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Forensic Medical Perspective on Child Brides and Child Marriages

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Review

History

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ABSTRACT

There are many definitions on the concept of "child" from past to present. According to the generally accepted view, individuals under the age of 18 are considered as "children" and marriages before the age of 18 are considered as "child marriages", while girls married before the age of 18 are considered as "child brides". The reality of child brides continues to increase as a global problem in our country and in many countries of the world. Early marriages performed without the informed consent of the child are a common form of sexual abuse of girls. Sexual abuse at a young age can cause many negative effects in the later stages of a child's life.
The problem of child brides is one of the most important social problems that need to be solved in our country. In approaching this problem, first of all, a common language should be created between legal regulations and institutions, and laws should be reorganized to ensure they do not leave the door open for marriages at a young age.
In this study, it is aimed to reveal the awareness of the issue of "child brides" and "child marriages", which continue to be a serious social problem in our country and all over the world, and to determine the necessary solution suggestions for the solution of the problem in the light of the literature.

Keywords: Child marriages, Early marriages, Child brides, Child abuse

Çocuk Gelinler ve Çocuk Evlilikleri Gerçeğine Adli Tıbbi Bakış

Derleme

Süreç

Geliş: 09/07/2024
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ÖZET

"Çocuk" kavramı üzerine geçmişten günümüze yapılan birçok tanım mevcuttur. Genel kabul gören görüşe göre; 18 yaş altı bireyler "çocuk" ve 18 yaşından erken yapılan evlilikler ise "çocuk evlilikleri" olarak değerlendirilirken 18 yaşından önce evlenen kız çocukları ise "çocuk gelinler" olarak karşımıza çıkmaktadır. Çocuk gelinler gerçeği ülkemizde ve dünyanın birçok ülkesinde küresel bir sorun halinde artarak devam etmektedir. Çocuğun bilinçli rızası olmaksızın gerçekleştirilen erken evlilikler kız çocuklarında görülen cinsel istismarın sık görülen şeklidir. Küçük yaşlarda maruz kalınan cinsel istismar çocuğun yaşamının ileriki dönemlerinde pek çok olumsuz etkiye neden olabilmektedir.
Çocuk gelinler sorunu, ülkemizde çözüm üretilmesi gereken önemli toplumsal sorunların başında gelmektedir. Bu soruna yaklaşımda öncelikle yasal mevzuat ve kurumlar arasında ortak bir dil oluşturulmalı, yasalar çocuk yaşta yapılan evliliklere açık kapı bırakmayacak şekilde tekrar düzenlenmelidir.
Bu çalışmada; ülkemizde ve tüm dünyada ciddi bir toplumsal problem olarak süregiden "çocuk gelinler" ve "çocuk evlilikleri" konusunun farkındalığını ortaya koymak ve literatür ışığında sorunun çözümü için gerekli çözüm önerileri belirlemek amaçlanmıştır.

Anahtar Kelimeler: Çocuk evlilikleri, Erken evlilikler, Çocuk gelinler, Çocuk istismarı

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Introduction

In its most basic definition, a child is defined as an individual who lives from birth to adulthood, but it is also defined as an individual who is physically, mentally and socially immature. In addition to the fact that there are many different definitions of the concepts of child and childhood, it is a controversial issue as to what age limits the childhood period covers.[1]

Entered into force in 1990, the United Nations Convention on the Rights of the Child, which is the human rights document ratified by the largest number of countries in a short period of time, is defined in Article 1 as "Every human being shall be deemed to be a child until the age of eighteen years, except when he or she attains majority at an earlier age according to the law applicable to the child." In Turkey, the concept of child is defined as "a person who has not yet attained the age of eighteen, even if he/she attains majority at an earlier age" in the Child Protection Law (CPL) adopted on July 3, 2005 and as "a person who has not yet attained the age of eighteen" in the Turkish Penal Code (TPC) No. 5237. According to Article 11 of the Turkish Civil Code (TCC), "Adulthood begins with the completion of 18 years of age." Individuals under the age of 18 are considered as children. Based on these definitions, the common opinion is that individuals under the age of 18 are children.[2]

Although there are various definitions of the concepts of "child" and "early marriage", the generally accepted view is that individuals under the age of 18 are considered "children" and marriages before the age of 18 are considered "early marriages".[3] The United Nations Children's Emergency Fund (UNICEF) defined child marriages as "marriages that take place before the age of 18 and before they are physically, physiologically and psychologically ready to bear the responsibility of marriage and childbearing".[2] Girls who marry before the age of 18 are "child brides", which has become a major problem in the society.

Early marriages are usually forced marriages that are performed without the consent of the child and by family decisions. These marriages performed without the informed consent of the child are a type of violation of human rights and children's rights and are a common form of sexual abuse seen in girls. Child marriages are also a major problem because they are socially associated with sexual abuse.[3,4]

Child Brides in the World

Although many studies have been carried out in the world on child marriages, which closely concern all segments of the society, such as the Universal Declaration of Human Rights, the Convention on the Elimination of All Forms of Discrimination against Women and the Convention on the Rights of the Child, child marriages are still a global problem in many countries of the world.[5]

In the report published by UNICEF on May 3, 2023, it was stated that according to current global figures, there are an estimated 640 million child brides worldwide.[6] The problem of child brides is widespread in many parts of the world, especially in regions with low levels of development such as Sub-Saharan Africa, North Africa, Central and Southeast Asia, Latin America and the Caribbean.[7,8] While Nigeria with 75%, Central African Republic 68%, Chad 68%, Bangladesh 66%, Guinea 63%, Mozambique 56% are the countries with the highest rates of child marriages, our country ranks second among European countries after Georgia with a rate of 14%.[9] Countries with high rates of early marriage have unequal consent laws for girls and boys, which reinforces the idea that it is appropriate for girls to marry early.[8,10] In patriarchal societies with underdeveloped educational and economic levels and traditional patterns, early marriages continue in perpetuity. In these societies, women and girls are turned into objects of sexual and economic exploitation.[5]

There are different rules on the legal age of marriage and minimum age of marriage around the world. While the official age of marriage is 18 in most countries, there are provisions that allow children under 18 to marry with the consent of their parents or the judiciary, leading to contradictions in practice. In Saudi Arabia and Yemen, there is no minimum age of marriage, while in Lebanon the minimum age of marriage is 9 for girls and 13 for boys. In Iran, the minimum age of marriage is 13 for girls and 15 for boys. In the United States, child marriage laws vary widely between states. In states other than Delaware and New Jersey, children under the age of 18 may be allowed to marry with parental or judicial consent. Delaware became the first state to ban marriages under the age of 18 without exception in 2018 by requiring children to reach the age of 18 to marry. New Jersey followed shortly after. In Norway in 2003 and in the UK in 2023, changes were made to the legal procedure to prevent child marriages. With these changes, the legal age of marriage was raised to 18, and children aged 16 and 17 were prohibited from marrying even with the consent of their parents.[5,11-13]

Child Brides in Turkey

Child marriages, which occur at high rates in underdeveloped countries, also occur at high rates in developing countries. The actual frequency of child marriages cannot be determined due to unofficial and unregistered marriages. Although official data on marriage in our country include only marriages performed with an official ceremony, even these data show that child marriages have a high rate among all marriages.[5,14]

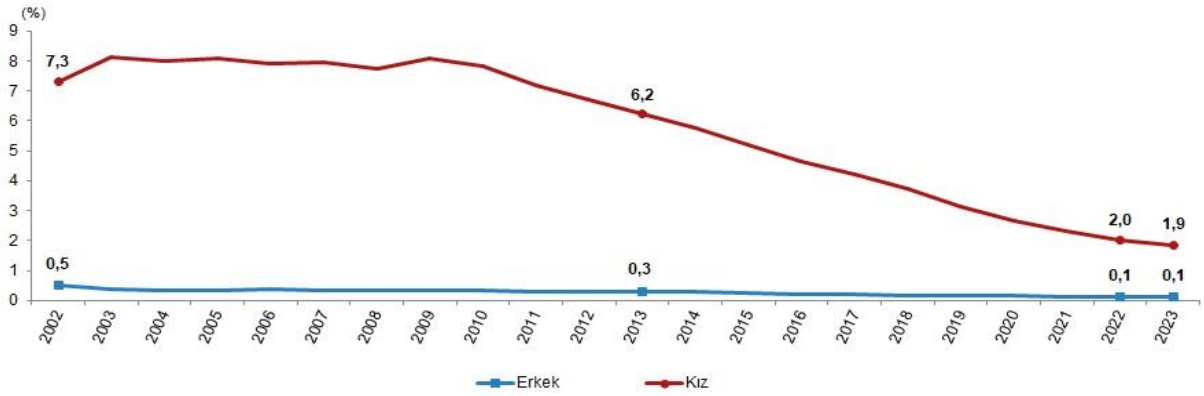
In our country where child marriages are observed at high rates, according to the results of Hacettepe University Institute of Population Studies Turkey Demographic and Health Survey (TNSA), 15.2% in 1998, 11.9% in 2003 and 9.6% in 2008 were observed in the 15-19 age group. When the statistics are analyzed, it is seen that the general trend is towards a decrease in marriages between the ages of 15-19.[15]

Table 1. Marital Status at Age 15-19 [5]

	Single	Married	Divorced	TOTAL
Male	3.194.698	15.543	102	3.210.343
Female	2.832.889	216.810	1604	3.051.303
TOTAL	6.027.587	232.353	1706	6.261.646

Table 2. Number of children married in the 16-17 age group by gender [16]

Year	Number of Married Boy Child	Number of Married Girl Child
2002	2.592	37.263
2023	706	10.471



Graph 1. Proportion of child marriages in total marriages by sex in 2002 and 2023 [16]

According to 2010 data from the Turkish Statistical Institute (TUIK), there are 6,261,646 children between the ages of 15 and 19 in Turkey, of which 3,210,343 are boys and 3,051,303 are girls. When the marital status of these children is analyzed, it is seen that there is a remarkable difference of approximately 14 times between the number of married girls and married boys.[5]

An analysis of the number of boys and girls married at the age of 16-17 according to TurkStat statistics for 2023 shows that there is a significant decrease in the total number of children married at the age of 16-17, while there is a 15-fold difference between the genders in 2002 and 2023. According to official data, the proportion of girls married at the age of 16-17 in total marriages decreased from 7.3% in 2002 to 1.9% in 2023. As can be seen in the available statistics, the problem of child marriages is concentrated on girls, who are numerically much higher.[16]

In the evaluation by provinces, it was reported that the provinces with the highest rate of girl child marriages in 2018 were Ağrı 14.8%, Muş 14.1% and Bitlis 12.5%, while the provinces with the lowest rates were Bolu 0.7%, Trabzon 0.9% and Artvin 0.9%.[17]

When the findings of a study conducted with a total of 5,036 women in 26 provinces in Turkey are examined, it is seen that the idea that child marriages are common only in Eastern Anatolia and South Eastern Anatolia regions is not correct. Although child marriages are observed at higher rates in these regions, it has become a problem that we encounter all over Turkey.[17] The reality of child brides continues to exist as a fundamental problem in our country and in the world.[18]

Legal Regulations on Child Brides in Turkey

With the January 1, 2002 Turkish Civil Code, the age of marriage, which was previously seventeen for men and fifteen for women, has been set at seventeen for both sexes by regulating the discrimination between the sexes. Accordingly, Article 124 of the TCC reads as follows: “A man or a woman cannot marry until they reach the age of seventeen. However, in extraordinary cases and for a very important reason, the judge may allow a man or woman who has reached the age of sixteen to marry. Whenever possible, the parents or guardian shall be heard before the decision.”[19] According to this article, it is not possible to marry minors under the age of seventeen, even with the consent of their parents, except in extraordinary circumstances. Generally, in extraordinary cases such as pregnancy or having a child, girls who have reached the age of sixteen may be allowed to marry a judge's decision.[5]

When the TPC No. 5237 is analyzed in terms of child marriages, articles related to sexual neglect and abuse of children are observed. Sexual offenses are defined in the TPC and the definition of "sexual abuse" is used when these offenses are committed against children, while the term "sexual assault" is used when these offenses are committed against adults.[5]

Article 103 of the TPC under the heading "Sexual abuse of children";

1. "The person who sexually abuses a child shall be punished with imprisonment from three to eight years. Sexual abuse is defined as

a. All kinds of sexual behavior against children who have not completed the age of fifteen or who have completed the age of fifteen but whose ability to perceive the legal meaning and consequences of the act has not developed,
b. Sexual acts committed against other children only on the basis of force, threat, deception or any other reason affecting the will are understood."

Article 103/2. regulates the crime of qualified sexual abuse, Articles 103/3. and 103/4. regulate the aggravating circumstances of the crime, and Articles 103/5. and 103/6. regulate the aggravation of the crime due to its consequences.[20]

In our society, it can be observed that families marry off girls under the age of fifteen. When this situation occurs, judicial action is taken against the defendant, the defendant's mother and father and the victim's mother and father.[15]

Article 104/1 of the TPC titled "Sexual intercourse with a minor" reads as follows: "A person who has sexual intercourse with a child who has completed the age of fifteen without force, threat or deception shall be sentenced to imprisonment from two years to five years upon complaint." Article 104/2 states that if the crime is committed by persons who are prohibited from marrying the victim, and Article 104/3 states that if the crime is committed by persons under the obligation of protection, care and supervision, it will be punished without a complaint.[20] This article defines sexual intercourse with a minor as an independent crime. However, the spouse who informally marries a child over the age of fifteen and has sexual intercourse with this child will not be punished unless the victim files a complaint.[5]

Article 3 of the Law No. 5395 on CPL is defined as "a child is a person who has not attained the age of eighteen, even if he/she becomes an adult at an earlier age".[21] Although the age limit of eighteen stated in the definition of child in this article is in line with international agreements, it contradicts with other laws in our country.[5]

According to the aforementioned articles in the Turkish legal system, the definition of child marriages and child brides varies across laws. Girls under the age of 15 are considered child brides in the TPC, girls under the age of 17 are considered child brides in the TCC, and girls under the age of 18 are considered child brides in the CPL. In our country, there are contradictions between laws in terms of the concept of "child bride".[15]

Causes of Child Marriage

There are various reasons why child marriages, which are an important health problem and a violation of human rights, continue. The incompatibility in the legal regulations on the subject is one of these reasons. When we look at the laws in force in our country, although children under the age of 18 are considered as children, marriages with children over the age of 15 are subject to the condition of 'complaint', and marriages with children over the age of 16 are officially permitted. This situation is one of the important reasons for child marriages between the ages of 15 and 18.[22]

In the May 2010 report of the Turkish Grand National Assembly (TBMM) Commission on Equal Opportunities for Women and Men on early marriages, the causes and consequences of child marriages are presented in detail. In this report, the underlying causes of child marriages are categorized as traditions, customs, lack of education, domestic violence, misperception of religious beliefs, social pressure and the language used, socio-economic reasons, traditional marriage types such as cradle to cradle and property.[15]

Studies on the subject show that there is a direct proportion between family poverty and the incidence of child marriages. In some families with low-income levels, girls are seen as a financial burden and therefore they are married off at an early age for reasons such as reducing this burden and earning money from bride price.[15] Some families also think that the marriage of their daughters will provide their salvation because of the economic difficulties they experience.[5] It has been observed that families with good economic status do not tend to marry their children at an early age in rural areas.[15] Early marriages are more common in families with poor sociocultural structure and low education levels. It is observed that there is a direct proportion between the level of education and the age at marriage. According to the Turkey Family Structure Survey (TAYA) conducted by the Ministry of Family and Social Policies in 2006, the rate of marriage under the age of 18 was 48% among illiterate individuals, while it was found to be 6% among those with undergraduate and graduate education.[18]

Traditional practices are another reason for early marriage of girls. Traditional practices such as berdel marriages, blood price marriages, cradle to cradle marriages and bride price practices, which are still observed in some segments of the society, lead to child marriages.[22] In berdel marriages, which are performed as an exchange of brides between two families, or cradle-kertmesi marriages, the marriage age of girls is not taken into account. In patriarchal societies, since the concept of honor is interpreted through women, it is thought that the risk of protection of honor will be eliminated by marrying girls at an early age.[2] In patriarchal societies, child marriages are normalized and legitimized. As a result, gender inequality is reinforced and life choices of girls are reduced.[5]

Table 3. Consequences of Early Marriage[15]

Medical Consequences	Psychosocial Consequences
Mothers	**Mothers**
Inadequate body weight gain	Failure to attend educational institutions
Obesity, excessive increase in body weight	Limitations in social activities
Preeclampsia	Loss of business opportunities
Anemia	Poverty
Sexually transmitted infections	Divorce and separation
Head-pelvis discrepancy	Social isolation
Severe hemorrhages	Stress/depression
Postpartum problems	Substance abuse
Frequent pregnancy	Frequent pregnancy
Deterioration of general well-being	
Maternal mortality	
Babies	**Babies**
Low birth weight	Development retardation
Premature birth	Behavioral disorders/substance abuse
Sudden infant death syndrome	School failure and dropout
Acute infections	Unemployment/poverty
Accidents	Unintended pregnancy
Infant mortality	

Consequences of Child Marriage

Early and forced marriages in adolescence, a period of physical, psychological and social growth and maturation, cause painful consequences for children and society in

The report of the TBMM Commission on Equal Opportunities for Women and Men on early marriages presents a table on the consequences of early marriage (Table 3). The consequences for the girls themselves and their babies, who appear as child brides, are categorized in medical and psychosocial terms. As seen in Table 3, child marriage causes many problems for both groups. Another striking point is that lack of education and poverty, which are among the leading psychosocial consequences of child marriage, are the main causes of child brides. Therefore, improvements in education and economic areas will be the key to solving the problem of child brides.[2]

Lack of education is both a cause and a consequence of child marriages. The rate of child marriages is higher among parents and families with low levels of education. Education is the main factor in preventing child marriages. In societies where education is not given sufficient importance, it becomes impossible to prevent child marriages. The education of children who are married at an early age is interrupted [(15)]. Since children whose education is interrupted and who are left ignorant cannot be expected to raise well-educated and conscious children in the future, early marriages cause generations-long educational problems and the continuity of similar traditions.[5]

Girls' education is interrupted by early marriages and as a result, they are prevented from having a profession, participating in production and enjoying their right to work. This situation then causes many problems. Women and girls who cannot have economic freedom are trapped in a cycle of lack of education, lack of money and dependency.[5] Inequality between women and men in society is also reinforced.[22] general. Over time, the problems seen in children due to early marriages turn from being a problem that concerns only children to a social problem and appear as heavy blows to the society. Child marriages lead to problems in many areas such as health, education and social life.[5]

Early marriages cause unwanted pregnancies and early motherhood for girls who do not have sufficient knowledge about family planning. Child brides, who have not yet completed their psychosocial development and whose bodies are not physiologically ready for childbearing, even if they have reached the level of physical development, especially in terms of reproduction, often experience difficulties before and after birth if they become pregnant. With early pregnancies and births, the individual who is herself a 'child' is also obliged to bear the responsibility of motherhood. Early pregnancies also pose a danger for mother and baby.[18,22] All adolescent pregnancies are considered a medically risky group. It is known that girls who give birth between the ages of 15 and 19 have a much higher risk of dying during delivery compared to women in their early 20s.[15]

Girls who marry early are at higher risk of intimate partner violence compared to adult women. Research shows an association between neglect, physical, emotional and sexual abuse in early childhood and poor health outcomes in adulthood. Girls who marry early report higher levels of depression and are at increased risk of somatic illness. Adults who were abused in childhood are more likely to repeat the cycle of violence and have higher rates of family dysfunction and mental health disorders. Studies have shown that child brides who are married off at an early age fail to develop physically, emotionally and spiritually, and in the following years they fall short in many areas and are unable to overcome the devastation. Child brides also develop a sense of worthlessness as a deeper problem due to marriage performed without their consent.[23-25]

By marrying off girls who have not completed their psychosocial development, children may experience problems in their social identities. With early marriages, children are expected to acquire domestic roles for which they do not yet feel ready.[5] In addition to the responsibilities imposed on the individual with early marriages and early motherhood, they are isolated and disconnected from their social environment as a result of the restrictions imposed by the society for married women. This situation may prevent children from acquiring social skills acquired during adolescence and healthy identity formation. It is a controversial issue how happy girls who are exposed to many restrictions by the society and their husbands, who cannot acquire social skills adequately and who cannot complete their social identity development will be in their marriages.[22]

Child Brides through the Perspective of Child Abuse

The World Health Organization defines child abuse as "behaviors done knowingly or unknowingly by an adult that negatively affect the child's health, physical and psycho-social development". Child abuse can be classified as physical abuse, sexual abuse, emotional abuse and neglect. These different types of child abuse appear as a social problem affecting all segments of society.[26,27]

Sexual abuse is the use of a child who has not yet completed psychosocial development by an adult for sexual stimulation. Since sexual abuse is the most difficult type of abuse to detect among the types of child abuse, it usually remains hidden.[26,28] Cases of sexual abuse are observed in all age and socioeconomic groups. It has been reported that sexual abuse is mostly experienced for the first time in children between the ages of 8-12.[29] Sexual abuse experienced at a young age may cause physical, psychosocial and behavioral negative effects in the later periods of the child's life. Early marriage of children is the most common form of sexual abuse in girls.[22,27] Exposure to physical, emotional, verbal and sexual violence can be observed frequently in girls who are married at an early age.[18]

Forensic Medical Approach to Child Abuse Cases

All situations in which the intent, negligence, imprudence or carelessness of another person/persons causes a person to become physically or mentally ill, and therefore involves a criminal element and is foreseen to take place in a trial process are considered as "forensic cases".[30]

Physicians frequently encounter forensic cases during their duties. Physicians have an obligation to report these forensic cases. Physicians who encounter a forensic case must report the crime to the competent authorities within the scope of Article 280 of the TPC. Accordingly, in the fight against the crime of neglect and abuse against children, the health professionals who have the slightest suspicion that the crime has been committed should report the situation to the competent authorities without delay.[31]

Articles 102-105 of the TPC No. 5237 deal with sexual offenses. Article 102 of the TPC includes sexual crimes against adults under the title of crimes against sexual immunity (sexual assault), Article 103 includes sexual abuse crimes against children, Article 104 includes sexual intercourse with minors (between the ages of 15-18) and Article 105 includes sexual harassment crimes.[29] As required by these articles of law, it is among the obligations of forensic physicians to interview the victim, collect biological evidence of the crime, determine the findings related to the crime by examining the victim and report them to the judiciary.[31]

The permission of the judicial unit (prosecutor or judge) is required for the internal body examination of cases of alleged sexual assault/abuse. In forensic medicine practices in our country, acute period examinations of cases subjected to sexual assault and sexual abuse are performed in hospitals affiliated to the Ministry of Health, University hospitals and Group Directorates and Branch Directorates affiliated to the Forensic Medicine Institute. Chronic period examinations of these cases are mostly performed at the 6th Specialized Board of the Istanbul Forensic Medicine Institute. The evaluation of sexual assault/abuse cases is a complex process involving history, physical examination, genital examination, treatment and rehabilitation. Since mental effects can be observed in cases of sexual abuse and assault in addition to physical and genital findings, a multidisciplinary approach including physical and mental health evaluation of the victim and laboratory examinations should be applied in such cases.[32,33]

Solution Suggestions

The problem of child brides is one of the most important social problems that our country needs to find solutions for.[5] Correction of this situation in our country, which has a very high rate of child brides compared to many developed countries, is extremely essential. Examining how developed countries tackle this issue may be enlightening for finding solutions in our country. For example, in the UK, which has a child bride rate of only 1.7%, various policies are implemented to further reduce this problem. In the UK, there is a 'Forced Marriage Unit' working to prevent forced marriages and this unit fights against forced child marriages.[22] In Norway, another developed country, some changes have been made in the legal procedure to prevent child marriages. In 2003, an article prohibiting forced marriage was added to the penal code and imprisonment of up to 6 years was deemed appropriate for forced marriages. In addition to this article, while marriages under the age of 18 were previously possible with the consent of the family, the provision that parents could no longer give consent for marriage was added to the child law to prevent such situations.[5]

Solutions should be sought as soon as possible to combat early and forced marriages. In approaching the problem of child marriages, a harmonized arrangement should be made between laws and institutions.[5] It is expected that the problem of early marriages will be reduced to a great extent with the implementation of legal regulations and severe penal sanctions in case of violation of prohibitions. Incompatibilities between the TCC No. 4721, the TPC No. 5237 and the CPL No. 5395 should be resolved. The laws should be reorganized in a way that does not leave the door open to child marriages. The deterrence of the relevant penalties in the TPC No. 5237 should be increased, and supervisory mechanisms should be established in the enforcement of the laws.[15]

Apart from increasing penalties, the most important step in the prevention of child marriages, is to ensure a change in the mentality of the society and to renew the patriarchal

perspective of the society.[17] Girls should be seen as a social "asset" rather than an economic "burden".[18] Awareness of child marriage should be raised in all segments of society.[17] Educational projects should be developed in schools, health centers and public education centers to raise awareness especially in rural areas. Written and visual media should be used to raise awareness against child marriage.[5]

As the level of education increases, the number of child marriages decreases. Especially in regions where child marriages are common, absenteeism of girls from education should be taken under control, teachers and school personnel should be given responsibility for ensuring attendance and inspections should be strictly implemented.[5] For children in formal education, issues such as the drawbacks of early marriages, maternal and child health and reproductive health should be adequately included in the curriculum. Due to the high number of illiterate women, trainings should be given to women in order to increase the literacy rate among women. In addition, in order to prevent early marriages, vocational training courses should be emphasized and opportunities should be provided for women to have a job.[15]

As a result, child marriage is a problem that prevents the exercise of human rights, reinforces inequality between men and women and deprives children of their basic rights such as education. These marriages are an area that should definitely be combated in terms of gender equality.[15] Child brides should be seen as a "serious problem" in the world and especially in developing countries like our country and effective solutions should be sought. Healthy and happy individuals will be realized as a result of the child undergoing a healthy socialization process, completing her/his education, getting to know herself/himself and making her/his own marriage decision.[17]

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Overview of Cardiorenal Syndrome

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Review

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ABSTRACT

Cardiorenal syndrome is a complex clinical condition affecting both the kidney and the heart. It is divided into 5 different subgroups according to various clinical features. However, in most clinical settings this is difficult to determine because the pathophysiology is complex, and the pathways are poorly understood. Given this complex clinical situation, many challenges arise in the management of both acute and chronic cardiorenal syndrome. In this review, the definition, classification, pathophysiology and treatment of cardiorenal syndrome were evaluated.

Keywords: Cardiorenal syndrome, kidney damage, heart failure

Kardiyorenal Sendroma Genel Bakış

Derleme

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ÖZET

Kardiyorenal sendrom hem böbrek hem kalbi etkileyen karmaşık bir klinik durumdur. Çeşitli klinik özelliklere göre 5 farklı alt gruba ayrılır. Ancak çoğu klinik ortamda patofizyolojinin iç içe geçmiş olması ve yolakların yeterince anlaşılmamış olması nedeniyle bunu belirlemek zordur. Bu karmaşık klinik durum göz önüne alındığında hem akut hem kronik kardiyorenal sendromun yönetiminde birçok zorluk ortaya çıkmaktadır. Bu derlemede, kardiyorenal sendromun tanımı, sınıflandırması, patofizyolojisi, tedavisi incelenmektedir.

Anahtar Kelimeler: Kardiyorenal sendrom, böbrek hasarı, kalp yetmezliği

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Introduction

The complex interdependent relationship between the kidney and the heart was described by Robert Bright in 1836. He identified distinct cardiac structural changes seen in those with severe kidney disease. Cardiovascular and kidney diseases have many common points of interaction. These are hemodynamic interactions between the heart and kidney in heart failure, the effects of atherosclerotic disease on both organ systems, neurohormonal activation, cytokines, biochemical perturbations along the anemia-inflammation-bone mineral axis in chronic kidney disease (CKD), and structural changes in the heart with progression of kidney disease. In addition, the term "cardiorenal syndrome" (CRS) encompasses a range of disorders involving the heart and kidneys in which acute or chronic dysfunction in one organ can lead to acute or chronic dysfunction in another organ.[1] This article focuses primarily on the definition, pathophysiology, and diagnostic and treatment strategies of CRS.

