target. Chemotherapeutic drugs, doxorubicin and anthracyclines, are able to trigger immunogenic cell death. Targeting critical pathways for the improvement of therapeutic efficacy by inducing antitumor immune responses has great potential to improve drug response (Kroemer et al., 2013; T. Wu & Dai, 2017).

Table 1: List of factors affecting drug resistance

Cells	Proteins/miRNAs	Resistance to
TAMs	MMPs	Anti-angiogentic therapy
	PGE2	Immunotherapy
	IDO	Immunotherapy
TANs	OSM	Anti-angiogentic therapy
	Bv8	Anti-angiogentic therapy
MDSCs	IL-10	Immunotherapy, Sunitinib
CAFs	sFRP2	Vemurafenib
	miRNA-21	Paclitaxel

CONCLUSION

Cancer is a complex disease and there is more than one factor in the formation of the disease. TME acts as a significant role in maintaining tumor heterogeneity and disease progression. Therapeutic resistance remains a major problem in cancer therapy. TME is effective in the response of tumor cells to drug treatment. An understanding of TME is essential for the development of new strategies that will allow new therapeutic approaches. The development of drug regimens that can target TME may offer a solution to prevent therapeutic resistance and improve outcomes for patients.

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CHAPTER 8

MICRORNA: A MAJOR PLAYER REGULATING EMT IN COLORECTAL CANCER

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INTRODUCTION

Colorectal cancer (CRC) accounts for 10% of cancer-related deaths all over the world and ranks second in women and third in men among cancer types (Dekker, Tanis, Vleugels, Kasi, & Wallace, 2019). Although great progress has been made in clinical diagnosis and treatment with emerging technologies in patients with CRC, the development of metastases still affects the prognosis of patients and reduces the survival rate. Surgery and chemotherapy are the first options for treatment strategies for these patients. Early detection has also resulted in an improvement in the 5-year survival rate in CRC patients. However, the development of metastases in CRC makes early diagnosis difficult and thus negatively affects the 5-year survival rate (Xie, Chen, & Fang, 2020). Although the 5-year survival rate in CRC patients is 90% in the early stage, this rate drops to 5% in distant metastases. Therefore, metastasis is thought to be the main cause of death in CRC patients (Park et al., 2017). The removal of tumor cells from the primary tumor microenvironment, their spread to various organs and colonization in the secondary region cause the formation of metastasis. Biological processes such as angiogenesis, escape from apoptosis, proliferation, invasion, and migration are essential steps for metastasis formation and colonization (Siddiqui, Al-Ghafari, Choudhry, & Al Doghaither, 2019). Although metastasis is the primary cause of cancer-related death, the timing and molecular markers of metastasis have not been fully defined. Hence, there is an urgent need to understand the mechanism of metastasis in CRC.

The epithelial-mesenchymal transition (EMT) is a differentiation process of cells in which epithelial cells lose their characteristic features such as polarity, cell-cell adhesion and immobile and transform to mesenchymal cells that have migratory and invasive properties. These primary tumor cells form a new tumor microenvironment by accumulating in different tissues and organs through the blood circulation (Fares, Fares, Khachfe, Salhab, & Fares, 2020; O'Brien et al., 2020). EMT mechanism, which is a natural process in the development of living things, can lead to metastasis when it occurs in tumor cells. In the EMT process, the transition from epithelial phenotype to mesenchymal phenotype occurs with variations in expression of certain genes (O'Brien et al., 2020). Some factors such as transcription and epigenetic mechanisms are thought to be responsible for this pathological condition. Many studies have demonstrated EMT is strongly associated with metastasis (Guangyuan et al., 2017). Therefore, knowing the molecular mechanism of

EMT and identifying the factors affecting this mechanism can significantly increase the survival chances of CRC patients.

MicroRNAs (miRNAs) are a small class of non-coding RNAs consisting of 18-20 nucleotides in length (Zhu et al., 2021). miRNAs post-transcriptionally regulate gene expression by inhibiting or degrading target mRNA. Emerging evidence has revealed a strong correlation between the level of miRNA expression and tumorigenesis. Abnormal expression of miRNAs is a critical point in the proliferation, invasion and metastasis of tumor cells (Du, Yu, Chen, & Cai, 2021; Florian et al., 2020; Zhu et al., 2021) . miRNAs may have an oncogenic or tumor suppressive function in regulating pathways leading to tumorigenesis (Thomas, Ohtsuka, Pichler, & Ling, 2015). Therefore, many recent researchers have focused on the potential use of miRNAs as therapeutic target and biomarker (Balacescu et al., 2018). In present review, we will outline EMT-associated miRNAs and their target genes, their role in inhibition or promotion of EMT, and their potential as therapeutic targets in colorectal cancer in light of recent developments

1. EMT-METASTASIS IN COLORECTAL CANCER

Metastasis is a multiple process in which primary tumors migrate from their microenvironment, accumulate in different tissues and organs, and form secondary tumors. Metastatic tumors (secondary tumors) can escape from the immune system and settle in secondary areas through the blood circulation (Fares et al., 2020). These tumors have a more aggressive profile than primary tumors and are the primary cause of mortality in approximately 90% of patients with CRC. Distant organs such as the liver, lung, and lymph nodes are generally the secondary tumor microenvironment for them in CRC (Xiao et al., 2020). While EMT is a biological process that occur under normal physiological conditions such as embryogenesis and wound healing, it is ab-normally activated in pathological conditions such as cancer (Skrypek, Goossens, De Smedt, Vandamme, & Berx, 2017). A variety of transcriptional growth factors con-tribute to the regulation of the EMT process. Various transcriptional growth factors contribute to the regulation of the EMT process. Transcription factors induce EMT by suppressing epithelial markers and activating mesenchymal markers. The effective-ness of these factors is also closely linked to the signaling pathways associated with EMT (D. H. Kim et al., 2018).

1.1. Molecular Markers: E-Cadherin/ N-Cadherin/ Vimentin

Certain molecular markers such as E-cadherin, N-cadherin and vimentin are involved in the induction of metastatic events. E-cadherin is a cell protein characterized by an epithelial phenotype, while N-cadherin and vimentin are characterized by a mesenchymal phenotype. Down-expression of E-cadherin and up-expression of N-cadherin and vimentin are important indicators in the activation of the EMT program. In particular, E-cadherin is a key protein in the migration and invasion of tumor cells. E-cadherin is encoded by the CD1 gene and its down-expression leads to loosening of intercellular tight junctions and increases cellular motility (Bruner & Derksen, 2018). E-cadherin inactivation promotes metastasis in tumor cells. For example, overexpression of miR-10b caused a decrease in E-cadherin protein level in patients with CRC, resulting in tumor invasion and metastasis (Abdelmaksoud-Dammak et al., 2017). On the other hand, miR-200a-3p, which acts as a tumor suppressor, inhibited the expression of FOXA1, YAP1 and N-Cadherin in colon cancer cells and increased the expression of E-Cadherin. The miR-200 family (miR-200a, -200b, -200c, -141 and -429) acts as an EMT and metastasis inhibitor in CRC (Di et al., 2020).

1.2. Transcription Factors

Many genes encoding transcription factors are now known to contribute to the EMT process. These transcription factors are involved in suppression of the epithelial phenotype and activation of the mesenchymal phenotype via hyper-methylation and histone de-acetylation. The most well-recognized transcription factors are SNAI (SNAI1/Snail and SNAI2/Slug), ZEB (ZEB1 and ZEB2), and TWIST (TWIST1 and TWIST2) nuclear proteins (Skrypek et al., 2017). The SNAIL family contains three members as SNAIL (SNAI1), SLUG (SNAI2), and SMUG (SNAI3), and is a zinc finger transcription factor that generally functions as a gene suppressor. SNAIL binds to the promoter region of E-cadherin and suppresses its expression in colon cancer. SNAIL immunoreactivity has been detected in most tumor cells that have undergone EMT in colorectal cancer (Brzozowa et al., 2015). TWIST (TWIST1-TWIST2) induces EMT by binding to the E-box site on the E-cadherin promoter and repressing E-cadherin transcription. Therefore, overexpression of TWIST in tumor cells is associated with a poor prognosis (Deng et al., 2016). The ZEB transcription factor contains two members, ZEB1 and ZEB2, and can repress or activate transcription by binding to regulatory gene sequences. These transcription factors suppress the expression

of the gene (CD1 gene) encoding E-cadherin and thus contribute to the mesenchymal phenotype. ZEB1 expression was found to be significantly associated with overall survival of patients with CRC, and overexpression of ZEB1 correlated with liver metastasis (G. J. Zhang, Zhou, Tian, Liu, & Xia, 2013).

1.3. Signaling Pathways

Molecular signaling pathways are systems that regulate the proliferation, differentiation and functions of healthy cells. Genetic changes in signaling pathways can lead to dysfunctions. Thus, the characteristic features of cancer cells, such as uncontrolled proliferation and reduced apoptosis, emerge in the cells (Cheng, Xu, Chen, Zhao, & Wang, 2019; Farooqi, de la Roche, Djamgoz, & Siddik, 2019). Wingless-type (Wnt) signaling pathway is one of the important signaling pathways in CRC. Wnt signaling pathway is divided into two groups as canonical and non-canonical pathways. However, the canonical pathway (also referred to as the Wnt/β-catenin signaling pathway) is more associated with CRC (Cheng et al., 2019). Inactivating mutations in Adenomatous polyposis coli (APC), a tumor suppressor gene, are thought to initiate tumorigenesis in all colorectal adenomas and carcinomas, resulting in activation of the Wnt/β-catenin signaling pathway (Schatoff, Leach, & Dow, 2017). Wnt hyperactivation is usually an oncogenic marker in CRC. Increased activation of Wnt signaling raises the level of Snail that suppresses E-cadherin and regulates EMT. In addition, Wnt signaling inhibits GSK3β activity, leading to increased Slug levels. As a result, Snail and Slug accumulation suppress E-cadherin transcription and induce EMT (N. Zhang et al., 2021). The Notch signaling pathway has a critical role in pathogenesis of CRC. This pathway includes 4 Notch receptors (Notch 1-4) and 2 different types of ligands ((delta-like; DLL1, DLL3, and DLL4 and jagged-like; Jagged-1 and Jagged-2). Both deregulation and overactivity in the NOTCH pathway promote CRC development. Upregulation of Jagged-1 mediated by β-catenin increases the expression level of Notch-1 in CRC. Especially, aberrant activation of Notch1 has been associated with aggressive CRC and EMT (Vinson, George, Fender, Bertrand, & Sigounas, 2016). Apart from these signaling pathways, it is known that various molecular pathways play an active role in the functioning of CRC such as NF-κB signaling pathway (Soleimani et al., 2020), PI3K/Akt signaling pathway (Pan et al., 2020) and p53-signaling pathway (Slattery et al., 2019).

2. miRNAs AS THERAPEUTIC TARGETS IN EMT

Considering the studies conducted in recent years, changes in miRNA expression profiles have been proven to be associated with many diseases, including cancer (Shi, Li, Yang, & Li, 2019; Si, Shen, Zheng, & Fan, 2019). Advancing molecular biological techniques (in vivo and in vitro) and especially the bioinformatics applications (in silico) have enabled the elucidation of the structure and functions of miRNAs, as well as the target genes of miRNAs, and the biological pathways in which they interact (K. S. Kim et al., 2019; Shuo Li, Liu, Fang, Wang, & Fei, 2019; Mao et al., 2019). Most of the researches on miRNA and metastasis showed that miRNAs regulated EMT by inhibiting or activating their target genes and signaling pathways in CRC. miRNAs function as a tumor suppressor or oncogene depending on their target genes and affects the EMT process. These regulators can interact directly or indirectly with molecular markers involved in the EMT process, such as E cadherin and N-cadherin. Therefore, miRNAs may contribute to the diagnosis and treatment of the disease and may be a potential therapeutic target. Research on the roles of miRNAs in the EMT process and their potential as therapeutic targets is summarized below.

2.1. EMT-inhibiting miRNAs

In recent studies, many miRNAs have been identified as EMT inhibitor in colorectal cancer. The miR-200 family members (miR-200a, miR-200b, miR-200c, miR-141 and miR-429) are well-known EMT inhibitor among the identified miRNAs. The miR-200 family members inhibit the induction of EMT by suppressing expression of ZEB1 and ZEB2 transcription factors (O'Brien et al., 2018). In addition, miR-708 (Sun et al., 2019) and miR-551b (K. S. Kim et al., 2019) are among the miRNAs that deregulate EMT by targeting ZEB1. In a study, it was shown that up-regulation of miR-4429 inhibited the EMT process in CRC. miR-4429 inactivates SMAD family member 3 (SMAD3) by targeting the Forkhead box protein M1 (FOXM1) gene and thus inhibits the EMT process (H. Li, Liang, Zhang, Shui, & Zhang, 2021). Another EMT inhibitor is miR-29b. While the expression level of miR-29b increases, the expression level of its target gene ETS translocation variant 4 (ETV4) decreases in colon cancer cells. The high expression level of miR-29b leads to blockade of the ERK signaling pathway, resulting in suppression of EMT. (Leng et al., 2021). In a similar study, downregulation of miR‑142‑3p suppressed cell proliferation, proliferation, and mesenchymal phenotype in CRC via the RAC1‑ERK signaling pathway (XIE et al., 2021). Activation of

the NF‑κB signaling pathway promotes metastasis in CRC cells by regulating the expression of EMT markers (E-cadherin, N-cadherin). miR-129 inactivates activation of the NF‑κB signaling pathway via targeting the SRY-Box Transcription Factor 4 (SOX4) gene (Chen et al., 2021). Wen et al. investigated the biological role and clinical value of miR-944 in CRC. miR-944 has been reported to function as a tumor suppressor in CRC tissues and cells. In addition, MET Transcriptional Regulator (MACC1) gene expression was found to be suppressed by miR-944. MACC1 is a gene associated with metastasis and promotes the ability of tumor cells to migrate and spread (Wen et al., 2017). However, overexpression of miR-944 inhibits expression of GATA Binding Protein 6 (GATA6), β-catenin protein and ZEB1 as well as EMT-related proteins (Tang et al., 2021). A previous study showed that miR-137-3p regulates EMT process in CRC cells by targeting Lysine Demethylase 1A (KDM1A) (Ding, Zhang, Feng, Tang, & Zhou, 2021).

In addition to the miRNAs mentioned above, many miRNAs have been found that function to suppress the EMT process including miR-148a-3p (Hu et al., 2021), miR-597-5p (Shuo Li et al., 2019), miR-4284 (Miao, Li, Zhang, & Wang, 2021) and miR-143-5p(X. Li, Zhang, Cui, Wu, & Wang, 2021). These findings indicated that miRNAs could be a potential therapeutic target with their role in suppressing the EMT process in CRC.

2.2. EMT-Promoting miRNAs

In addition to the miRNAs mentioned above, various miRNAs act as activators in the EMT process. For example, miR-410-3p is an oncogenic miRNA in CRC cells and directly targets the zinc finger CCHC-type containing 10 (ZCCHC10) gene. The upregulation of ZCCHC10 is induces cell migration and proliferation. miR-410-3p activates the EMT process by repressing the ZCCHC10 target gene via NF-κB signaling pathway (Ma, Shi, & Wan, 2021) . In addition, the low expression level of miR-410 caused a decrease in β-catenin and phosphorylated glycogen synthase kinase‑3β (p-GSK-3β) protein levels and promoted the dickkopf‑related protein 1 (DKK-1) expression. As a result, upregulation of miR-410 led to downregulation of the DKK1 gene and activates the EMT process via the Wnt/β‑catenin signaling pathway in CRC cells (W. Wang, He, Rui, & Xu, 2019). This pathway is dysregulated in a large proportion of patients with colorectal cancer, contributing to the development of CRC. It promotes the expression of Snails and Twist inhibiting E cadherin. In this signaling pathway, the development

of EMT occurs by binding of TCF/LEF transcription factors to nuclear β-catenin. Zhou et al demonstrated that Ras association domain family member 6 (RASSF6) is a tumor suppressor gene and inhibits cell migration, proliferation and EMT progression through the WNT signaling pathway in CRC cells. miR-496 activates the WNT signaling pathway by suppressing the expression of RASSF6, thereby promoting the EMT process in the CRC (H. Wang et al., 2020). Similarly, miR-576-5p induces the Wnt/β-catenin signaling pathway by targeting wingless-type MMTV integration site family member 5A (Wnt5a) and consequently acts as an EMT promoting factor (LUO et al., 2021). Moreover, WNT5A and PTEN are suppressed by miR-26, enhancing CRC metastasis (Fan et al., 2018).

In a research relating to CRC cell migration, invasion and EMT, upregulation of miR-645 has been shown to induce EMT by targeting the 3'-UTR region of ephrin-A5 (EFNA5) mRNA (Shuai Li et al., 2020). SRY-related HMG-box 30 (SOX30) is a tumor suppressor gene targeted by miR-645. An inverse relationship was found between mir-645 expression and SOX30 expression, and it was reported to have a moderate effect on SOX30 in CRC cells. Downregulation of SOX30 promotes EMT-associated metastasis (Guo et al., 2017). A previous study showed that increased expression level of miR-103 is associated with poor prognosis and worse survival in patients with CRC. Furthermore, miR-103 directly targeted the LATS2, a tumor suppressor gene, and sup-pressed its expression. As a result, overexpression of miR-103 enhanced tumor development and metastasis in CRC tissues (Zheng et al., 2016).

Although most of the miRNAs in CRC inhibit the EMT process via signaling pathways and target genes, there are miRNAs stimulating this process. As mentioned above, miRNAs promoting EMT silence tumor suppressor pathways or genes, thereby leading to metastasis in CRC cells.

CONCLUSIONS

Considering the biological process of metastasis and EMT in colorectal cancer, it is possible to target EMT to develop new therapeutic strategies. To this end, EMT-related signaling pathways, miRNAs, and certain genes may serve as potential therapeutic targets to suppress the EMT process and revert EMT to MET for the treatment of colorectal cancer. Many studies conducted in recent years have effectively demonstrated the roles of miRNAs in tumor development and metastasis and contributed to the elucidation of cancer pathogenesis. In this review, we emphasized the EMT process and

EMT-related miRNAs and their mechanisms of action for CRC in the light of current studies. EMT-associated miRNAs mostly inhibit the EMT process by targeting specific genes via signaling pathways. Therefore, miRNAs are considered to have a therapeutic potential in colorectal cancer. However, further studies are needed before miRNAs can be used as therapeutic targets in clinical applications.

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CHAPTER 9
BORON-CONTAINING COMPOUNDS: POTENTIAL APPLICATIONS IN CANCER THERAPY

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INTRODUCTION

Boron (B) – atomic number 5- is a naturally occurring semi-metal element and the only electron-deficient nonmetallic element in nature (Das et al., 2013). B has a high affinity, especially for oxygen which is an electronegative atom. Because of that, Borates have strong boron–oxygen bonds. Boron is an abundant element in the oceans, sedimentary rocks, coal, shale, and some soils. B is an essential micronutrient for plants found in all foods, such as fruits, vegetables, and nuts (Howe, 1998).

1. Boron in life

According to previous studies, it was essential for plant metabolism (Sommer & Lipman, 1926; Warington, 1923), while B was not considered a useful or necessary element for animals and humans. However, in a 1981 study, Boron was beneficial against arthritic syndromes, while in another study, it was observed that it exacerbated gross bone abnormalities in chicks in case of deprivation (Hunt & Nielsen, 1982; Newnham, 1982). B is necessary to complete the life cycle of all organisms because B deprivation causes impaired growth, development, or maturation (Fort et al., 2002; Rowe & Eckhert, 1999). The amount of Boron is 3-20 mg in the body, with different volumes in different tissue in the form of boric acid or borates. The highest boron content is found in bone and hair, the tissue containing creatine (Devirian & Volpe, 2003). Apart from plants, B plays a vital role in animal and human metabolism, such as hormone production, psychological activities, wound healing, and bone development and maintenance (Nielsen & Meacham, 2011). Besides, the blocking capacity of Boron in over adiposity has been proven (Dogan et al., 2017).

2. Usage of boron

B has unique properties and is preferred as an active compound in various industries such as agriculture, chemistry, material science, energy research, electronics, and life science (Das et al., 2013). Boron-containing compounds have strong antimicrobial effects and are used as bactericide, fungicide, and insecticide (Klotz et al., 1994). B is also used to produce heat-resistant glass (borosilicate glass) and ceramics (Smedskjaer et al., 2011). Because of their antimicrobial effects, Boron compounds are preferred for household laundry and cleaning products (Richold, 1998). Another structural feature of B is that it is a metalloid. Because of this feature, it is used in the computer and electronics industries. Besides, B is useful in the optical industry for producing optical and nuclear probes (Lewis, 1986). It has been

proven that Boron-containing compounds have the potential anti-cancer effects against several cancer types (Figure 1).

3. Boron for cancer therapy

Due to the biological properties of B, its importance is increasing by the day. B was used as a chemotherapeutic agent in cancer studies against lung cancer (Devirian & Volpe, 2003), breast cancer (Scorei et al., 2008), prostate cancer (Cui et al., 2004), colon cancer (Dick & Fleming, 2010), neuroblastoma (Ciofani et al., 2010), endometrial cancer (Tülüce et al., 2017) ovarian cancer (Psurski et al., 2019) and cervical cancer (Korkmaz et al., 2007). B causes apoptosis through the inhibition of critical pathways. Nowadays, Boron-containing compounds are preferred for treating high malignancy and inoperable cancers. Boron-based therapies are being developed and exhibit different mechanisms of action. Types of cancer exposed to B reveal different biochemical and molecular responses (Cebeci et al., 2022). Boron displays inhibitory effects on various enzymatic activities such as serine proteases, NAD-dehydrogenases, and mRNA splicing. Because of the chemical structure of B, it may bind to important cellular molecules such as proteosomes, proteases, and peptidases and may inhibit their functions (Acerbo & Miller, 2009; Barranco & Eckhert, 2004). Besides, B competitively binds to the receptors and triggers apoptosis. B has strong chemopreventive properties. A study on smoking women showed B significantly decreased lung cancer risk. In addition, dietary usage of B caused a decrease in prostate and cervical cancer rates (I Scorei & Popa, 2010).

Figure 1: Boron has potential anti-cancer effects against several cancer types.

More mechanisms have been discovered about the potential anti-cancer effects of boron-containing compounds. For example, their administration may regulate the release of hormones that cause cancer progress. Besides, they can induce inhibition of proliferative gene expressions while triggering the anti-cancer mechanisms in the cancer cells (Gallardo-Williams et al., 2004). A strategy for cancer therapy is the usage of chemicals that have hormone-like biological effects. In the patient's body, they may influence the synthesis, secretion, transport, metabolism, binding, action, and catabolism of natural hormones. Besides, the chemicals with anti-estrogenic, estrogenic, anti-androgenic, or androgenic effects trigger functional abnormalities of the immunity system, nerve system, and reproductive system by breaking up the endocrine system, disrupting the molecular signaling interaction. In a recent study, boric acid displayed an estrogen-like effect against the human breast cancer cell line *in vivo* (Gallardo-Williams et al., 2004).

Reactive oxygen species are overexpressed within the tumor microenvironment, and in another cancer therapy strategy, anti-cancer agents target there. Boronic acid (and ester) prodrugs are a type of this strategy. Anti-cancer boron-containing prodrugs have been commonly used with tumor targeting properties leading to the release of the parent drug (Maslah et al., 2020).

4. Boron in drug development

Since B was initially thought to be toxic, it was not preferred in drug development strategies. However, B is in the same line of the periodic table as carbon and nitrogen that make up the skeleton of life and is one of the important candidates that should be preferred in designs with bioactivity (Fernandes et al., 2019). In recent studies, it has been shown that B toxicity is related to dose and time, and it is beneficial for the creation of biologically active products in drug design due to its unique chemical properties. It has been reported that boronic acid-containing compounds are biologically active, and in recent years, they have been commonly used against infectious diseases and cancer as new therapeutic agents (Fernandes et al., 2019). Boronic acid-containing compounds exhibit anti-cancer activity not only as proteasome inhibitors but also as acting on different targets. According to a study with a fulvestrant derivative containing Boron, this compound is a promising agent in cancer treatment. It is a selective estrogen receptor down-regulator (SERD) and competitively binds to Estrogen receptor alpha (ERα) and downregulates it in tamoxifen-sensitive and tamoxifen-resistant breast cancer cells (Robertson, 2002).

The first time usage of a boronic acid-containing compound for cancer treatment is Bortezomib (PS-341), trade name Velcade (Kane et al., 2003). It was developed against multiple myeloma and mantle cell lymphoma, acting as a proteasome inhibitor. Proteasome - sophisticated enzyme complex- works as a protein inhibitor and degrades ubiquitin-labeled protein (Gollob & Sciambi, 2007; San Miguel et al., 2006). Moreover, this drug has been used alone or in combination for the treatment of carcinomas, including breast, lung, colon, prostate, and pancreas (Das et al., 2013). The underlying molecular mechanism of the anti-cancer activity of Brotezomib is the inhibition of nuclear factor-kB (Sartore-Bianchi et al., 2007). According to *in vitro* study, it has triggered the cell cycle blockade and apoptosis. Besides, tumor growth is inhibited by Brotezomib in *in vivo study* (Megli et al., 2005).

Talabostat (PT100) is a methanesulfonate salt of l-valinyl-l-boroproline and is able to block Dipeptidyl peptidase 4 (DPP4), including tumor-associated fibroblast activation protein alpha (FAP). The immune response is crucial to fighting cancer in the body. Talabostat produces specific cytokines and chemokines as well as modulates the activity of immune cells (Cunningham, 2007).

Benzoxaboroles are biologically active compounds with better hydrolytic resistance and are more soluble in water than boronic acids because

of their chemical properties (Adamczyk-Woźniak et al., 2009). In the beginning, they have been used in organic synthesis, glycopeptide recognition, and supramolecular chemistry (Nocentini et al., 2018). However, they have become more interesting to scientists after the remarkable biological activities exhibited by some of their varieties. A benzoxaborole shown potential anti-cancer agent character against SKOV3 (ovarian carcinoma), MDA-MB231 (breast cancer), and HCT116 (colon colorectal carcinoma). In a study with a different type of benzoxaborole, it displayed both anti-proliferative and pro-apoptotic behavior by blocking the cell cycle in the A2780 ovarian cancer cell line (Psurski et al., 2019).

In a recent study, synthesized non-peptide boronic acid derivatives were used against various cancer cell lines such as HL60, RPMI 8226, A549, MDA-MB-231, and A2780 and displayed high anti-proliferative activity. IC50 values were calculated at minimal concentrations such as nanomoles. Although different results were obtained as cells and agents dependent, non-peptide boronic acid derivatives showed summarily anti-cancer activities (Ge et al., 2017).

Usage of B with different aims is interesting for cancer therapy, such as Boron nitride nanotubes (BNNTs) which is a boron-containing nanomolecule. They have unique physical and chemical properties including chemical inertness, high thermal stability, and optimal resistance to oxidation. These nanomolecules can be used as a nanovector for various applications, including cancer therapy. Actually, studying with a nanomolecule has some difficulties, however BNNTs' bioapplicability is high in cells and organisms. In cancer treatment, one of the important problem is adverse effects of anti-cancer compounds against healthy cells. BNNTs may also help in this point and provide targeted therapy (Ferreira et al., 2015).

Boron, especially 10B isotope, has unique chemical properties. It is able to absorb neutron to initiate a nuclear reaction with the release of energetic particles such as a- and Li-particles. Boron-based therapeutic nanomaterials, including boron neutron capture therapy (BNCT) are used in radiation therapy against malignant brain tumors and other cancers. BNCT is used in binary treatment and selectively transported into tumor cells. Nanomaterials, also called nanovectors, are preferred as a drug carrier in various pharmaceutical applications, including cancer therapy with advances in nanobiotechnology. At this point, boron-containing nanomolecules differ from classical small agents, which are not boron-containing. Because of its anti-cancer potential, targeted delivery of BNCT into the cancer cell is critical

for more effective therapy. Due to obtaining successful results, various boron-containig nanomolecules with different nanoscales have been produced and tested such as boron-enriched magnetic nanoparticles (MNPs), nanoparticles, nanotubes and liposomes (Yinghuai & Hosmane, 2013)

5. Boron deprivation and overconsumption in the body

B deprivation causes various comorbidities, including lipid, mineral, bone, energy metabolism, and endocrine function. Boron level in the body is also related to brain function and orthopedic diseases (Nielsen & Meacham, 2011). B deprivation during embryonic development has led to high mortality and malformations in frogs and zebrafish. At the same time, overconsumption of B resulted in severe defects in early embryonic development and raised fetus mortality (Nielsen & Meacham, 2011). Overconsumption of Boron may cause harmful consequences. According to previous studies, raised Boron intake has caused reproductive and developmental failure. Dietary intake of 26 mg boron/kg B caused infertility, while 248 mg boric acid/kg led to developmental disorders (Devirian & Volpe, 2003; Fail et al., 1998). Accumulating B in muscle, bone, brain, liver, kidney, hypothalamus, blood, lymph node, prostate, and adrenal tissue because of overuse has led to inflammation, unhealthy weight gain, edema, irregular metabolism, and alopecia (Eren et al., 2012). Moreover, it is reported that exposure to high-dose B decreased the crucial molecules in the body, such as glucose, lactic acid, glycogen, and ATP.

CONCLUSION

The atomic number of B is 5, and it is a naturally occurring semi-metal element. For all organism, including plants, animals, and humans, B is essential and play a critical role in metabolism. Because of its unique chemical and physical properties, Boron-containing compounds are preferred for producing new agents against various diseases such as cancer. B shows anti-cancer effects against lung, breast, prostate, colon, neuroblastoma, endometrial, ovarian cancer, and cervical cancers. Besides, B can be used for targeted cancer therapy as a nanovector. The effect of boron varies depending on concentration and dose. Moreover, the deprivation and overconsumption of boron cause adverse effects on the body. For this reason, it is critical to adjust the application dose and time in boron studies.

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CHAPTER 10

VITAMIN E AND ALZHEIMER'S DISEASE

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1. Vitamin E

1.1. The History of Vitamin E

Vitamin E was discovered, in 1922, by Herbert M. Evans and K. Scott Bishop as an essential dietary component for the normal course of reproductive functions. Evans et al. called this molecule as “Factor X”, which they concluded to cause sterility in rats in its deficiency (Evans et al., 1922). By 1924, it was determined that this factor was a vitamin, and factor X was called “vitamin E” since A, B, C, and D were used in the nomenclature of vitamins until that date (Sure, 1924). Vitamin E, also known as tocochromanols, are amphipathic compounds consisting of a polar chromanol ring and a lipophilic isoprenoid chain. The main vitamins E are tocopherols and tocotrienols, the difference between them is due to the number of double bonds in their side chains. Both tocopherols and tocotrienols each have four subgroups (α-, β-, γ-, and δ-), and the position and number of methyl groups in the chromanol ring led to their formation (Strobbe et al., 2018; Fig. 1).

Tocochromanol type	R^1	R^2
α-Tocopherol	CH_3	CH_3
α-Tocotrienol	CH_3	CH_3
β-Tocopherol	CH_3	H
β-Tocotrienol	CH_3	H
γ-Tocopherol	H	CH_3
γ-Tocotrienol	H	CH_3
δ-Tocopherol	H	H
δ-Tocotrienol	H	H

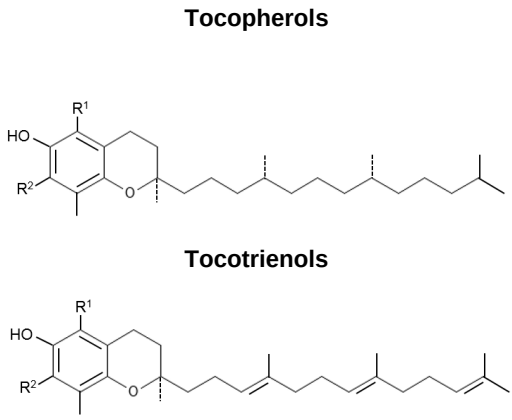

Figure 1: Chemical structures of tocochromanols.

First, α-tocopherol was discovered by concentrating on wheat germ in 1936, and the discovery of other tocopherols was completed by 1950 (Evans et al., 1936; Kundu et al., 2021). Because of these positive effects on the reproductive system, the ancient Greek words "Tocos" meaning "birth" and "Phero" meaning "to bring" were combined and called Tocopherol (Packer et al., 2002). Vitamin E is an essential molecule for humans. The main plant sources rich in vitamin E are; wheat germ oil, sunflower, safflower and

soybean oil, sunflower seeds, almonds, peanuts, beetroot, kale, spinach, red bell pepper, asparagus, mango, and avocado (Abushita et al., 1997; Aksoz et al., 2020).

1.2. Pharmacokinetics of Vitamin E

The absorption of vitamin E by the organism varies depending on dietary fats, bile salts, and pancreatic enzymes. After oral intake, vitamin E joins the chylomicrons in the intestinal epithelium and reaches the circulation through the lymphatic system. Chylomicrons advancing in the systemic circulation are hydrolysed by lipoprotein lipase (LPL) enzyme found in the surface epithelium of various organs and tissues, and the lipids and vitamin E they contain are transferred to the tissues (Traber et al., 1985). While 70% of vitamin E metabolism occurred in the liver, the rest occurred in the intestine and kidney, which has CYP4F2 activity (Bardowell et al., 2012). Different forms of vitamin E are metabolized by different CYPs, eg α-tocopherol is primarily metabolised by CYP3A4 (Birringer et al., 2001) and γ-tocopherol is metabolised by CYP4F2 (Sontag et al., 2002).