Definitions and Phenotypes

The National Heart, Lung, and Blood Institute Working Group defined CRS in 2004 as a condition in which interactions between the kidneys and the circulatory system increase circulating volume, worsening symptoms of heart failure (HF), and causing disease progression. The working group emphasized that severe cardiorenal derangement leads to CRS and that treatment of congestive HF in this setting is limited by the decline in renal function.[2] This cardiology-centered perspective plays a fundamental role in understanding CRS, especially in acute heart failure. The Acute Dialysis Quality Initiative (ADQI) summarized the approach in 2008, dividing CRS into 2 main groups, cardiorenal and renocardial syndromes, taking into account the triggering factor of the disease process. Accordingly, the disease was further divided into 5 subtypes based on the sequential involvement of organs and their severity, and these are summarized in Table 1.[3] Although the ADQI CRS Classification has overcome some of the initial ambiguities in the definition of CRS, determining the initial injury and subsequent events and understanding the processes leading to decompensation of acute or chronic CRS/renocardiac syndrome can be challenging.[4]

Pathophysiology

Part of the difficulty in identifying and treating CRS stems from the involvement of multiple complex pathophysiologic processes. The traditional explanation for the development of CRS focuses on the failure of the heart to produce adequate output, resulting in prerenal hypoperfusion.[1] The renin-angiotensin-aldosterone system (RAAS) plays an important role in the progression of renal damage and worsening of HF. Inadequate renal blood flow or perfusion pressure triggers renin release by the juxtaglomerular cells of the afferent arterioles via pressure-sensing baroreceptors in the ascending limb of the loop of Henle. Increased renin levels increase the production of angiotensin II (Ang II).[5] Ang II has various adverse effects on the heart, blood vessels, and kidneys. Ang II increases the filtration fraction by vasoconstricting the efferent arterioles in the kidney. It increases sodium reabsorption via aldosterone in the distal tubules. Ang II may lead to kidney damage by increasing the synthesis of endothelin 1, a potent vasoconstrictor and pro-inflammatory peptide.[6] Ang II causes transforming growth factor-β1 (TGF-β1) mediated hypertrophy in cardiac myocytes. It causes contraction of vascular smooth muscle on AT1 receptors. It also increases oxidative stress and inflammation. Left ventricular dysfunction in heart failure patients activates the sympathetic nervous system (SNS) to maintain perfusion. This results in increased contractility and systemic vasoconstriction. These mechanisms support perfusion in the short term but may exacerbate cardiac and renal dysfunction in the long term.[7] Elevated intra-abdominal pressure (IAP) can lead to intra-abdominal hypertension (IAH) and abdominal compartment syndrome in severe cases. IAP elevations are often seen as surgical complications.[8] In addition, it is increasingly common in the pathophysiology of CRS. IAP is high in 60% of patients with advanced chronic HF. While normal IAP values in healthy individuals are between 5-7 mmHg, IAP values between 8-12 mmHg in these patients are associated with kidney damage and this may lead to the development of Type 2 CRS.[9] HF causes volume overload and increased central venous pressure (CVP). Elevated venous pressures weaken the flow gradient in the renal circulation. This leads to congestion, glomerular dysfunction, and decreased urine output. Several studies have shown that elevated IAP results in decreased GFR and renal plasma flow, and an elevated CVP is significantly associated with decreased renal function.[10,11]

Pulmonary vascular resistance is in constant interplay with right ventricular function. In pulmonary hypertension, the stressed heart tries to balance pre-load and afterload to accommodate increased pulmonary vascular resistance. Resultant neurohormonal activation (endothelin, arginine vasopressin) leads to water and salt retention, worsening venous congestion, and further reduced cardiac output. This may cause a decrease in GFR.[1]

Anemia plays a major role in the pathophysiology of CRS. Failure to provide oxygen to an already stressed heart or a damaged kidney can cause ischemic damage that can result in progressive cell death in both organs. Red blood cells contain many antioxidants and therefore anemia can lead to increased oxidative stress.[12]

Table 1. CRS Classification

Phenotype	Naming	Definition	Clinical Examples
Type 1 CRS	Acute CRS	Heart failure leading to acute kidney injury (AKI)	Cardiogenic shock and AKI after acute coronary syndrome (ACS), AKI after acute heart failure (AHF)
Type 2 CRS	Chronic CRS	Chronic heart failure (CHF) leading to CKD	Chronic heart failure
Type 3 CRS	Acute Renocardiac Syndrome	AKI leading to AHF	Heart failure during AKI resulting from volume overload, inflammatory attack and metabolic disorders in uremia
Type 4 CRS	Chronic Renocardiac Syndrome	CKD leading to CHF	Left ventricular hypertrophy (LVH) and heart failure resulting from cardiomyopathy associated with CKD
Type 5 CRS	Secondary CRS	A systemic process leading to both heart and kidney failure	Amyloidosis, sepsis, cirrhosis

Biomarkers and Diagnosis

Biomarkers

Biomarkers contribute to the diagnosis of CRS. Cardiac biomarkers, B-type natriuretic peptide (BNP) and its inactive form, pro B-type natriuretic peptide (NT-proBNP), are helpful in the diagnosis and prognosis of both acute and chronic HF**.** BNP values are significantly higher in patients with acute HF without renal failure.[1] Studies have shown that better results are obtained in acute HF and NT-proBNP levels decrease in patients with decreased renal function after treatment. High NT-proBNP has been shown to contribute predictively to CRS risk stratification by BNP in patients with acute HF before the development of renal dysfunction.[13] High-sensitivity cardiac troponins I and T are established diagnostic and prognostic markers in acute myocardial infarction. Troponins increase with decreasing GFR, and a sustained elevation is associated with a higher risk of death.[14] In addition, suppressor of tumorigenicity 2 (ST2) measurements are valuable in predicting heart failure-related deaths and hospitalizations and are not affected by renal function.[15] Serum galectin-3 levels have also been shown to be independent predictors of cardiovascular mortality.[16]

Renal biomarkers, serum creatinine and changes in urine output are late signs of acute kidney injury, defining renal function. Cystatin C is a sensitive marker of GFR and has prognostic value as an indicator of hospitalization and mortality from acute heart failure.[17] Cystatin C, unlike creatinine, is less affected by age and non-renal factors.[18] Tubular damage markers include insulin-like growth factor binding protein 7 (IGFBP-7), tissue inhibitor of metalloproteinase-2 (TIMP-2), neutrophil gelatinase-associated lipocalin (NGAL), and kidney injury molecule-1 (KIM-1), but further studies are needed for these markers.[19,20]

Imaging Modalities

Non-invasive imaging modalities play an important role in detecting markers of venous congestion and forward flow impairment in CRS and are easily accessible clinical tools at the bedside. Echocardiography can help diagnose congestive status with hemodynamic parameters such as CVP, systolic pulmonary artery pressure, pulmonary capillary wedge pressure/left atrial pressure, and cardiac output (CO).[21] In addition to CVP, there are other useful echocardiographic measurements such as lateral and septal wall longitudinal motion (E') in relationto mitral in flow velocity (E). The E/E' ratio is directly related to the pulmonary capillary wedge pressure; E/E' >15 means that the pulmonary capillary wedge pressure is ≥18 mmHg.[22,23] Decreased left ventricular ejection fraction, increased pulmonary artery pressure, and larger right ventricular diameter have been in dependently associated with an increased incidence of CRS.[24] Renal ultrasonography and intrarenal venous flow patterns are emerging tools for determining renal venous congestion and its clinical significance in CRS. Other renal hemodynamic parameters, such as renal arterial resistive index and renal perfusion index, are not used as predictors of clinical outcomes in CRS, despite the ircorrelation with CVP, mean arterial pressures, and effective renal plasma flow.[25] Renal ultrasonography provides information on the chronicity of the disease by assessing renal size, echogenicity, cortical thickness, and abnormal corticomedullary ratios. This is useful in determining whether AKI or CKD is the primary disorder in the clinical presentation of CRS.[26]

Treatment

Due to the complex and heterogeneous pathophysiology of CRS, there are many difficulties in its treatment and method. The drugs used in the treatment of CRS have not been fully investigated in randomized controlled trials. Therefore, there is no consensus on the treatment strategies of CRS patients.[27] There are many drug groups and strategies used in the treatment of cardiorenal syndrome. Diuretics and ultrafiltration together with inotropic agents, beta-blockers (BB), angiotensin-converting enzyme inhibitors (ACEI), angiotensin receptor blockers (ARBs) or angiotensin receptor neprilysin inhibitors (ARNI), mineralocorticoid receptor antagonists (MRA) and sodium glucose transporter inhibitors (SGlT2i) are some of these drug groups.[28] Other treatment options include implantable defibrillator therapy (ICD) and cardiac resynchronization therapy (CRT).[29]

Decongestive Therapy

Acute management of the patient with venous congestion often focuses on rapid correction of hypervolemia to aid symptomatic relief. There are effective treatments that result in decongestion, but none have been found to improve survival or reduce disease progression.[30] Similarly, although sodium restriction is recommended to prevent hypervolemia, its positive effect has not been demonstrated.[31] Loop diuretics (furosemide, bumetanide, torasemide and ethacrynic acid) are the preferred diuretics in acute or chronic HF.[32] The duration of action of loop diuretics is short, lasting 2 to 3 hours and up to 6 hours for intravenous bolus and oral administration, respectively. Intravenous furosemide has greater bioavailability than oral furosemide.[33] Torasemide has a longer half-life and therefore requires less frequent dosing. It has been shown that torasemide may be more effective in decongestive treatment of HF compared to furosemide.[34] Although diuretic synergy is effective in patients with acute HF, its effect in CRS is a matter of debate. It may also cause further deterioration of renal function.[35] Deterioration of renal function in type 1 CRS leads to higher hospitalization rates and mortality.[36] However, studies with high and standard doses of loop diuretics have not shown any significant difference in their effects on renal function. However, high-dose loop diuretics have been shown to provide better symptomatic relief. This suggests that loop diuretics may not contribute to kidney damage and that a decrease in eGFR may be an indicator of the severity of heart disease.[30,37,38]

The effectiveness of diuretics in decongestive therapy decreases with increasing severity of HF.[39] Impaired absorption, decreased renal blood flow, azotemia, and proteinuria cause decreased diuretic concentrations in the tubular lumen, leading to diuretic resistance. Diuretic resistance can be defined as continuing congestion despite increasing diuretic doses equivalent to 80 mg/day furosemide, less than 0.2% sodium excretion; and failure to excrete 90 mmol sodium in the next 72 hours despite taking 160 mg furosemide twice daily. Clinically, inadequate improvement in patients' symptoms, increased mortality after discharge, and rehospitalization are indicators of diuretic resistance. Although some pharmacological agents have been used in diuretic resistance, they have not been successful in the long term.[40] Thiazide-type diuretics do not show sufficient efficacy in CRS. In addition, another diuretic group, potassium-sparing diuretics such as spironolactone, has been tried but has not been shown to be beneficial.[41]

If fluid overload persists despite the appropriate maximal use of pharmacological treatment tools and/or renal replacement therapy (RRT) is required due to uremic indications and electrolyte disturbances, patients may receive invasive decongestive treatments such as ultrafiltration (UF) and RRT.[42] Intrafiltration is a mechanical process that removes isotonic fluid and low molecular weight molecules from the circulation and eliminates hypervolemia without neurohormonal activation. Different studies have addressed the effectiveness of ultrafiltration in patients with CRS. The RAPID-CHF study found better outcomes in CRS patients using ultrafiltration instead of pharmacological treatment.[43] In the UNLOAD study, patients with acute HF who underwent ultrafiltration were associated with a lower readmission rate 90 days after hospital discharge, despite no improvement in renal function.[44] In contrast, the CARRESS-HF study examined type 1 CRS patients with renal dysfunction in a randomized controlled manner. In this study, patients who underwent UF were found to have more side effects and less weight loss than those who used diuretics.[42] This difference was thought to be related to the worse renal function of patients in the CARRESS-HF study. A large-scale meta-analysis emphasized that UF was more effective and safe in the treatment of CRS without worsening renal function compared to diuretic therapy.[45]

Inotropic and Vasodilator Therapy

In patients with type 1 CRS, the effects of inotropic agents and vasodilators to improve cardiac output and increase renal perfusion and provide diuresis may be beneficial. The most notable of these drugs are nitroglycerin and nesiritide. These two drugs have been shown to be more beneficial than inotropic agents such as dopamine and dobutamine.[28] Among inotropes, dopamine improves renal blood flow through its cardiac inotropic effect and its effects on β- and α-adrenergic receptors and renal dopaminergic receptors. Although some studies suggest a renal protective effect of low-dose dopamine in acute HF, a long-term benefit has not been demonstrated.[46] Few and sparse data are available on the use of other inotropes in CRS.[47]

Beta Blocker Treatment

BB's have been included in the first-line treatment of chronic HF because of their ability to improve HF prognosis and mortality. However, a direct benefit has not been proven in patients with acute decompensated HF or CRS.[27]

Renin Angiotensin Aldosterone Inhibitors Treatment

Inhibition of the renal angiotensin aldosterone system (RAAS) is the cornerstone of HF treatment. They are treatments that reduce mortality in HF. ACE inhibitors and ARBs have been shown to reduce mortality in HF and CRS, even in patients with severe renal impairment. However, close monitoring is recommended in these patients, especially for potassium levels.[28] Aliskiren, a direct renin inhibitor, has not been shown to be beneficial in improving hospitalization and mortality rates.[48]

In recent years, many studies have been conducted with sacubitril/valsartan, a combination of angiotensin receptor blocker and neprilysin inhibitor. ARNI caused less renal failure compared to other RAAS inhibitors. It also reduced mortality and hospitalization rates.[49,50]

Sodium Glucose Transporter-2 Therapy

SGLT-2 reabsorbs glucose and sodium in the proximal tubule of the kidney. Blockade of SGLT2 improves overall survival, improves cardiovascular outcome, and has clinical benefits by reducing HF hospitalizations and renal failure.[29,51] The use of SGLT2i has been recommended as first-line therapy in guidelines for heart failure as well as for diabetic kidney disease and other subtypes of proteinuric glomerular disease.[52]

New Therapeutic Approaches

There are different therapeutic approaches that are being tried and are being investigated in CRS. Studies with tolvaptan, a selective antagonist of the V2 arginine vasopressin receptor, have shown that this drug does not reduce the risk of cardiovascular events and HF-related hospitalizations.[53,54] However, it has been shown to provide cardiovascular benefits in patients with hyponatremia.[55] In recent years, activation of the erythropoietin receptor in the heart of patients with HF has attracted attention because activation of this receptor may play a protective role against apoptosis, fibrosis, and inflammation and may lead to improvement of cardiac structure and function.[56] Both improved cardiovascular mortality and modest improvement in renal function have been demonstrated in patients with gout treated with the uric acid-lowering agents allopurinol or probenecid. Probenecid has been shown to have inotropic properties and may be useful in HF as monotherapy or in combination with hydrochlorothiazide to increase diuresis.[56,57]

Implantable cardiac defibrillators (ICDs) are thought to be beneficial not only in HF but also in CRS.[29] However, it has been suggested that the effectiveness of these devices is reduced in patients with impaired renal function.[58] In addition to these approaches, there are many newly developed mechanical and non-pharmacological treatment methods. However, no clear benefits have been shown and further studies are needed for their development.[40]

Conclusion

CRS is a group of diseases that can be chronic or acute, affecting the kidney and heart. Venous congestion, low arterial perfusion, and neurohumoral activation affect both organs, and if appropriate treatment is not given, a vicious cycle begins. Given the complex pathophysiology of CRS, many challenges arise in the management of both acute and chronic CRS. There are proven treatments, but there are also promising new approaches. Better knowledge of the pathophysiology and treatment options of CRS and a multidisciplinary approach will reduce mortality and morbidity in these patients.

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Diagnosis and Management of Dissemine Intravascular Coagulation

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Review

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ABSTRACT

The primary objective of this review is to define disseminated intravascular coagulation (DIC), which is a complication associated with a range of traumatic and non-traumatic conditions, and to provide insights into its underlying pathophysiology. Additionally, this review aims to emphasise the importance of early recognition of DIC, particularly in cases of thrombotic or haemorrhagic complications, and to highlight the benefits of prompt anticoagulant, blood product, and cryoprecipitate treatment in improving prognosis.

Keywords: Dissemine intravascular coagulation, Thrombosis, Coagulation, Bleeding

Dissemine İntravasküler Koagulasyonun Tanı ve Yönetimi

Derleme

Süreç

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ÖZET

Bu derlemedeki öncelikli olarak amacımız birçok travmatik veya nontravmatik hastalıklara eşlik eden ve mortaliteyi arttıran dissemine intravasküler koagulasyonu tanımlayarak patofizyolojisi hakkında bilgi verip, erken dönemde tanınmasını sağlamak. Trombotik veya kanamalar ile seyreden dissemine intravasküler koagulasyonun erken dönemde tanınması antikoagulan veya kan ürünleri ve kriyopresipitat tedavisinin erken başlanması prognozu olumlu etkilemektedir.

Anahtar Kelimeler: Dissemine intravasküler koagulasyon, Tromboz, Koagulasyon, Kanama

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Introduction

The initial account of disseminated intravascular coagulation (DIC) was documented by Dupuy in 1834. He observed that animals died immediately following the intravenous administration of brain material and that autopsies revealed the presence of widespread intravascular clots.[1] Thirty years later, Trousseau identified a predisposition to thrombosis in advanced cancer patients.[2] In 1873, Naunyn demonstrated that intravascular clots frequently formed following the intravenous administration of a solution containing erythrocytes, and Wooldridge demonstrated that procoagulant factors were located in erythrocytes.[3–5] A definitive description and pathogenesis of DIC was made in 1955 by Ratnoff et al. in pregnant women and fetuses, who died as a result of amniotic fluid embolism. In 1961, Lasch and colleagues provided the first comprehensive explanation of how DIC might result in bleeding. Their work introduced the concept of consumptive coagulopathy, which remains a fundamental tenet of modern understanding of this condition.[6,7]

The International Society of Thrombosis and Haemostasis defined DIC as " a pathological process involving the systemic activation of the blood coagulation cascade, that ultimately results in microvascular thrombosis and simultaneous depletion of platelets and clotting factors, resulting in life-threatening bleeding".[8] Disseminated intravascular coagulation is observed in approximately 1% of patients admitted to hospital.[9] The incidence rate can be increased according to the underlying conditions. In the oncology population, the estimated incidence was 6.8% in patients with solid tumors, and 83% in patients with sepsis.[10,11]

Etiology and Classification

DIC is typically a secondary complication of underlying diseases. The primary causes of DIC are diverse and include infections, solid tumours, haematologic neoplasms, pregnancy, vascular disease, neonatal pathologies, traumatic or non-traumatic internal-external tissue damage, and chemical or biological agents.[12] Acute promyelocytic leukaemia is the most prevalent neoplastic disease seen in DIC.[13] Mucin-producing tumours, including stomach, ovary and pancreas cancers, play an important role in the development of DIC through the secretion of enzymes or necrotic tissue that can stimulate the coagulation cascade.[14] The emergence of cytokine release syndrome as an adverse event associated with the use of chimeric antigen receptor T cells for the treatment of haematological malignancies such as leukaemia or lymphoma has also been associated with DIC. It is hypothesised that procoagulant factors released as a consequence of endothelial inflammation are the underlying cause of DIC in this patient group. The likelihood of a patient developing DIC is directly correlated with the severity of cytokine release syndrome.[15]

Pathophysiology

Part In simple terms, the pathophysiology of DIC can be described as follows: depending on the underlying disease, procoagulant activation is so strong that a surplus of thrombin exerts excessive influence on anticoagulant regulatory mechanisms, such as protein C, antithrombin, and tissue factor pathway inhibitor, thereby overwhelming their capacity to maintain control. This permits the formation of thrombosis to occur at any point along the vessels.[16] In DIC, there is an inherent conflict between a heightened thrombin state, which clinically presents as microvascular occlusion by thrombosis, emboli, and fibrin thrombi, leading to a gradual failure of multiple organ systems from tissue ischaemia, and a haemorrhagic state can result due to the use of platelet and clotting factor, and/or accelerated plasmin generation.[17] Laboratory abnormalities in this condition, which can be described as 'consumption coagulopathy', include prolonged aPTT and PT/INR, decreased platelets and fibrinogen, and increased fibrin degradation products.

Acute Disseminated Intravascular Coagulation

Acute DIC is a condition characterised by consumptive coagulopathy, whereby the production of thrombin exceeds the natural anticoagulants levels in plasma. It is typically triggered by a significant release of tissue factor into the intravascular space, resulting in the widespread deposition of fibrin thrombi within small vessels. The subsequent is the development of multi-organ dysfunction. Multi-organ failure most commonly affects the lungs and kidneys. Other common sites included are the brain, heart, liver, spleen, adrenal glands, pancreas and gastrointestinal tract.[18,19]

Procoagulant effects of Thrombin (Figure 1):

1. Conversion of fibrinogen to fibrin
2. Activation of factors V, VIII and XI to promote further thrombin generation
3. Activation of factor XIII to promote fibrin cross-linking
4. Thrombocyte aggregation, which induces the clotting system to produce more thrombin.

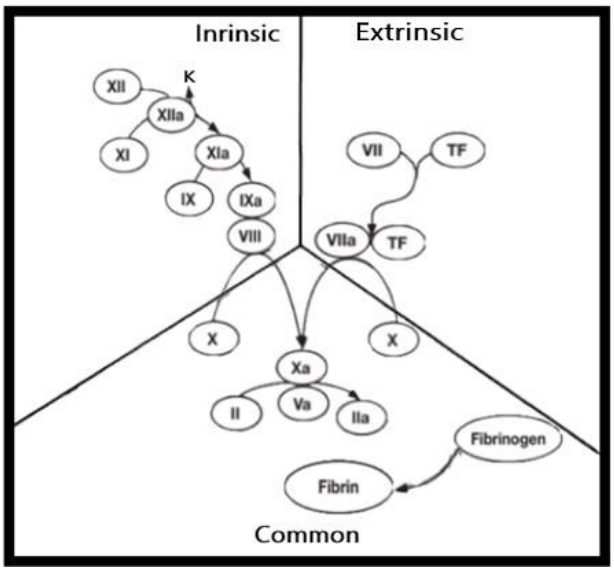

Figure 1. Coagulation cascade pathways

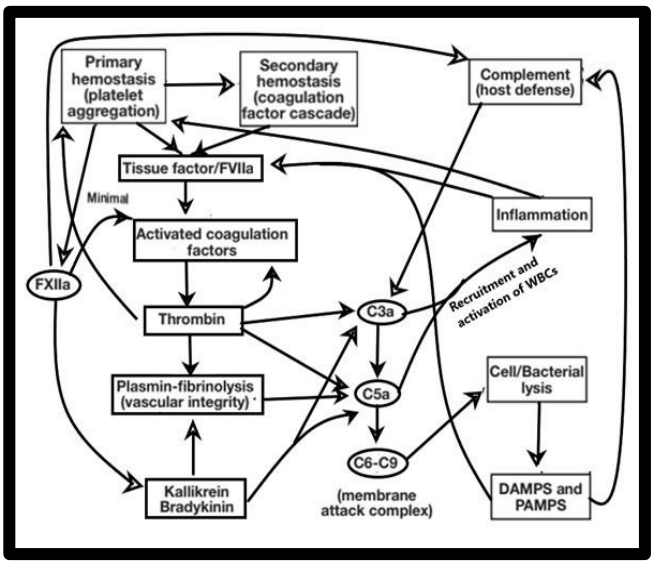

Figure 2. Primary, secondary hemostasis and response of complement system

The effects of thrombin result in the further activation of clotting factors, thereby producing an increased level of thrombin and, consequently, a greater number of fibrin clots. This fibrin thrombus are degraded by plasmin and are converted to fibrin degradation products. When present within the intravascular space, these fibrin degradation products have the potential to interact with the glycoprotein IIb/IIIa receptor, which is located on the surface of thrombocyte and fibrinogen, thereby inhibiting the polymerisation of fibrin and the aggregation of platelets.[17]

The fibrin degradation products, contribute to bleeding, the most common symptom observed in acute DIC, together with the consumption of platelets, fibrinogen and coagulation factors. Other conditions induced by thrombin increase the activity of the coagulation cascade. Thrombin stimulates protease-activated receptors on platelets and increases the intracellular interleukins (IL-1 and IL-6). Their release further enhances proinflammatory activity by increasing thrombocyte activation and leukocyte adherence.[13]

Thrombin is responsible for the release of plasminogen activator inhibitor-1 from endothelial cells and the subsequent activation of thrombin-activatable fibrinolysis inhibitor within the plasma. This ultimately results in a reduction in plasmin-mediated clot lysis. Additionally, DIC results in the decrease of antithrombin and the down-regulation of the Protein C system, thereby reducing the body's capacity to eliminate thrombin. Following the onset of multiorgan failure, the production of anti-thrombin by the liver is reduced. While anti-thrombin in the environment is degraded by enzymes released from neutrophils, this results in the continuation of DIC process. It is not uncommon for shock to occur in DIC. It has been observed that shock can precipitate DIC and may contribute to its prolonged course. This, in turn, effects the macrophages of the reticuloendothelial system clearance ability, for the synthesised tissue factor, activated coagulation factors and fibrin degradation products (Figure 2).[17]

As a consequence of the rapid depletion of platelets and clotting factors associated with acute DIC, the platelet count may be below 50,000/L at admission time, which occurs in 10-15% of cases, with PT and aPTT exhibiting marked prolongation, and D-dimers demonstrating elevated levels. [18, 20] The results of intrinsic and extrinsic (PT, aPTT) and common pathways, are indicative of the concentration of factors X, V, II and fibrinogen (see Figure 1). This is the "common" pathway for both extrinsic and intrinsic pathways, resulting in the activation of thrombin and the conversion of fibrinogen to fibrin. Additionally, Prothrombin level serves as an indicator of the factor VII concentration within the extrinsic way. In this pathway, factor VII is activated by tissue factor and then proceeds through the common pathway, leading to thrombin activation and fibrin clot formation. The aPTT level serves to reflect the levels of factors VIII, IX, XI and XII present within the intrinsic pathway. The intrinsic pathway is initiated by collagen or polyphosphate activating Factor XII, which subsequently activates the common pathway and forms a fibrin clot. The prolonged prothrombin time and activated partial thromboplastin time observed in DIC are a consequence of the consumption of clotting factors. Thrombin activity is increased in acute DIC, due to three factors: increased consumption of antithrombin, increased degradation of antithrombin by neutrophil

elastase release, and reduced synthesis of Antithrombin by the liver due to microvascular thrombosis. A reduction in protein C levels is also observed as a consequence of a decline in thrombomodulin levels, which is attributable to the delivery of tumour necrosis factor α, IL-1 and IL-6 as acute phase reactants. This, in turn, results in a diminution in thrombin inactivation and an enhancement in the formation of thrombosis.[16]

Diagnosis

There is no definitive biochemical marker to diagnose DIC. Diagnosis of DIC is a clinical and laboratory diagnosis based on laboratory findings of coagulopathy and/or fibrinolysis following an underlying clinical condition (e.g. sepsis, malignancy). According to the parameters in the algorithm defined by the International Society on Thrombosis and Haemostasis, following the modifications made to the algorithm by Taylor et al, DIC is diagnosed with 93% sensitivity and 97% specificity when scored 5 or more points (Table1).[21]

The Scientific Committee of the Japanese Association for Acute Medicine has proposed another algorithm for acute DIC. The scoring system is based on the presence/absence of systemic inflammatory response syndrome, degree of thrombocytopenia, amount of elevated fibrin degradation products and whether INR is above 1.2. (Table1).[22]

Management of DIC

Treatment of the primary disease

Treatment of DIC is a topic of ongoing debate within the medical community. However, a set of treatment guidelines has been published, which provide broadly similar recommendations. The primary objective of treatment should be to address the underlying disorder. If the underlying condition is effectively managed and treated, DIC has been observed to resolve spontaneously in a significant number of cases. Most guidelines agree on this point, despite the lack of high-quality evidence on the effectiveness of treating the underlying condition. However, early intervention may be necessary to prevent complications from haemorrhage and thrombosis during the treatment of the underlying disease.[23,24]

Transfusion therapy with platelets and fresh frozen plasma

Significantly low platelet counts, coagulation factors and in particular fibrinogen levels have been shown to be associated with an increased risk of bleeding. Current guidelines recommend that patients with disseminated intravascular coagulation who have active bleeding or are at high risk of bleeding and require invasive procedures should receive platelet concentrate and fresh frozen plasma. However, there is currently a lack of high-quality evidence to support this recommendation. The decision to transfuse platelets depends on the clinical signs. Generally platelet concentrate transfusion is indiacted to the patients with active bleeding and a platelet count below 50×10^9/l or non-bleeding DIC patients with a platelet count below 20×10^9/l. A transfusion of fresh frozen plasma is typically indicated in cases of massive or DIC bleeding also. The correction of coagulation defects associated with prolonged APTT or PT (more than 1.5 times the normal value) or decreased fibrinogen level (less than 1.5 g/dl) requires the administration of large volumes of fresh frozen plasma. It is recommended that an initial dose of 15-30 ml/kg of fresh frozen plasma be administered for treatment purposes.[23,25-27]

Administration of smaller volumes of prothrombin complex concentrate may be beneficial in cases where volume overload may potentially lead to complications in patients.