Two main metabolites carboxyethyl hydroxychromanol (CEHC) and 13'-caroboxychromanol (13′-COOH) are formed in the liver by cytochrome P450 (CYP) mediated ω oxidation and subsequent β oxidation. While CEHCs are excreted through plasma and urine, 13′-COOHs are excreted in faeces via biliary secretion (Jiang, 2022). Moreover, each form of vitamin E is metabolized at different rates. For instance; since α-tocopherol is mostly incorporated into the VLDL structure via its specific transporter, α-tocopherol transport protein (α-TTP), a small amount of α-CEHC metabolites (only <1% of total α-tocopherol) is formed (Leonard et al., 2005); in contrast, γ-tocopherol is largely metabolized to γ-CEHC (Galli et al., 2003). Circulating VLDL is catabolized by LPL and converted to forms VLDL residues (IDL) which are taken up by the liver via the apoE receptor, and LDL, which also enables the transport of vitamin E to peripheral tissues. Besides during this hydrolysis process, VLDL transfers the vitamin E to HDL as well as to the tissues (Kayden et al., 1993). It has been claimed that there is probably a similar transfer also between vascular endothelium and blood cells (erythrocytes and leukocytes), like vitamin E transfer between circulating lipoproteins (Traber et al., 1992). It has been reported that tocopherols reach the peak plasma concentration (C_{max}) in approximately 6 hours (T_{max}) following oral administration with meals, while tocotrienols reach the peak concentration after 3 to 4 hours. In the same study, it was shown that α-

tocopherol reached a much higher plasma peak concentration (C_{max}) than tocotrienols (1.82–2.92 μM and 0.89–1.92 μM, respectively) (Qureshi et al., 2015; Qureshi et al., 2016). Moreover, in a previous investigation, much lower plasma concentrations were obtained following intramuscular and intraperitoneal tocotrienol administrations compared to oral administration in rats. Chylomicrons formed after oral administration contribute to both increased absorption and decreased elimination of tocotrienols (Yap et al., 2003). When different administration routes are compared, T_{max} of tocotrienols in parenteral use is reached 1 hour after intramuscular injection (Satyamitra et al., 2012), while this time extends up to 3-4 hours in oral administration (Yap et al., 2001). The elimination half-life ($t_{1/2}$) of α-tocopherol in humans is approximately 20 hours (Julianto et al., 2000); however, $t_{1/2}$ for different isomers of tocotrienol has been reported to vary between 2.3 and 4.4 hours. Due to the short half-life of tocotrienols, it is recommended to be used twice a day to maintain bioactive levels in plasma (Mahipal et al., 2016).

1.3. Effects of Vitamin E

The most well-known effect of Vitamin E is to prevent lipid peroxidation thanks to its antioxidant activity. Vitamin E gives the hydrogen in the hydroxyl group in the chromodal ring to free radicals and ensures they reach a stable state. Oxidized vitamin E becomes stable again through various antioxidant mechanisms, especially vitamin C and thiol antioxidants (glutathione and lipoic acid) (Packer et al., 2001; Engin, 2009). However, some forms of vitamin E and its metabolites show anti-inflammatory effects by inhibiting eicosanoids via COX-2/5-LOX, suppressing pro-inflammatory NF-κB and JAK-STAT6 signalling pathways, or blocking ionophore-mediated Ca^{++} influx (Jiang, 2014).

Vitamin E regulates the expression of various gene locations by binding to some nuclear receptors. Pregnane X receptor (PXR) and the peroxisome proliferator-activated receptor (PPAR) are two main groups of nuclear receptors modulated by vitamin E. PXR plays an important role in the biotransformation of toxic foreign compounds and drugs, especially by regulating various xenobiotic pathways, including CYP enzymes (Traber, 2004; Moore et al., 2006). PPARs are receptors associated with gene regions related to lipid and glucose metabolism, cell proliferation, and apoptosis. It is thought that vitamin E provides anticancer and hypocholesterolemic effects

through these receptors (Michalik et al., 2006; Traber et al., 2011; Abraham et al., 2019).

Because of these properties, vitamin E supplements have been used alone or in combination with other antioxidants such as vitamin C in many diseases such as Alzheimer's disease, Parkinson's disease, Metabolic Syndrome, and Rheumatoid Arthritis in which oxidative damage or inflammation plays an important role in the pathogenesis (Zaffarin et al., 2020; Azzi, 2021).

1.4. Vitamin E and the Central Nervous System

Vitamin E is an essential vitamin for human and animal species. Especially α-tocopherol deficiency also known as a primary α-tocopherol deficiency causes a neurological disease by a mutation in α-TTP and is called AVED (Ataxia with vitamin E deficiency) which is a disease characterized by ataxia, retinitis pigmentosa, and loss of proprioception, and is also known as primary α-tocopherol deficiency (Lee et al., 2019). On the other hand, secondary α-tocopherol deficiency mainly develops as a result of fat absorption disorders such as Steatorrhea, Cystic Fibrosis, Cholestasis, and Biliary Atresia (Sokol, 1988; Lee et al., 2019).

α-tocopherol must cross the blood-brain barrier (BBB) to be effective in the central nervous system (CNS). Although this has not been fully elucidated, several theories attempt to explain the transport of α-tocopherol to the CNS. The most common theory is that vitamin E, which is mostly carried by HDL and LDL in the blood, uses the lipoprotein receptors as a carrier protein for the transition to the CNS. Especially α-tocopherol-loaded HDLs cross the BBB, via their receptor, Scavenger Receptor Class B Type 1 (SRB1) (Goti et al., 2001; Balazs et al., 2004). Indeed, studies with porcine brain endothelium showed a positive correlation between α-tocopherol and SRB1 levels (Goti et al., 2000). In addition, in a study with SRB1(-/-) knockout mice, it was determined that although α-tocopherol levels were high in the blood, brain α-tocopherol levels were quite low (Mardones et al., 2002). This observation showed that the HDL receptor is of great importance in the transition to α-tocopherol CNS. In the same study, normal monitoring of liver, kidney, and spleen α-tocopherol levels led to the conclusion that SRB1 is not effective in passing to these organs. In another similar study performed with LDLR (-/-) knockout mice for the LDL receptor, no decrease was observed in the brain tocopherol level (Lee et al., 2019).

Crossing the BBB, α-tocopherol is delivered to a special group of astrocytes that express α-TTP, called Bergmann glial cells, located close to this barrier (Hosomi et al., 1998). In addition, the expression of α-TTP in different parts of the brain is thought to vary depending on neuronal health. For example, it has been noticed that α-TTP expression is also present in Purkinje cells and CA2 pyramidal cells of the hippocampus in patients with Alzheimer's disease, Down syndrome, and Abetalipoproteinemia, but not in healthy individuals (Copp et al., 1999). It is thought that this occurs for the modulation of α-tocopherol transport to regulate the increased oxidative stress in the mentioned diseases (Thakur et al., 2010; Ulatowski et al., 2012). In addition, it has been reported that α-tocopherol transfer within the CSF and among the various neurons in the CNS occurs mainly by apoEs synthesized in astrocytes (Bhatia et al., 2002; Lee et al., 2019).

2. Alzheimer's Disease

2.1. The History of Alzheimer's Disease

Alzheimer's Disease (AD) is a neurodegenerative disease responsible for 80% of dementia cases and is clinically characterized by a progressive deterioration in memory and cognitive functions (Ashraf et al., 2016; Scheltens et al., 2016). In the brain tissue of individuals affected by AD, severe atrophic lesions are observed, especially due to hippocampal and cortical synapse and neuron losses (Gómez-Isla et al., 1996; Mouton et al., 1998). Histopathological distinctive findings include neurofibrillary tangles (NFTs), amyloid beta (Aβ) plaques, and atrophy. Although its pathogenesis has not been fully elucidated, the most accepted hypothesis is the protein accumulation theory. It has been shown that protein accumulation in the brain is triggered by many environmental and genetic factors, especially oxidative stress (Grimm et al., 2012; Grimm et al., 2013; Drachman, 2014; Herrup, 2015).

2.2. Oxidative Stress and Alzheimer's Disease

Reactive oxygen species (ROS) form in the process of reducing O_2 to H_2O in the mitochondria and can interact with the surrounding biological molecules. Oxidative stress is an imbalance between ROS and antioxidant mechanisms (Sies, 1986). Although the human brain constitutes 2-3% of the total body weight, it accounts for 20-25% of the total basal metabolism and has the highest O_2 consumption. Hence, the brain has the highest ROS production in the body (Mosconi et al., 2008).

Oxidation of all macromolecules has been found in the brain of Alzheimer's patients, even in the very early stages of the disease. For example; the glycation end products (AGEs) formed as a result of the glycation reaction between reduced sugars and protein side chains cause the formation of senile plaque and neurofibrillary tangles in neurons (Castellani et al., 2001). In addition, lipid peroxidation impairs cell membrane movements and functions, while oxidized proteins and nucleic acids accumulate intracellularly and cause loss of function in neurons (Sayre et al., 1997; Smith et al., 1998; Nunomura et al., 1999).

All these have led to the research of antioxidants, especially vitamin E, in studies on the pathogenesis and treatment of Alzheimer's disease.

3. VITAMIN E and ALZHEIMER'S DISEASE

3.1. The Effects of Vitamin E on Alzheimer's Disease

Vitamin E is thought to be important for brain health due to its antioxidant, anti-inflammatory, and hypocholesterolemic effects (Brigelius-Flohé & Traber, 1999; Reiter, 2007; Jiang, 2014). Several studies have been conducted to investigate the effects of vitamin E on AD. These studies have reported that vitamin E affects both protective and triggering mechanisms related to AD. Since vitamin E is a powerful antioxidant, it provides a protective contribution to AD. *Previous in vitro* studies have shown that oxidative stress triggers neurofibrillary tangle formation by hyperphosphorylating tau proteins. Particularly; 4-hydroxynonenal (4HNE), formed as a result of lipid peroxidation, directly activates mitogen-activated protein (MAP) kinase and p38, and this leads to tau hyperphosphorylation (Mattson, 2004; Giraldo, 2014; Alavi Naini & Soussi-Yanicostas, 2015). In addition, oxidative stress increases glycogen synthase kinase-3 beta (GSK3) activity, which triggers tau hyperphosphorylation (Hernandez et al., 2013). Vitamin E is effective against lipid peroxidation and shows antioxidant activity by blocking the peroxidation of polyunsaturated fatty acids in the cell membrane (Brigelius-Flohé, 2009). Thus, the formation of many neurotoxic or triggering molecules that leads to hyperphosphorylation of tau protein is prevented. Vitamin E has greater antioxidant potential against peroxyl radicals than other antioxidants such as glutathione or β-carotene (Xu, 2009).

Another beneficial effect of vitamin E is its hypocholesterolemic effect. Various animal models have shown a strong correlation between increased cholesterol due to the direct stimulation of β and γ-secretase enzyme activities in the amyloidogenic APP pathway and Aβ level (Sparks et al., 1994; Refolo

et al., 2000; Maulik et al., 2013). Vitamin E prevents this stimulation by lowering the cholesterol level by different mechanisms and ensures the healing of AD (Oriani et al., 1997; Grimm et al., 2008; Grimm et al., 2016).

Aβ peptides form as a result of the amyloidogenic pathway aggregate to form Aβ plaques. In addition to disrupting interneuronal communication in the extracellular matrix, these plaques also show neurotoxic effects by inducing hyperphosphorylation of tau proteins in neurons. In a study, it was shown that particularly α-tocopherol and α-tocopherol quinine forms inhibit the formation of Aβ plaques by inhibiting Aβ aggregation (Shea et al., 2005; Dai et al., 2007; Wang et al., 2016).

On the other hand, vitamin E may cause neurotoxicity by triggering some AD-related protein accumulation by three main mechanisms. One of these mechanisms is protein kinase C (PKC) inhibition by particularly α-tocopherol inhibits (Tasinato et al., 1995). Since PKC regulates the α-secretase activity required in non-amyloidogenic APP processing, inhibition of PKC is thought to disrupt the non-amyloidogenic pathway (Skovronsky et al., 2000). Besides, vitamin E increases the synthesis of arachidonic acid from membrane phospholipids by upregulating the expression of phospholipase A2 (PLA2) (Tran et al., 1996). Arachidonic acid causes the ceramide/sphingomyelin ratio to increase in favour of ceramide by activating the sphingomyelinase (nSMase) enzyme, which provides the breakdown of sphingomyelins, an important membrane phospholipid for CNS (Robinson et al., 1997). Consequently, an increase in ceramide in the CNS gives rise to proapoptotic and neurotoxic effects by activating the amyloidogenic APP pathway (Dawson et al., 1998; Toman et al., 2002). Moreover, in a study conducted in 2015, it was claimed that α, γ-, and δ tocopherols activate protein accumulation by increasing the gene expression of β and γ-secretin, which are amyloidogenic pathway enzymes, and inhibiting the degranulation of the Aβ (Grimm et al., 2005).

3.2. Clinical Impact of Vitamin E on Alzheimer's Disease

Conflicting results have been reported in clinical correlation studies on vitamin E and AD to date. In the first clinical study published in 1989, it was reported that plasma vitamin E levels of 55 patients with Alzheimer's disease have low levels compared to the control group (Jeandel et al., 1989). Similar results have been reported in many clinical studies (Table 1). In addition, 80 studies on micronutrients and AD were examined in a meta-analysis study. Consequently, it has been suggested that vitamin E is at lower plasma levels

in AD patients and this is not associated with disease-induced malnutrition. (da Silva et al., 2014). Another meta-analysis study published in 2017 confirmed that vitamin E levels were also significantly lower in CSF and brain tissue in AD patients (de Wilde et al., 2017). In contrast to these findings, however, it was shown that there was no difference in vitamin E levels of AD patients compared to the control in a few studies (Liu et al., 2018).

Table 1. Studies about the relation between Alzheimer's disease and reduction of vitamin E levels.

Authors, Year	**Number of Patients**	**Findings**
Zaman et al., 1992	10 AD patients	Low levels of plasma α-tocopherol
Jimenez-Jimenez et al., 1997	44 AD patients	Decreased levels of vitamin E both in serum and in CSF
Sinclair et al., 1998	25 AD patients	Low levels of plasma α-tocopherol
Foy et al., 1999	79 AD patients	Low levels of plasma α-tocopherol
Bourdel-Marchasson et al., 2001	20 AD patients	Low levels of plasma α-tocopherol
Polidori et al., 2002	35 AD patients	Low levels of plasma α-tocopherol
Rinaldi et al., 2003	25 MCI patients and 63 AD patients	Low levels of plasma α-tocopherol
Mecocci et al., 2002	40 AD patients	Low levels of plasma α-tocopherol
Giavarotti et al., 2013	23 AD patients	Lower plasmatic levels of α-tocopherol
Mullan et al., 2017	251 AD patients	AD Lower levels of α-tocopherol but γ-tocopherol higher in serum of AD patients

MCI; Mild cognitive impairment, AD; Alzheimer's Disease.

In addition to clinical correlation studies, some clinical trials have also been conducted on this topic. In the first clinical study in 1997, 341 AD patients were followed and found that 2000 IU/d vitamin E supplementation for two years slowed down the progression of AD (Sano et al., 1997). In a clinical trial conducted eight years after this study, 769 AD patients received 2000 IU/d vitamin E supplementation for three years, but no beneficial effect was observed on cognitive functions (Petersen et al., 2005). In another study published in 2014, 613 AD patients received 2000 IU/d vitamin E supplements for 6 months to 4 years and reported cognitive function loss slowed down in AD patients (Dysken et al., 2014). Moreover, asymptomatic 7540 elderly men received a low dose (400 IU/day) of vitamin E supplementation for 6 years, which was found ineffective prophylactic in preventing the development of dementia (Kryscio et al., 2017).

In another clinical study, 800 IU/day vitamin E supplement was given to 33 AH patients for 6 months. Among these patients who received the same vitamin E supplementation, cognitive functions improved in those with improvement in oxidative stress parameters, while cognitive functions worsened in those without improvement in oxidative stress parameters. Hence, the authors emphasised that if vitamin E does not reduce oxidative stress, it may worsen the symptoms of AD (Lloret et al., 2009).