In cases of massive haemorrhage in DIC due to fibrinogen deficiency, purified fibrinogen concentrates or cryoprecipitate are recommended. The majority of prothrombin complex concentrates contain vitamin K-dependent factors (II, VII, IX and X) and anticoagulants like protein S, protein C and antithrombin. Nevertheless, they are deficient in crucial coagulation factors, such as FV. Vitamin K represents a useful alternative for the correction of vitamin K-dependent clotting factors; however, its impact will not be significant until more than six hours have elapsed.[28] The administration of fibrinogen, whether as a fibrinogen concentrate or cryoprecipitate, may be of particular importance in cases where fibrinogen is deficient. The aim is to maintain fibrinogen levels above 1.5g/dl in patients with bleeding.[24] However, for women with concurrent postpartum haemorrhage, a higher level (above 2.0g/dl) is recommended.[29] Giving 30 mg fibrinogen concentrate per kg bodyweight is associated with a 1 g/dl increase in fibrinogen.[30]

Heparin as Anticoagulan Therapy

Kongstad et al. reported that heparin treatment was beneficial in DIC and especially in early DIC. [31] A well-evaluated retrospective cohort study was conducted to investigate the efficacy of heparin and LMWH in patients with coronavirus. The results showed that those who failed to receive heparin were significantly more likely to die.[32]

Table 1. Dissemine intravascular coagulation diagnosis according to International Society on Thrombosis and Haemostasis and scientific committee of the Japanese Association for Acute Medicine

Parameters	Points	*International Society on Thrombosis and Haemostasis*	*Japanese Association for Acute Medicine*
		Laboratory result	Laboratory result
Platelet count	3		<80.000
			Reduction more than ≥50% in 24hour
	2	<50000	
	1	≥50000 - <100000	≥80000 - <120000
			Reduction more than ≥30% in 24hour
D-Dimer	3	Strong increase	≥25 µg/mL
	2	Moderate increase	
	1		≥10, <25 µg/mL
PT	2	≥6 sec	
	1	≥3sec, <6 sec	INR≥1.2
Fibrinogen	1	<100	
SIRS score	1		>3
SOFA score	2		
	1		
Total Score		≥5	≥4

In a further cohort study with a lower quality evaluation, mortality rates were found to be 83% in patients treated with heparin and 86% in those not treated with heparin. This suggests that there is no significant difference between the two groups. Nevertheless, in this study, the majority of patients developed multiple organ failure with DIC, and the number of cases was relatively low.[33]

A retrospective analysis of patients with sepsis who developed DIC in 2022 revealed that the administration of unfractionated heparin at prophylactic or therapeutic doses via subcutaneous or continuous intravenous infusion was associated with a reduction in 28-day mortality and hospital mortality rates, as well as a favourable safety profile, particularly in relation to intracranial and gastrointestinal bleeding.[34] A further retrospective analysis of a comprehensive database demonstrated that the early commencement of prophylactic doses of unfractionated heparin was linked to a reduction in hospital mortality.[35] In a randomised controlled study conducted by Jaimes et al. on patients with sepsis, 28-day mortality was examined, and no significant difference was found between the heparin-treated group and the control group. Similarly, Liu et al. observed that the administration of low-molecular-weight heparin had no impact on mortality rates (31.8% to 40%).[36, 37]

The International Consensus on Thrombosis and Haemostasis does not recommend thromboprophylaxis in patients with DIC who are bleeding or have a platelet count of less than $20x10^9$/L. For patients with acute promyelocytic leukaemia, thromboprophylaxis is indicated, with a lower threshold for platelet transfusion of 20x109/L.[38]

As bleeding is the predominant feature of obstetric disseminated intravascular coagulation (DIC) patients, it is advisable to initiate treatment in those with predominant thrombotic findings.[39] Patients diagnosed with DIC and presenting with purpura fulminans, acral ischaemia, or venous thromboembolism should be initiated on a therapeutic dose of heparin. [24,28]

Conclusion

Despite recent advances in understanding the pathogenesis of DIC, the prognosis for patients remains poor. Mortality rates are increasing, especially in cases where the diagnosis is made late. The lack of a definitive laboratory biomarker increases the importance of the physician in the diagnosis of DIC. In the early period after an underlying disease, confirmation of the diagnosis of DIC using scoring systems and early initiation of anticoagulant therapy for thrombotic complications and replacement therapy for bleeding will make a positive contribution to reducing mortality.

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Special types of breast cancer: Clinical, Histological Features and Survival Outcomes

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ABSTRACT

Objective: In this study, we investigated the clinical characteristics and survival outcomes of patients diagnosed with special types of breast cancer who presented to our clinic.
Methods: The demographic, clinicopathological, and survival characteristics of all rare, histologically special subtype breast cancer patients who applied to Sivas Cumhuriyet University Oncology Center between 2010 and 2020 were retrospectively reviewed.
Results: The records of 1198 patients with invasive breast cancer were examined, and 104 of them (8%) were identified as having other histological special subtypes. Of these, 19 (8%) had apocrine cancer, 19 (8%) had mucinous type, 17 (7%) had invasive cribriform, 15 (6%) had invasive papillary, 11 (4%) had metaplastic type, 9 (4%) had invasive micropapillary, 6 (2%) had neuroendocrine, 3 (1%) had tubular type, 3 (1%) had microinvasive type, and 2 (1%) had undifferentiated carcinoma. The majority of these patients, 102 (98%), were female, with a median age of 52 years (range 26-82). Of the women, 60 (59%) were postmenopausal, and 42 (41%) were premenopausal. The ECOG Performance Score (PS) was 0 in 79 (76%) patients, 1 in 17 (16%) patients, and 2 in 8 (8%) patients. Upon evaluation, 50 patients (48%) had comorbid conditions, and 26 patients (25%) had a family history of breast cancer. At diagnosis, 25 patients (24%) were stage I, 50 (48%) were stage II, 26 (25%) were stage III, and 3 (3%) were stage IV. Histopathologically, 75 patients (72%) were estrogen receptor (ER)-positive, 69 (66%) were progesterone receptor (PR)-positive, and 26 (25%) were HER2-positive. An intraductal component was detected in 54 (60%) patients, and multicentricity was observed in 15 (16%) patients. A modified radical mastectomy was performed on 56 (54%) patients, while breast-conserving surgery was performed on 45 (43%) patients. Adjuvant chemotherapy was administered to 76 (73%) patients, hormonal therapy to 73 (70%), and radiotherapy to 72 (68%). The median follow-up period was 54 months (range 1-201). During follow-up, metastasis was detected in 13 patients (13%), and recurrence was detected in 7 patients (7%). The 5-year and 10-year overall survival rates were 86% and 77%, respectively, while the 5-year and 10-year event-free survival rates were 79% and 70%, respectively.
Conclusion: In our study, the majority of patients with special type breast carcinoma were non-metastatic, and histopathologically, they were hormone receptor-positive with low grade. There was no statistically significant difference in 5-year and 10-year overall survival or event-free survival among the special types.

Keywords: Breast Cancer, Special types of breast cancer, overall survival, event-free survival

Özel Tip Meme Karsinomları: Klinik, Histolojik Özellikleri ve Sağkalım Sonuçları

Araştırma Makalesi

Süreç

Geliş: 16/10/2024
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ÖZET

Amaç: Bu çalışmada kliniğimize başvuran özel tip meme kanseri tanılı hastaların klinik özelliklerini ve sağkalım sonuçlarını araştırdık.
Yöntem: Sivas Cumhuriyet Üniversitesi Onkoloji Merkezi'ne 2010-2020 yılları arasında başvuran meme kanserli tüm nadir, histolojik olarak özel alt tip hastalarının demografik, klinikopatolojik ve sağkalım özellikleri retrospektif olarak incelenmiştir.
Bulgular: Çalışmada 1198 invaziv meme kanserli hastaların dosyaları incelenmiş ve bunlardan 104'ünün (8%) diğer histolojik özel alt tipinde olduğu tespit edilmiştir. Apokrin kanser 19 (8%), musinöz tip 19 (8%), invaziv kribriform 17 (%7), invaziv papiller 15 (%6), metaplastik tip 11 (%4), invaziv mikropapiller 9 (%4), nöroendokrin 6 (%2), tubuler tip 3 (%1), mikroinvaziv 3 (%1), undifferansiye 2 (%1) hastada saptanmıştır. Bu hastaların büyük bir kısmı 102 (98%)' si kadın olup median yaşı 52 (26-82) bulunmuştur. Kadınların 60 (%59)'u postmenopozal, 42 (41%)'isi de premenopozaldir. 79 (76%) hastanın ECOG Performans skoru (PS) 0, 17 (16%)'sinin ECOG PS 1, 8 (8%)'inin ECOG PS 2 olarak izlenmiştir. Hastalar sorgulandığında 50'sinde (48%) komorbid hastalıklar olduğu, 26'sinde (25%) ailede meme kanseri öyküsü olduğu görülmüştür. Tanıda 25 (24%) hastanın evre I, 50 (48%) hastanın evre II ve 26 (25%) hastanın evre III, 3 (%3) hastanın evre IV olduğu tespit edilmiştir. Histopatolojik değerlendirmelere göre hastaların 75'inde (72%) estrogen reseptörü (ER) pozitif, 69'ünde (66%) progesterone reseptörü (PR) pozitif, 26'sında (25%) HER2-pozitif olarak bulunmuştur. İntraduktal component 54 (60%) hastada tespit edilmiştir. Multisentrisite 15 (16%) hastada izlenmiştir. 56 (54%) hastaya modifiye radikal mastektomi, 45 (43%) hastaya meme koruyucu cerrahi uygulanmıştır. 76 (73%) hastaya adjuvant kemoterapi, 73 (70%) hastaya hormonterapi ve 72 (68%) hastaya radyoterapi verilmiştir. Medyan takip 54 (1-201) ay olup takipte 13 (13%) hastada metastaz, 7 (7%) hastada nüks tespit edilmiş. Hastaların 5 ve 10 yıllık overall survival sırasıyla 86% ve 77% olup, 5 ve 10 yıllık event-free survival sırasıyla %79 ve %70 olarak bulunmuştur.
Sonuç: Çalışmamızda özel tip meme karsinomlu hastaların tamamına yakını nonmetastatik olup histopatolojik olarak hormon reseptörü pozitif ve düşük gradelidir. Özel tipler arasında, 5 ve 10 yıllık overall survival/ event-free survival istatistiki olarak anlamlı bulunmamıştır.

Anahtar Kelimeler: meme kanseri, özel tip, genel sağkalım, olaysız sağkalım

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Introduction

Breast cancer is a heterogeneous disease and according to the WHO classification, breast cancer can be categorized into 21 different histological types based on their varying morphological and growth features.[1] Invasive ductal carcinoma is the most common histological type. Among the histological special subtypes of breast carcinoma are tubular carcinoma (2%), medullary carcinoma (1%), papillary carcinoma, metaplastic carcinoma (less than 1%), and other epithelial tumors such as squamous cell carcinoma, as well as mesenchymal and stromal tumors/fibroepithelial tumors. Major studies that have defined molecular subtypes have almost exclusively focused on invasive ductal breast cancers, without including rare histological subtypes.[4] Although clinical, pathological, and epidemiological differences between ductal and lobular carcinomas have been examined in numerous studies, information regarding rarer histological subtypes such as mucinous, tubular, medullary, and papillary carcinomas is still not well understood.[2-5] Our understanding of these subtypes primarily relies on various case reports and studies based on small clinical series.[2,3] Histopathological classification has prognostic significance. For instance, tubular carcinoma is associated with a good prognosis, while metaplastic cancer is linked to a poor prognosis.[2,3] In this study, we examined the clinical, histopathological, and survival characteristics of patients diagnosed with special types of breast cancer who presented to our results.

Materials and Methods

This study was conducted with 104 patients diagnosed with special types of breast cancer among 1166 patients treated and followed up between 2010 and 2020 at Sivas Cumhuriyet University department of radiation oncology. This study was performed in accordance with the principles of the Declaration of Helsinki and approved by the the local ethics committee of Sivas Cumhuriyet University.

Patient Selection

In this study for patient selection, female patients >18 aged, diagnosed with special types of breast cancer at all stages were included. Clinicopathological data were obtained from the patients' files and medical records. Information regarding age at diagnosis, comorbidities, family history, menopausal status, follow-up, treatments, recurrence site and vital status were gathered from the files and medical records. Patients who were amenorrheic for more than 6 months before the diagnosis of breast cancer, those receiving hormone replacement therapy, or those who were at least 50 years old and whose menopausal status was not indicated in the medical records were considered postmenopausal.

At the time of diagnosis, all patients were staged according to the 8th Edition of the American Joint Committee on Cancer staging manual. The performance status of the patients was assessed according to Eastern Cooperative Oncology Group (ECOG) scoring system.

HER2 testing was performed using immunohistochemistry (IHC) or in situ hybridization (ISH).[6] Hormone receptor for ER and PR was used the method specified in the guidelines.[7] The subgroup classifications of patients as luminal type A and B, HER2 overexpression type, and triple-negative were according to the St. Gallen International Expert Consensus.[8]

The overall survival (OS) was defined as the time from diagnosis to the last follow-up or death. The time from diagnosis to recurrence/distant metastasis, death or last follow-up in those without recurrence/metastasis was defined as event-free survival (EFS).

Statistics

Statistical analyses of all data were conducted using "IBM SPSS Statistics for Windows, (Statistical Package for the Social Sciences, IBM Corp., Armonk, NY, USA) Version 23.0" Student's T test (normal distribution) or Mann-Whitney U test (abnormal distribution) were used to compare the groups for non-categorical variables. Chi-square test was used to compare categorical variables. Kaplan-Meier test was used to determine survival times. A p-value < 0.05 was considered statistically significant.

Results

In this study, 1198 breast cancer patients were reviewed, with 5.5% (n=104) identified as having special histological subtypes. The median age of these patients was 52 years (range 26-82). At the time of diagnosis, 60% were postmenopausal. Comorbidities were present in 50% of the patients and 25% had a family history of breast cancer. At diagnosis, 48% of the patients were in stage II. The demographic and clinical characteristics of the patients were presented on the table 1.

ER positivity was present in 72% of the patients, 66% were progesterone receptor (PR)-positive, and 25% were HER2-positive. The median Ki67 index was 20%. The molecular subtypes were distributed as follows: 31% were luminal A, 26% were luminal B HER2-negative, 17% were luminal B HER2-positive, 8% were HER2-positive, and 18% were triple-negative. An intraductal component was observed in 60% of patients, and in 65% of these patients, the intraductal component accounted for less than 25% of the tumor. Multicentricity was detected in 16% of the patients. Table 2 was summarized the histopathological characteristics of the patients.

Seven different histological types of breast tumors were evaluated. The demographic and clinical characteristics of the more common (>5) breast cancer subtypes were presented in table 1. Treatments were administered according to the standards of our center. Adjuvant chemotherapy (CT) and radiotherapy (RT) were applied based on risk factors (age, tumor size, regional lymph node involvement, etc.). Accordingly, 54% of the patients underwent modified radical mastectomy (MRM) and 69% underwent axillary dissection. The majority of patients received adjuvant therapies (chemotherapy, hormonotherapy and radiotherapy were administered to 73%, 70% and 68% of patients, respectively). Among early-stage patients, local recurrence was observed in 7% during follow-up, while the metastasis rate was 13%, with bone being the most common site of metastasis. The treatments administered to the patients and the patterns of recurrence and metastasis were presented in table 3. The median follow-up period was 54 months (range 1-201). Survival outcomes of the patients were presented in tables 4 and 5. Accordingly, the 5-year OS and EFS were 86% and 79%, respectively, while the 10-year OS and EFS were 77% and 70%, respectively.

Table 1. Demographic and clinic characteristics of patients

	Number of patients n=104	%
ECOG Performance status		
0	79	76
1	17	16
2	8	8
Menopausal status		
Premenopausal	42	41
Postmenopausal	60	59
Comorbidity	50	48
Diabetes mellitus	20	19
Hypertension	36	35
Heart disease	11	11
Family history+	26	25
T stage		
T1	34	33
T2	45	43
T3	18	17
T4	4	4
Tx	3	3
N stage		
N0	58	56
N1	22	21
N2	16	15
N3	8	8
Stage		
I	25	24
II	50	48
III	26	25
IV	3	3

ECOG Performance status: Eastern Cooperative Oncology Group Performance status

Table 2. Pathological characteristics of patients

	Number of patients n=104	%
ER Status		
Negative	29	28
Positive	75	72
PR Status		
Negative	35	34
Positive	69	66
Ki67 (Median, %)	20	0-95
HER2 Status		
Negative	78	75
Positive	26	25
Histological Subtypes		
Luminal A	32	31
Luminal B (HER2-negative)	27	26
Luminal B (HER2-positive)	18	17
HER2-positive	8	8
Triple-negative	19	18
Grade		
1	44	42
2	37	36
3	23	22
Lymphovascular invasion (n=91)		
Negative	60	66
Positive	31	34
Perineural invasion (n=88)		
Negative	72	82
Positive	16	18
Intraductal component (n=90)		
No	36	33
Yes	54	67
Intraductal component ratio (n=54)		
<%25	35	65
≥%25	19	35
Multifocality (n=96)		
No	81	84
Yes	15	16
Tumor necrosis (n=82)		
No	50	85
Yes	32	15
Extracapsular invasion (n=100)		
No	76	76
Yes	24	24

ER: Estrogen receptor, PR: Progesterone receptor, HER2: Human epidermal growth factor receptor 2

Table 3. Treatments received by patients and recurrence-metastasis patterns

	Number of patients n=104	%
Surgery		
No	3	3
MRM	56	54
BCS	45	43
Axillary Surgery		
No	3	3
SLNB	29	28
AD	72	69
Adjuvant Treatments		
Chemotherapy	76	73
Hormonoterapi	73	70
Radiotherapy	72	68
Local relapse	7	7
Metastasis	13	13
Metastasis sites		
Bone	11/13	11
Brain	5/13	5
Lung	3/3	3
Liver	3/3	3

MRM: Modified Radical Mastectomy, BCS: Breast Conserving Surgery, SLNB: Sentinel Lymph Node Biopsy, AD: Axillary Dissection

Table 4. Overall Survival outcome of patients

Number of patients n=104	5 yıllık Overall survival %	10 yıllık Overall survival %	p value
Apocrine	74	65	
Neuroendocrine	80	60	
Mucinous	88	81	
Metaplastic	82	72	0.714
Invasive Cribriform	88	76	
Invasive Micropapillary	78	78	
Invasive Papillary	78	62	

Table 5. Event-free survival outcome of patients

Number of patients n=104	5-year Event-free survival %	10-year Event-free survival %	p value
Apocrine	68	52	
Neuroendocrine	63	42	
Mucinous	81	81	0.490
Metaplastic	72	72	
Invasive Cribriform	88	66	
Invasive Micropapillary	56	56	
Invasive Papillary	80	64	

Discussion

Special types of breast cancers are rare and due to their infrequency, comprehensive clinical evaluations have not been conducted, leading to a limited understanding of their distinct clinical features. Most of the available data on treatment strategies come from small series and case reports, leaving clear recommendations for clinical management still lacking. As a result, current international guidelines generally recommend the use of chemotherapy regimens typically applied for invasive ductal carcinoma, where indicated, in patients with special types of breast cancer. However, this recommendation reflects the absence of robust prognostic data. This study has identified the characteristics of special type breast tumors. The correlation between histological type and prognosis is well established. The good prognosis group; cribriform, tubular, mucinous and the intermediate prognosis group; invasive micropapillary and medullary carcinoma, the poor prognosis group; mixed ductal and solid lobular, metaplastic and high-grade neuroendocrine carcinoma.[1] In this study, the histological types with the best prognosis were mucinous and tubular carcinomas, while the worst OS was observed in the apocrine and neuroendocrine carcinoma groups.

Apocrine carcinoma is a neoplasm predominantly composed of apocrine-type epithelium.[9] In a published retrospective analysis involving more than 6,800 invasive ductal carcinoma cases and 72 cases of apocrine carcinoma. Apocrine carcinoma was independently associated with a poorer disease-free survival (DFS), whereas invasive ductal carcinoma breast cancer showed similar outcomes.[10] In our study, apocrine carcinoma accounted for 0.15% of breast cancer cases. The 5-year OS and EFS were 74%, 68%.

Mucinous carcinomas represent 1-4% of all breast cancers and they are typically diagnosed at older ages.[11] In our study, this carcinoma subtype comprised 0.15% of breast cancer cases. Mucinous carcinomas are generally luminal type, low-grade, and associated with a favorable prognosis. Previous studies have reported a 5-year OS rate of 94% and a 10-year survival rate of 89%.[11] In our study, the 5-year OS was 88%, and the 10-year OS was 81%, consistent with prior findings.

Invasive micropapillary carcinoma is a rare breast tumor characterized by clusters of cells without fibrovascular cores within stromal spaces and is associated with a poor prognosis. Lymphovascular invasion is commonly observed.[12-13] In our cohort, 9 cases were identified, with a 10-year OS of 78%. In the literature, a study with 98 cases reported a 10-year OS of 48%. [13]

Metaplastic carcinomas are tumors that include sarcoma-like spindle cell areas, squamous differentiation, and chondroid or osseous differentiation along with adenocarcinoma.[14-17] These are typically high-grade tumors, with a prognosis worse than triple-negative invasive ductal carcinoma.[15] In our study, the 5-year OS was 82%, and the 10-year OS was 72%. The incidence of metaplastic breast

carcinoma ranges from 0.2-0.6%, and in our study, this rate was 0.09%.

Neuroendocrine carcinomas of the breast are defined by the presence of neuroendocrine features with widespread expression of neuroendocrine markers. Their prevalence can reach up to 0.5% of breast cancers.[18] In our study, the incidence was 0.05%. Neuroendocrine tumors typically have a favorable prognosis; however, the high-grade small cell variant is associated with a poor prognosis. In our study, the 5-year OS was 80%, and the 10-year OS was 60%, with clinical-pathological features aligned with literature data.

Invasive cribriform carcinomas (ICC) represent 0.3-0.8% of breast cancers.[20] In our study, ICC comprised approximately 0.14% of cases, with a 5-year OS of 88% and a 10-year OS of 76%. These tumors are typically ER+, low-grade, and exhibit low proliferation, reflecting a favorable prognosis.

Overall, in our study, the majority of patients presented at stage 2 and stage 3. Early-stage patients constituted 72% of the entire cohort.

Limitations

Retrospective analysis, small groups of patients, lack of more detailed new molecular and genetic testing were limitations of this study.

Conclusion

The clinical management of special histological types of breast cancer presents a genuine challenge, and clear guidelines are still lacking. These tumors represent a heterogeneous group with rare, diverse behaviors and prognoses, making prospective studies impractical. It remains debated whether patients with special type breast carcinomas have a better prognosis. Clinical experience is important, as each case contributes to understanding the characteristics of these tumors and helps in making the most appropriate treatment decisions. Our results were generally consistent with data from other studies, except for some cases. These discrepancies may be related to variations in pathological assessment and environmental factors.

Declarations

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Availability of data and material: The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Author's contribution: All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by EDA ERDİŞ. The first draft of the manuscript was written by EDA ERDİŞ and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Ethics approval: The present study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of University of Sivas Cumhuriyet (Date:19.09.24, No: 2024/09-34).

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Assessment of the *In Vitro* Antimicrobial Activity of *Ficus alba* and *Ficus carica* Latex

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Research Article

History

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ABSTRACT

Objective: *Ficus alba* Linn. and *Ficus carica* Linn., both belonging to the Moraceae family, have been utilized in traditional medicine for many years due to their antimicrobial, antifungal, and antioxidant properties. In the present study, the antimicrobial activities of latex samples from *Ficus alba* L. and *Ficus carica* L. were evaluated against various microorganisms, including the Gram-negative bacteria *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 70063), *Pseudomonas aeruginosa* (ATCC 27853), and *Acinetobacter baumannii* (ATCC 17978). Additionally, the study assessed the activity against Gram-positive bacteria such as *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212), as well as the yeast-like fungus *Candida albicans* (ATCC 10231).
Methods: Both of the latex samples were collected from the south-western region of Türkiye. The microbial inoculums were adjusted according to the McFarland 0.5 standard and antimicrobial activity was assessed according to the standard disc diffusion method of European Committee on Antimicrobial Susceptibility Testing (EUCAST).
Results: Both types of latex samples of *Ficus species (Ficus sp.*) exhibited strong antimicrobial activity. The most potent antimicrobial activity was observed against *C. albicans* (15.3 mm). The strongest antibacterial activity was observed against *E. coli* (13.3 mm) among the six different bacterial species tested, while the weakest antibacterial effect was observed against *A. baumannii* (8.3 mm). The antimicrobial activity of *F. carica* latex was found to be more potent than that of *F. alba* latex.
Conclusion: The findings support the potential use of this latex as natural antimicrobial agents, particularly in the battle against pathogens that are developing antibiotic resistance. It is recommended that further investigation be conducted into the phytochemical components of these plants and latex, with a view to their potential future use in the pharmaceutical industry.

Keywords: Antimicrobial activity, *Ficus alba, Ficus carica*, Latex.

Ficus alba ve *Ficus carica* Latekslerinin *In Vitro* Antimikrobiyal Aktivitesinin Araştırılması

Araştırma Makalesi

Süreç

Geliş: 03/10/2024
Kabul: 01/11/2024

ÖZET

Amaç: Moraceae familyasında yer alan *Ficus alba* Linn. ve *Ficus carica* Linn. bitkileri antimikrobiyal, antifungal ve antioksidan özellikleri sayesinde geleneksel tıpta uzun yıllar boyunca kullanılmaktadır. Bu çalışmada *Ficus alba* L. ve *Ficus carica* L. lateks örneklerinin antimikrobiyal aktivitesi Gram-negatif bakterilerden *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 70063), *Pseudomonas aeruginosa* (ATCC 27853), *Acinetobacter baumannii* (ATCC 17978) ve Gram-pozitif bakterilerden *Staphyloccoccus aureus* (ATCC 29213), *Enterococcus faecalis* (ATCC 29212) ve maya mantarlarından *Candida albicans* (ATCC 10231)'a karşı test edilmiştir.
Yöntem: Lateks örnekleri Türkiye'nin güney-batı bölgesinden toplanmıştır. Mikrobiyal inokulumlar McFarland 0.5 standartına uyarlanarak, antimikrobiyal aktivite European Committee on Antimicrobial Susceptibility Testing (EUCAST)'in standart disk difüzyon metoduna göre araştırılmıştır.
Bulgular: Her iki *Ficus* türüne *(Ficus sp.*) ait lateks örneği de güçlü antimikrobiyal aktivite göstermiştir. En güçlü antimikrobiyal aktivite *C. albicans*'a (15.3 mm) karşı gözlemlenmiştir. Altı farklı bakteri türü arasında en güçlü antibakteriyel aktivitenin *E. coli*'ye (13.3 mm) karşı olduğu, en zayıf antibakteriyel etkinin ise *A. baumannii*'ye (8.3 mm) karşı olduğu tespit edilmiştir. *F. carica* lateksinin antimikrobiyal aktivitesinin *F. alba*'nın lateksine göre daha güçlü olduğu belirlenmiştir.
Sonuç: Sonuçlar, özellikle antibiyotik direnci gelişen patojenlerle mücadelede, bu latekslerin doğal antimikrobiyal ajanlar olarak potansiyel kullanımını desteklemektedir. Gelecekteki farmasötik uygulamalar için bu bitkilerin ve latekslerinin fitokimyasal bileşenlerinin daha detaylı araştırılması gerektiği vurgulanmıştır.