These contradictory effects of Vitamin E on AD; may depend on individual variations such as gender, genetic polymorphisms, and even smoking or diet type (Lloret et al., 2019). Furthermore, Brewer (2010) suggested that the conflicting effects of Vitamin e supplementation may result from the wrong time, wrong amount/dose, and unbalanced one-way use. He argued that the dose of vitamin E supplement used should be calculated individually due to the absorption, metabolism, and redox potentials caused by personal differences. In addition, it has been reported that vitamin E supplementation is more appropriate to give to MCI patients due to irreversible neuronal loss in advanced AD patients. Eventually, although Vitamin E is an antioxidant molecule, it becomes unstable by taking up reactive oxygens. Therefore, combined use of Vitamin E with other antioxidants such as vitamin C and glutathione which play a role in regenerating mechanisms of vitamin E may be more beneficial (Brewer, 2010).

In conclusion, vitamin E is an essential supplement for CNS. However, its therapeutic effects on Alzheimer's have not been yet fully established and further researches are necessarily on this subject.

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CHAPTER 11

ROLE OF CHEMOKINES IN HCV-RELATED COMPLICATIONS

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INTRODUCTION

Hepatitis C virus (HCV) is an important infectious agent that invades the innate and adaptive immune systems of the host. With the chronicity of hepatitis C infection, the disease picture may turn into fibrosis, cirrhosis, and hepatocellular carcinoma (HCC). HCV has developed successful mechanisms to achieve viral persistence by both modulating immunity and developing avoidance strategies (Brass and Brenndörfer, 2014). Chemokines play an important role in the recruitment of antiviral immune cells to the liver and in the pathogenesis of hepatic injury. HCV-mediated modulation of hepatic immune cell chemotaxis significantly influences tissue damage and antiviral immunity (Charo et al., 2006; Xue et al., 2021). This chapter summarizes the roles of chemokines and their receptors in hepatitis C infection, as well as associated hepatic complications and HCV-mediated modulation of these complications. In the future, with the clarification of HCV-mediated modulation of chemokines and their receptors, the role of chemokines in mediating hepatic fibrosis would be understood more clearly and effective immunotherapies can then be designed.

1. Hepatitis C Virus

Infection caused by the hepatitis C virus (HCV) is a serious health problem worldwide and according to the recent statistical predictions, more than 177 million people globally are carriers of this virus (Petruzziello et al., 2016). HCV, belonging to the Hepacivirus genus of the Flaviviridae family, is a non-cytopathic, enveloped, hepatotropic RNA virus with a diameter of 40-70 nm (Barth et al., 2006). HCV RNA consists of three structural proteins (core, E1, E2) and seven non-structural proteins (p7, NS2, NS3, NS4A, NS4B, NS5A, NS5B). These proteins play important roles in the life cycle and replication of HCV (Roger et al. 2021). Approximately 70-80% of individuals exposed to this pathogen develop chronic hepatitis C (CHC) infection, which can cause serious liver complications such as fibrosis, cirrhosis, and HCC (Lavanchy et al., 2011). Treatment of hepatitis C infection, for which there is no preventive vaccine yet, relies solely on antiviral drugs (Mohd Hanafiah et al., 2013). Until recently, the main/standard treatment option for HCV infection was a combination of 24-48 weeks ribavirin (RBV) and pegylated interferon (Peg-IFN). Recently, with the development of Direct-Acting Antiviral Agents (DAA), which are used for the treatment of hepatitis C, treatment regimens without IFN and/or ribavirin have come into question (Oruç and Ersoy, 2016). The primary target of DAA

drugs are gene groups that synthesize the HCV replicase complex from non-structural proteins. DAAs effect by inhibiting one of the NS3/4A, NS5A, and NS5B proteins of HCV (Feeney et al., 2014). DAAs fall into four categories: (i) NS3/NS4 protease inhibitors (ii) NS5A (nucleoside-type) protein inhibitors (iii) NS5B polymerase inhibitors and (iv) NS5B (non-nucleoside-type) polymerase inhibitors (Gümüş and Tunçbilek, 2016).

Viral RNA in hepatocyte cytoplasm can be detected during acute HCV infection. When HCV-associated molecular patterns (PAMPs) bind to intracellular pattern recognition receptors (PRRs) (Loo et al., 2006), this intermolecular interaction lead to the activation of transcription factors (such as nuclear factor κB (NFκB) and interferon regulatory factor (IRF) 3) and induce antiviral activity (Saito et al., 2008; Li et al., 2012). Plasmacytoid dendritic cells (pDC) can detect circulating HCV RNA and produce IFN-α. IFN-I and IFN-γ released by pDC and hepatocytes during viral infection induce NK (natural killer) cells and directly suppress HCV replication (Nellore et al., 2011). NK cells are an important part of the innate immune response, involved in the cytolytic destruction of acute HCV-infected hepatocytes, as well as in the release of cytokines that stimulate the adaptive immune response. Activated NK cells produce IFN-γ and tumor necrosis factor (TNF-α), leading to dendritic cell maturation and the release of interleukin 12 (IL-12), thus inducing an adaptive immune response by differentiation of CD4 and $CD8^+$ T cells (Chigbu et al., 2019; Xu et al., 2016).

2. Chemokine and Chemokine Receptors

Chemokines, 8-12 kDa in size and a member of the cytokine family, are chemoattractant glycoproteins that control lymphoid organ development and immune cell movements (Viola and Luster, 2008). Chemokines are divided into two groups: homeostatic and proinflammatory. Inflammatory chemokines are formed in response to stimuli during inflammation and control the activity and chemotaxis of leukocytes in the processes of infection, inflammation or tissue damage. Structurally expressed homeostatic chemokines play a role in the development of the immune system, angiogenesis, and tumor development (Vandercappellen et al., 2008; Zlotnik et al., 2011). However, several chemokines have also been identified, termed 'dual-function' chemokines, which can perform both functions simultaneously. Homeostatic and dual-function chemokines show higher selectivity towards their targets than inflammatory chemokines (Moser et al., 2004). The main function of chemokine and chemokine receptors is to recruit

leukocytes to the immune response site. Chemokines have four receptor groups CXC (α), CC (β), CX (γ), and CX3C (δ) according to their amino acid structure (Choi and An, 2011). Most alpha (α)-chemokines are important mediators that act on neutrophil migration, innate immunity, type 1 helper T cell (Th1) inflammation, and acute inflammation. On the other hand, beta (β)-chemokines exert their effects mainly on monocytes and T-lymphocytes, and some on eosinophils, basophils, and natural killer (NK) cells and play a role in chronic inflammation, parasitic infections, and allergy (Vilgelm and Richmond, 2019).

Chemokines exert their functions by binding to chemokine receptors on the cell membrane. Chemokine receptors containing seven transmembrane helices, a cytoplasmic carboxy-terminal rich in threonine and serine residues, and an extracellular ligand binding site are included in the rhodopsin-like GPCR superfamily. (Choi and An, 2011; Allen et al., 2007). Chemokines activate chemokine receptors via the Ca^{2+} metabolic pathway, which is one of the cell signal transduction mechanisms, and enzymatic reactions occur within the cell (Legler et al., 2016). These reactions cause an increase in Ca^{2+} concentration, release of intracellular mediators from the endoplasmic reticulum (ER), and functionalization of the target protein. When chemokine receptors are stimulated by chemokines, they regulate the chemotaxis of T cells and phagocytic cells to the site of inflammation. Receptor expressions may vary in different cell types (Legler et al., 2016; Mellado et al. 2001). Table 1 summarizes the receptor, ligand, and target cells of the chemokines.

Table 1. Chemokine and Chemokine Receptors

Chemokine receptor	**Ligands**	**Target cells**
CC chemokines		
CCR1	CCL3, CCL4,CCL5, CCL7,CCL8, CCL13-16, CCL23	T cells, basophils, monocytes, eosinophils
CCR2	CCL2, CCL7, CCL8, CCL13, CXCL16	Memory T cells, DC, HSC, monocytes,
CCR3	CCL11, CCL13, CCL7, CCL5, CCL8, CCL13,	Mast cells, eosinophils, Th2 cells, platelets, basophils
CCR4	CCL17, CCL22	Platelets, Th2 cells, basophils, DC, macrophages
CCR5	CCL3-5,	T cells, Treg, NK, immature DC

	CCL8,CCL13, CCL16	monocytes, NKT, neutrophil, Th1, CTL, naive T cell,
CCR6	CCL20	T cells, DC, B cells
CCR7	CCL19, CCL21	T cells, DC
CCR8	CCL16	KC, NK, memory T cell, Th1, Th2
CCR9	CCL25	Plasma cells, T cells
CCR10	CCL27, CCL28	T cells
CXC chemokines		
CXCR1	CXCL8, CXCL6	Monocytes, neutrophils
CXCR2	CXCL8, CXCL1, CXCL2, CXCL3, CXCL5, CXCL6	Neutrophils, vascular endothelial cells, monocytes
CXCR3	CXCL9, CXCL10, CXCL11	Mast cells, Th1 cells, mesangial cells
CXCR4	CXCL12	Expressed in multiple cells
CXCR5	CXCL13	Macrophages, lymphocytes, Follicular DC
CXCR6	CXCL16	Biliary epithelial cell, hepatocyte, portal endothelium,
CXCR7	CXCL11, CXCL12	Hepatocyte, LSEC, widely expressed
CX3C chemokines		
CX3CR1	CX3CL1	Smooth muscle cells, macrophages,
XC chemokines		
XCR1	XCL1, XCL2	NK cells, T cells

Treg, regulatory T cells; HSC, hepatic stellate cells; NK, natural killer; NKT, natural killer T cell; cytotoxic T lymphocyte; LSEC, liver sinusoidal endothelial cell; DC, dendritic cells; KC, Kupffer cell; Th, T helper

3. The Role of Chemokine/Chemokine Receptors in HCV Infection

Chemokines, which are considered biomarkers and therapeutic targets in CHC infection, play important roles in the pathogenesis of CHC and associated hepatic inflammation (Fallahi et al., 2017). In the liver, chemokines not only traffic leukocytes to the immune response site, but are also secreted by cells such as hepatocytes and hepatic stellate cells (HSCs), thus exerting both pro-fibrotic and anti-fibrotic effects (Wasmuth and Tacke, 2010). Chemokines are very important in lymphocyte migration to the liver in HCV infection and they lead to liver damage that occurs during infection (Zeremski et al., 2007). The inflammatory CXC and CC chemokine levels were found to be highly expressed in blood and liver associated with CHC infection (Wald et al., 2007). The ligands capable of binding to Th1-related CXCR3 and

CCR5 chemokine receptors can be detected in the peripheral blood of HCV-infected patients after approximately 2-8 weeks (Thimme et al., 2012).

3.1. CXCR3-Associated Chemokines in HCV Infection

The levels of CXCR3 ligands such as CXCL9 (MIG), CXCL10 (IP-10), and CXCL11(I-TAC) are increased in the livers of CHC patients. It was also revealed that these ligands are involved in chemotactic events through their common receptors CXCR3 during CHC infection and lead to T lymphocyte trafficking in the liver (Zeremski et al., 2008; Harvey et al., 2003). Th1, NK, and cytotoxic $CD8^+$ T cells, which play a critical role in host defense against viruses, also carry CXCR3 chemokine receptors on the cell membrane. Therefore, expression of the CXCR3 receptor and its related ligands provides an advantage in virus evasion (Brass and Brenndörfer, 2014). Previous studies have shown that HCV proteins differentially modulate chemokine levels. When chemokines CXCL10, CXCL9, and CCL5 were induced by HCV proteins (such as NS5A and nucleus) their levels were increased in hepatocytes, whereas it was determined that the levels of CCL5 and CXCL10 chemokines were decreased (Larrubia et al., 2007; Sillanpää et al., 2008). In the early stages of HCV infection, TLR3 and RIG-I activation causes the expression of CXCR3 ligands in infected hepatocytes and that expression is further potentiated by type I and III IFN induction (Brownell et al., 2013). In addition, some HCV proteins can block IFN signaling and PRR activation (Bode et al., 2008). Specifically, the HCV NS3/4A protease has been shown to inhibit retinoic acid inducible gene-I (RIG-I) and TLR3 signaling by cleavage of mitochondrial antiviral signaling protein (MAVS) and TIR-domain-containing adaptor-inducing-interferon-β (TRIF) (Meylan et al., 2005; Li et al., 2005; Li et al., 2007). CXCR3-related chemokines induced by

HCV replication are predicted to be further enhanced by the secretion of Th1-type cytokines IFN-γ and TNF-α in the inflammatory environment (Helbig et al., 2009). CXCR3-related chemokine gradients are important for the transport and attachment of T cells from peripheral blood to the chronically infected liver. However, although CXCR3 positive T cells are involved in HCV clearance, they can cause tissue damage when the infection becomes chronic (Helbig et al., 2009).

3.2. CCR1/CCR5-Associated Chemokines in HCV Infection

CCR1 and CCR5 chemokine receptors are expressed by Th1/Tc1 cells, $CD8^+$ T cells, NK cells, memory T cells, and antigen-presenting cells. CCL3 (MIP-1α), CCL4 (MIP-1β), CCL5 (RANTES), and CCL8 ligands' receptors

are CCR1 and CCR5. CCR5 positive T cells can be detected with high frequency during acute HCV infection as they are an important part of antiviral immunity (Gruener et al., 2001). Differential expression of CCR5 and CCR1 chemokine receptors has been detected in the peripheral blood and intrahepatic T cells of HCV-infected individuals. Expression of CCR5 and CCR1 in HCV-infected subjects compared to healthy subjects increased in intrahepatic $CD4^+$ T cells, but significantly decreased in peripheral blood-derived T cells (Lichterfeld et al., 2002). Furthermore, intrahepatic levels of CCL3-5 ligands have been shown to be elevated during CHC infection. (Zeremski et al., 2007; Apolinario et al., 2002). A higher concentration of CCR5-related chemokines in portal pathways in CHC patients may retain these cells in the liver (Neumann-Haefelin et al., 2008). In addition, the level of $CD8^+$ T cells with surface expression of CCR1 and CCR5 may be decreased in this infection (Lichterfeld et al., 2002). HCV proteins have been shown to have opposite effects on CCL5 expression in a number of experimental models. Nattermann et al. showed that the interaction of HCV E2 protein with CD81 resulted in an increased expression of CCL5 by $CD8^+$ T cells (Nattermann et al., 2006). In addition, it has been determined that the regulatory role of HCV core on CCL5 expression may vary depending on endogenous transcription factors. In this context, CCL5 expression can be induced or inhibited (Boisvert et al., 2003). Also, HCV NS3/4A protein has an inhibitory effect on CCL5 expression (Sillanpää et al., 2008).

3.3. Other Chemokines Associated with HCV Infection

CXCR6, involved in immune system regulation, mediates the ligand CXCL16, which is present in both transmembrane-bound and soluble forms (Tabata et al., 2005). The soluble form CXCL16 is involved in the migration of immune cells such as $CD8^+$, $CD4^+$, T cells, NK, natural killer T cell (NKT), and monocytes through the CXCR6 receptor in conditions such as hepatic cell inflammation (Tabata et al., 2005; Izquierdo et al., 2014). The CXCR6-CXCL16 interaction is required for the migration and recruitment of effector cells to the liver (Geissmann et al., 2005).

T cell subpopulations express the chemokine receptor CCR4. It is known that the expression of the CCR4 ligands CCL17 and CCL22 is increased by the HCV protein NS3/4A. The experiment with NS3/4A-transgenic mice showed increased expression of CCL17 and CCL22 ligands and associated increased numbers of CCR4 positive $CD4^+$ T cells in the livers of mice. (Brenndörfer et al., 2012; Riezu-Boj et al., 2002).

Another chemokine receptor whose expression is increased in HCV infection is CCR2, which is expressed on macrophages, monocytes, DCs, and T cells (Zhdanov et al., 2008). CCL2 (MCP-1), CCL7 (MCP-3), CCL8 (MCP-2), and CCL13 (MCP-4), all expressed in the hepatic environment, are ligands of CCR2 (Nattermann et al., 2006; Oo et. al., 2010). In particular, the CCL2 ligand is significantly increased in livers infected with the proteins of HCV NS3/4A and NS5A, and this increase has been associated with progressive hepatic inflammation (Brenndörfer et al., 2010; Soo et al., 2002).

The expression of CXCL8 (IL-8), the ligand of the chemokine receptor CXCR1/2, which plays a role in the migration of monocytes and neutrophils to the liver, is increased in HCV-infected patients (Polyak et al., 2001). Some HCV proteins can modulate CXCL8 expression. HCV core, NS4A/B, and NS5B have been shown to induce CXCL8 expression, while NS3/4A protein has been shown to reduce CXCL8 expression (Kadoya et al., 2005; Sillanpaa et al., 2008). Increased CXCL8 expression levels have also been associated with resistance to interferon therapy (Polyak et al., 2001).

4. Chemokines in HCV-Related Hepatic Diseases

In CHC infection, necro-inflammatory damage accumulates over years, and as a result of this accumulation, fibrosis is thought to be initiated by direct activation of hepatic stellate cells (HSC). Activated HSCs respond to retinoid loss and mitogenic and chemotactic stimuli, causing them to proliferate and migrate to sites of hepatic injury (Mormone et al., 2011). HSCs are involved in the secretion of profibrotic and proinflammatory cytokines and play a dominant role in liver fibrosis with increased accumulation of extracellular matrix proteins (Fahey et al., 2014). Transforming growth factor-β (TGF-β) plays important role in liver fibrogenesis (Dooley S and ten Dijke, 2012). By various mechanisms, TGF-β promotes myofibroblast and liver fibrosis activation of HSCs. It has been shown that HCV-derived $CD8^+$ T cells may contribute to HSC activation by causing a decrease in IFN-γ expression and an increase in TGF-β (Muhanna et al., 2008).

Cirrhosis, a result of fibrosis, may develop in one out of every three patients infected with chronic hepatitis (Lingala et al., 2015). Cirrhosis, defined as the progression of regenerative nodules embedded in fibrous strips, occurs in response to hepatic injury, causing portal hypertension and liver failure. Cirrhosis is one of the most important risk factors for the development of HCV-related HCC (Schuppan and Afdhal, 2008). Indirect mechanisms such as long-duration hepatic inflammation and the presence of cirrhosis, are

likely to contribute to cancer development. (Okuda et al., 2002). In the pathogenesis of hepatitis C infection and its complications (fibrosis, cirrhosis, HCC), a number of chemokines/receptors are modulated in the blood and liver. This condition regulates angiogenesis, carcinogenesis, inflammation and metastasis, and anti-tumor immunity (Capece et al., 2013; Huang et al., 2010). The expression of CCL2, a crucial chemokine for the initiation of liver fibrogenesis, is increased in HCV-infected patients, and this increase in expression is associated with the stage of fibrosis (Asselah et al., 2005). In addition, a CCL2 promoter polymorphism (at position -2578) may affect the prognosis of the disease, with more severe hepatic inflammation and increased fibrosis in hepatitis C infection (Mühlbauer et al., 2003). Interestingly, it has been shown that high expression of CCL2 chemokine may be a marker for rapid progression to cirrhosis, whereas high expression of CCL4 chemokine level may be a marker for slow progression (Farci et al., 2012).

Serum levels of the CCR6-CCL20 axis are increased in CHC and related fibrosis, cirrhosis, and HCC (Northfield et al., 2008). High CCR6 mRNA levels have been detected in Th17 cells and regulatory T cells (Tregs), therefore, it is thought to have an important role in the mechanisms of carcinogenesis, proliferation, and metastasis associated with hepatitis C infection (Chen et al., 2011).

CXCR3 ligands are another important group of chemokines associated with HCV-associated fibrosis and cirrhosis (Coulon et al., 2006). In a study in chronic liver patients, an association was observed between serum levels of CXCL9, CXCL10, and CXCL11 chemokines and liver fibrosis. In the same study, researchers reported that the CXCR3 chemokine receptor is expressed differently according to different stages and etiologies of chronic liver diseases (Tacke et al., 2011). Although its level is increased in fibrosis, it has been shown in an *in vitro* model that CXCL9 chemokine has an inhibitory effect on TGF-β and collagen expression. Therefore, this chemokine is thought to have an anti-fibrotic role (Heydtmann et., 2005). In contrast, HSC-mediated binding of CXCR3-CXCL10 causes activation of the mitogen-activated protein kinase (MAPK) pathway and plays a role in HCC development, thus CXCL10 is thought to have a proliferative role (Giatromanolaki et al., 2007).

The CXCR4 receptor, which uses only CXCL12 as a ligand, is known for its profibrotic functions in the liver and is expressed by different immune cells such as monocytes, T, and B cells (Ding et al., 2014). CXCL12-CXCR4

interaction increases HSC activation, leading to fibrogenesis (Hong et al., 2009). The hepatic expression of CXCL12 is further increased with the development of HCV-induced cirrhosis (Ding et al., 2014). In addition, CXCR4 receptor and CXCL12 chemokine levels were found to be correlated with intrahepatic tumor cell proliferation. Expression of CXCL12 and CXCR4 has been shown to be higher in HCC tissue compared to cirrhotic liver (Li et al., 2007). Besides, CXCL11 and CXCL12 share a newly discovered common receptor, CXCR7 (Burns et al., 2006). The CXCR7 chemokine receptor, which is important for angiogenesis and vascular homeostasis, is located on liver sinusoidal endothelial cells (LSECs) (Ding et al., 2014). Signaling pathway activation of this chemokine receptor results in increased proliferation in the cell (Chalin et al., 2018).

CXCL16/CXCR6 activation is known to promote liver fibrosis progression (Wehr et al., 2015). It has also been reported that CXCL16 accelerates lipid aggregation, removal of extracellular matrix from the cell, and formation of reactive oxygen species (ROS) in liver cells (Ma et al., 2018). Wehr et al. have shown that liver NKT cells act together with CXCR6 in early-stage damage and contribute to liver fibrosis progression by creating inflammatory reactions (Wehr et al., 2013).

With the increased expression of IL-8 induced by TNF-α in HCV-infected liver, the uptake of neutrophils increases, and the necro-inflammatory process is exacerbated (Polyak et al., 2001). In connection with this situation, the intrahepatic increase of IL-8 chemokine correlates with the advanced stage of fibrosis. The increase in IL-8 has been shown to be significant in patients with a higher degree of neutrophil infiltration, impaired liver function, and cirrhosis (Neuman et al., 2007). In addition, the upregulation of IL-8's receptor, CXCR2, in HCC tissue is thought to play important roles in tumor proliferation (Liu et al., 2011).

CONCLUSIONS

Chemokines play an important role in the control of HCV replication and mediate the inflammatory response. The role of chemokines is vital for spontaneous clearance and elimination by immunotherapy in hepatitis C infection. Marker potentials have been demonstrated in predicting the prognosis of infection, including stages of HCV-related fibrosis (Tacke et al., 2011). Their angiogenic/angiostatic effects and modulating roles in HCV-associated metastasis have been demonstrated (Xue et al., 2021). Because of these vital roles of chemokines in hepatitis C infection, further investigation

will provide a clearer understanding of their role in HCV immunopathogenesis. Thus, it will be possible to develop biomarkers that can predict the clinical prognosis of the disease and new therapeutic targets for this difficult-to-treat infection.

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CHAPTER 12

HYSTERECTOMY AND SEXUALITY

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INTRODUCTION

A hysterectomy is briefly defined as the surgical removal of a woman's uterus (Centers for Disease Control and Prevention., 2022). Hysterectomy can be subtotal or total (Clayton, 2006). Hysterectomy can be performed in different ways. These are abdominal hysterectomy, laparoscopic assisted vaginal or total hysterectomy, and robotic assisted hysterectomy (Amarin, 2015). It is recommended that women should avoid sexual intercourse until the upper part of the vagina has fully healed for about 6-8 weeks after hysterectomy (Vomvolaki et al., 2006).

Hysterectomy

One of the most common surgeries performed on women worldwide is hysterectomy. Nine out of every ten women undergoing hysterectomy have this surgery for a non-malignant and non-life-threatening reason. Therefore, hysterectomy indication policy should be re-evaluated in line with new treatments (Shimizu, 2011).

Considering the hysterectomy rates, it can be seen that hysterectomy is common in many countries in the world. For example, in the United States, 1 in 3 women has been reported to have a hysterectomy by the age of 60 (Centers for Disease Control and Prevention., 2022). It has been reported that approximately 3.1 million hysterectomies were performed between 2000 and 2004 in the United States. When this is considered according to the annual average, it has been found to be approximately 600 000 per year (Committee on Gynecologic Practice, 2009; Whiteman et al., 2008).

Hysterectomy, one of the most frequently performed surgical procedures in the UK, is performed approximately 100 000 each year (Clayton, 2006). Similarly, hysterectomy, which is the most frequently performed gynecological operation in Canada, is performed 50 000 each year (Innie et al., 2014).

Since hysterectomy, which is a frequently encountered operation, is an operation with a high probability that women encounter, it is important to investigate how women's lives are affected after hysterectomy. In particular, knowing its effect on sexuality is important in supporting women after hysterectomy and even before. Therefore, this chapter focuses on women's experiences of sexuality after hysterectomy.

Hysterectomy and Impact on Sexuality

This chapter explores the sexuality of women after hysterectomy. There are not many studies examining the experiences of women with hysterectomy surgery. Another gap in the literature is what women want to know about sexuality after hysterectomy and how to provide women with this information (Vomvolaki et al., 2006). The effect of hysterectomy on sexuality has not been fully explained (Zobbe et al., 2004). As each person's reaction to everything may differ, the effects that women will experience on their sexual functions after hysterectomy surgery may differ within themselves. Furthermore, many women are feared that hysterectomy might influence their sexual attraction (Vomvolaki et al., 2006).

Positive or no negative impact on sexuality

Studies have shown that hysterectomy has positive aspects on sexuality or has no effect (Danesh et al., 2015; Dragisic & Milad, 2004; El-Toukhy et al., 2004; Ellström et al., 2003; Farrell & Kieser, 2000; Lonnée-Hoffmann & Pinas, 2014; Roovers et al., 2004; Zobbe et al., 2004). For instance, the systematic review of hysterectomy on sexuality in 2000 showed that much of the research was actually poorly designed. It reported that the studies included in the review did not adversely affect the sexuality of women after hysterectomy (Farrell & Kieser, 2000). As a result of a another review, the literature of the last 10 years of hysterectomy was examined and according to the result obtained, hysterectomy for benign disease has a positive effect on sexual function. On the other hand, between 10 and 20 percent of women experience some different sexual function problems such as dyspareunia (Lonnée-Hoffmann & Pinas, 2014). In the narrative review, which consists of studies examining the changes in the sexual functions of women after hysterectomy, which was carried out for a similar purpose, it was concluded that the problems of sexual disorders were resolved after hysterectomy for benign diseases in the uterus. On the other hand, radical hysterectomy performed as a result of cancer indication may cause negative effects on sexual function (Danesh et al., 2015).

According to the results of a study conducted with 75 women who had undergone hysterectomy, examining the effect on sexual functioning after hysterectomy, it was found that most patients did not experience any change in sexual desire or orgasm. Moreover, it has been shown that hysterectomy can have a positive effect on reducing the pain felt during sexual intercourse (Dragisic & Milad, 2004).

In a study conducted with 413 women who underwent different types of hysterectomy in the Netherlands, it was found that sexual pleasure improved in all patients regardless of the type of surgery (Roovers et al., 2004). In a study conducted with 319 women who had a hysterectomy in a multicenter study in Denmark, a decrease in the frequency of dyspareunia was found 12 months after hysterectomy. Except for dyspareunia, there was no difference in sexual outcomes (Zobbe et al., 2004). There was no difference between the two patient groups in terms of changes in sexuality, according to the study findings of a total of 74 women who had laparoscopic or abdominal hysterectomy surgery performed in Sweden, evaluating the changes in sexuality one year later (Ellström et al., 2003). This study implied that sexuality would not be affected by surgical techniques. Similarly, another study showed that vaginal, abdominal or laparoscopic hysterectomy for benign disease has no adverse effects on sexual function (El-Toukhy et al., 2004). The results of those studies indicate that there may be a link between the indication of hysterectomy and its effect on sexual function.

However, as a result of the study examining the effect of abdominal, vaginal or laparoscopic methods for a hysterectomy on female sexuality, it has been shown that the effect of vaginal and laparoscopic hysterectomy on female sexuality is less than abdominal hysterectomy (Ayoubi et al., 2003). The results of this study show us that there is a need for more research on this subject.

Negative impact on sexuality

The evidence has shown the negative effects of hysterectomy on sexuality (Celik et al., 2008; Goetsch, 2005; Katz, 2003; Peterson et al., 2010; Reis et al., 2008; Rodrguez et al., 2012). Using the female sexual function index, female sexuality was assessed after hysterectomy in a study of 100 women who had a hysterectomy in Spain. As a result of the study, it was found that there is a negative effect on female sexual function after hysterectomy (Rodrguez et al., 2012). In a similar study in Turkey, the effects of hysterectomy on female sexual function index in post-menopausal women were examined on 92 women and it was found that hysterectomy had negative effects on sexual functions in the first 6 months after the operation (Celik et al., 2008). In the study conducted in Turkey (Celik et al., 2008), which is an important point emphasized by the authors, it was emphasized that all of the participants who participated in the study were in the postmenopausal period, as they obtained the opposite results of the previous studies in the literature.

This may imply that the effects of hysterectomy surgery on sexuality may be different in the postmenopausal and premenopausal periods.

Findings in the second stage of the study, which showed that sexual problems developed after hysterectomy, showed that the frequency of sexual problems increased in direct proportion as the time spent after hysterectomy increases (Peterson et al., 2010). These findings imply the importance of evaluating women against the possibility of increasing complaints of women who have sexual problems after hysterectomy.

In a study conducted in the USA between 1990 and 1992, although it was stated that sexual satisfaction increased after hysterectomy, it was noted that some women, albeit in small numbers, had worsened sexual functions (Goetsch, 2005). From this point of view, it should not be forgotten that there is a risk that women may be adversely affected in their sexual functions, albeit a little. Supporting the treatment and care of these women in this direction should not be overlooked.

According to the results of a qualitative study conducted with 31 women who had a hysterectomy in Turkey, it was found that more than half of the women were of the opinion that their sexual life would be adversely affected (Reis et al., 2008). Contrary to studies showing that sexuality will be positively affected after hysterectomy, this result indicates that the loss of an organ in women's bodies, such as the uterus, may cause sexual concerns for them. For example, it is remarkable that one of the participants compared the situation of how you smell without a nose to this situation, as well as the absence of a uterus (Reis et al., 2008). At this point, we can see that women give importance to body integrity. In a study examining the literature on sexuality studies after hysterectomy and discussing the roles of nurses, she reported that there are very few nursing studies on the subject. In this case, attention was drawn to the fact that it may indicate that sexual assessment and education not enough be included in clinical practice (Katz, 2003).

IMPLICATIONS and CONCLUSION

Nurses encounter women many times throughout their lives. For this reason, they are an important position to listen to sexual problems and evaluate sexuality as a part of routine care (Katz, 2003). It is important to provide counselling services to women in the hysterectomy process. Also, It should also be made more accessible for nurse-led preoperative counseling (Mokate et al., 2006).

It is stated that sexual function after hysterectomy is multifactorial It should not be forgotten that there are different influences factors of sexuality after hysterectomy. For example, partnering sexual function or support, hormonal status, mental health, and physical ability are also important factors affecting sexuality after hysterectomy (Helström, 1994). In addition, the fact that women are depressed, anxious, have a poor body image, and have an unsatisfied close relationship may have an impact on sexual dysfunction. For this reason, evaluation of the psychosocial variables of women before and after the surgery and providing support for this may be beneficial for them in terms of sexuality after hysterectomy (Peterson et al., 2010).

Therefore, it would be beneficial to cover not only women but also their partners in counselling (Mokate et al., 2006). Individual and couple therapy before or immediately after hysterectomy can positively affect post-hysterectomy sexuality and relationship outcomes (Peterson et al., 2010). It is important to know that women who will have a hysterectomy may be worried about their sexual life (Mokate et al., 2006). The patients cannot get enough information about their sexual health before hysterectomy due to some cultural reasons and embarrassment due to both patients and physicians. It is recommended that physicians of the same sex be fully informed in order to reduce the communicative problems of women before and after the surgery on sexual issues (Danesh et al., 2015).

It is important to consider methodological differences in explaining the differences in the results of the studies (Bayram & Beji, 2010). In addition, taking into account the culture in which the studies were conducted and the concept of gender in the society of women may be useful in analyzing the results. Health care providers should take into account the variables of women. The importance given to the uterus of the woman and the close relationship can be considered the main factors to be considered.

A study on the effect of education on the potential sexual consequences of hysterectomy with 204 women who had had a hysterectomy showed that preoperative sexual counselling positively affected satisfaction with the results of hysterectomy (Bradford & Meston, 2007). The result of this study indicates the importance of providing education and counseling for women who have had a hysterectomy.

It is recommended to provide psychological support to the patients before and after the surgery to help them adapt more easily to the problems that may occur after the surgery. Moreover, training is recommended for women to be prepared in advance for facing sexual problems after the surgery

and to assess their needs when they have sexual function problems themselves (Danesh et al., 2015). Furthermore, it is recommended that services be developed to support men whose wife has a hysterectomy. Men should be given the opportunity to discuss the problems of their husbands with a hysterectomy and it is recommended that they be encouraged in this regard. In addition, it may be beneficial to share information through mass media to meet the needs of patients and their families (Chou et al., 2006).