Anahtar Kelimeler: Antimikrobiyal aktivite, *Ficus alba, Ficus carica*, Lateks

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Introduction

The *Ficus* species, belonging to the Moraceae family, includes species *Ficus alba* L. (*F. alba* L., white fig) and *Ficus carica* L. (*F. carica* L., black fig). These plants hold significant importance among medicinal species due to their diverse biological activities.[1] *Ficus* species produce '*latex*', a rubber-like vascular fluid.[2] The latex is a viscous white liquid flowing from the immature fruit stalks of *F. alba* and *F. carica*. The antimicrobial, antifungal, and antioxidant properties discovered through studies on the latex of these plants have led to their extensive utilization in traditional medicine for many years. The biological activities of *Ficus sp.* latex are attributed to the flavonoids, polyphenols, proteolytic enzymes, and other secondary metabolites in their biochemical structure.[1,3] The latex of *F. alba* reported to exhibit antimicrobial activity against Gram-positive and Gram-negative bacteria.[4] Research indicates that the latex of *F. carica* exhibits strong antimicrobial activity against pathogens such as *Staphylococcus aureus* and *Escherichia coli*.[1] Additionally, it is effective against fungal infections and show antifungal activity.[5] Furthermore, it has been demonstrated that the latex of these plants exhibits synergistic effects with traditional antibiotics against multidrug-resistant (MDR) pathogens, thereby enhancing the efficacy of antibiotics.[6] Understanding the potential antimicrobial effects of latex derived from *F. alba* and *F. carica* is considered a crucial step for future pharmaceutical applications.

Microorganisms develop antimicrobial resistance in response to the uncontrolled use of conventional antimicrobial agents for treating infectious diseases. Additionally, the side effects and rare complications associated with certain antimicrobial agents have led researchers to scrutinize the antimicrobial efficacy of medicinal plants. Given the rising prevalence of antimicrobial resistance and the diminishing effectiveness of traditional antibiotics, investigating the antimicrobial effects of these plant latex is crucial for the discovery and development of natural antimicrobial agents. This study aimed to evaluate the *in vitro* antibacterial and antifungal activities of the latex derived from *Ficus alba* and *Ficus carica*. There are numerous studies conducted both globally and within our country that investigate the antimicrobial activity of leaf extracts from *Ficus species*.[7,8] Most research has been conducted on the *F. carica* species, whereas studies involving *F. alba* species are limited. A study conducted in our country evaluated the antimicrobial activity of *F. alba* and *F. carica* against foodborne pathogens.[9] It can be asserted that our research is one of the pioneering studies investigating the antimicrobial activity of *F. alba* and *F. carica* against medical pathogenic bacteria.

Materials and Methods

Materials

The latex samples of *F. alba* and *F. carica* used in this study were collected in August-2024 from the south-western region of Türkiye (Ortaca, Muğla, Türkiye: 36° 50' 20" N, 28° 45' 52" E). The latex fluids were obtained by squeezing the sap from the stems of unripe green fruits. The samples were stored in two separate brown glass bottles with droppers at +4°C in a refrigerator until the experimental analysis was completed.

Test Microorganisms

In the present study, the bacteria recommended by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) and known for their sensitivity were used.[10] Gram-negative bacteria; *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 70063), *Acinetobacter baumannii* (ATCC 17978), and *Pseudomonas aeruginosa* (ATCC 27853); Gram-positive bacteria; *Staphylococcus aureus* (ATCC 29213) and *Enterococcus faecalis* (ATCC 29212) ant the yeast-like fungus *Candida albicans* (ATCC 10231) were used.

Microorganism Inoculums

The bacterial cultures were prepared overnight on 5% sheep blood agar at 37°C, while yeast cultures were cultivated on yeast peptone agar at the same temperature for 48 hours. Bacterial colonies from these young cultures were diluted in sterile saline solutions (0.9% NaCl) to adjust the McFarland standard of No. 0.5 (~10^8 colony-forming units (CFU)/mL) for bacterial suspensions and (~10^7 CFU/mL) for yeast suspensions.

Antimicrobial Test Discs

The latex samples, stored in a refrigerator (Arçelik, Türkiye) at +4°C, were allowed to reach room temperature for 30 minutes, alongside blank antibiotic discs (Bioanalyse, Türkiye). The latex samples were then filtered through sterile filters with a pore size of 0.22 µm (IsoLab, Germany). Subsequently, 200 µL of each latex sample was applied to sterile antibiotic discs with a diameter of 6 mm, which were placed in sterile petri dishes (FıratMed, Türkiye). The discs were permitted to absorb the latex fluids at room temperature for 15 minutes before being stored at +4°C for an additional 45 minutes.[11]

Antimicrobial Susceptibility Testing

The standard disc diffusion method recommended by EUCAST was employed to assess the antibacterial and antifungal activities.[11,12] Bacterial suspensions were inoculated onto Mueller Hinton Agar (MHA) (Merck,

Germany) medium. After a 15-minute absorption period, latex-impregnated discs were placed on the inoculated media, with four discs placed in each culture plate. Antibiotic discs, including Ampicillin (20 µg/disc) (Bioanalyse, Türkiye), and Trimethoprim (25 µg/disc) (Bioanalyse, Türkiye), served as positive controls for bacterial growth, while Fluconazole (25 µg/disc) (Bioanalyse, Türkiye) was utilized as a positive control for yeast. Blank antibiotic discs acted as negative controls. All plates were incubated at 37°C for 18 to 24 hours. Each test was conducted in triplicate. Figure 1 illustrates the experimental protocol for testing the antimicrobial activity of *F. alba* and *F. carica* latex.

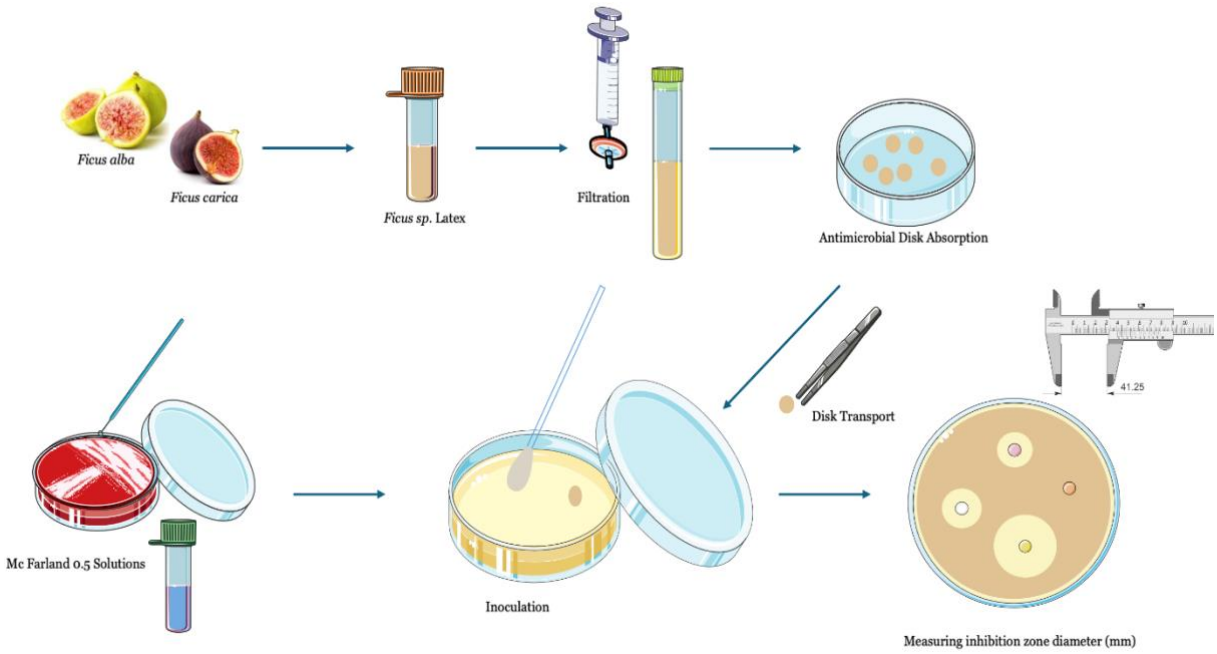

Figure 1. Schematic diagram of the antimicrobial activity test protocol for F. alba and F. carica latex. (The figure was created by Servier and is licensed under a Creative Commons Attribution 4.0 Unported License).

Table 1. Antimicrobial Activities of Ficus alba and Ficus carica Latex (Mean ± SEM)

Microorganisms	Diameter of Inhibition Zone* (mm)							
	Ficus alba				*Ficus carica*			
	Z_1	Z_2	Z_3	$Z_{\bar{x}}$	Z_1	Z_2	Z_3	$Z_{\bar{x}}$
Escherichia coli	12	14	14	13.3 ±1.15	14	13	10	12.3±2.08
Staphylococcus aureus	13	11	11	11.6±1.15	11	12	13	12±1.0
Klebsiella pneumoniae	9	10	8	9±1.0	11	11	9	10.3±1.15
Acinetobacter baumannii	8	9	8	8.3±0.57	9	9	10	9.3±0.57
Pseudomonas aeruginosa	10	10	9	9.6±0.57	11	9	10	10±1.0
Enterococcus faecalis	9	10	8	9±1.0	10	10	8	9.3±1.15
Candida albicans	14	16	14	14.6±1.15	15	15	16	15.3±0.57

Z: Zone, $Z_{\bar{x}}$: Arithmetic mean of zones, (Mean ±SEM, mm), *Diameter of inhibition zones including the diameter of the disc (6 mm)

Evaluation of Test Results

After the incubation period, the inhibition zone diameters surrounding the latex discs were measured in millimeters (mm) using a ruler. The diameter of the discs (6 mm) was factored into the measurements. The average inhibition zone for each type of latex was then calculated.

Statistical Analysis

The data obtained from this study were analyzed using the GraphPad-Prism (v10.0) software (Boston, USA). Arithmetic means and standard deviations were used to report the results.

Results

In the present study, it was observed that the latex of *F. alba* and *F. carica* exhibited significant antimicrobial activity against the tested microorganisms. The inhibition zone diameters for *F. alba* were as follows: *E. coli* (13.3 mm), *S. aureus* (11.6 mm), *K. pneumoniae* (9 mm), *A. baumannii* (8.3 mm), *P. aeruginosa* (9.6 mm), *E. faecalis* (9 mm), and *C. albicans* (14.6 mm). The inhibition zone diameters for *F. carica* were as follows: *E. coli* (12.3 mm), *S. aureus* (12 mm), *K. pneumoniae* (10.3 mm), *A. baumannii* (9.3 mm), *P. aeruginosa* (10 mm), *E. faecalis* (9.3 mm), and *C. albicans* (15.3 mm). The results of the antimicrobial activity are presented in Table 1.

The strongest antimicrobial activity for both types of latex was observed against *C. albicans.* Among the bacterial species tested, the most potent antibacterial activity was noted against *E. coli*, while the weakest activity was observed against *A. baumannii*. The antimicrobial activity of *F. carica* latex was stronger than that of *F. alba*. The antimicrobial activities of *F. alba* and *F. carica* latex are shown in Figures 2 and 3.

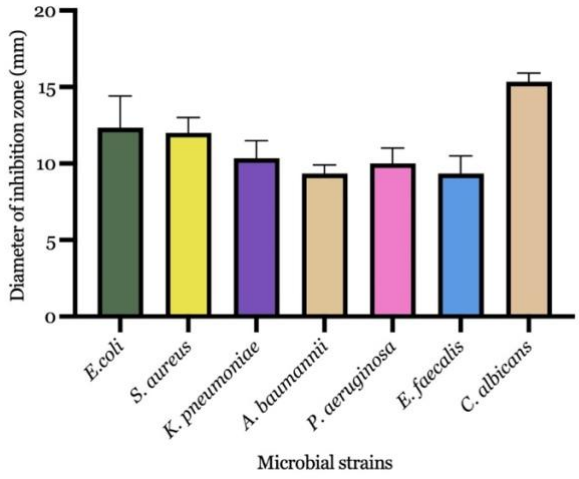

Figure 2. Antimicrobial Activities of Ficus alba Latex Samples. The values represent the means ± standard deviation of data independent experiments.

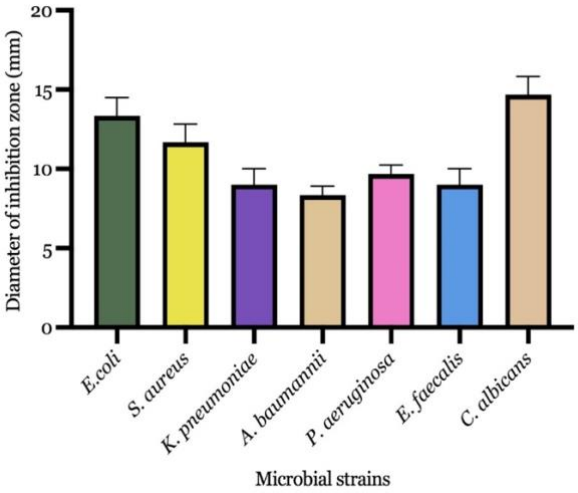

Figure 3. Antimicrobial Activities of Ficus carica Latex Samples. The values represent the means ± standard deviation of data independent experiments.

Discussion

The development of antimicrobial resistance to conventional antimicrobial agents used in the treatment of infectious diseases is an escalating concern. The rise in antimicrobial resistance among pathogenic bacteria complicates infection management, prolongs hospital stays, and increases morbidity and mortality rates. Consequently, this situation has resulted in significant economic losses for healthcare systems. The resistance to conventional antibiotics, coupled with the lengthy and costly process of developing new antimicrobial drugs, has prompted scientists to explore alternative agents with antimicrobial potential. In particular, the emergence of MDR pathogens has spurred the investigation of a variety of alternative antimicrobial agents for therapeutic applications. In this context, agents with minimal side effects and toxicity are being prioritized in research as potential alternative antimicrobial solutions.[13,14]

In this study, the antibacterial and antifungal activities of *F. alba* and *F. carica* latex samples were investigated using the disc diffusion method. The findings indicated that the antimicrobial activity of *F. carica* latex was stronger than that of *F. alba*. For both types of latex, antifungal activity was observed to be higher than antibacterial activity. The results of this study are largely consistent with both national and international research.[15-18] In a study investigating the antibacterial activity of *F. carica* latex using the disc diffusion method, the standard test microorganisms were employed, similar to our study, and an inhibition zone diameter ranging from 10.16 to 14.5 mm was observed. It has been stated that *F. carica* latex can be regarded as an antibacterial agent against infections caused by test organisms.[19] Raskovic et

al. tested the antifungal activities of *F. carica* latex at different times of the year, reporting that latex collected in the spring exhibited higher antifungal activity than that collected in the summer.[20]

Aref et al. reported significant antimicrobial inhibition against human pathogens for four distinct species of *F. carica* in their study. Similar to our study, they demonstrated the most potent antimicrobial activity against *C. albicans*.[6] In a study investigating the antibacterial and antibiofilm activities of *F. carica* extracts formulated as nanoparticles, the most significant antibacterial effect was observed against *P. aeruginosa*, which contrasts with the findings of our study.[21] Rashid et al. investigated the latex and leaf extracts of *F. carica* and observed strong antibacterial and antifungal activity, which is consistent with our study. They reported that the antifungal activity was higher than the antibacterial activity.[8]

In the literature, it has been observed that there are more studies on the biological properties of *F. carica* compared to *F. alba.*[22] Akarca and Tomar reported that the antimicrobial activity of *F. carica* was higher than that of *F. alba* in their research involving the latex of both species, which is consistent with the findings of our study.[9] Studies have reported that *F. carica* has a higher glucose content than *F. alba*.[23] Nafis et al. investigated the combinational antimicrobial activity of *F. carica* leaf essential oil and conventional antibiotics in their study. They found that, in the presence of this combination, the Minimum Inhibition Concentration (MIC) value decreased significantly by 1 to 16-fold, particularly against Gram-positive bacteria, indicating a synergistic interaction.[4]

Limitations

The phytochemical composition of plants comprises various bioactive components. Although this study demonstrated the antimicrobial potential of *F. alba* and *F. carica* latex, a phytochemical analysis of these latexes should be conducted to identify the specific biological components responsible for their antimicrobial activity. Further research is necessary to ascertain the phytochemical composition of *Ficus sp.* latex.

Conclusion

The results of this study demonstrate the significant antimicrobial activity of *F. alba* and *F. carica* latex. Given the rising issue of antimicrobial resistance, these latexes may serve as alternative agents in the treatment of microbial infections. In the present era, where the search for alternative agents to conventional commercial antimicrobials continues unabated, it is critical to determine the phytochemical content and active biochemical components of these latexes through chemometric analyses. Further studies should investigate the phytochemical composition, potential toxic effects and possible combinational-synergistic interactions with conventional antibiotics.

Ethics Committee Approval

This study does not require approval from an Ethics Committee.

Acknowledgment

The figure was created using pictures from Servier Medical Art (Servier Medical Art; https://smart.servier.com/) by Servier is licensed under a Creative Commons Attribution 4.0 Unported License (https://creativecommons.org/licenses/by/4.0/).

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Unveiling the Prognostic Potential of *SLC2A* Gene Family in Glioblastoma Multiforme Using Bioinformatics Approaches

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ABSTRACT

Objective: Glioblastomas (GBMs) are invasive and metastatic cancers with very low overall survival rates. Therefore, it is very important to propose a new biomarker for GBM diagnosis and prognosis. For this purpose, we aimed to investigate the prognostic potential of the *SLC2A* gene family, which has great importance in cancer, in GBM.

Methods: *Solute carrier 2A* (*SLC2A*) gene family expression levels, methylation and overall survival rates were analyzed with TCGA, GEPIA and UALCAN databases. Mutations were evaluated with Kaplan-Meier Plot and UCSC Xena database. Protein-protein interactions were analyzed with String database.

Results: No statistically significant mutation was detected in the *SLC2A* gene family. As a result of the analysis, high expression in *SLC2A1* and *SLC2A5* genes and decrease in *SLC2A6* gene expression were found to be statistically significant. Hypermethylation was detected in the promoter regions of *SLC2A1*, *SLC2A2*, *SLC2A3* and *SLC2A5* genes, while hypomethylation was detected in *SLC2A4* and *SLC2A6* genes. The increase in *SLC2A3* gene expression was associated with the overall survival rate of the patients.

Conclusion: *SLC2A1*, *SLC2A5* and *SLC2A6* gene up-regulation may be a biomarker in the diagnosis of GBM, and *SLC2A3* may be a marker in prognosis.

Keywords: Glioblastoma, *SLC2A* Genes, Bioinformatics

Biyoinformatik Yaklaşımlar Kullanılarak Glioblastoma Multiform'da *SLC2A* Gen Ailesinin Prognostik Potansiyelinin Ortaya Çıkarılması

Araştırma Makalesi

Süreç

Geliş: 13/12/2024
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ÖZ

Amaç: Glioblastomalar (GBM'ler) çok düşük genel sağkalım oranlarına sahip invaziv ve metastatik kanserlerdir. Bu nedenle, GBM tanısı ve prognozu için yeni bir biyobelirteç önermek çok önemlidir. Bu amaçla, kanserde büyük öneme sahip olan *SLC2A* gen ailesinin GBM'deki prognostik potansiyelini araştırmayı amaçladık.

Yöntemler: *Solute taşıyıcı 2A* (*SLC2A*) gen ailesi ekspresyon düzeyleri, metilasyon ve genel sağkalım oranları TCGA, GEPIA ve UALCAN veri tabanları ile analiz edildi. Mutasyonlar Kaplan-Meier Plot ve UCSC Xena veri tabanı ile değerlendirildi. Protein-protein etkileşimleri String veri tabanı ile analiz edildi.

Bulgular: *SLC2A* gen ailesinde istatistiksel olarak anlamlı bir mutasyon saptanmadı. Analiz sonucunda *SLC2A1* ve *SLC2A5* genlerinde yüksek ekspresyon ve *SLC2A6* gen ekspresyonunda azalma istatistiksel olarak anlamlı bulundu. *SLC2A1*, *SLC2A2*, *SLC2A3* ve *SLC2A5* genlerinin promotör bölgelerinde hipermetilasyon, *SLC2A4* ve *SLC2A6* genlerinde ise hipometilasyon saptandı. *SLC2A3* gen ekspresyonundaki artış hastaların genel sağ kalım oranı ile ilişkili bulundu.

Sonuç: *SLC2A1*, *SLC2A5* ve *SLC2A6* geninin yukarı regülasyonu GBM tanısında bir biyobelirteç olabilir ve *SLC2A3* prognozda bir belirteç olabilir.

Anahtar Kelimeler: Glioblastoma, SLC2A Genleri, Biyoinformatik

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Introduction

Glioblastomas (GBMs) are the most common and malignant brain tumors in the world. Patients have a low survival rate[1]. Especially the 5-year rate of survival of glioblastoma multiforme (GBM) is low in elderly patients. The prognosis of GBM varies according to age and pathological type. The World Health Organization (WHO) has classified central nervous system (CNS) gliomas as low-grade and high-grade[2]. It has a higher prevalence in men than in women and in Caucasians than in other ethnicities[3]. Despite the development of modern approaches to the treatment of GBM, it remains a fatal disease with an extremely poor prognosis[4]. A large number of different genetic and molecular changes occur in GBM during its development. There are many important signaling pathways that lead to the growth and progression of the brain tumor[5].

The increased glucose uptake by cancer cells is called the Warburg effect. Numerous investigations have demonstrated that many cancers overexpress glucose transporter proteins[6]. Overexpression of glucose transporter proteins meets the energy requirements of tumor cells. It also provides cancer cells with sufficient precursor molecules for aerobic glycolysis. Thus, the ATP needs of tumor cells are met[7]. In human cells, glucose transport is mediated by the solute carrier 2A (SLC2A) family. SLC2As are also called the glucose transporter or GLUT family[8]. Based on sequence similarity, the SLC2A1-SLCA4 (GLUT1-4), and SLC2A14 (GLUT14) are group I GLUTs; SLC2A5 (GLUT5), SLC2A7 (GLUT7), SLC2A9 (GLUT9), and SLC2A11 (GLUT11) are group II GLUTSs; and SLC2A6 (GLUT6), SLC2A8 (GLUT8), SLC2A10 (GLUT10), SLC2A12 (GLUT12), and SLC2A13 (GLUT13) are group III[9]. SLC2A family proteins have 12 transmembrane regions, one N-linked glycosylation site, and a cytoplasmic linker domain[10]. Almost all cells in the human body expression the *SLC2A1* gene. The most crucial glucose transporter in the muscle, neurological system, brain and other tissues and organs is this protein. Maintaining a balanced physiological metabolism is crucial for human health[11]. Nevertheless, SLC2A1 is also crucial for the metabolic function of cancer cells. For cancer cells to continue growing and spreading, they must ingest an excessive amount of glucose. High protein expression of SLC2A1 in cells may mean carcinogenesis[12]. SLC2A1, SLC2A2, SLC2A3, and SLC2A4 proteins stimulate glucose uptake by cancer cells, erythrocytes, pancreatic β-cells, neurons, cells of the blood-brain barrier, endothelial, fat and muscle cells[13]. Cancers of the endometrium, liver, breast, lung and stomach can grow and spread as a result of *SLC2A1* overexpression [14-18]. In one study, it was predicted that SLC2A1 might also be associated with GBM prognosis[19]. It has been observed that elevated *SLC2A2* expression is linked to insulin secretion, glucose concentration, autonomic nervous system activity, and the control of body temperature and feeding[20]. According to earlier research, tumor cells use the SLC2A protein to transfer glucose to intracellular reserves in order to meet their high energy requirements. This implies that the invasiveness and development of tumors may be related to the expression of distinct *SLC2A* subtypes[21]. Higher overall survival in cases of breast and liver cancer as well as other cancers is positively correlated with overexpression of *SLC2A2*[22]. The SLC2A3 protein is of major importance in intracellular glucose transport in glycolysis. Glycolytic activity results in an elevated metabolic rate and increased glucose uptake, meeting the increased energy demands of tumor cell proliferation[23,24]. Overexpression of *SLC2A3* has been associated with poorer clinical outcomes, including increased invasion, larger tumor size, advanced pathological stage, tumor recurrence, and vascular embolization[25]. In addition, SLC2A3 leads to changes in the tumor microenvironment through activation of macrophage infiltration, worsening the prognosis in gastric and breast cancer[26]. *SLC2A3* has a high affinity for glucose and has been found to have increased expression in patients with brain tumors[27]. *SLC2A3* expression level has a significant association with the pathological grading of glioma tumors[28]. The association between SLC2A4 overexpression and many types of cancer remains unclear[29]. However, according to the TCGA database, SLC2A4 is a favorable prognostic factor for breast cancer[30]. Davidson et al (1992) reported that *SLC2A5* is expressed in the brush border membrane of human small intestinal enterocytes[31]. Burant et al (1992) stated that SLC2A5 is a fructose transporter and may be responsible for fructose uptake from the lumen of the small intestine[32]. Doege et al (2000) showed that *SLC2A6* was overexpressed in COS-7 cells and had high glucose transport activity[33].

In this study, we aimed to investigate the expression and methylation levels of *SLC2A1- SLC2A6* genes belonging to the *SLC2A* family in human GBM tissue and healthy tissue samples using The Cancer Genome Atlas (TCGA) and UALCAN databases, to determine the mutation rates in these genes using Kaplan-Meier Plot and UCSC Xena databases, and finally to determine protein-protein interactions using String databases. There is no study on GBM and *SLC2A* gene families in the literature.

Material and Methods

Sampling and Data Extraction

This is a bioinformatics study planned to reveal the relationship between GBM and *SLC2A1*, *SLC2A2*, *SLC2A3*, *SLC2A4*, *SLC2A5* and *SLC2A6* genes and proteins belonging to the *SLC2A* gene family. Data from GBM patient and control groups were obtained using TCGA (https://www.cancer.gov/tcga) databases for analysis in the study. Ethics committee approvals of the patients were obtained within the scope of the Cancer Genome Project. Access to GBM patient and control group data was provided from the TCGA database on 08.12.2024.

Gene expression, Methylation and Survival analysis

Expression, methylation and survival data of GBM patient and control groups were analyzed using TCGA (https://www.cancer.gov/tcga), GEPIA database (http://gepia.cancer-pku.cn/), UALCAN database (https://ualcan.path.uab.edu/analysis.html).

Mutation analysis

Mutations from GBM patients were analyzed using Kaplan-Meier Plot (https://kmplot.com/analysis/) and UCSC Xena databases (https://xena.ucsc.edu/).

Protein-Protein Interaction

The interactions of SLC2A1-SLC2A6 proteins with each other and with different proteins were analyzed using the String database (https://string-db.org/).

Statistical Analysis

In the evaluation of the data of our study, the expression relationship between GBM and control tissues was evaluated with One-Way ANOVA test using UALCAN databases. Methylation analyses were analyzed with Student's t-test using UALCAN databases. Survival rates of patients were evaluated using UALCAN database. Mutation analyses were analyzed using Kaplan-Meier Plot and UCSC Xena databases. A log-rank *p*-value below 0.05 was considered statistically significant.

Results

Expression level of SLC2A family genes in GBM

Expression levels of *SLC2A1-SLC2A6* genes in GBM tumor and healthy control tissues were analyzed using TCGA and GEPIA databases. As a result of the analysis, no statistically significant relationship was observed in *SLC2A2*, *SLC2A3* and *SLC2A4* genes. However, the difference between GBM tumor tissue and healthy tissue in *SLC2A1*, *SLC2A5* and *SLC2A6* genes was found to be significant (T=163, N=207, $p<0.005$) (Figure 1). The expression level of *SLC2A1* gene in tumor tissue was determined to be higher compared to the healthy control group, but this significant difference in expression level was not found to be associated with the survival rate of the patients ($p=0.95$) (Figure 2). When we evaluated *SLC2A2* gene expression, it was determined that the expression level in tumor tissue was similar to the healthy control tissue and no significant relationship was detected. The patient's survival rate was not shown to be substantially correlated with this relationship in expression level ($p=0.68$) (Figure 2). *SLC2A3* gene expression was found to be higher in tumor tissue compared to the control group. There was no statistical significance in this difference. However, the survival rates of patients with high *SLC2A3* expression were found to be statistically significant ($p=0.033$) (Figure 2). *SLC2A4* gene expression level was lower in tumor tissue compared to the control group, but this difference was not statistically significant ($p=0.96$) (Figure 2). *SLC2A5* gene expression level was found to be higher in tumor tissue. This difference was statistically significant. This statistical difference in expression level is not associated with the survival rate of the patients (p=0.60) (Figure 2). When we evaluated the *SLC2A6* gene, although the decrease in expression level in the tumor tissue was found to be significant, it was not found to be associated with the survival rate of the patients ($p=0.35$). (Figure 2).

Expression levels of *SLC2A1-SLC2A6* genes and other different genes associated with GBM are given in Figure 3. According to Microarray analysis results, *SLC2A1*, *SLC2A3* and *SLC2A5* gene expression levels were determined to be higher in GBM tumor tissue compared to the control group (Figure 3).

Methylation level of SLC2A family genes in GBM

Promoter methylation levels of the *SLC2A* gene family in GBM tumor tissue and healthy control tissue are given in Figure 4. While hypermethylation was observed in *SLC2A1*, *SLC2A2*, *SLC2A3* and *SLC2A5* genes in GBM tumor tissue, hypomethylation was observed in *SLC2A4* and *SLC2A6* genes.

Mutation Analysis

Somatic mutation (Single Nucleotide Polymorphisms and small INDELS-Ensemble somatic mutation Variant) analysis in *SLC2A1*, *SLC2A2*, *SLC2A3*, *SLC2A4*, *SLC2A5* and *SLC2A6* genes in GBM patients was performed in 381 individuals. Five patients with mutations in the SLC2A1 gene and two patients with mutations in *SLC2A2*, *SLC2A3*, *SLC2A4* and *SLC2A5* genes were identified. Only one patient with mutations in the *SLC2A6* gene was identified. No statistical significance was found between those with or without mutations ($p<0.05$) (Figure 5).

Protein-Protein Interaction

The interactions of SLC2A1-SLC2A6 proteins with other proteins were analyzed using the String database (Figure 6). As a result of the analysis, the proteins with the highest homology scores with these proteins were Cellular tumor antigen p53 (Tp53), Hexokinase-4 (GCK), Solute carrier family 2, facilitated glucose transporter member 14 (SLC2A14), Ras-related protein Rab-10 (RAB10), Carbonic anhydrase 6 (CA6), MFS domain-containing protein (SLC2A11-2), respectively (Table 1). When the molecular function of SLC2A family proteins was examined, it was determined that they had the highest Glucose transmembrane transporter activity, the highest Glucose import during the biological process, and when evaluated in terms of KEGG pathways, they were associated with the highest rate of insulin resistance (Figure 7).

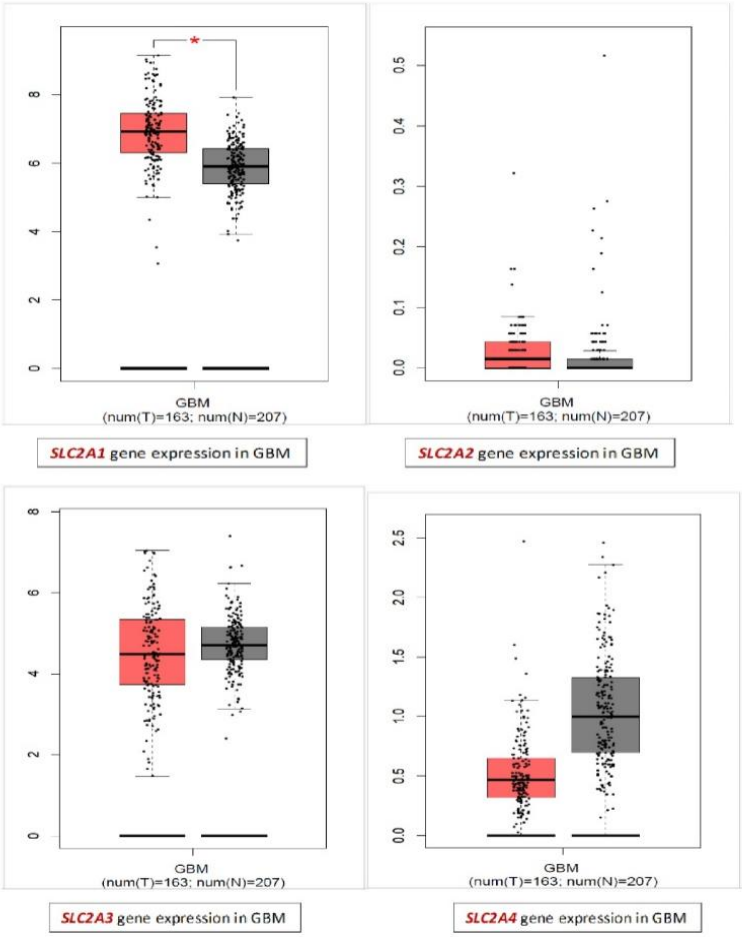

Figure 1.2. Comparison of UALCAN of the high and low expressions of SLC2A1-SLC2A6 in TCGA GBM cohort ($p<0.05$).

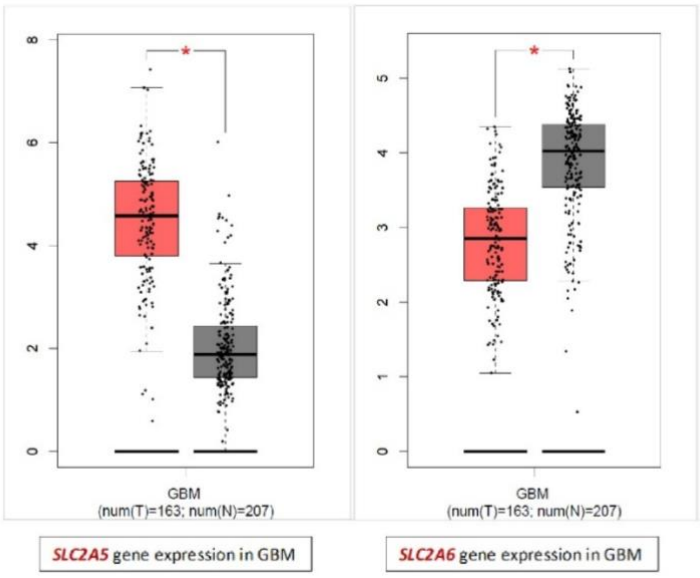

Figure 1.2.

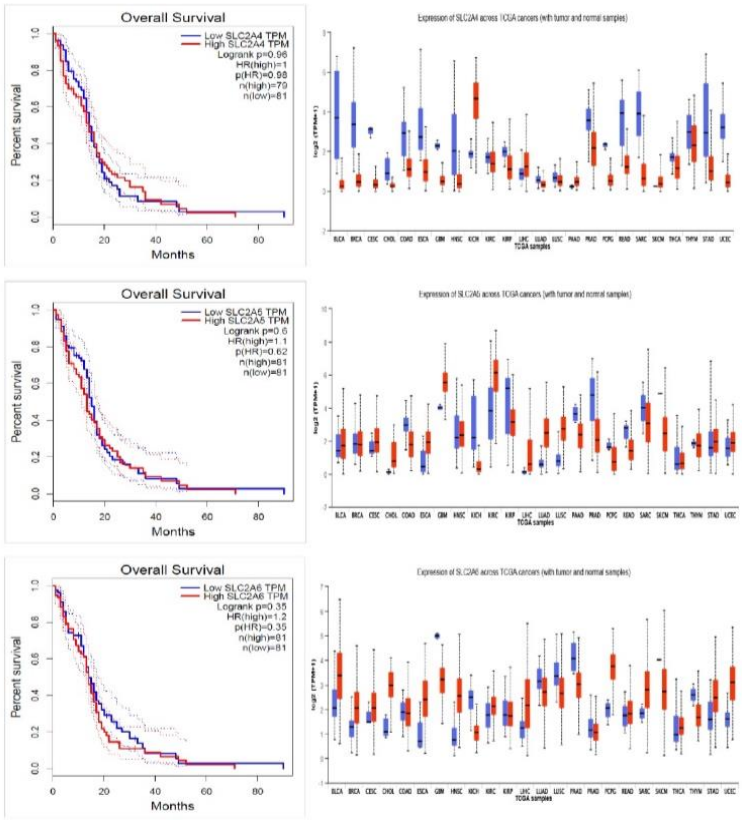

Figure 2.1. A) Comparison of UALCAN survival curves of the high and low expressions of SLC2A1- SLC2A6 in TCGA GBM cohort (p<0.05). Red line indicates the high expressions of mRNA; green line indicates the low expressions of mRNA. B) Expression of SLC2A gene family across TCGA tumors. Red column: GMB, Blue column: normal tissue.

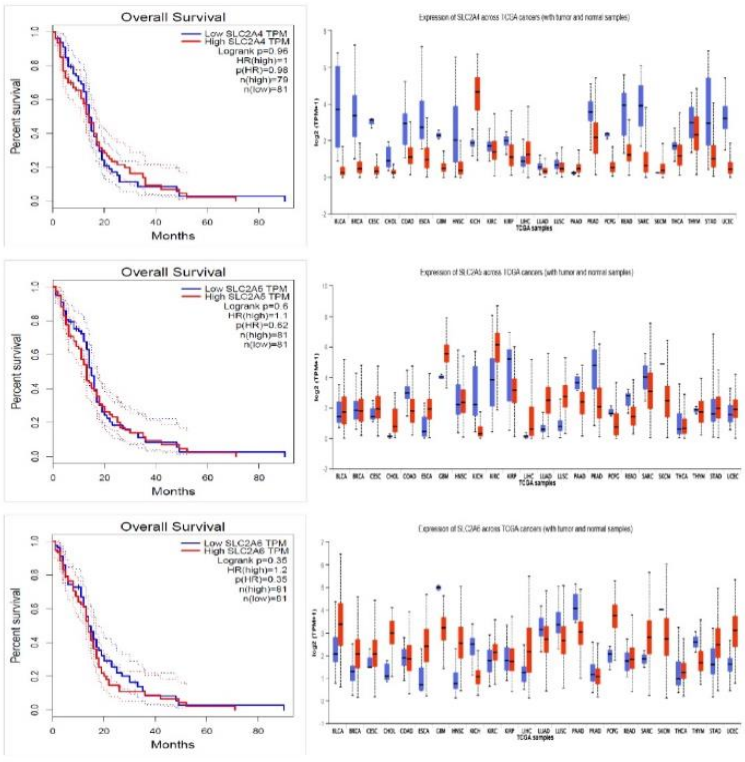

Figure 2.2

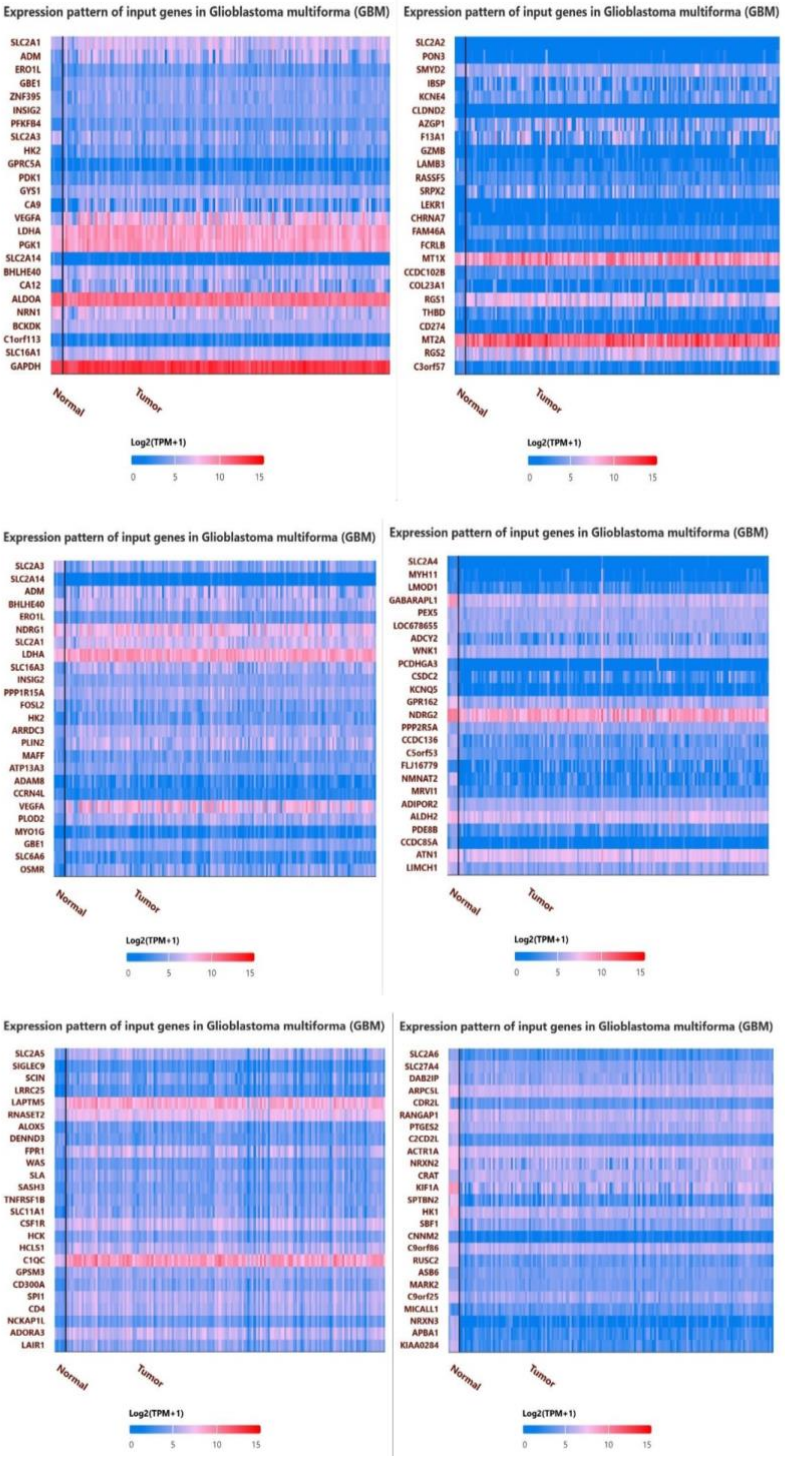

Figure 3. Microarray analysis results of SLC2A1-SLC2A6 gene expressions in relation to other genes. Data were analyzed according to UALCAN and TCGA database.

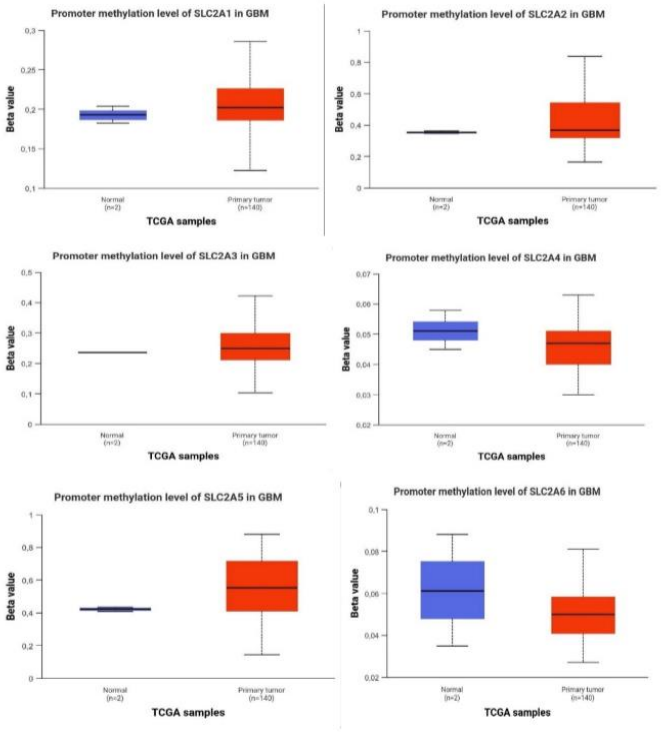

Figure 4. Promoter methylation level of SLC2A- SLC2A6 genes.

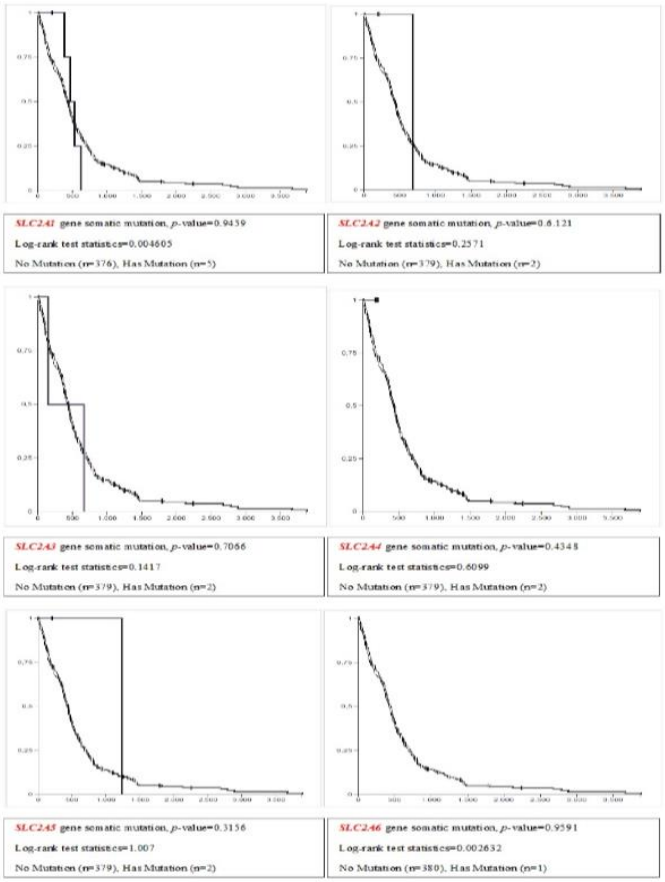

Figure 5. SLC2A1- SLC2A6 genes were analyzed with Kaplan Meier Somatic Mutation (Single Nucleotide Polymorphisms (SNPs) and Small INDELs)-Ensemble Somatic Variant.

Table1. Predicted Functional Proteins Associated with SLC2A1-SLC2A6 Proteins.

Proteins	Proteins Associated	Predicted functional proteins	Homology score
SLC2A1	TP53	Cellular tumor antigen p53	0.967
SLC2A1	SLC2A4	Solute carrier family 2, facilitated glucose transporter member 4	0.909
SLC2A1	SLC2A2	Solute carrier family 2, facilitated glucose transporter member 2	0.906
SLC2A1	HIF1A	Hypoxia-inducible factor 1-alpha	0.902
SLC2A1	BSG	Basigin	0.900
SLC2A1	EPAS1	Endothelial PAS domain-containing protein 1	0.888
SLC2A1	STOM	Erythrocyte band 7 integral membrane protein	0.880
SLC2A1	SLC5A1	Sodium/glucose cotransporter 1	0.874
SLC2A1	LDHA	Lactate dehydrogenase A	0.863
SLC2A1	CA9	Carbonic anhydrase 9	0.849
SLC2A2	GCK	Hexokinase-4	0.961
SLC2A2	HNF1A	Hepatocyte nuclear factor 1-alpha	0.954
SLC2A2	INS	Insulin A chain	0.942
SLC2A2	TP53	Cellular tumor antigen p53	0.926
SLC2A2	SLC2A1	Solute carrier family 2, facilitated glucose transporter member 1	0.906
SLC2A2	SLC2A5	Sodium/glucose cotransporter 1	0.899
SLC2A2	GCG	Glicentin-related polypeptide	0.876
SLC2A2	NEUROD1	Neurogenic differentiation factor 1	0.866
SLC2A2	NKX6-1	Homeobox protein Nkx-6.1	0.862
SLC2A2	NEUROG3	Neurogenin-3	0.854
SLC2A3	SLC2A14	Solute carrier family 2, facilitated glucose transporter member 14	0.868
SLC2A3	CREB1	Cyclic AMP-responsive element-binding protein 1	0.817
SLC2A3	MECP2	Methyl-CpG-binding protein 2	0.781
SLC2A3	HK1	Hexokinase-1	0.737
SLC2A3	HIF1A	Hypoxia-inducible factor 1-alpha	0.736
SLC2A3	PFKFB3	6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3	0.736
SLC2A3	LDHA	Lactate dehydrogenase A	0.730
SLC2A3	SLC16A3	Monocarboxylate transporter 4	0.730
SLC2A3	HK2	Hexokinase-2	0.711
SLC2A3	PKM	Pyruvate kinase	0.689
SLC2A4	RAB10	Ras-related protein Rab-10	0.984
SLC2A4	INS	Insulin A chain	0.977
SLC2A4	RAB14	Ras-related protein Rab-14	0.973
SLC2A4	TBC1D4	TBC1 domain family member 4	0.969
SLC2A4	RAB8A	Ras-related protein Rab-8A	0.968
SLC2A4	ASPSCR1	Tether containing UBX domain for GLUT4	0.968
SLC2A4	RAB2A	Ras-related protein Rab-2A	0.955
SLC2A4	VAMP2	Vesicle-associated membrane protein 2	0.945
SLC2A4	IRS1	Insulin receptor substrate 1	0.940
SLC2A4	PPARG	Peroxisome proliferator-activated receptor gamma	0.934
SLC2A5	CA6	Carbonic anhydrase 6	0.866
SLC2A5	SLC5A1	Sodium/glucose cotransporter 1	0.861
SLC2A5	KHK	Ketohexokinase	0.796
SLC2A5	CA3	Carbonic anhydrase 3	0.764
SLC2A5	ENO1	Alpha-enolase	0.745
SLC2A5	SLC22A12	Solute carrier family 22 member 12	0.731
SLC2A5	SLC15A1	Solute carrier family 15 member 1	0.664
SLC2A5	TAS1R3	Taste receptor type 1 member 3	0.618
SLC2A5	G6PC3	Glucose-6-phosphatase 3	0.611
SLC2A5	G6PC2	Glucose-6-phosphatase 2	0.607
SLC2A6	SLC2A11-2	MFS domain-containing protein	0.849
SLC2A6	SLC2A11	Solute carrier family 2, facilitated glucose transporter member 11	0.525
SLC2A6	SLC2A3	Solute carrier family 2, facilitated glucose transporter member 3	0.512
SLC2A6	SLC22A8	Solute carrier family 22 member 8	0.475
SLC2A6	SLC2A7	Solute carrier family 2, facilitated glucose transporter member 7	0.472
SLC2A6	SLC2A1	Solute carrier family 2, facilitated glucose transporter member 1	0.469
SLC2A6	SLC2A2	Solute carrier family 2, facilitated glucose transporter member 2	0.467
SLC2A6	DHDH	Dihydrodiol dehydrogenase	0.466
SLC2A6	SLC2A5	Solute carrier family 2, facilitated glucose transporter member 5	0.465
SLC2A6	NFKBIE	NF-kappa-B inhibitor epsilon	0.463

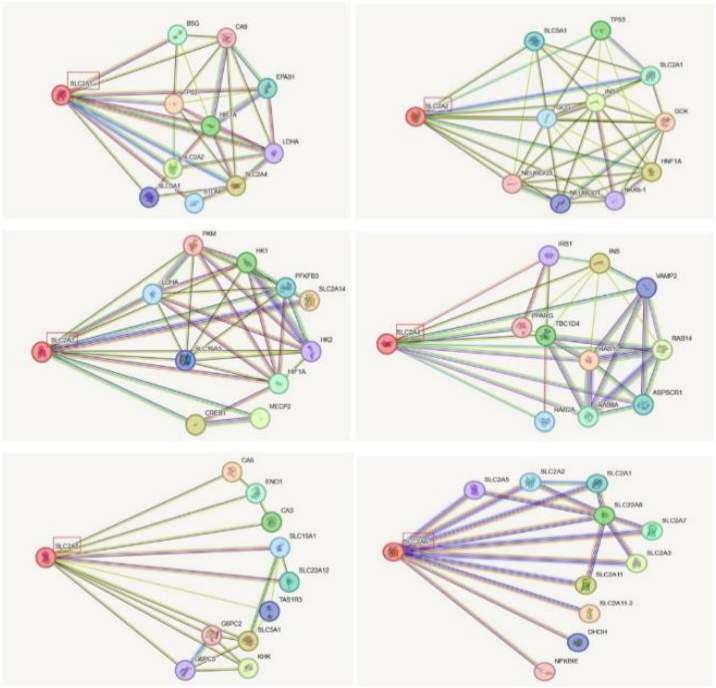

Figure 6. String analysis of known and predicted protein-protein interactions with proteins SLC2A-SLC2A6. Red line indicates evidence of fusion; green line indicates neighborhood evidence; blue line indicates association evidence; purple line indicates experimental evidence; yellow line indicates text mining evidence; light blue line indicates database evidence; black line indicates co-expression evidence.

Figure 7. *Diagrams of the molecular function, biological process, and KEGG pathways of SLC2A1-SLC2A6 proteins*

Discussion

Gliomas are classified as grades I to IV according to the level of malignancy determined by their histopathological type. Gliomas with a grade I malignancy level have low proliferative potential and are related to lesions that can be treated with surgical procedures. In contrast, grades II to IV gliomas are highly malignant and invasive. GBM is the most aggressive, invasive and undifferentiated tumor type. GBM is defined as grade IV by WHO[34,35]. Since GBM is aggressive and invasive, early diagnosis is necessary to increase the survival rate of patients. Therefore, new potential biomarkers are needed for the diagnosis and prognosis of GBM. The *SLC2A* gene family may be an important biomarker in GBM. This study is significant since it is the first to use the TCGA database to ascertain the level of expression of SLC2A family genes in 207 normal tissues and 163 GBM tumor tissues. Based on our research, *SLC2A1*, *SLC2A2*, *SLC2A3*, and *SLC2A5* genes were significantly upregulated in GBM as a result of gene expression analysis. However, *SLC2A4* and *SLC2A6* genes were downregulated. Expression levels of *SLC2A* family members are increased in different tumors, thus indicating the potential oncogenic effect of the *SLC2A* family[36]. According to studies, SLC2A1 has a strong affinity for mannose, galactose, and glucose. Additionally, this transporter has been demonstrated to be strongly expressed at the blood-brain barrier, where it controls the rate at which glucose enters the brain. In addition, high expression of *SLC2A1* has been detected in erythrocytes, which rely solely on glycolysis for ATP production, and in the placenta, where SLC2A1-null mice have been shown to utilize glucose extensively, resulting in embryonic lethality[37]. The positive expression rate of *SLC2A1* can approach 50% in a variety of malignant tumor cells, such as those found in the breast, liver, pancreas, ovary, lung, esophagus, brain, kidney, skin, endometrial, colon and cervical regions. Thus, the degree of hypoxia, invasion, and metastasis, as well as the proliferation of malignant tumors, may be associated with SLC2A1[38]. As a result of the analysis, we found that *SLC2A1* gene expression levels were significantly higher in GBM patients. In addition, we detected hypermethylation in the promoter region. Nevertheless, there was no correlation between the patients' survival rate and this elevated expression or hypermethylation. The primary hepatic tissue sugar transporter, SLC2A2, has a decreased affinity for glucose[39]. In a study conducted by Yun et al. (2017) in patients with hepatocellular cancer, SLC2A2 was determined to be associated with clinical stages and was independently associated with the survival rate of patients[40]. In our analysis, the increase in *SLC2A2* gene expression level in GBM tumor tissue was not found to be significant. However, hypermethylation was detected in the promoter region. The increase in expression level was not associated with the survival rate of the patients. Recent studies have shown that SLC2A3 levels are increased in circulating tumor cells that tend to metastasize to the brain. In addition, SLC2A3 is essential for tumor cells to survive in the brain[41]. Additionally, it has been noted that a higher risk of metastasis in head and neck and breast malignancies is positively connected with elevated *SLC2A3* gene expression[42]. In the analysis, the increase in *SLC2A3* gene expression level in GBM tumor tissue was not significant and hypermethylation was detected in the promoter region. Although it was not significant, the increase in expression level was found to be associated with the survival rate of the patients. In the study of Shi et al., *SLC2A4* expression was significantly reduced in breast cancer and hypermethylation in the promoter region was detected[43]. Similarly, we noted a decrease in GBM tumor tissue in our study. However, this decrease was not significant. This decrease in expression level was not found to be associated with the survival rate of the patients. Groenendyk et al. reported that they stopped cell proliferation, migration and metastasis by blocking SLC2A5 fructose transport. In addition, they found that the localization and structure of mitochondria in cancer cells with suppressed *SLC2A5* gene played a role in the metastasis of cancer cells[43]. *SLC2A5* expression is elevated in metastatic liver lesions, lung tumors, brain, colon, testicular, uterine and breast carcinoma[44]. In this study, *SLC2A5* gene expression level was significantly increased in GBM tumor tissue. Hypermethylation was detected in the promoter region. However, the increase in expression level was not found to be associated with the survival rate of the patients *SLC2A6* overexpression can cause mitochondrial damage, stop cancer cells from proliferating, and cause tumor cells to undergo apoptosis[45]. *SLC2A6* gene expression level was significantly decreased and hypomethylation was detected in the promoter region. However, the increase in expression level was not found to be associated with the survival rate of the patients.