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CHAPTER 13

VASCULAR SURGERY AND ARTIFICIAL INTELLIGENCE

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INTRODUCTION

Artificial intelligence (AI) is the study of the mathematical formulas that enable machines to reason and carry out cognitive tasks like decision-making, word recognition, and problem-solving. (Hashimoto et al.,2018). The broad field of AI focuses on comprehending and developing systems that exhibit traits of human intelligence, including as reasoning, adaptation, interaction, learning, and sensory comprehension (URL 1).In actuality, AI refers to a machine's or a device's capacity to decide on its own, based on information gathered (Dey et al., 2019).

Since the 1970s, AI and robotics have advanced in a variety of industries, from manufacturing automation, where assembly lines' speed and efficiency were greatly increased, data processing, and many other enterprises. With the exception of a few robotic tools like the CyberKnife, this led to advancements in robotics that have largely bypassed the medical sector (Accuray, Sunnyvale, California, USA) (Adler, 2005; Weidlich V &Weidlich GA, 2018). Significant progress in AI has been made since it was first introduced at the Dartmouth College conference in 1956, resulting in uses in daily life in a variety of fields, such as education, finance, media, telecommunications, including industry, transport, marketing, or computer science. The first uses of AI in medicine date back to the 1970s, and as machine learning (ML) techniques have advanced, AI has raised hopes and opened up new possibilities for usage in clinical practice (Raffort et al.,2020).

As years of study have finally reached knowledge thresholds that have quickly produced useful applications, such as International Business Machine's (Armonk, NY) IBM Watson and Tesla's (Palo Alto, CA) autopilot, AI has increasingly been a topic of both popular and scholarly literature (URL 2). The four basic subfields of AI presented below have received most of the recent attention. I. Machine Learning (ML), II. Natural Language Processing, III. Artificial Neural Networks and IV. Computer Vision (Hashimoto et al.,2018).

In contrast to conventional ML, deep learning (DL) learns representations from the raw data. In actuality, DL enables computational models built from several neural network processing layers to learn representations of input with different levels of abstraction (LeCun et al.,2015).

The study of certain computer algorithms used to produce predictions or choices using sample data and a mathematical algorithm model is known as machine learning (ML). DL is far more complicated than ML and uses

representation learning and artificial neural networks. DL is associated with a hierarchy of growing complexity and abstraction whereas ML algorithms continue to take a linear approach since it may also be viewed of as a way to automate predictive analytics (Khalsa et al.,2021).

DL enables the learning of representations of data at multiple levels of abstraction in computational models with numerous processing layers. The state-of-the-art in many other fields, including drug discovery and genomics, object detection, visual object recognition, and speech recognition has been significantly improved by these techniques. By using the backpropagation approach to suggest changes to a machine's internal parameters that are used to calculate the representation in each layer from the representation in the previous layer, DL may identify detailed structure in large data sets. Recurrent nets have shed light on sequential data types such as text and speech while deep convolutional networks have improved the processing of images, video, voice, and audio (Farabet et al.,2013; LeCun et al.,2015; Leung et al.,2014).

In medicine, this typically entails the use of data (health records or information extracted from images) to determine the best course of treatment, predict a likely diagnosis, or discover a new disease (Darcy et al., 2016; Szolovits et al.,1988).

In order to prevent future undesirable clinical outcomes or staff shortages, trends will be identified and predictive algorithms will be created for intensive care units, patient monitoring equipment, diagnosis, surgical equipment use, preventative measures, and vital patient information. AI will assist with patient admissions, triage procedures, selecting the best medication and dosing for patients, contacting care professionals, and keeping logs based on the patient's symptoms and vital signs (Weidlich V &Weidlich GA, 2018).

Therefore, it is crucial for surgeons to have a foundational understanding of AI in order to comprehend how it may effect health care and to think about potential interactions with this technology. The purpose of this writing is to introduce the present state of AI in vascular surgery.

Artificial intelligencein surgery

Over the past two decades, there have been a significant increase in papers on AI and surgery. The accelerating increase in computer power accessible to the greatest AI training runs can help to partially explain this phenomenon. There are numerous therapeutic applications for AI that have the potential to be used in the surgical context. AI will progressively play a significant role in surgical clinical practice (Mangano et al.,2020).

Way surgery steps were carried out in the operating room (OR) has undoubtedly changed as a result of the enormous and quick technological advancements that humans have made in the past ten years. The modern OR is a high-tech workplace that has integrated novel computational systems into the clinical workflow with the goal of streamlining procedures and assisting the surgical team. AI is becoming more and more crucial for surgical decision-making in order to address a variety of information sources, including patient risk factors, disease natural history, anatomy, patient values, and cost, and help surgeons and patients predict the outcomes of surgical decisions more accurately (Dias et al.,2020).

The history of electronic health and artificial intelligence in surgery in the United States, as well as the application of AI in robotics to examine the benefits and cost-effectiveness of such strategies used in pre-operative, intra-operative, and post-operative care circumstances, can be traced back to a French patient who virtually underwent a laparoscopic cholecystectomy with a New York surgeon in the year 1996 (Zemmar et al.,2020).Also DL, increased during the Coronovirus 2019 pandemic, according to a research by Salman et al. in Washington, where surgical departments used the idea to minimize human touch (Salman et al.,2020).In an effort to replace human surgeons in treatments involving animals, John Hopkins University students developed the smart tissue autonomous robot (STAR), which incorporated algorithms oriented toward doing an even better job than they could. Furthermore, according to researchers, the use of AI in surgical operations is limited and that in order to facilitate proper integration, all surgeons must approach the subject with a healthy skepticism (Hashimoto et al.,2020).

As the key players in the use of AI-based technologies for surgical care, surgeons should search for opportunities to work with data scientists to gather innovative forms of clinical data and aid in developing meaningful interpretations of that data (Weber et al.,2014).Engineers can offer automated, computational solutions to data analytics challenges that would otherwise be too expensive or time-consuming for manual procedures. Engineers also have the clinical experience to help data scientists and engineers answer the relevant questions with the right data (Hashimoto et al.,2018).

Artificial intelligence in vascular surgery

Cardiovascular clinical care is currently confronted with real-world difficulties such as cost reductions in prevention and treatment, low cost-effectiveness, overutilization, subpar patient care, high readmission and

mortality rates, and low cost-effectiveness (Krittanawong et al.,2017). AI's potential in medicine is just beginning to be explored. It is necessary to launch the earliest perception of AI and its prospective uses in vascular surgery as well (Raffort et al.,2020). The unique potential for AI to drive precision cardiovascular medicine is enabled by the ability to transform massive data into tools for cognitive computing, DL, and ML (Krittanawong et al.,2017). AI-based decision-making tools are used by a number of fields, including vascular surgery, to enhance clinical performance (Zarkowsky DS &Stonko DP, 2021).

Vascular surgery will place more emphasis on new digital technology. There are numerous potential applications. Endovascular procedures can benefit from simulation-based training since it can shorten procedure timeframes and enhance procedure-specific parameters. Radiation dosage reduction is also made possible by the use of intraoperative image-guided navigation and robots. Risk classification and individualized treatment plans are both possible with artificial intelligence. The usage of health apps can enhance patient follow-up treatment (Wolk et al.,2020).

Imaging is a crucial part of patient treatment in vascular surgery. Imaging enables the confirmation of the diagnosis, assessment of the prognosis, and choice of surgical surgery. Because veins exhibit a high degree of variation in morphology, size, and curvature, vascular segmentation is particularly difficult. AI approaches can be used to improve pattern recognition and image segmentation (Ali et al.,2022).

Cardiovascular imaging is likely to undergo significant changes as a result of data science. There are issues with timing, effectiveness, and missed diagnoses throughout the entire imaging chain. AI applications rely on reliable data, the use of suitable computational methods or tools, and the validation of their clinical use in automated measurement, image segmentation, and eventually automated diagnosis. AI may reduce costs and enhance value at the image capture, interpretation, and decision-making phases. Additionally, the accuracy provided by cardiovascular imaging, along with "big data" from pathology and the electronic health record, should help to better characterize disease and tailor treatment (Dey et al., 2019).

AI-assisted detection, diagnosis, and interpretation can make treating vascular disorders easier. The use of Computed Tomography Angiography (CTA) and Magnetic Resonance Angiography (MRA) images, which can be explained by AI strategies, can result in the development of a robust clinical decision-support system. For peripheral arterial occlusive diseases (PAOD)

and carotid artery stenosis, AI can identify the site of the occlusive or stenotic lesion, the length and degree of stenosis, plaque features, the formation of collaterals, inflow vessels, and the distal run off. AI can also indicate the degree of calcification, hemodynamically severe stenosis, and the existence of atherosclerosis (Ali et al.,2022).

With the aid of pictures of precisely selectable lesions, practitioners can aid in patient identification, diagnosis, and therapy planning. Computer-aided diagnosis (CAD) and clinical decision support systems can help the field of vascular surgery (Buschmann et al.,2018; Takahashi R & Kajikawa Y, 2017; van Ginneken et al.,2011).

AI may support in vascular surgery will be the driving factor in reduced morbidity and death, preventing handicap and reliance statuses in society (Rathore et al.,2016). By integrating AI into the present Vascular Surgery facility, the probability of improved patient management will be elevated. The healthcare facility's dependability will also increase. An key development in the education of future surgeons will be the convalescent's ability to adapt to contemporary technology from the start (Buschmann et al.,2018; Takahashi R & Kajikawa Y, 2017; van Ginneken et al.,2011; URL 3).

In addition, while AI has introduced innovative approaches to medical education and training, it can also be used to assess the abilities of healthcare professionals. AI offers the chance to track the behavior of the surgeon, physiological data, environmental data, or verbal communication using natural language processing. AI has been used to automatically grade the performance of trainees during a suturing task and evaluate surgical psychomotor abilities during laparoscopic procedures (Alonso et al.,2016; Oquendo et al.,2018).However, in a study from Turkey, it was determined that students' understanding of artificial intelligence was insufficient. Students' education may include more material on AI, which may have future uses (ÖntürkAkyüz et al.,2021). Young surgeons should be ready to incorporate AI into their future practices as it will undoubtedly influence medical education and training. Every patient is now a source of enormous data because to advancements in technology. International registries, like Vascunet, which has the support of the European Society for Vascular Surgery, are in the process of being created and will encourage cooperation and data exchange across many nations to enhance the caliber, security, and efficacy of vascular healthcare. The techniques for data collection, administration, and analysis will undoubtedly alter and revolutionize as a result of AI, allowing for the

potential of drawing conclusions from extremely huge datasets (Modarai B., 2019; URL 4).

Furthermore, computational methods like 3D printing have for the first time made it feasible to create artificial blood vessels and their networks with any complex form and connections. The printed vascular arteries can be employed in organ transplantation or as grafts to alleviate poor blood flow (Han et al.,2016).

Limitations Currently Existing and Future Directions

Although AI-derived technologies have interesting potential uses in both clinical practice and medical research, there are still a number of difficulties. The first technical issue is data-related. In order to train an efficient and reliable model, DL needs to have access to enormous amounts of data. The highly heterogeneous nature of medical data makes it possible for errors or omissions. They may also change over time and be difficult to interpret (Raffort et al.,2020).

Additionally, there is a great deal of variability in terms of quality, formats, resolutions, dimensions, and scales because data are produced by multiple vendors and maintained in numerous different registries. To create big, multicenter databases, a significant amount of standardization work is required. The privacy and security of data are additional problems to tackle for data sharing with the rise of AI in health models (Wang, F., &Preininger, A., 2019).

A wide range of medical issues that could benefit from DL's capabilities have not been thoroughly evaluated. DL has many features that could be useful in the healthcare industry, including its superior performance, end-to-end learning model with integrated feature learning, ability to handle complex and multi-modality data, and more. The DL research community as a whole needs to address a number of issues related to the characteristics of health care data (i.e., sparse, noisy, heterogeneous, and time-dependent), as well as the need for improved techniques and tools that allow DL to interface with clinical decision support workflows (Raffort et al.,2020).The algorithms don't provide any evidence of a causal link. Major obstacles to algorithms' implementation in clinical practice include their accountability and the verifiability of the analysis they provide (Cabitza et al.,2017).Not least of all, developing AI for medical applications needs a coordinated environment with specific platforms and infrastructures that have sufficient computing power. Future therapeutic applications of AI-derived technologies will require both

political will and financial backing to build out such technological infrastructure (Vuong et al.,2019).

Due to variations in data analysis, DL is superior to ML. Data is inputted into a model in machine learning (ML), which labels the data, finds features, and finally produces an output. For instance, photographs of carotid vessels can be input, classified as carotid, and then specific features (such as carotid atheromatous plaques) recognized to produce a classification of symptoms or asymptomatic conditions based on the images (Shiraishi et al., 2011).

CONCLUSION

The management and analysis of medical data, the creation of expert systems for forecasting and making decisions, or the evolution of equipment are just a few of the numerous potential uses of AI in medicine that can be used in vascular surgery. It might be applied in a variety of contexts, including as patient care, surgical education and training, healthcare information and surveillance systems, research, or the development of evidence-based medicine. Although AI holds a lot of promise, there are still numerous obstacles to overcome and mysteries to uncover beneath the "algorithmic iceberg." In order to direct and assist data scientists and industrials in developing pertinent applications and ensure a safe and suitable usage in clinical practice, the involvement of surgeons and medical experts in these technological advances is of the utmost importance.

The use of AI methods in vascular surgery is showing encouraging signs of improvement in terms of clinical, organizational, and educational issues. AI-based technologies have demonstrated impressive effectiveness in enhancing preoperative patient assessment, assisting in decision-making, improving surgical performance, and streamlining operating room scheduling. However, there are still some issues with data supply, protection, and transparency, so more research and precise consensus guidelines are required to certify these technologies for everyday common practice.

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CHAPTER 14

THE IMPLEMENTATION OF BLOCKCHAIN TECHNOLOGIES FOR A FUTURE OF INTEGRATED AND SECURE HEALTHCARE

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INTRODUCTION

Famously known as the data structure behind the success of bitcoin, blockchain is a decentralized bookkeeping system that collectively validates and stores data of transactions; synchronized among its parties. Transactional information or data is stored in blocks that are then appended onto the blockchain. With all parties having access to the same chain of blocks and the ability to add to it, whilst consensus and validation processes are done to ensure data integrity.

With its use immediately gaining popularity in the financial sector, a lot of its usages are still mainly adapted to financial transactions. However, with greater understanding and recognition of blockchain technologies' functionality and transparency as a decentralized network, many are gearing towards an economic reform of using blockchain technology in every aspect of the economy. It is believed that blockchain 3.0, a more information-security oriented application that is used in non-financial sectors such as justice, healthcare, and other governmental institutions is inevitable [8].

One of the movements in which blockchain technology can be utilized is in realizing and improving the health information exchange (HIE) models. The HIE is a push for electronic data exchange mechanisms amongst healthcare entities, for the purpose of improving care coordination and reducing medical errors [1]. Various models are in use to realize these efforts: direct model, query-based model, and patient-centered exchange model [1]. However, the models mentioned are considered inefficient and insecure due to the nature of its centralized dependency. Given that, blockchain technology has recently been proposed to overcome these challenges in order to gain patients' trust to support HIE for an optimized healthcare system.

This paper reports a review with the purpose of discussing the implementation of blockchain technologies in healthcare and the main components to a blockchain framework that plays a role in the efforts of pushing forward the health information exchange. As well as a short discussion on the potential for blockchain integrated health care to be implemented into premature medical systems of low to middle-income economies.

The Blockchain

With the recent, 2009, outbreak of the redeeming cryptocurrency, Bitcoin, an escalating interest in blockchain technologies came about. But what *is* blockchain really? And how does it work?

The foundations of blockchain technology lie in the concept of a decentralized database, by which no single entity holds control over and rather is collectively owned, controlled and monitored by all its users. Its nature of a synchronized and decentralized database means that every user within the network has access to the same permanent and transparent chain of blocks that record arrays of transactions or input. These 'blocks' are made up of the data itself and a header, which contains the previous block's hash value and a hash value specific to that block's contents and predecessor block hash value. This specific and sensitive hash value generation makes it very difficult to tamper with old data and transactions as data alteration changes the hash value of a block. Therefore, causing a discrepancy within the chain, where a block's hash value does not correlate with its proceeding blocks. There are two main divides in blockchain approach, the permissionless and permissioned, the two differ in means of validation, network accessibility, etc.

Permissionless blockchain networks are typically open-source software, available for anyone. This type of network allows all users to read and write into the chain. Because of this more open and loose user access, more rigorous and cumbersome means of validation when writing onto the chain, in order to prevent unwanted malicious users. This process involves there being an agreement or consensus protocol. Some of the derived consensus models include proof of work and proof of stake [9]. These are oftentimes motivated by providing monetary incentives or losses to ensure non-malicious behaviour.