Conclusion

Consequently, we believe that *SLC2A1*, *SLC2A5* and *SLC2A6* may be useful prognostic biomarkers for GBM by showing the association of *SLC2A* family genes expression with GBM in this study. Although the increase in expression level was not significant, *SLC2A3* expression was found to be associated with the survival rate of patients. Therefore, it is thought that the increase in *SLC2A1*, *SLC2A5* and *SLC2A6* gene expression may be a biomarker in the diagnosis of GBM, and SLC2A3 may be a marker in prognosis.

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Global Research Trends of Gut Microbiota in Gestational Diabetes Mellitus: A Bibliometric and Visualized Analysis

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Research Article

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ABSTRACT

Objective: Gestational Diabetes Mellitus (GDM) is a metabolic condition that imposes a great economic burden on the global scale and poses great risks to the health of both mothers and babies. Studies on the role of intestinal microbiota in diabetes are remarkable. This study aims to analyze the trends and basic components in studies investigating the relationship between diabetes and intestinal flora.
Methods: Publications on intestinal microbiota in GDM were obtained from the Web of Science Core Collection database (WoS) on February 14, 2024, covering the period from 2009, when the first study on this subject was conducted, to the present. RStudio (Biblioshiny) and VOSviewer (1.6.17) software were used in the bibliometric analysis. Editorial material, reviews, and letters were not included in the study.
Results: A total of 254 articles were obtained in this study. Although the number of publications has been increasing since 2017, the most studies on the subject were conducted in 2022 with 56 scientific articles. Endocrine metabolism is the field with the most publications. The most frequently used terms, as determined by the commonality analysis, are gut microbiota, obesity, and pregnancy.
Conclusions: This is the first bibliometric analysis on gut microbiota in GDM. Studies on gut microbiota in GDM have only been conducted in the last 16 years. Research on GDM and gut microbiota is increasing and attracting more and more attention from researchers. In the future, gut microbiota is expected to remain a central focus in diabetes research.

Keywords: Bibliometric study, gut microbiota, gestational diabetes mellitus

Gestasyonel Diyabet Mellitus'ta Bağırsak Mikrobiyotasının Küresel Araştırma Eğilimleri: Bibliyometrik ve Görselleştirilmiş Analiz

Araştırma Makalesi

Süreç

Geliş: 15/11/2024
Kabul: 17/12/2024

ÖZET

Amaç: Gestasyonel Diyabet Mellitus (GDM), küresel anlamda büyük ekonomik yük getiren ve hem annelerin hem de bebeklerin sağlığında büyük riskler oluşturan metabolik bir durumdur. Bağırsak mikrobiyotasının diyabetteki rolü üzerine yapılan araştırmalar dikkat çekicidir. Bu çalışma, diyabetin bağırsak florasıyla bağlantısını araştıran araştırmalardaki eğilimleri ve temel bileşenleri analiz etmeyi amaçlamaktadır.
Metot: 14 Şubat 2024'te Web of Science Core Collection veritabanından (WoS) GDM'deki bağırsak mikrobiyotası üzerine yayınlar elde edilerek, bu konudaki ilk çalışmanın yapıldığı 2009 yılından günümüze kadar olan dönemi kapsamaktadır. Bibliyometrik analizde, RStudio (Biblioshiny) ve VOSviewer (1.6.17) yazılımları kullanıldı. Editöryal materyal, derlemeler, mektuplar çalışmaya dahil edilmemiştir.
Bulgular: Bu çalışmada toplam 254 makale elde edildi. Yayın sayısı 2017'den bu yana artış eğilimi gösterse de, konuyla ilgili en fazla çalışma 2022'de 56 bilimsel makale ile yapılmıştır. Endokrin metabolizması en fazla yayın yapan alandır. Ortak varlık analiziyle belirlendiği üzere en sık kullanılan terimler bağırsak mikrobiyotası, obezite ve gebeliktir.
Sonuçlar: Bu, GDM'de bağırsak mikrobiyotası üzerine yapılan ilk bibliyometrik analizdir. GDM'de bağırsak mikrobiyotası üzerine çalışmalar yalnızca son 16 yıldır yürütülmektedir. GDM ve bağırsak mikrobiyotası üzerine yapılan araştırmalar artışta olup araştırmacıların giderek daha fazla ilgisini çekmektedir. İleriye bakıldığında, bağırsak mikrobiyotasının diyabet araştırmaları alanında merkezi bir odak olmaya devam edeceği açıktır.

Anahtar Kelimeler: bibliometric analiz, bağırsak mikrobiyotası, Gestasyonel diyabet

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Introduction

Gestational diabetes mellitus (GDM) is the term for a glucose intolerance that is not noticeable before pregnancy and is identified during the second or third trimester of pregnancy [1]. It is an especially prevalent metabolic condition affecting women during pregnancy [2]. Also, approximately 20 million infants are affected by GDM, which is on the rise worldwide and particularly so in low- and middle-income nations [3]. GDM is linked to poor outcomes for both mothers and infants. After giving birth, mothers with GDM are prone to experience long-term hyperglycaemia or type 2 diabetes [4]. Additionally, preeclampsia, prenatal hypertension, and additional cardiovascular disorders during pregnancy are linked to GDM co-morbidities [5]. It is widely believed that the pathophysiological foundation of GDM arises from an aberrant up-regulation of insulin release in relation to the degree of enhanced insulin resistance that is inherent to pregnancy. Multi-endocrine and neurological pathways combine to regulate glucose, leading to a complicated process [6]. It is commonly recognized that gut dysbiosis affects women who have gestational diabetes mellitus (GDM) [7]. Against this backdrop, we will examine the research on the association between gut microbiota and GDM.

'Gut microbiota' refers to beneficial microbial populations that have colonized the gastrointestinal (GI) system. The gut microbiota is more prevalent in the distal colon, in particular [8]. The creation of short-chain fatty acids, vitamin synthesis, mucosal barrier function, and food digestion are all supported by the gut microorganisms [9]. Crucially, the gut microbiota's relations with host cells regulate immunological response and host metabolism [10]. Furthermore, a growing body of research suggests that the host's native gut microbiota influences intestinal permeability and could be involved in the emergence of a long-lasting, low-grade inflammatory state that aids in the appearance of chronic metabolic disorders [11]. A population imbalance in the gut microbiota, characterized by a rise in pathogenic bacteria and a reduction in beneficial flora, can lead to a number of underlying health conditions, including neurological disorders, allergy diseases, metabolic syndrome, and some kinds of cancer [12]. The connection among microbiota in the intestine, gut dysbiosis, inflammation, obesity, and resistance to insulin during gestation was initially noted by Koren et al [13].

In recent years, research on the relationship between gut microbiota and GDM has gained popularity. In the past, the only methods available for evaluating the gut microbiota for this purpose were bacteriological culture techniques. However, in more recent times, a variety of sophisticated approaches have started to be employed. These days, the methods that clinicians use include bacterial culture, temperature gradient gel electrophoresis, denaturing gradient gel electrophoresis, fluorescence in situ hybridization, DNA microarrays, terminal restriction fragment length polymorphism, microbiome shotgun sequencing, and cloned 16S rRNA gene sequencing for the direct sequencing of 16S rRNA amplicons. [14]. Furthermore, the ELISA technique is used to measure the concentrations of different biomolecules that influence intestinal permeability and upset the balance of microbiota [15].

Bibliometric analyses are statistical techniques for conducting quantitative investigations using a general literature database [16]. By making predictions about the future frontiers and creating an information map, they may be used to illustrate how a particular knowledge subject has evolved [17]. The literature contains bibliometric analyses of GDM. Nevertheless, there are not any research that particularly look at the gut microbiota in GDM in these types of analyses. The bibliometric study we are now undertaking will contribute the first information to the literature in this regard.

Methods

Data Sources and Search Strategy

The present investigation used a bibliometric analyse approach to examine papers published in the Web of Science (WoS) database on gut microbiota in GDM patients. This study's data was collected from the sub-database of the Science Citation Index Expanded (SCI-E), Emerging Sources Citation Index (ESCI) and Social Science Citation Index (SSCI) sourced from the Web of Science Core Collection (WoS) database on 14.02.2024. The search technique included the search query '(TS=(gestational diabetes mellitus) AND TS=(gut microbiota)', and a Boolean search was conducted. A total of 247 publications were found, but when the exclusion criteria were applied, 243 publications that comprised the study were discovered. The data were examined using Vosviewer (1.6.17) software and RStudio (Biblioshiny) [18].

Inclusion Criteria

The data evaluation was undertaken to span the time frame from 2009, that the first research published in this subject, until now in order to attain a holistic interpretation incorporating keywords, titles, and abstracts. The language of publishing was not differentiated, and only "articles," "reviews," and "early presence" research were chosen.

Exclusion Criteria

The analysis of data was carried out by excluding publications from the research, including proceeding paper, letters, corrections, and editorial materials.

Data Statistics and Indicators

Bibliometric mapping is a visual presentation of scientific literature based on quantitative bibliometric data [19]. We used the VOSviewer program, developed by Van Eck and Waltman at the Centre for Science and Technology Research at Leiden University, to construct bibliometric networks to determine the relationships of keywords, authors, institutions and documents. Using Microsoft Excel 2020, the pattern of publication numbers by year was investigated. Diverse nodes in the network graph stand in for diverse factors, including authors, countries, and institutions. The larger the circles, the more frequently publications there are; the circles represent the frequency or quantity of publications. The centrality of a node indicates its location in the knowledge network as well as its effect on other nodes. A high centrality index increases the likelihood that key nodes will form in the network [20, 21].

Results

Database Overview

Descriptive analyses were conducted utilizing Biblioshiny. The primary data insights are depicted in Figure 1.

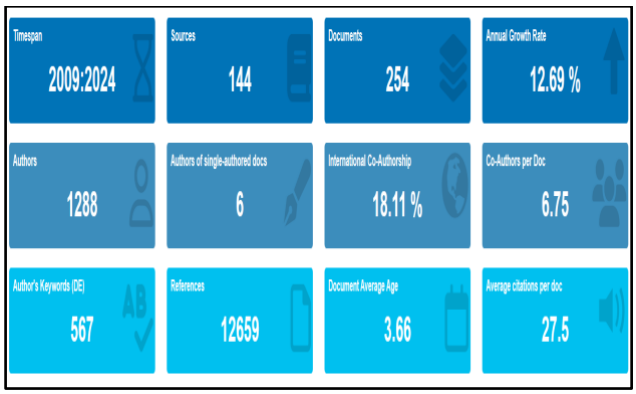

Figure 1. Main information

A comprehensive analysis of the study of gut microbiota in GDM encompassed 254 publications from 144 distinct sources (including journals, books, etc.) between 2009 and 2024, sourced from the Web of Science (WoS) database. The rate of publication growth stands at 12.69% annually, with an average document age of 3.66 years. On average, each document receives 27.5 citations. Among the 1288 authors, only 6 have published as single authors. Regarding author collaboration in documents, the prevalence of international co-authorship stands at 18.11%.

The Annual Publication Distribution Map Index

Figure 2 illustrates the temporal evolution of document growth in the bibliometric analysis of studies concerning gut microbiota in GDM and an increasing global scientific research interest in.

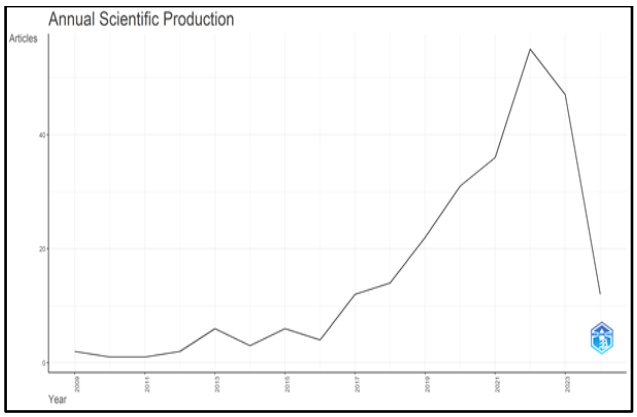

Figure 2. The annual number of published papers

The publication volume has shown an upward trajectory over the years, with 56 papers already published in 2022, 43 in 2023, 38 in 2021, 30 in 2020, 22 in 2019. Notably, the year 2022 experienced the most pronounced surge in gut microbiota in GDM research.

The distribution of research articles on the intestinal microbiota in GDM patients scanned in SCI-E, ESCI and SSCI published in WoS between 2009 and 2024 and their numbers sorted according to the index type they were scanned are shown in Figure 3. There have been 232 SCI-E, 11 ESCI, and 9 SSCI papers published thus far.

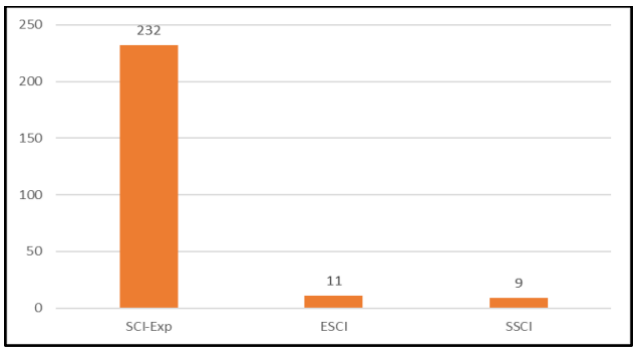

Figure 3. Number of indexes in which publications are scanned

Status of Average Annual Citations

The bibliometric analysis of studies focusing on gut microbiota in GDM included an examination of annual citation trends, as depicted in Figure 4.

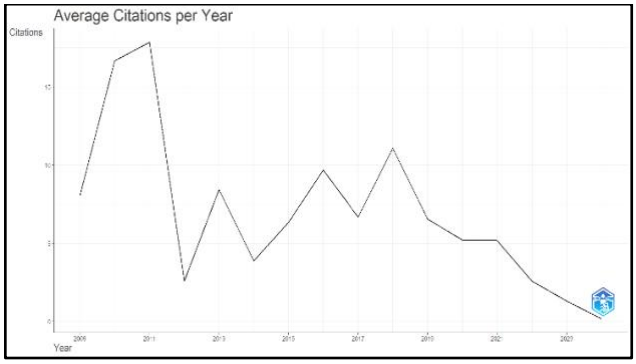

Figure 4. The average citations per year

According to Figure 4, there are differences in the number of citations made by year. While 2011 was the year with the highest number of citations on average, this average tended to decrease as the years progressed.

Sankey Diagram

In the diagram referred to as the "Three-Axis Graph," three parameters (journal, author, and country) have been configured within the program for correlation, and the top performers for each parameter are provided in Figure 5.

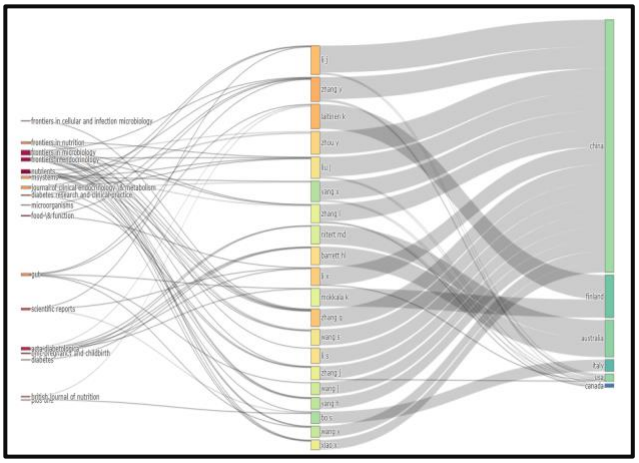

Figure 5. The Sankey diagram for the journal, author and country

In Figure 5, the magnitude of the relationship between parameters is represented by the size of the tiles. The size of the boxes in this diagram signifies the prominence of parameters in the literature. Notably, the foremost journal is "Frontiers in Microbiology," helmed by leading author "Zhang Y.", with China emerging as the leading country in the field.

Distribution of Country and Country Based Citation Relationship

In this research area, the most productive countries in Table 1 and the relationship map between the countries in Figure 6 are extracted from the RStudio software. Because there are 42 nations in this category, only the top ten countries in terms of number of publications are considered.

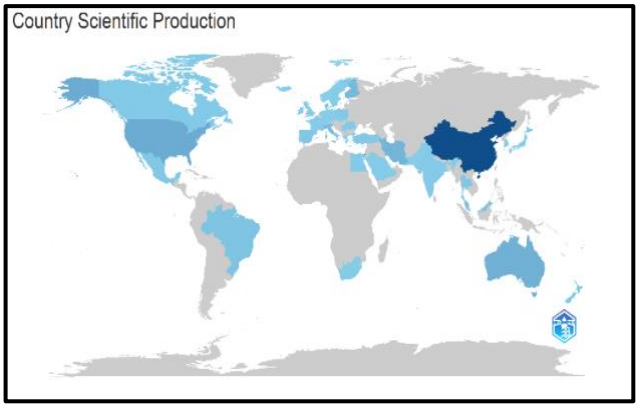

Figure 6. Country scientific production.

On the map, the colours navy blue, blue and grey mean the country that broadcasts the most, the country that broadcasts less and the country that does not broadcast, respectively.

Table 1. Most productive countries

Country	Article	Total Citation
CHINA	513	1779
USA	129	896
AUSTRALIA	108	690
IRAN	93	410
ISRAEL	82	96
FINLAND	77	777
DENMARK	68	333
ITALY	62	362
MALAYSIA	48	112
BRAZIL	40	226

The ten countries that collaborate the most by several documents are shown in Table 2. the world cooperation map for these values are shown in Figure 7.

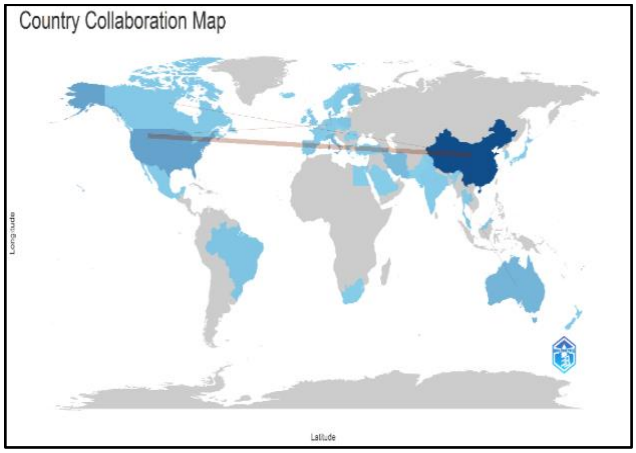

Figure 7. Country based collaboration map

China-USA ranks first with 10 documents among the countries with the most cooperation

Table 2. Number of documents from the 10 most cooperating countries.

From	To	Frequency
CHINA	USA	10
AUSTRALIA	MALAYSIA	2
CHINA	CANADA	2
CHINA	SINGAPORE	2
DENMARK	GERMANY	2
USA	GERMANY	2
AUSTRALIA	NEW ZEALAND	1
AUSTRALIA	SWEDEN	1
AUSTRALIA	UNITED KINGDOM	1
BRAZIL	FRANCE	1

Most Published Authors Institutions and Their Collaborations

According to the data in figure 8, the Chinese Academy of Medicine and the University of Turku in Finland are the two universities where scientists who produce the most academic publications on the gut microbiota in patients with GDM work or are funded (11 papers). In second place are Nanjing Medical University, Peking Union Medical College, Peking University and University of Queensland (10 studies). In third place is Royal Brisbane Women's Hospital with 9 studies.

Most Cited Document and Author Citation Relationship

The most cited document refers to the research article/paper that has accumulated the highest number of total citations from other scholarly works. In other words, it's the paper that has been referenced the most by other researchers and accordingly received the highest cumulative number of citations. The ten most cited documents are presented in Table 3.

Citation relationship based on the author are analysed in Figure 9, and "Effects and Mechanisms of Probiotics, Prebiotics, Synbiotics, and Postbiotics on Metabolic Diseases Targeting Gut Microbiota: A Narrative Review" published by Li, Zhu, Gan et al. in 2021 [22], and Hasain, Mokhtar Kamaruddin et al.'s 2020 work "Gut Microbiota and Gestational Diabetes Mellitus: A Review of Host-Hut Microbiota Interactions and Their Therapeutic Potential" [23] had the most cited publications with 89 citations. The paper "Diet-Gut Microbiota Interactions and Gestational Diabetes Mellitus (GDM)" published by Ponzo, Fedele, Goitre et al. [24] in 2019 comes in second with 73 citations. Liu, Pan, Lv et al.'s 2019 paper "Alterations of Gut Microbiota and Blood Lipidome in Gestational Diabetes Mellitus with Hyperlipidaemia" [25] has 64 citations and is the third most cited study.

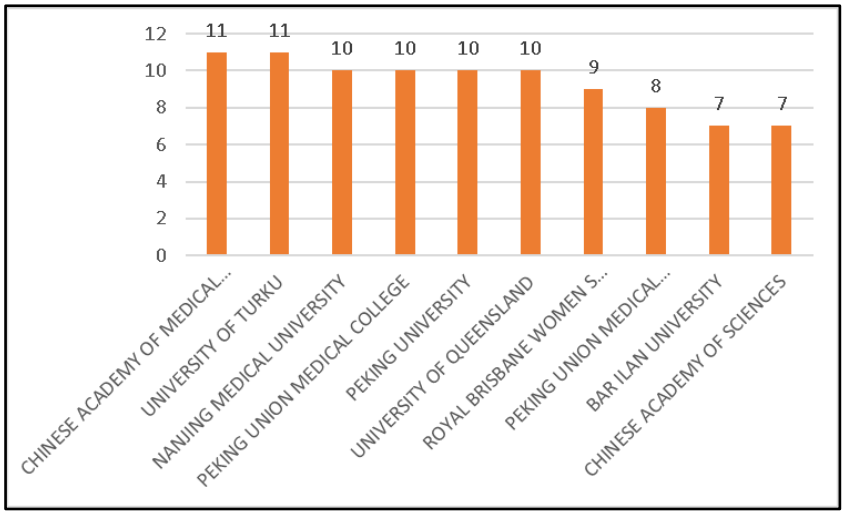

Figure 8. Institutions of the most published authors

Table 3. Most cited documents

Paper	DOI	TC	TC per Year	Normalized TC
Catalano Pm, 2011, Am J Obstet Gynecol	10.1016/j.ajog.2010.11.039	250	17,86	1,00
Luoto R, 2010, Br J Nutr	10.1017/S0007114509993898	250	16,67	1,00
Crusell Mkw, 2018, Microbiome	10.1186/s40168-018-0472-x	246	35,14	3,17
Wang J, 2018, Gut	10.1136/gutjnl-2018-315988	246	35,14	3,17
Laitinen K, 2009, Br J Nutr	10.1017/S0007114508111461	243	15,19	1,88
Cho Ce, 2013, Am J Obstet Gynecol	10.1016/j.ajog.2012.08.009	208	17,33	2,05
Gomez-Arango Lf, 2016, Diabetes	10.2337/db16-0278	191	21,22	2,19
Hu J, 2013, Plos One	10.1371/journal.pone.0078257	172	14,33	1,70
Wesolowski Sr, 2017, Nat Rev Gastro. Hepatol	10.1038/nrgastro.2016.160	142	17,75	2,66
Ferrocino I, 2018, Sci Rep	10.1038/s41598-018-30735-9	139	19,86	1,79

(TC: total citations)

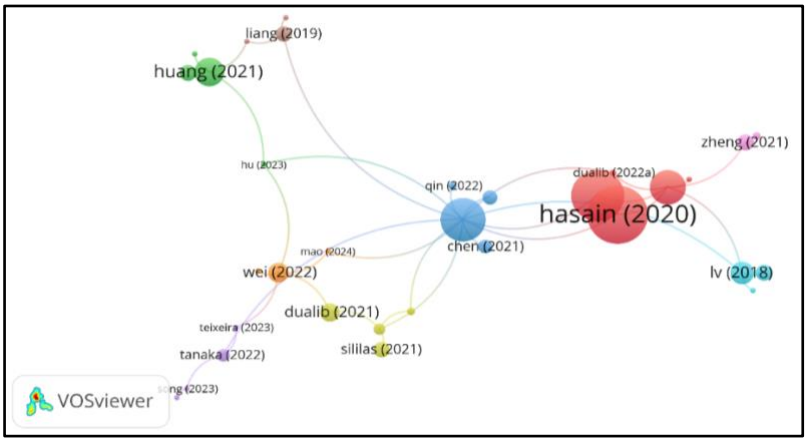

Figure 9. Citation relationship based on the author

Most Cited Local Sources

Examining the most cited local sources that publish research on gut microbiota in patients with GDM reveals that, that Diabetes Care has the highest number of citations (587) followed by Plos One (516), Nutrients (439), Nature (417), and SCI Re-UK (363) (Figure 10).

Sources Dynamics

If the dynamics of the resources are examined over the years, 'Scientific Reports' and 'Nutrients' started to conduct the first studies on this subject for the first time in 2009, and the number of studies started to increase as of 2017 and peaked with the beginning of 2024 (Figure 11).

Academic Research Categories

When academic articles published in WOS are classified into research fields, the top five are Endocrinology Metabolism, Nutrition Dietetics, Microbiology, Gynaecology, and Biochemistry-Molecular Biology (Table 4).

Trend Topics

Trending topics about the gut microbiota in GDM, literature from 2009 to 2024 by year are given in Table 5.

During the first quarter of 2017, second quarter of 2020, and third quarter of 2022, the dominant trending theme was "gut microbiota." Then, the trend topic "pregnancy" came to the fore in the first quarter of 2020, the second quarter of 2021 and the last quarter of 2022. "Gut microbiota", "pregnancy" and "obesity" continue to be the most talked about topics in this field. The developed keyword TreeMap is shown in Figure 12.

Common Presence Analysis

Using the VOSviewer software, the technique of "Common presence analysis" has been employed to incorporate keywords with distinct colours corresponding to the publication year (Figure 13). The colours of items are based on the duration elapsed since their publication. The average (yellow) publication year for recently introduced terms is 2023 for the analysis performed in this paper. Figure 13 illustrates the timeframe spanning from 2020 to 2023, represented by the progression of blue-green-yellow colours.

While terms such as "gut microbiome", "16 rRNA sequencing", "neonate", problems", and "oral microbiota" were most commonly used in 2020 and 2021, "gestational diabetes mellitus", "pregnancy", "obesity", "probiotics" had become popular as of the second half of 2021. After 2023, the keywords "hormone metabolism," "metabolic biomarker," and "behaviour change techniques" began to appear often in academic publications.

Thematic Analysis

Using author-defined keywords, a thematic investigation of gut microbiota in GDM publications was undertaken, employing bibliometric methods. The network representation in Figure 14 reveals a bifurcation of gut microbiota in GDM studies into four thematic clusters.

These encompass investigations concerning " gestational diabetes" within the overarching or fundamental theme of literature, and examinations of "intestinal microbiome" within a more specific domain.

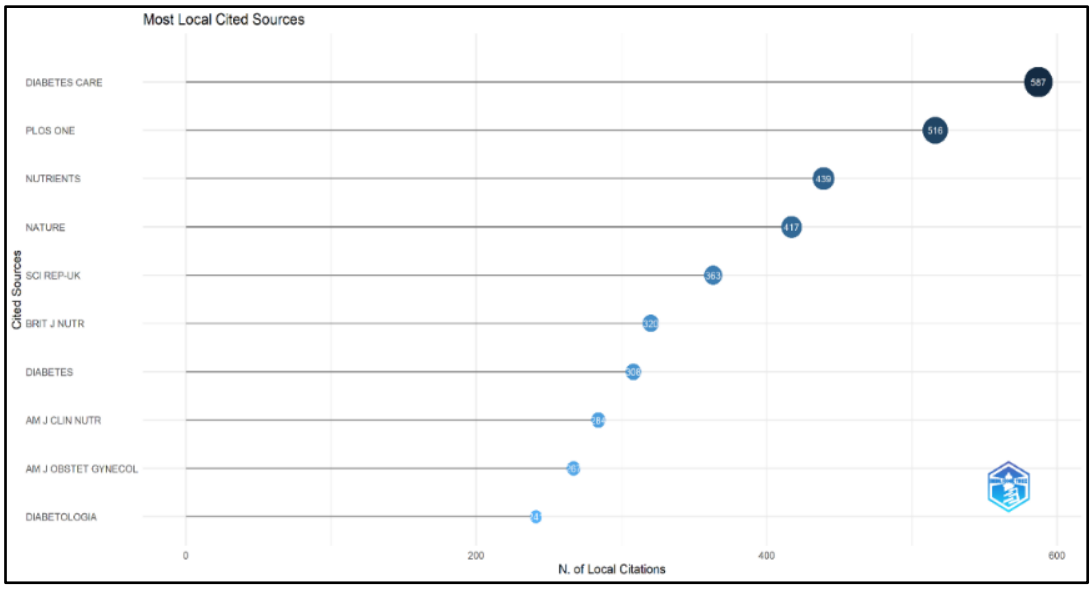

Figure 10. Most local cited sources

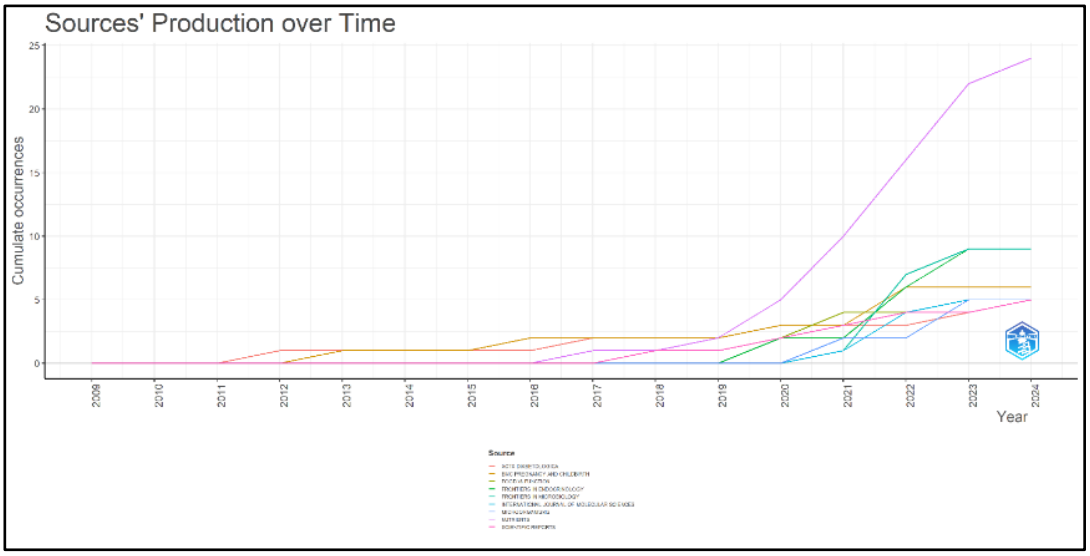

Figure 11. Sources' production over time

Table 4. The number of papers published by academic categories

Research categories	Number of papers
Endocrinology Metabolism	52
Nutrition Dietetics	44
Microbiology	34
Obstetrics and Gynaecology	24
Biochemistry and Molecular Biology	21
Food Science Technology	12
Research Experimental Medicine	12
General Internal Medicine	11
Chemistry	9
Science Technology Other Topics	9

Table 5: Trending topics by year

item	freq	year_q1	year_med	year_q3
pregnant-women	17	2018	2019	2022
oxidative stress	13	2018	2019	2022
birth-weight	6	2018	2019	2020
gut microbiota	86	2017	2020	2022
obesity	63	2018	2020	2022
gestational diabetes-mellitus	34	2018	2020	2023
pregnancy	71	2020	2021	2022
double-blind	50	2018	2021	2022
association	35	2019	2021	2022
risk	37	2019	2022	2023

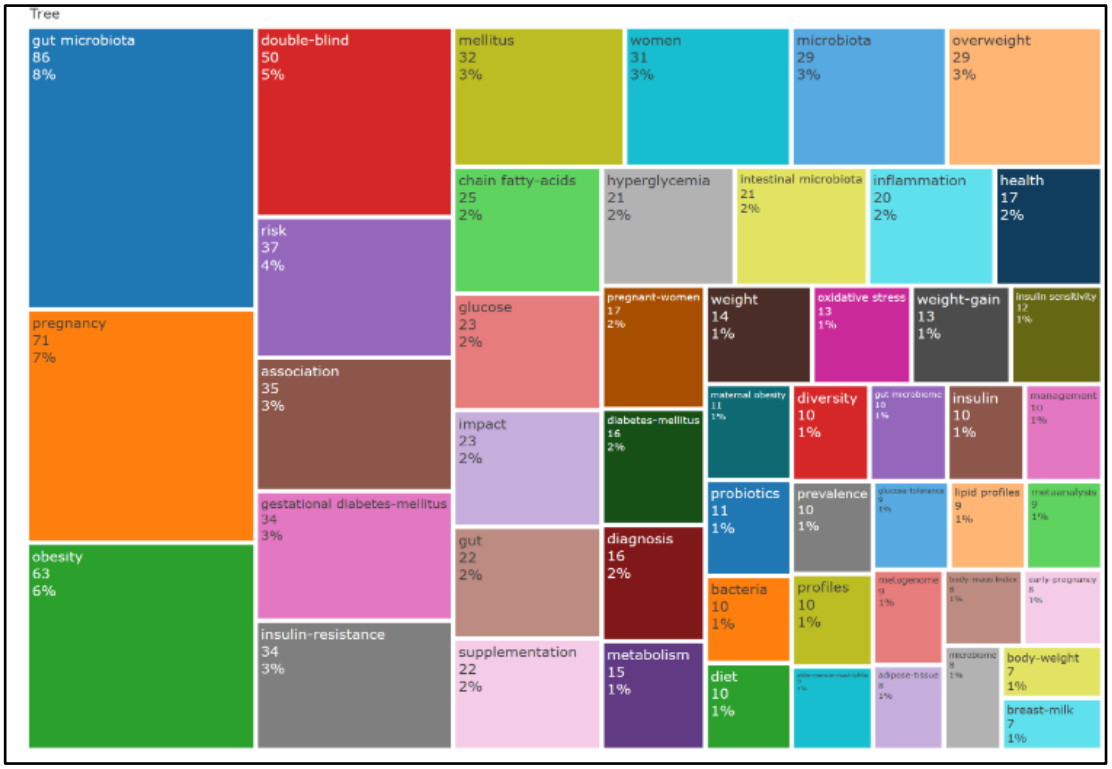

Figure 12. TreeMap of keywords

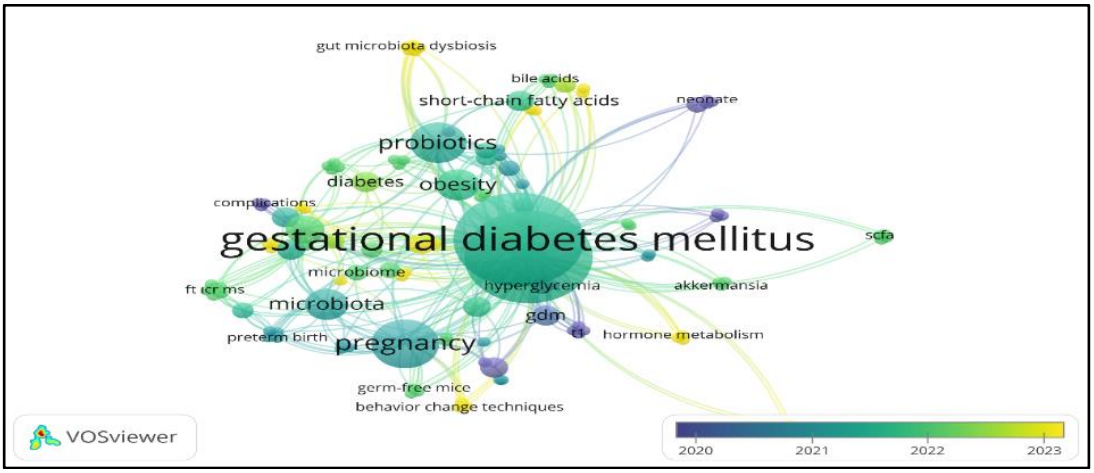

Figure 13. Common asset analysis

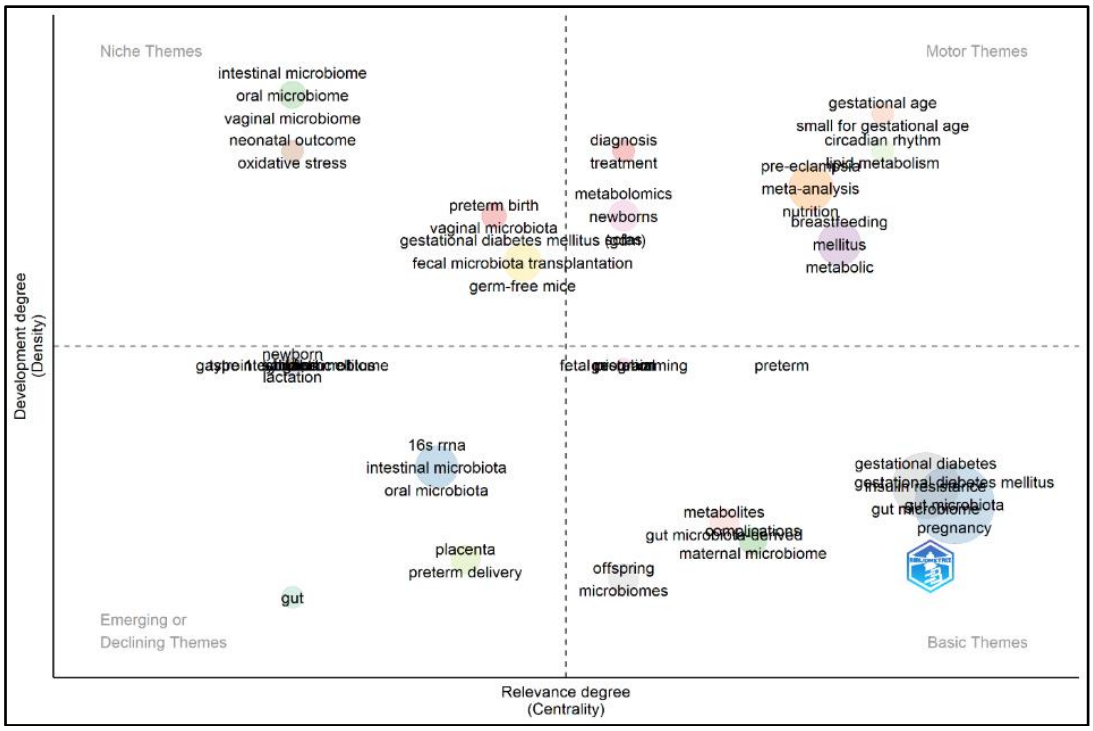

Figure 14. Thematic analysis based on keywords.

Discussion

As the volume of scientific literature is increasing rapidly, researchers resort to bibliometric analysis tools to identify trends in research areas, identify keywords, and understand interdisciplinary relationships. The current bibliometric analysis research is one of the first in the literature to investigate the gut microbiota in GDM. This study, which aims to systematically examine studies on gut microbiota in patients diagnosed with GDM, looked at various topics such as author profile, co-citation status, research areas of the examined publications, journals in which the studies were published, and the results were presented. Our findings indicate that GDM and gut microbiota is a rapidly expanding topic with huge promise. There is no question that this study topic represents a significant step into the future, and we are confident that GDM and gut microbiota will keep evolving. The trend is likely to raise worldwide consciousness of GDM and gut microbiota.

The examination of the quantity of publications and citations indicates that this topic of study was identified fifteen years ago, and since 2017, it has been receiving increased attention. We discovered that in the upcoming years, it will become more significant. Additionally, it has a high significance, which suggests that nations and areas with strong publication opportunity share research. The top 10 nations are divided among three Asian countries, three North and South American countries, three European countries, and the remaining two European countries. China tops the list of the ten most successful institutions, with six, followed by Australia in second place with three institutions. It is clear that, the dominance of Chinese research in this field may be attributed to increased funding opportunities, advanced research infrastructure, and government policies prioritizing scientific innovation in public health domains.

Chinese and Malaysian writers share the top spot with 89 citations between the most cited authors based on the high impact and high citation connection, and Italian authors coming in second. China is generally seen as a pioneer in the research on the gut microbiota in GDM.

Ultimately, although the first studies on this topic was mostly keyword-focused on "probiotics" and "obesity" until 2020, it began to focus genetic research on "16rRNA sequencing" between 2020 and 2023. By of 2023, the GDM gout microbiota has grown more cognizant of pathophysiological and metabolic issues by exploring hormone metabolism, its association with metabolic biomarkers, and markers for habit change.

Furthermore, we expect more thorough and excellent works with various approaches to studying the gut microbiota to be published in this subject in the upcoming years.

Conclusion

The creation of a bibliometric study can contribute to this situation, because this sort of research allows finding and evaluating the current literature in an academic field, measuring its influence, accessibility, trends, and cooperation in the scientific community. More high-quality research on GDM and gut microbiota requires improved collaboration across nations with diverse economies.

Strengths and Limitations

We believe that our work represents the first bibliometric analysis of the knowledge domain and recent research trends on gut microbiota and GDM. We also partially recognized potential research trends, hotspots, and borders in this field of study. As with all academic studies, this study also has a limitation. Foremost, we included only SCI-E, ESCI, and SSCI

from the WoS database in our investigation, which may have resulted in the exclusion of additional high-quality literature in databases in this field. To address this limitation, future studies should consider integrating alternative databases and exploring grey literature to encompass a broader spectrum of research. Differentiating the keywords to be used in subsequent projects, databases, reviewing the diversity and different data types of the index will contribute to its comprehensive expansion and generalization.

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Conflict of interest: No conflict of interest was declared by the authors.

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Investigation of the Synergistic Effect of *Allium Polyanthum Schult* Extract and Docetaxel on Apoptosis-Related Genes in Colorectal Cancer Cells and Bioinformatics Analysis

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Research Article

History

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ABSTRACT

Objective: Colorectal cancer (CRC) is a type of cancer with high mortality and widespread metastasis. The aim was to determine the expression levels of apoptosis-related genes in CRC cells treated with *Allium polyanthum Schult* plant extract and the combination of this plant extract with docetaxel.

Methods: Expression analyses of *caspase-2* (*CASP2*), *nuclear factor NF-kappa-B1* (*NFKB1*), apoptosis regulator *BAX* and proto-oncogene *MYC* genes that play a role in apoptosis in the CRC cell line HT-29 and the healthy colon cell line CCD-18Co, which were treated with only *Allium polyanthum Schult* plant extract and docetaxel together with this plant extract, were performed using the real-time polymerase chain reaction (RT-PCR) method. Bioinformatics analysis of relevant genes was performed using various databases.

Results: *CASP2*, *MYC*, *NFKB1* and *BAX* gene expression was significantly decreased in CRC cells treated with the combination of *Allium polyanthum Schult* extract and docetaxel compared to healthy cells. Accordingly, only the extract of *Allium polyanthum Schult* plant significantly reduced the expression of apoptosis-related genes in HT-29 cells compared to the extract combined with docetaxel. As a result of bioinformatics analysis, it was found that CASP2, MYC, NFKB1 and BAX proteins interact with each other and the expression levels of their genes are associated with survival. In addition, the methylation status of *CASP2* and *NFKB1* has the potential to change protein levels by affecting epigenetic mechanisms in CRC.

Conclusion: According to the information in the literature, it has been reported that *Allium* species affect genes in apoptotic pathways. Accordingly, *Allium polyanthum Schult* alone and in combination with docetaxel should be supported by further studies.

Keywords: *Allium polyanthum Schult*, apoptosis, colorectal cancer, docetaxel, gene expression

Allium Polyanthum Schult Ekstraktı ve Dosetakselin Kolorektal Kanser Hücrelerindeki Apoptozla İlgili Genler Üzerindeki Sinerjik Etkisinin Araştırılması

Araştırma Makalesi

Süreç

Geliş: 13/12/2024
Kabul: 25/12/2024

ÖZET

Amaç: Kolorektal kanser (CRC), yüksek mortalite ve yaygın metastazlı bir kanser türüdür. CRC'nin erken teşhis edildiği durumlarda kemoterapi ve cerrahi uygulamalar esastır. Docetaxel, taksan grubunda bir antikanser ajandır. Ayrıca, tıbbi aktiviteye sahip bitkilerin CRC üzerinde koruyucu bir etkiye sahip olduğu bilinmektedir. Amaç, Allium polyanthum Schult bitki özütü ve bu bitki özütünün dosetaksel ile kombinasyonu ile tedavi edilen kolorektal kanser hücrelerinde apoptozisle ilişkili genlerin ifade düzeylerini belirlemekti.

Yöntem: Sadece Allium polyanthum Schult bitki ekstresi ve bu bitki ekstresiyle birlikte docetaxel uygulanan CRC hücre hattı HT-29 ve sağlıklı kolon hücre hattı CCD-18Co'da apoptozda rol oynayan kaspaz-2 (CASP2), nükleer faktör NF-kappa-B1 (NFKB1), apoptoz düzenleyici BAX ve proto-onkogen MYC genlerinin ekspresyon analizleri, gerçek zamanlı polimeraz zincir reaksiyonu (RT-PCR) yöntemi kullanılarak gerçekleştirildi. Çeşitli veritabanları kullanılarak ilgili genlerin biyoinformatik analizi gerçekleştirildi.

Bulgular: CASP2, MYC, NFKB1 ve BAX gen ekspresyonu, Allium polyanthum Schult özütü ve docetaxel kombinasyonu ile tedavi edilen CRC hücrelerinde sağlıklı hücrelere kıyasla önemli ölçüde azaldı. Buna göre HT-29 hücrelerinde sadece Allium polyanthum Schult bitkisinin ekstraktı, dosetakselle kombine olan ekstrakta göre apoptozla ilgili genlerin ekspresyonunu oldukça düşürmüştür. Biyoenformatik analiz sonucunda, CASP2, MYC, NFKB1 ve BAX proteinlerinin birbirleriyle etkileşime girdiği ve genlerinin ekspresyon seviyelerinin hayatta kalma ile ilişkili olduğu bulundu. Ayrıca, CASP2 ve NFKB1'in metilasyon durumu, CRC'de epigenetik mekanizmaları etkileyerek protein seviyelerini değiştirme potansiyeline sahiptir.

Sonuç: Literatürdeki bilgilere göre, Allium türlerinin apoptotik yollardaki genleri etkilediği bildirilmiştir. Buna göre, Allium polyanthum Schult'un tek başına ve docetaksel ile kombinasyonu daha fazla çalışma ile desteklenmelidir.

Anahtar Kelimeler: *Allium polyanthum Schult*, apoptoz, docetaksel, gen ekspresyonu, kolorektal kanser

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Introduction

CRC is a cancer with high mortality and incidence worldwide. According to GLOBOCAN statistics for 2022, CRC ranks fourth in terms of incidence and third in terms of mortality.[1] Due to the increasing incidence of CRC, which is usually diagnosed at an advanced age, it is predicted that these cases will increase according to the estimates for the year 2035. It is estimated that CRC cases will increase especially in underdeveloped countries.[2, 3] The term CRC refers to the uncontrolled proliferation of glandular epithelial cells within the colon, including the rectum.[4] CRC is diagnosed by staging. These stages are classified as Stage 0-IV. While surgical treatment is applied in Stage 0 to II, adjuvant therapy is recommended in addition to surgery in Stage III. In Stage IV, advanced surgical applications and the use of targeted therapies are included.[5] For CRC, as in all cancers, there is a strong relationship between apoptosis signaling and cell survival. Abnormal functioning of apoptosis pathways in CRC disrupts homeostasis of colorectal epithelial cells. Suppression of apoptotic pathways has been explained by resistance to chemotherapeutic agents. The fact that apoptosis is different in normal cells compared to cancer cells and that apoptosis increases the cytotoxic response in cancer has helped develop various strategies in this regard.[6] In addition, apoptosis includes genetic factors that may be effective in the proliferation and recurrence of cancer cells Investigating the genes and proteins involved in apoptotic pathways has the potential to help shed light on the disease.[7]

Although there are various chemotherapeutic applications that increase the survival of CRC, drug resistance is a possibility. In addition, the controversial reliability of synthetic drugs directs attention to traditional medical applications. In this context, the effectiveness of many plants with medical importance for many diseases, including cancer, has been reported from past to present. The fact that natural medical practices are safer in addition to chemotherapy has helped increase studies on this subject. [8-10] Medicinal aromatic plants are important in terms of their medical effectiveness in cancer. The anti-cancer properties of plants in the *Allium* genus have been investigated and in addition, their anti-microbial, anti-oxidant and anti-inflammatory activities have been revealed. There are many plants identified as belonging to the *Allium* genus, such as garlic, leek and onion.[11-13] *Allium* plants belong to the *Amaryllidaceae* family and contain many bioactive components, which explains their biological activity. The discovery of many therapeutic activities of *Allium* plants has made the research of plants belonging to this genus important.[14]

Materials and Methods

Preparation of Allium polyanthum Schult extract

Allium polyanthum Schult plant was obtained from Zara, Sivas, Turkey. The plant was collected in May and September. Essential oils were extracted according to the method determined by Alkan et al. According to the procedure, the essential oils of *Allium polyanthum Schult* plant were obtained by hydrodistillation for three hours from flowering stem parts and only stem parts with the help of Clevenger apparatus. The temperature of the cooling water was adjusted to 4°C by connecting the Clavenger apparatus to the microhiller device. The isolated essential oil was purified from water by Na_2SO_4. The samples were stored at -20°C.[15]

Cell culture

HT-29 (ATCC, HTB-38) was used within the scope of CRC cell line. In addition, CCD-18Co (ATCC, CRL-1459) cell line is healthy colon epithelial cells. These cells were cultured at 37ºC in a humidified environment containing 5% CO_2. Roswell Park Memorial Institute 1640 medium (RPMI, Biological Industries) was used as HT-29 medium, and Minimum Essential Medium (MEM, Sigma-Aldrich) was used as CCD-18Co cell line medium. 10% Fetal bovine serum (FBS, Capricorn Scientific) and 1% penicillin/streptomycin (Sigma-Aldrich) were applied to the media.

Expression of apoptosis-related genes

RT-PCR method was used for expression analysis of *CASP2*, *NFKB1*, *BAX* and *MYC* genes in HT-29 and CCD-18Co cells. First, cell lines were seeded in six-well plates for gene expression analysis. The cytotoxic dose of *Allium polyanthum Schult* plant extract and additionally the cytotoxic dose (IC_{50}) of docetaxel drug together with extract were applied to these cells.[16] IC_{50} doses of 0.190 µM for docetaxel, 0.043 µM for *Allium polyanthum Schult* + docetaxel, and >300 µM for *Allium polyanthum Schult* alone were applied to the CCD-18Co cell line separately. IC_{50} doses of 0.077 µM for docetaxel, 0.009 µM for *Allium polyanthum Schult* + docetaxel, and >300 µM for *Allium polyanthum Schult* alone were applied to the HT-29 cell line. After 48 hours of incubation, RNA isolation from cells was performed according to the kit procedure. cDNA was generated from the RNAs obtained using the cDNA synthesis kit. Primers for genes are provided in the form

of primer assays. Gene expression analysis was performed using Syber Green Master mix.

Bioinformatics analyses

Analysis of protein-protein interactions of CASP2, NFKB1, BAX and MYC was provided by the Stringv12 database (https://version-12-0.string-db.org/cgi/network?networkId=bBuQ7cSnMJMh). The GEPIA database is an effective tool for analyzing the expression levels of many genes in tumor tissues compared to normal tissues (http://gepia2.cancer-pku.cn). UALCAN is a database that provides transcriptomic data for all cancer types (http://ualcan.path.uab.edu/index.html). Using this database, survival rates and methylation levels of *CASP2*, *BAX*, *NFKB1* and *MYC* genes were analyzed separately in colon and rectum compared to normal tissues.[17] According to the database, methylation levels between 0.7-0.5 indicate hypermethylation, and between 0.3-0.25 indicate hypomethylation.[18, 19] Using the MuTarget database, which identifies mutations in cancer, the somatic mutations in *CASP2*, *BAX*, *NFKB1* and *MYC* genes were analyzed to determine which genes' expression was affected in colon adenocarcinoma (https://www.mutarget.com).[20]

Statistical Analysis

RT-PCR data were obtained using Rotor-Gene 6000 Series Version 1.7 software. The $\Delta\Delta C_T$ method was used for gene expression analysis. Expression analysis was completed with the RT^2 profiler RT-PCR Array Data Analysis version 3.5 application (https://geneglobe.qiagen.com/us/analyze). ANOVA tests was applied and Tukey's multiple comparison test was used. Three replicates were used to obtain data. Significance value was accepted as $p<0.05$.

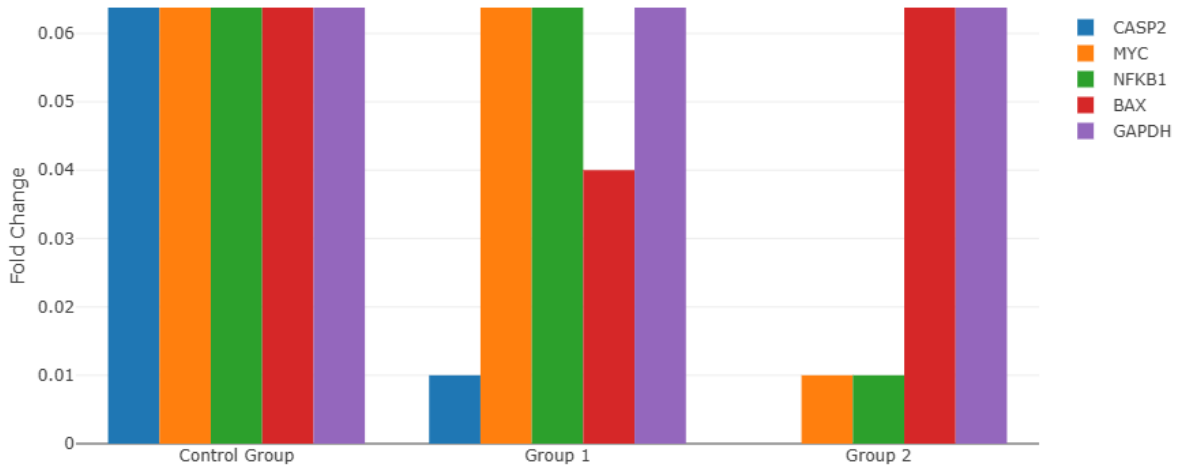

Figure 1. Fold change graph for expression levels of *CASP2*, *NFKB1*, *BAX* and *MYC* genes in HT-29 cells (Group 1: Combination of *Allium polyanthum Schult* extract and Docetaxel ; Group 2: *Allium polyanthum Schult* extract)

Table 1. Fold change, fold regulation and p values of *CASP2*, *NFKB1*, *BAX* and *MYC* genes in HT-29 cells (Group 1: Combination of *Allium polyanthum Schult* extract and Docetaxel ; Group 2: *Allium polyanthum Schult* extract)

Genes	**Group 1**			**Group 2**		
	Fold change	**Fold regulation**	***p*-value**	**Fold change**	**Fold regulation**	**p-value**
CASP2	0.01	-117.78	0.001*	0.00	-310.83	0.001*
MYC	0.08	-13.27	0.001*	0.01	-97.01	0.001*
NFKB1	0.07	-14.42	0.001*	0.01	-78.25	0.001*
BAX	0.04	-27.67	0.001*	0.42	-2.39	0.001*

Results

Expression level of apoptosis-related genes

The expression levels of *CASP2*, *NFKB1*, *BAX* and *MYC* genes were evaluated in HT-29 and CCD-18 cells with CRC. The effectiveness of *Allium polyanthum Schult* plant extract and its combination with docetaxel was evaluated. Accordingly, *CASP2*, *NFKB1*, *BAX* and *MYC* gene expressions were significantly decreased in HT-29 cells to which *Allium polyanthum Schult* plant extract was applied and the combination of plant extract and docetaxel was applied compared to HT-29 cells to which nothing was applied (Figure 1) (Table 1).