Permissioned or federated blockchain modelnetworks, appropriately named, require users to request for permission or authorization from an entity in order to gain access to the ledger. This type of network does not require an as complex consensus as permissionless networks, because identities are known thus malicious behaviour brings no incentive and is easily traceable [9].

Methods

Research papers published between 2018 and 2020 surrounding themes of blockchain technology, its applications in healthcare, and medical record sharing, as well as patient responses to the concept, were searched and collected. Each paper was categorized by its corresponding theme, then its main ideas and findings were highlighted. Together, these ideas were extrapolated to answer the questions mentioned above which are further elaborated below:

The push for health information exchange

How blockchain technologies can be able to serve as a secure system by which medical record data can become an open-source network and be easily transferred amongst institutions, may it be research or healthcare entities.

Implementation in developing countries' healthcare systems

A brief outlook on the potential benefits of the implementation of blockchain technologies in underdeveloped medical systems of low to middle income countries.

Results

A total of five papers were selected, three of them focused on its own blockchain architecture. The components that were mutually significant for the purpose of healthcare integration were identified and later discussed. Two other papers focused on the response and evaluation of blockchain in healthcare were also selected to understand the market's reaction towards blockchain implementation in healthcare.

Architecture

A Blockchain Framework for Patient-Centered Health Records and Exchange (HealthChain) [4]

- Permissioned, Smart contracts, proxy re-encryption
- The proposed HealthChain patient-centered health record and exchange framework operate under a patient-centric framework. It provides users with a view of their medical records and serves as interface bridging patients and other parties such as hospitals and insurance companies. With the hope of its service and security being enough incentive for patients to continuously

Secure and Trustable Electronic Medical Records Sharing using Blockchain [1]

- Permissioned, smart contracts
- There are three main purposes for blockchain use in healthcare:
- Primary patient care - access all your medical information at any hospital. Focus typically on institution-based, which implies a network between institutions or caregivers and patients can access

their data on their respective nodes. Or case-specific which is used to connect involved parties and ensure transparency during a patients' treatment (especially long term).

- Data aggregation for medical research - use of blockchain ensures patient privacy and provides means of traceability of data source and thus transparency.
- better patient care - a network of healthcare entities responsible for the patient care, such as hospitals, insurance companies and pharmacies
- An oncology-specific framework, that is composed of three parts, membership service, databases (off-chain) and nodes managing consensus.
- ***Enabling trust in healthcare data exchange with a federated blockchain-based architecture [6]***
- Permissioned, smart contracts
- The blockchain serves as a door to the off-chain information. Operations within the blockchain are executed via a series of smart contracts
- Its architecture was built upon the means for automated processes, data traceability, decentralization, auditability and GDPR compliance, and trust

Evaluation and response [10]

Benefits of Blockchain Initiatives for Value-Based Care: Proposed Framework

- Built a framework for the means to evaluate the financial and non-financial performance of value-based care supported by innovative technologies such as blockchain.
- Points of evaluation:
  - Financial - how it increases revenue and reduce costs
  - Customer - how it provides easy access to records, value for money, provide customer satisfaction
  - Internal - how it is able to improve internal processes, delivering accurate medical services
  - External - how it helps distributed transaction systems with smart contracts to ensure security and privacy matters, interoperable systems with external parties to ensure reduced costs.

- Learning and innovation - how it allows for system growth and improvement, evolve and innovate.

The Potential of Blockchain Technology for Health Information Exchange: Experimental Study From Patients' Perspectives [2]

- Patients play a crucial role in health information exchange by providing the necessary consent. Thus, identifying and building public support is necessary as efforts without so, will fail in providing healthcare providers with reliable, useful, and integrated health information as a result of unspirited providing of complete and updated data.
- Health information exchange scenarios were set: 4 models - 2 policy strengths - 2 types of information = 16 scenarios
- Results indicate favorable attitudes towards the implementation of blockchain-based exchange mechanisms for privacy, coordination and information exchange.

DISCUSSION

Secure health information exchange

As highlighted by Dubovitskaya, Xu, Ryu, Schumacher, and Wang, the three identified purposes of blockchain implementation are in primary patient care, data aggregation for medical research and to increase connectivity between the stakeholders of healthcare. Stemming from issues within said categories; most of them rooted in the sensitive nature of medical records and data, thus the careful evaluation of access to records, creating a time-consuming process of accessing data. As well as reluctant data sharing due to patient trust issues in the means of exchange [1]. Successful implementation of blockchain technology for these three purposes can alleviate that lack of trust by creating a heavily encrypted and cross-validated ecosystem in which data is shared and viewed. With blockchain operating as a decentralized ledger, its security, and privacy are optimal for healthcare information exchange to serve the purposes mentioned above. Based on the papers found some of the consistently present attributes within the proposed frameworks and its contribution to making blockchain technology a viable means for healthcare information exchange were identified.

Decentralization: When defining the concept of blockchain, its pivotal feature lies in its elaborate decentralization of data authority. This concept lays the very foundation for blockchain technology. With data being collaboratively owned and users having the power to add data once

collectively approved. This reduces the risk of malicious or unintentional data tampering that is commonly found in centralized data structures in which single entities have power over massive amounts of data. The concept of the blockchain having a distributed 'power' or control over a database gives a sense of levelled responsibility and at the same time a structure resilient to cyber-attacks. Additionally, distributed ownership means that in order to effectively tamper with the data, access or control over the majority (>=51%) of the network nodes is required. Making it especially difficult if not near impossible in overtaking massive blockchains.

Permissioned: Amongst the types of blockchain models, the permission or federated blockchain model is more often observed in proposed healthcare implementations [6, 2, 4]. As previously mentioned, a permissioned blockchain model is a network where only trusted parties such as hospitals and research institutions have power to manipulate the blockchain. It is still a decentralized network; however, it is one open to identified and affiliated parties. In the framework found in reference [4], within their hyperledger, it was mentioned that there exists a membership service consensus. This category manages identity, privacy and confidentiality on the network. It functions to differentiate user roles and defines the functionality of the chain code available to the user. It proposes to crosscheck registering clinician/practitioners to the available national database, strengthening the validity of members and reducing the potential for malicious users. Each registered member is given a public and private key for signing and encryption and patients are given an additional symmetric encryption key that is made to encrypt/decrypt data corresponding to a patient. Similar to reference [2]'s proposed framework, where validation is extended to patients who must be validated by a consortium member in order to create an account. These consortium members are made up of trusted parties with the authority of blockchain manipulation. A required validation prior to joining the network, although is not necessary for a blockchain, can provide greater trust and assurance when utilizing the platform [4]. Additionally, because of the permissioned nature of the network, the tendency for malicious users is decreased as identification is easily traceable.

Proxy re-encryption (PRE): The use of proxy re-encryption (PRE) in securing private data when sharing decryptable files was used in Hylock and Zeng's blockchain framework, HealthChain. The PRE enables delegator to delegate decryption rights to a delegate through an intervening proxy. Protecting the sender's private key as delegates decrypt the sent proxy re-

encrypted form of the original message which is encrypted using the proxy's private key. This method can be utilized to secure patient data and identities. Moreover, following the advancements in quantum computing, the PRE's elliptic curve cryptography is proposed to be updated by implementing PRE using lattices. With the rapid growth in the technological field, continuous cryptosystem improvements are expected. Thus, staying up to date with its evolution is necessary to always guarantee patient privacy.

Efforts towards a blockchain integrated healthcare system through permissioned blockchain models along with implementations of smart contracts and identity encryption mechanisms aim to empower patients by having better control over their own data, easing and making transparent cross-hospital or institution data transfer and usage. By providing a safe means of data transfer, evident in blockchain models, comes trust and willingness of patients to share their data [1] as its mechanisms are proven safe and secure. As a result of proving secure data control and transfer; evaluation of willingly provided patient data of a blockchain integrated healthcare system would allow for the building of a more value-for-money-oriented healthcare service that aims for the highest ratio of quality to cost of medical services [10]. Creating a cost-efficient healthcare ecosystem whilst providing optimum care.

Low to middle income countries' healthcare reform

Ultimately the governmental provision of quality and accessible universal healthcare in sovereign states has been a standard for many years, however not realized for some, mainly in low to middle-income countries (LMICs)[5]. One of the main hindrances for citizens in LMICs is the high % of out-of-pocket expenditure, 48% as of 2014, needed in health financing [7]. In addition to that, some issues internal the healthcare care system include underqualified, underpaid and unmotivated staff as well as inadequate drugs and medical supplies [7]. Mills also mentions the inadequacies within the management of various parties within the health sector. These include its overly centralized system, inadequate regulation of private sectors adjunct to the healthcare system, weak incentives to improve service quality and speed. And last but not least the flaws within the governments themselves, corruption. According to the pooled hindrances and inadequacies in the healthcare system of low to middle-income countries, a permissioned blockchain healthcare system geared towards providing value for money services seems to be a suitable solution.

With a blockchain system, a decentralized, immutable and transparent system is built. Some examples of its effects include in situations given poor management and unfavorable government representatives, implementing blockchain into the healthcare system distributes their control, reducing malicious or corrupt tendencies. Thus, moving forward financially as potential leaks are being monitored and identities traceable, reducing losses [10]. Another example would be creating smart contracts with external private sector parties such as pharmaceutical and insurance companies, integrating and automating the processes of transactions. For LMICs, the ultimate goal is to achieve optimum value for money to be able to reduce 'out of pocket' expenses made by its citizens whilst providing quality care. This can be done by cooperation and balance between medical accuracy, integration and innovation.

Limitations

Negative perceptions of blockchain due to its association with many fraudulent cryptocurrencies as well as the lack of public understanding regarding its mechanisms can potentially slow-down the implementation of blockchain technology in fields outside of the financial one. Additionally, limitations and possible setbacks in its implementation, especially in LMICs, include however are not limited to the lack of monetary funds, lack of acceptance by governments and inability to grasp the intricacies of the system. Moreover, further feasibility studies of blockchain-healthcare integration in lower to middle-income countries can and should be done to more accurately determine its projected outcomes.

CONCLUSION

The future of blockchain integration into healthcare seems like a promising and necessary step to ultimately fulfil a secure means of healthcare information exchange. It is a fair barter, in order for patients to willingly provide their data, the healthcare sector must do their part in ensuring its security. Beyond information exchange, the nature of a permissioned blockchain with smart contracts and a strong cryptosystem along with a balance of financial, customer, internal, external and innovative evaluation would allow for the development and management of a value-for-money oriented healthcare services creating a cost-effective, integrated and secure healthcare system. With the various issues and limitations found in government healthcare availability and management in low to middle-income

countries, converting to a blockchain integrated healthcare system to push for value for value-for-money oriented medical service may be an executable solution.

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CHAPTER 15

COMMON BIOINFORMATICS TOOLS AND DATABASES IN MEDICAL MYCOLOGY

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INTRODUCTION

Bioinformatics is a field of applications that combines modern biology and medicine with the basic sciences of mathematics, computer science, physics, chemistry and data engineering. Over the past few decades, the need for databases and bioinformatics has basically been driven by large scale and huge data producing projects such as the human genome project (Watson, 1990) and following high throughput DNA sequencing based projects of other organisms (Turnbaugh et al, 2007). The complexity of analysis and interpretation of the increasing biological datasets have made bioinformatics an evolving discipline.

The developments in hardware and computer programming have a huge potential to make the theories and algorithms of basic sciences working more integrated and rapidly. At this point, bioinformatics could interpret the raw biological data about nucleic acid and protein sequences using statistical and computational techniques.

Bioinformatics uses many diverse algorithms from data mining, computer simulation, image processing to recently machine learning via artificial intelligence. Through the databases and algorithms, the field is able to store, retrieve, classify the data, and most importantly to predict and understand the molecular processes. Today, the major applications in the field consist of sequence and protein alignment, gene prediction, DNA-RNA-protein interactions, protein structure modeling, genome-wide evaluations, system biology, pathway analysis, drug-cell interaction, drug discovery and design, and so on.

Increasing knowledge about microbial communities was a complicated mission because of the broad diversity of microorganisms, and it was hard to discover most of the novel microbial groups via conventional techniques such as cultivation. Like discoveries in all biology sciences such as human genetics, next generation sequencing (NGS) technologies have also provided revolutionary data about the diversity of microorganisms. With the indispensable support of bioinformatics, most of the bottleneck-like challenges during developments in microbial genetics have been solved rapidly. Starting from the Human Microbiome Project (Turnbaugh et al, 2007; Integrative HMP [iHMP] Research Network Consortium,2019) with the support of NGS, improvements have continued to provide microbiological data not only about diversity of microorganisms, but also suggests new insight about their metabolic capabilities, microbiome dynamics, coevolution within the hosts, and host-pathogen interaction.

Medical mycology as a branch of clinical microbiology has also been evolving during the bioinformatics era. In this chapter, the main databases and bioinformatics tools used in medical mycology will be focused.

1. Towards the database need in medical mycology

From the last three decades, fungal infections have significantly increased. The infections due to the fungal species have a range from superficial to invasive infections that threaten the public health, especially for the increasing immunocompromised patients (Irinyi et al, 2015). About 1.7 billion people have been affected by superficial fungal infections. Moreover, today, invasive fungal infections cause a high mortality of more than 50%. Within the fungal groups, the genera of *Candida*, *Aspergillus*, and *Cryptococcus* are responsible for 90% of fungi-related deaths (Brown et al, 2012; Irinyi et al, 2015). Therefore, an urgent need has increased for high discriminating identification and rapid diagnosis especially for invasive fungal infections.

DNA barcoding and Sanger sequencing techniques revolutionized in identification and diagnosis. From the first proposal of DNA barcoding for biological identification (Hebert et al, 2003), the concept of species identification has prioritized culture independent, accurate, fast, cost-effective, and universal identification techniques. For this concept, in contrast to time-consuming and more expertise requiring conventional morphology and physiology dependent techniques, targeting DNA has been mostly accepted due to being more feasible and providing high quality resolution and information about fungal species (De Hoog et al, 2013).

The first sets of data convenient for databases about fungi were generated from genomic data. At this point, ribosomal DNA (rDNA) firstly became an unique genomic region due to have a significant discriminatory power and also common sequences through the fungi that enables rDNA targetable via some universal primers (e.g., ITS1, ITS4, etc.). Thanks to the pioneer techniques, the data of universal fungal genetic loci such as rDNA (e.g., the internal transcribed spacer [ITS], Large Subunit (LSU) of the rDNA), and protein-coding genes (e.g., translation elongation factor 1-α, RNA polymerase I and II, β-tubulin) began to be established via databases (De Hoog et al, 2019).

In time, the first generation of genomic datasets of Sanger sequencing have been mostly co-dominated by NGS data as second genomic datasets over the last decade. In addition, thanks to the development in high throughput

nucleic acid sequencing, databases have not only included DNA data, but also RNA and protein data. In addition to these mostly disconnected datasets, the novel datasets are now originating from the knowledge of intermolecular interactions. Together with the ever-increasing novel datasets of large scale projects from all around the world, databases have been growing and need to collect and share their data and systems by creating open-access and public computer networks for massive scale and more interactive data analysis.

2. Common public databases and web portals in medical mycology

Databases are required for data storage as an organized collection. Today, public databases mostly have collections from small to large scale researches including bidirectional data flow from both user to system, and system to user. Thus, the data always expands via computer networks, is assembled via algorithms, and becomes mostly open-access for data search and analysis.

Over the last decade, a large number of databases have been established for the usage in microbiology including medical mycology. Today, database infrastructures have been mostly organized on web-based platforms. As one of the largest scale infrastructures, the databases housed on National Center for Biotechnology Information (NCBI, www.ncbi.nlm.nih.gov) are basically related to biotechnology and biomedicine (Sayers et al, 2021). The NCBI platform works as various popular diverse modules, as well as under an integrated search module, Entrez system. The presented information through these databases are mainly classified under six categories including literature, genes, genomes, proteins, chemicals, and clinical (Sayers et al, 2022) (Table 1). Under these groups, Nucleotide, BLAST, Pubmed, Gene, Genome, Assembly, Popset, Protein, Taxonomy, Structure, PubChem are some of the most popular databases/tools of NCBI. Moreover, the system is also able to extract information from some external popular databases of Protein Data Bank (PDB, www.rcsb.org) and European Bioinformatics Institute (EMBL-EBI, https://www.ebi.ac.uk/).

According to the recent annual report of NCBI, the system contained about 3.6 billion records under the 35 diverse sets of databases (Sayers et al, 2022). From a search on the NCBI (Genetic database excluded; accession date:17/07/22) via a query using the words "fungi", "*Candida*" and "*Aspergillus*", the system was found to contain a total of 98 million, 2.5 million and 8.8 million records for the query words of fungi (in general),

Candida and *Aspergillus*, respectively. Thus, it seems that about 5.4% of all records on NCBI were related to fungi. Of these, *Candida* and *Aspergillus* composed 3% and 9% of the all records about fungi. Moreover, the majority of records were realized to come from nucleic acid and protein data within the 32 databases (Table 1).

Table 1. Description of NCBI databases and records related to medical mycology

Database	**Description**	**Records***		
Genomes		Fungi	Can	Asp
Nucleotide	DNA and RNA sequences	17880005	528839	1190803
BioSample	Biological source materials	743945	13365	10502
SRA	High-throughput DNA/RNA sequence read archive	456835	18506	11137
Taxonomy	Taxonomic classification	1	2	1
Assembly	Genome assembly information	12625	150	958
BioProject	Biological projects	36106	1553	3407
Genome	Genome sequencing projects	4140	55	190
BioCollections	Museum, herbaria, and biorepository collections	35	-	-
Genes		Fungi	Can	Asp
GEO Profiles	Gene expression and molecular abundance profiles	1126172	54244	67704
Gene	Collected information about gene loci	4681014	123075	659632
GEO DataSets	Functional genomics studies	217367	7223	4685
PopSet	Sequence	47087	2358	2888
HomoloGene	Homologous gene sets	9093	-	1
Proteins		Fungi	Can	Asp
Protein	Protein sequences	44516343	1198879	5344371
Identical Protein Groups	Protein sequences	25227803	397801	1417923
Protein Clusters	Sequence similarity-based protein clusters	-	-	-
Structure	Biomolecular structures	11953	618	1233

Protein Family Models	Conserved domain architectures	5048	224	403
Conserved Domains	Conserved protein domains	2183	46	94
Chemicals		Fungi	Can	Asp
PubChem Substance	Deposited substance and chemical information	12	73	263
PubChem Compound	Chemical information with structures, information and links	3	4	28
PubChem BioAssay	Bioactivity screening studies	12776	25766	12111
BioSystems	Molecular pathways with links to genes, proteins and chemicals	159	158	0
Literature		Fungi	Can	Asp
PubMed	Scientific and medical abstracts/citations	1968092	78802	56384
PubMed Central	Full-text journal articles	980947	111512	90169
NLM Catalog	Index of NLM collections	11011	248	108
Bookshelf	Books and reports	19016	1628	1054
MeSH	Ontology used for PubMed indexing	9	655	656

** Accession date: 17.07.2022. Can, Candida; Asp, Aspergillus*

From these remarkable numbers, NCBI databases are seen to pioneer the field by composing and providing an extensive amount of data about medical mycology. Because the system consists of approximately 20-fold more records than those about fungi, it may be possible over time to become obsolete in terms of quality of data. For instance, it was estimated that the Genbank database of NCBI could contain over 10% inaccurate ITS sequences (Nilsson et al, 2006). Therefore, some additional mycology-specific web user Interfaces (WUI) have been established by more expert teams of mycology including International Society for Human and Animal Mycology (ISHAM) Barcoding Database, The Westerdijk Fungal Biodiversity Institute, Saccharomyces Genome Database (SGD), Candida Genome Database (CGD), The Barcode of Life Data Systems (BOLD), Doctor Fungus, Mycology Online, Index Fungorum, FungiDB, MycoBank, Aspergillus Genome Database (AspGD), Fusarium Database, and so on (Prakash et al, 2017)**.** Many of these databases widely involve data of genes and genome, thus

presenting bioinformatics tools mostly about identification and taxonomy. Not all but some also include WGS data (Table 2).

ISHAM Barcoding Database (https://its.mycologylab.org/) focuses on mostly ITS sequence data, subsequently *TEF1α* gene sequences have also been integrated for especially polyphasic identification and taxonomy (Irinyi et al, 2016). Users can query their ITS and *TEF1α* sequences alone or in combination. In addition to this, International Fungal Multi Locus Sequence Typing Database (https://mlst.mycologylab.org/page/Home1) is another integrated database to make sequence analysis of seven to ten housekeeping genes as a Multilocus Sequence Typing (MLST)-based identification. The polyphasic identification module and database access of the Westerdijk Fungal Biodiversity Institute are more sophisticated. The Mycobank Polyphasic Identifications Databases housed on The Westerdijk Fungal Biodiversity Institute website (https://www.mycobank.org/page/Polyphasic_identification) provide species or group-restricted (e.g., yeast, *Fusarium*, dermatophytes, etc.) locus accession, MLST search, information of primers and the origin and features of strains as well as information of conventional techniques such as the minimum inhibitory concentrations (MICs) of the selected strains and media formulations for specific fungi groups (Crous et al, 2015). In comparison to the NCBI database, the above mentioned databases seem not to provide an open and self-employed submission system for independent researchers to upload their own sequence data. Thus, they are mostly contributed by more expert researchers of several institutes. This may provide more controllable and accurate data.

Thanks to the expanding further data about molecular cell biology of medically related fungal species, identification/phylogenetic analysis purpose databases above consequently have been broadened their collection covering more about gene functions, gene interactions, and metabolic pathways with the support of various known databases such as those of NCBI, EMBL-EBI and more. The Saccharomyces Genome Database (SGD, https://www.yeastgenome.org/) funded by US National Institutes of Health provides a huge amount of data either about usual genome sequence for identification/taxonomy, and also very deep and pure data of gene sequences and interactions, expression profiles, biochemical pathways related to *S. cerevisiae* (Skrzypek et al, 2015). The SGD retrieves data from numerous research articles and also from other comprehensive popular databases such as NCBI and EMBL-EBI modules including Uniprot (UniProt Consortium,

2019), (https://www.uniprot.org/), Interpro (http://www.ebi.ac.uk/interpro/), AlphaFold (Varadi et al, 2022; Jumper et al, 2021) (https://alphafold.ebi.ac.uk/) and EnsemblFungi (https://fungi.ensembl.org/tools.html). The Candida genome database (CGD, http://www.candidagenome.org/) that is related to SGD similarly provides a resource about gene, genome and protein information for primarily *C. albicans* and related species.

The clinically important fungal species have taken part in system biology related databases such as BioCyc, KEGG, Yeastrack+, and many others. In this context, the BioCyc (https://biocyc.org/) is one of the comprehensive data collections of genome databases and pathways for search and analysis tools (Karp et al, 2019). The BioCyc integrates genome data with metabolic and regulatory networks from nearly 20,000 databases. It provides bioinformatics tools about omics (e.g., transcriptomics, metabolomics) data analysis, comparative genomics and pathways through several organisms such as model eukaryotes and various microbes including bacteria, fungi and parasites. The BioCyc is a central portal for the system, and includes some sub-modules such as HumanCyc, MetaCyc, EcoCyc, YeastCyc, BsubCyc (specified on *Bacillus subtilis*), and more according to the examined organisms. Unfortunately, the system requires a paid license even for usage by research purposes for further analysis.

The Kyoto Encyclopedia of Genes and Genomes (KEGG, https://www.genome.jp/kegg/) as another comprehensive collections of genome, pathways and regulatory networks comprises main modules of genes and protein, pathway, chemical, disease, drug and medicus (Kanehisa and Goto, 2000). Thanks to the infrastructure, the system can collect records from distinct databases in a subject related manner. For instance, when one makes a search about *C. albicans*, the system presents related records under several categories such as gene, genome, 3D structure or domain of proteins, chemical substance and reaction.

The Yeastrack+ database (Monteiro et al, 2020) (http://yeastract-plus.org/) is a good example of a more specific collection for regulatory association analyses about target genes, transcription factors (TFs) and ontologie information between the pathogenic *Candida* species including *C. albicans*, *C. parapsilosis*, *C. glabrata*, *C. tropicalis* and *C. auris*. The records are mostly extracted from Gene Ontology (GO) (Gene Ontology Consortium, 2021; Kanehisa et al, 2000) (http://geneontology.org/) and previously mentioned databases of CGD and SGD. The Yeastrack+ database is organized

as four distinct, but interconnected databases of Yeastract (about *S. cerevisiae*; http://yeastract-plus.org/yeastract/scerevisiae/), PathoYeastract (Monteiro et al, 2020) (about pathogenic species of *Candida*) (http://yeastract-plus.org/pathoyeastract/), N.C. Yeastract (Godinho et al, 2021) (about non-conventional yeast such as *Yarrowia lipolytica*, *Kluyveromyces lactis*, *Kluyveromyces lactis*, *Kluyveromyces marxianus* and *Zygosaccharomyces bailii*) (http://yeastract-plus.org/ncyeastract/), and CommunityYeastract (Godinho et al, 2021; Oliveira et al, 2021) (about *Saccharomyces boulardii* and *Rhodotorula toruloides*) (http://yeastract-plus.org/community/). The Yeastrack+ database includes interactive modules for TF analysis via providing genes with promoter sequences, amino acid and DNA binding sequences of TFs, and via comparing TFs and promoter regions. The database also renders possible network analysis approaches via searching inter-species homologous network of genes.

Unlike others, the Expasy resource portal (https://www.expasy.org/) housed at the Swiss Institute of Bioinformatics provides a huge pool for the bioinformatics tools via presenting a brief introduction about selected tools and securely connecting to related web-site or open-source platforms (Wilkins et al, 1999; Schneider et al, 2004). A numerous bioinformatics tools are categorized as genes/genomes including genomics, metagenomics and transcriptomics; as evolution & phylogeny including evolution biology and population genetics; as structural biology including structural analysis, drug design and medicinal chemistry; and also categories as proteins and system biology including glycomics, lipidomics and metabolomics.

Some common public databases and web portals in medical mycology are listed in Table 2.

Table 2. Common online databases for medical mycology

Databases	Links	Type of records
National Center for Biotechnology Information (NCBI)	https://www.ncbi.nlm.nih.gov/	N, G, GN, T, WGD, P, Pst
International Society for Human and Animal Mycology (ISHAM-ITS)	http://its.mycologylab.org/	N, T
International Society for Human and Animal Mycology (ISHAM-	http://mlst.mycologylab.org/	M

Databases	Links	Type of records
MLST)		
Centraalbureau voor Schimmelcultures (Westerdijk Institute) Databases	https://www.mycobank.org/page/Polyphasic_identification	N, G, T
Saccharomyces Genome Database (SGD)	https://www.yeastgenome.org/	N, G, GN, T, WGD, Ps, Pw
Candida genome database (CGD)	http://www.candidagenome.org	N, G, GN, WGD
European Bioinformatics Institute (EMBL-EBI)	https://www.ebi.ac.uk/ http://www.ebi.ac.uk/interpro https://alphafold.ebi.ac.uk https://fungi.ensembl.org/tools.html	N, G, GN, P, Ps, Pt, Om, Ot
Broad Institute Databases	http://www.broadinstitute.org/scientific-community/data/	N, G, GN, T, WGD, P, Ps
FungiDB	http://fungidb.org/fungidb/	N, G, GN, Pt, Ot
Aspergillus and Aspergillosis Website	http://www.aspergillus.org.uk/	N, G, M
Fusarium Databases	https://fusarium.mycobank.org/ https://www.fusarium.org/	N, G
Kyoto Encyclopedia of Genes and Genomes (KEGG)	https://www.genome.jp/kegg/	GN, P, Ps, Pt, Om, Ch, Ot
BioCyc *YeastCyc*	https://biocyc.org/ https://yeast.biocyc.org/	GN, P, Ps, Pt, Om, Ch, Ot
Yeastrack+	http://yeastract-plus.org/ http://yeastract-plus.org/pathoyeastract	G, GN, WGD, P, Pt, TF

N, Nucleotide sequence; G, Gene; GN, Genome; M, MLST data; T, Taxonomy; P, Protein sequence; WGD, Whole genome data; Ps, Protein structure; Pt, Pathway data; TF, Data of transcription factors; Om, Omics data; Ch, Chemical data; Ot, Others

3. Bioinformatics in NGS data analysis

New generation sequence techniques have created a new perspective in medical mycology. NGS data has been commonly used for amplicon and whole-genome sequencing targeting both DNA and RNA. NGS data of DNA is mostly used for high-throughput amplicon sequencing such as microbiome analysis, and whole genome sequencing whereas NGS data of RNA is used for whole-transcriptome analysis via total RNA sequencing (Goodwin et al, 2016). NGS techniques provide high-resolution gigabase (gb)-lengths sequencing outputs. Therefore, all NGS platforms (Illimuna, Ion-Torrent, Pacific-Bioscience, Nanopore, etc.) have to provide broader database systems and tools for further NGS raw-data analysis. Nevertheless, NGS data analysis needs more expertise in comparison to Sanger-based sequencing. Some popular databases such as NCBI have integrated NGS data to their systems for public usage. The NCBI platform provides both raw NGS data and tools for comparison analysis of short-sequence queries. With the Assembly module on the system (Kitts et al, 2016), one could make a BLAST analysis and primer design throughout the gigabytes of whole genome data. In addition, in the BLAST module (Boratyn et al, 2019) one can perform comparison in the NGS data such as whole genome shotgun contigs, transcriptome shotgun assembly and high throughput genomic sequences. However, web-based interfaces such as the above systems suggest no common platform yet for processing raw NGS data, gene/protein mining or inter-species genome-wide comparison analysis. At this point, NGS tools go beyond the scope of Web User Interfaces (WUI) and enter the edges of more complex interfaces of Graphical User Interface (GUI), Command Line Interface (CLI) and Application Programming Interface (API) (Baker et al, 2020) that may be hard to make analysis by non-PC specialists such as clinicians or wet lab researchers. Some of these non-WUI software packages can generally operate on high performance personal computers or alternatively on comprehensive cloud computing platforms such as Google (https://cloud.google.com/), Amazon Web Services(https://pages.awscloud.com/AWS-Innovators-Amazon.html), and Microsoft Azure (https://azure.microsoft.com/en-us/get-started/azure-portal/). Open-source and more specialistic non-WUI tools for computational molecular biology have gained importance in the expanding database era.

4. Non-WUI software packages for data analysis

Non-WUI analysis tools are mostly distributed as GUI-, CLI-, API-based systems. Unlike the more user-friendly WUI-based tools, especially CLI -based tools mostly require special skills for not only usage of tools, but also the related operating systems and programming languages. Related to some genomic analysis such as sequence analysis and alignment, mutation and restriction profile analysis, evolutionary genetics analysis, vector design, and so on, the software of the MEGA, Bioedit, SnapGene or Vector NTI can be given as most popular GUI–based programs that are compatible with some or all operating systems (OS) of Windows, Mac and Linux. Besides these mostly simple and free or trial versions of the bioinformatics tools, Schrödinger platform (https://www.schrodinger.com/platform) is an example of paid GUI– and CLI–based and more complicated physics-based software for discovery of novel drug candidates. The Schrödinger platform enables analysis such as docking, molecular dynamics, generation of small chemical libraries and virtual screening for drug discovery.

The comprehensive GUI-based bioinformatics tools of Windows OS are not mostly freeware in contrast to those of Linux OS. However, in Linux OS, developers largely prefer CLI-based software that requires more skills for writing code during analysis preparation. Besides the used OS, the existing different code languages is another challenge of CLI-based software. Despite the relatively complexity, CLI-based software seems to dominate the bioinformatics field.

One of the exhaustive portals for code collaboration of CLI developers is Microsoft's Git-Hub platform (https://github.com/). The platform is freely open for individual users that have accounts. The Git-Hub platform mostly includes a wide variety of CLI-based bioinformatics tools coded with several languages such as Python, R, Jupyter Notebook, HTML, Shell, JavaScript, C++, and more. According to the languages, installation and application codes become variable. The Git-Hub platform now encloses about 12.000, 2000 and 400 repositories about genomics, proteomics and system biology, respectively (Access date: 20/07/2022). Because the per-application on the platform is not able to work properly equally, the best working ones could be filtered according to sorting choices. The open source project platforms of Bitbucket, GitLab, SourceForge, Google Cloud Source Repositories, AWS Code Commit and Phabricator can be given as alternatives to the Git-Hub platform. Some of the Python and R based programs on Git-

Hub are also deposited on Anaconda Cloud (https://anaconda.org/) that presents more pure packages for scientific computing.

CONCLUSION

In this chapter, some common databases and bioinformatic tools primarily related to medical mycology were aimed to be introduced. The bioinformatics field is precisely more than the reviewed due to the interdisciplinary nature. Herein, the suggested collection of databases and tools for use in medical mycology were selected according to some basic criteria such as data scale related to fungi, ease of use, open-accessibility, sustainability and accuracy of data. The importance of databases and bioinformatics tools has been increasing in health sciences including medical mycology. Ever-growing databases and accelerated computer systems will make it possible to apply a more accurate bioinformatics analysis from quaternary protein structure prediction based on sequence data to drug design, and to better understand more complicated biological processes.

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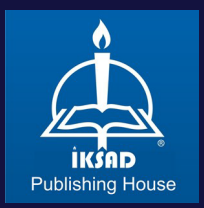

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