The combination of plant extract and docetaxel increased *CASP2*, *MYC* and *BAX* gene expression and decreased *NFKB1* gene expression in CCD-18Co cells compared to cells without any treatment. In addition, *CASP2* and *NFKB1* expressions were decreased, while *MYC* and *BAX* gene expressions were decreased in cells treated with only plant extract (Figure 2) (Table 2).

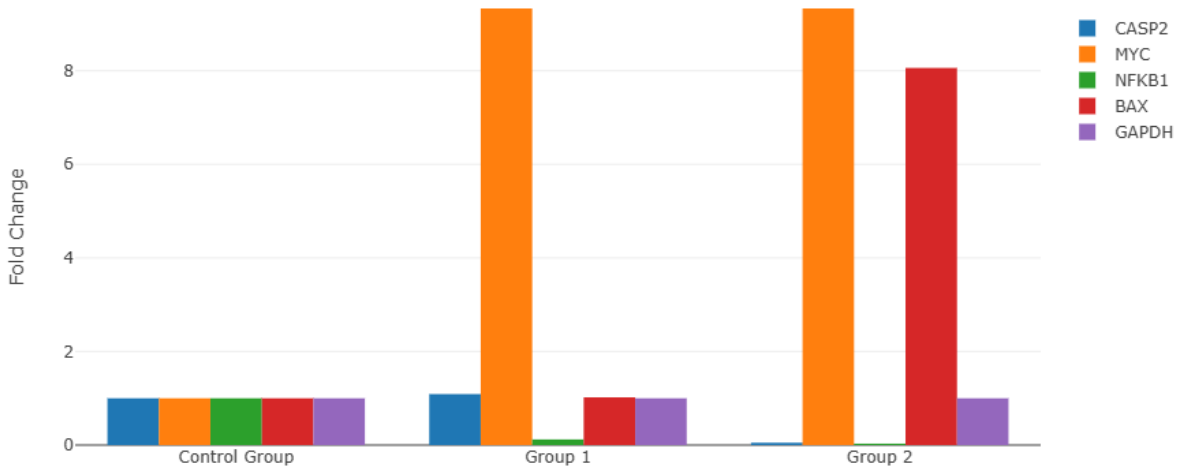

Figure 2. Fold change graph for expression levels of *CASP2*, *NFKB1*, *BAX* and *MYC* genes in CCD-18Co cells (Group 1: Combination of *Allium polyanthum Schult* extract and Docetaxel ; Group 2: *Allium polyanthum Schult* extract)

Table 2. Fold change, fold regulation and p values of *CASP2*, *NFKB1*, *BAX* and *MYC* genes in CCD-18Co cells (Group 1: Combination of *Allium polyanthum Schult* extract and Docetaxel; Group 2: *Allium polyanthum Schult* extract)

Genes	**Group 1**			**Group 2**		
	Fold change	**Fold regulation**	***p*-value**	**Fold change**	**Fold regulation**	**p-value**
CASP2	1.09	1.09	0.001*	0.05	-21.26	0.001*
MYC	19.03	19.03	0.001*	163.14	163.14	0.001*
NFKB1	0.12	-8.57	0.001*	0.03	-33.13	0.001*
BAX	1.02	1.02	0.001*	8.06	8.06	0.001*

A significant difference was observed in terms of *CASP2*, *NFKB1*, *BAX* and *MYC* expressions in CCD-18Co cells and HT-29 cells to which the combination of *Allium polyanthum Schult* extract and Docetaxel was applied. When CCD-18Co cells and HT-29 cells were compared, it was found that only *Allium polyanthum Schult* extract caused a change in the expression levels of *CASP2*, *NFKB1*, *BAX* and *MYC* genes ($p<0.05$) (Figure 3). It was determined that *CASP2*, *MYC*, *NFKB1* and *BAX* gene expressions were significantly decreased in HT-29 cells to which only *Allium polyanthum Schult* extract was applied compared to control cells. In addition, a decrease in *CASP2*, *MYC*, *NFKB1* and *BAX* gene expressions was observed in HT-29 cells to which *Allium polyanthum Schult* was applied together with docetaxel compared to control cells. However, the decreases in the expression of these genes were more pronounced in cells to which *Allium polyanthum Schult* was applied alone.

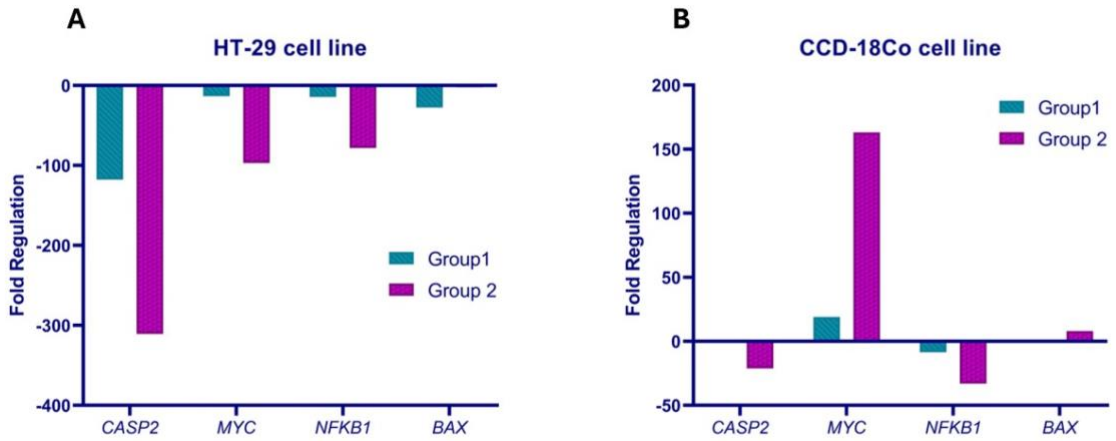

Figure 4. The combination of *Allium polyanthum Schult* extract and Docetaxel and only *Allium polyanthum Schult* extract increased apoptosis genes expression in HT-29 (A) and CCD-18Co (B) cells (Group 1: Combination of *Allium polyanthum Schult* extract and Docetaxel ; Group 2: *Allium polyanthum Schult* extract)

As a result, *CASP2*, *MYC*, *NFKB1* and *BAX* gene expression was significantly decreased in CRC cells treated with the combination of *Allium polyanthum Schult* extract and docetaxel compared to healthy cells. It was determined that the decrease in these genes was more in the cells given only the plant extract than in the CRC cells to which the plant extract and drug combination was applied.

Bioinformatics analyses

Bioinformatic analyses were performed within the scope of the study. The protein-protein interactions of CASP2, MYC, BAX and NFKB1 were analyzed using the String v12 database. Accordingly, it was determined that CASP2, MYC, BAX and NFKB1 were related to each other. In addition, CASP2, MYC, BAX and NFKB1 were seen to interact with many other proteins given in Figure 5. The interaction levels of these proteins were found to be significant. When the interaction scores of the proteins were examined, the highest interaction was found to be between NFKB1 and MYC proteins (Table 3).

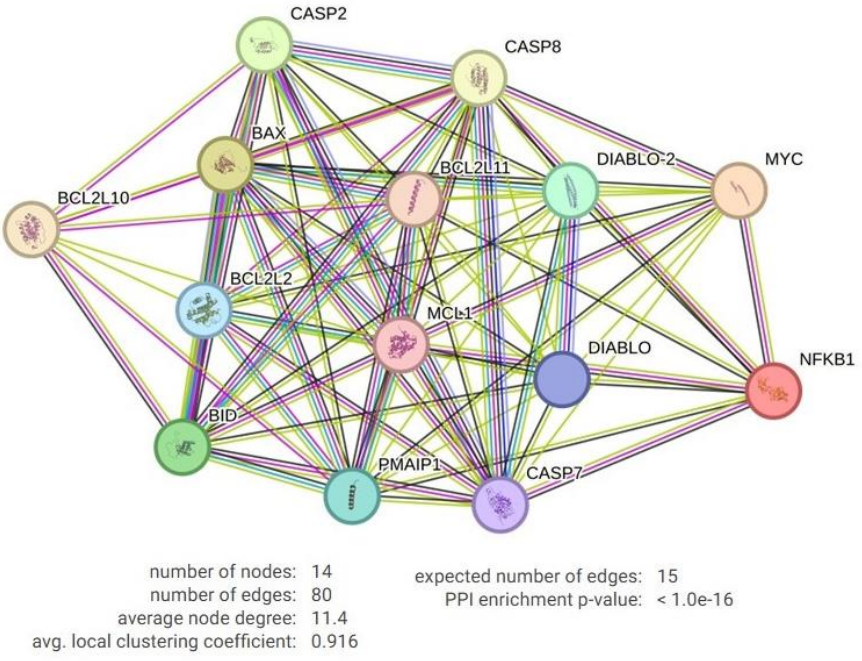

Figure 5. Illustration of interactions that CASP2, BAX, NFKB1, and MYC proteins share with other proteins

Table 3. Interaction scores of CASP2, BAX, NFKB1, and MYC proteins based on the STRING database

Node 1	Node 2	Node 1 accession	Node 2 accession	score
BAX	CASP2	ENSP00000293288	ENSP00000312664	0.447
BAX	MYC	ENSP00000293288	ENSP00000478887	0.415
MYC	NFKB1	ENSP00000478887	ENSP00000226574	0.917

The biological processes in which CASP2, BAX, NFKB1, and MYC proteins and additionally their associated proteins in 14 nodes participate according to the STRING database are given in Figure 6. Accordingly, it was determined that 12 proteins were highly related to extrinsic apoptotic pathways. In addition, it was recorded that 9 genes played a role in the intrinsic apoptotic pathway and 12 genes were generally effective in apoptotic pathways.

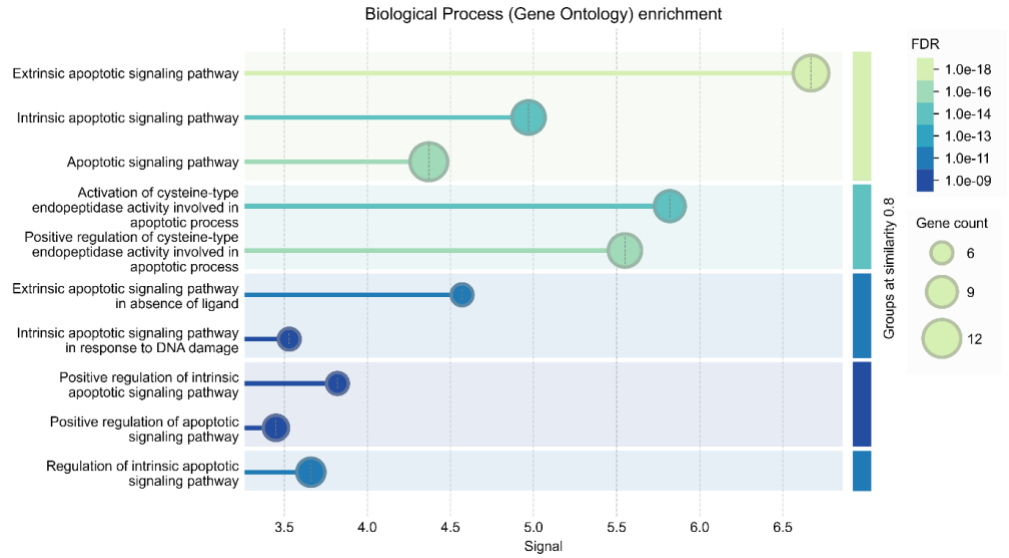

Figure 6. Common pathways of CASP2, NFKB1, BAX and MYC proteins in biological processes

Expression levels of *CASP2*, *NFKB1*, *BAX* and *MYC* genes were determined in 349 colon adenocarcinoma (COAD) patient tissues and 275 normal tissues within the scope of TCGA and GTEx data using GEPIA database. Accordingly, *CASP2*, *BAX* and *MYC* expression were significantly increased in COAD tissues compared to normal tissues. While *NFKB1* expression level was increased in COAD compared to normal tissues, this increase was not found to be significant (Figure 7).

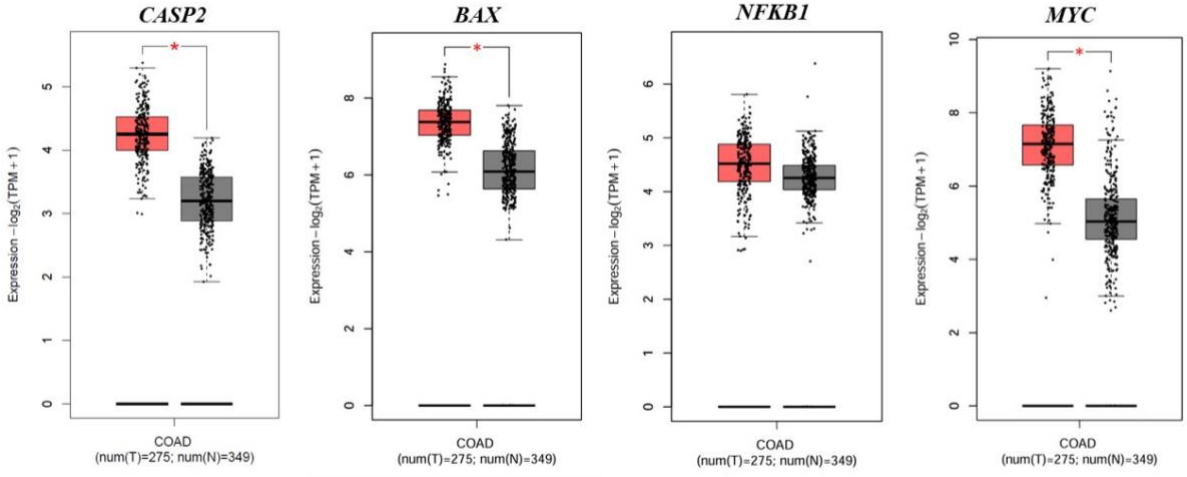

Figure 7. Box plot graph of *CASP2*, *BAX*, *NFKB1* and *MYC* gene expression in COAD compared to normal tissues obtained from GEPIA database (Red: Tumor tissue; Gray: Normal tissue) ($p<0.01$)

The expression of various genes has been associated with survival in cancer. According to the TCGA data of patients with COAD, the median of *CASP2*, *BAX*, *NFKB1* and *MYC* genes was taken and the effect of high and low expressions on survival was investigated. According to the findings, *BAX* and *CASP2* gene expression was closely associated with the survival of COAD patients, while *NFKB1* and *MYC* genes were not (Figure 8).

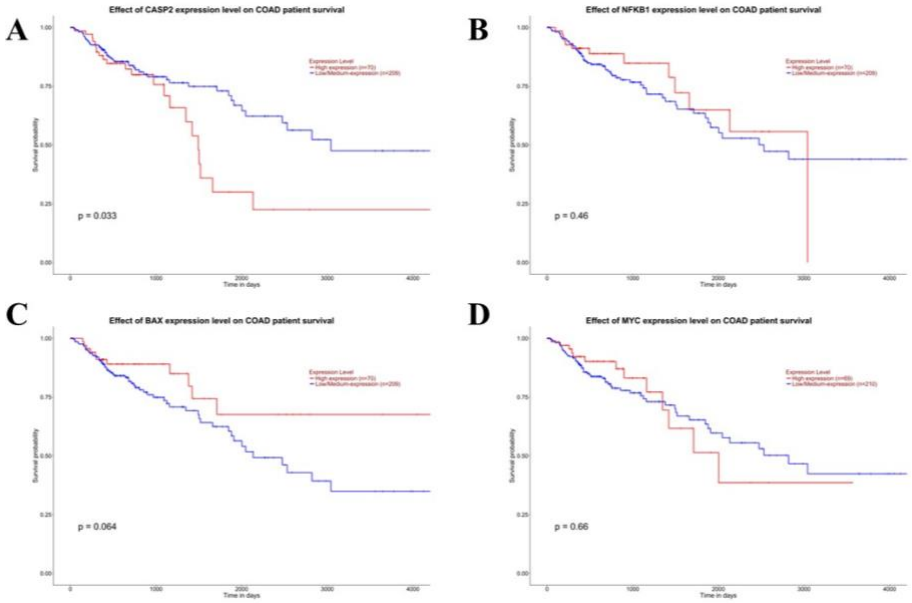

Figure 8. Kaplan–Meier curve showing the effect of *CASP2*, *NFKB1*, *BAX* and *MYC* gene expression on COAD survival ($p<0.05$)

Promoter methylation levels of *CASP2*, *NFKB1*, *BAX* and *MYC* genes were obtained from UALCAN database. Accordingly, methylation levels were examined in 313 primary COAD patient tissues and 37 normal tissues. Accordingly, it was found that *CASP2* gene was hypomethylated in COAD tissues compared to normal tissues, while *NFKB1* gene was hypermethylated. Promoter methylation levels of *BAX* and *MYC* genes were not found to be significant in COAD (Figure 9).

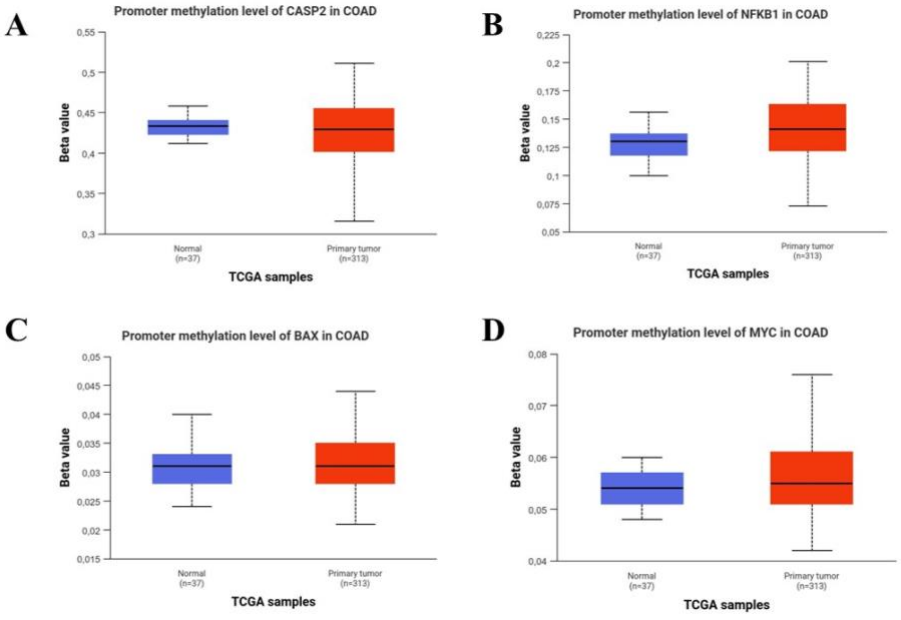

Figure 9. Levels of *CASP2* (A), *NFKB1* (B), *BAX* (C), and *MYC* (D) promoter methylation in COAD patients' tissue compared to normal tissues

All somatic mutations in *CASP2, NFKB1, BAX* and *MYC* genes in colon adenocarcinoma were examined using the muTarget database. It was determined how the mutations in our genes in the database affected the cancer hallmark genes determined by the system.[21] Somatic mutations in *CASP2*, *NFKB1*, *BAX* and *MYC* genes increased the expression of *hypoxia-inducible factor-1* (*HIF1A*), *dipeptidyl peptidase IV* (*DPP4*), *Neuropilin 1* (*NRP1*) and *janus kinase 2* (*JAK2*) which are among the cancer hallmark genes, and decreased the expression of *chemokine (C-X-C motif) ligand 14* (*CXCL14*). Accordingly, it has been determined that mutations in these genes related to apoptosis increase and decrease the expression of various genes that play a role in cancer (Figure 10).

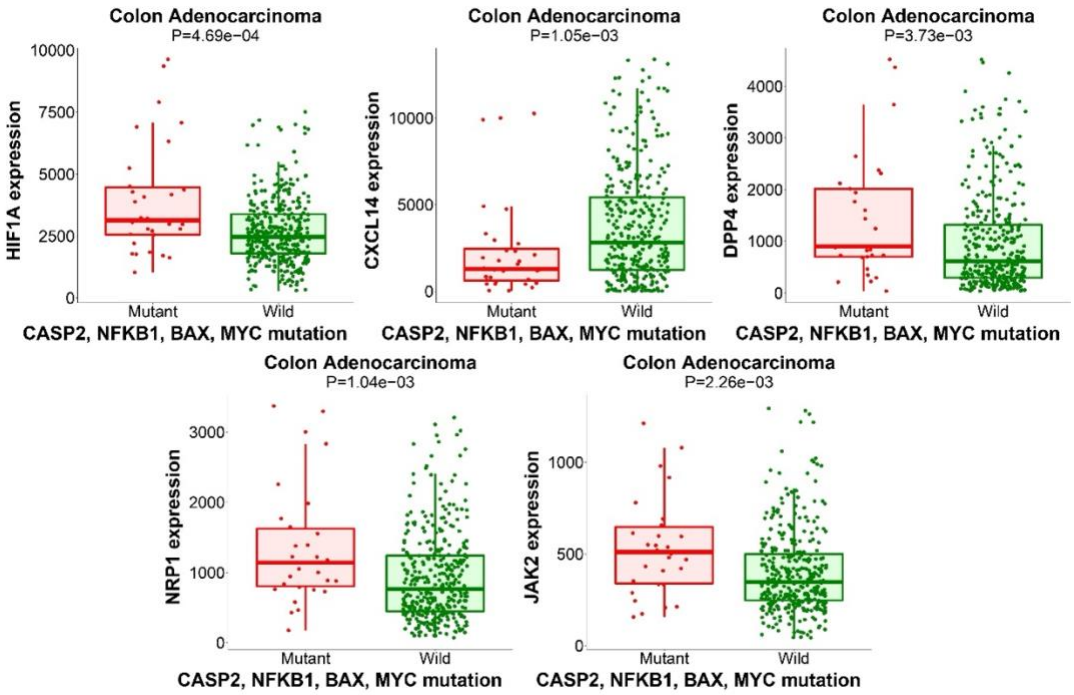

Figure 10. Effect of all somatic mutations of *CASP2*, *NFKB1*, *BAX* and *MYC* genes on the expression of certain cancer hallmark genes in colon adenocarcinoma ($p<0.05$)

Discussion

CRC has a high incidence rate globally and is the leading cause of cancer deaths.[22] CRC is a heterocomplex disease and therefore the effectiveness of recommended treatment options may vary.[23] In addition, studies have reported that the effectiveness of chemotherapeutic drugs applied for CRC is not fully effective due to many reasons, including drug resistance.[24] The drug docetaxel is also used in various types of cancer. Although this chemotherapeutic agent increases survival, it has been noted that it has many side effects.[25] Accordingly, within the scope of the study, the effect of *Allium polyanthum Schult* plant, which was extracted to create a synergetic effect by supporting the efficacy of docetaxel, on the expression of *CASP2*, *MYC*, *NFKB1* and *BAX* genes involved in apoptotic pathways was investigated.

Many plants belonging to the *Allium* genus have been reported to have various biological activities.[11] Because plants belonging to this genus contain many biologically active components, including high levels of sulfur, phenolic compounds and antioxidants.[26] Its biological activities can be listed as antiviral, antiasthmatic, antimotility,antidiabetic, antihypertensive, hypocholesterolemic, antiprotozoal, antiplatelet, antibacterial, antihelmintic, antiproliferative. In addition, the traditional use of this plant, which has cytotoxic roles in cancer, has highlighted its anticancer activity.[27-30] Abdel-Hady et al. reported anti-cancer activity of *A. ampeloprasum* strain in HepG2 and Caco-2 cells.[31] In another study, the cancer inhibitory properties of the affine type were demonstrated in OVCAR-3 cells, an ovarian adenocarcinoma cell line, due to its cytotoxic activity.[30] Cytotoxic activity of *A. atroviolaceum Boiss* methanol extract was proven by inducing apoptosis in MCF7, HeLa, MDA-MB-231 and HepG2 cells.[32, 33] In several studies, it has been reported that methanol or aqueous extracts of all parts of the *A. ursinum* plant have cytotoxic activity on gastric cancer cells, inhibiting cancer development in association with apoptosis, and have antioxidant properties.[34-37] Glycosides and saponin components isolated from *Allium schoenoprasum* plant

have been reported to have anticancer activity in HCT 116 and HT-29 colon cancer cells.[38] In the study conducted by Mskhiladze et al., the anticancer activity of furostanol and spirostanol and fractions obtained from *A. leucanthum* species was investigated in A549 lung cancer and DLD-1 colon cancer cell lines. Accordingly, it was discovered that *A. leucanthum* was cytotoxic in these cancer types.[39] Alshammari et al. proved the inhibitory effect of *Allium porrum* methanol extract on the proliferation of cancer cells via apoptotic pathways in colon cancer cells.[40] Since apoptotic pathways are associated with the development and progression of CRC cancer [41], the activity of *CASP2*, *MYC*, *NFKB1* and *BAX* genes was evaluated within the scope of the study.

Apoptosis, which is programmed cell death, ensures homeostasis of cells. However, impaired apoptotic processes can be activated by chemotherapy and targeted therapies. Caspases activate apoptosis. There are many members of the caspase family identified in mammals. CASP2, a member of the caspase family, is the caspase with the most conserved structure.[42] In a study involving 48-hour incubation of taxane group chemotherapeutic drugs, it was determined that *CASP2* activity in breast cancer cells increased 15-fold.[43] In this study, when *Allium polyanthum Schult* total extract was administered together with docetaxel, *CASP2* levels in CRC were decreased. In CRC cells administered only *Allium polyanthum Schult* total extract, a relatively greater decrease in *CASP2* expression was observed compared to healthy cells. The activation of *CASP2* indicates the activation of the apoptotic pathway.[44] Here, it is thought that the application of *Allium polyanthum Schult* extract alone may have anti-apoptotic activity. However, the fact that *CASP2* expression in CRC cells applied with only plant extract was relatively significantly more significant than in combined application suggests that combined application may have a more effective role in activating the apoptotic pathway. Additionally, bioinformatics analysis revealed that *CASP2* expression and promoter methylation increased with increasing COAD.

In the study conducted by Khazaei et al., the efficacy of the methanol extract of the flower parts of *Allium atroviolaceum* was tested on the apoptotic pathway in breast cancer cell line. Accordingly, it was reported that apoptosis was induced in the study in which various caspases were also included. NFKB plays a role in many cellular processes in cancer, both in the apoptotic pathway and in inflammation. NFKB inflammation has been frequently studied in relation to CRC. Upregulation of *NFKB* has been found to inhibit apoptosis and increase angiogenesis and cell proliferation.[45] Unlike cancer, it was determined that the *NFKB* gene was suppressed by *Allium sativum L.* extract in ulcerative colitis and had a protective effect on the colon.[46] According to the data obtained from the study, it was determined that there was a significant decrease in the *NFKB1* gene in CRC cells to which only *Allium polyanthum Schult* extract was applied. Accordingly, it is thought that extract may play a role in apoptosis by reducing *NFKB1* expression. According to bioinformatic data, it was determined that the expression of *NFKB1* increased in COAD and this increase was not significant. In addition, *NFKB1* was hypermethylated in COAD tumor tissues compared to COAD control tissues.

It is known that high expression of *BAX*, which has a proapoptotic function, helps in scavenging reactive species and inflicting cytotoxic damage on cancer cells. BAX has been reported to be suppressed in cancer.[47] In a study, it was reported that *BAX* was an effective prognostic factor in patients who underwent surgery for CRC. It was noted that higher mortality was seen in CRC patients in whom *BAX* expression was not observed.[48] In this study, it was determined that *BAX* expression was excessively decreased in CRC cells treated with only *Allium polyanthum Schult* and combined treatment with docetaxel. Accordingly, it is thought that there may be an adverse effect related to *BAX*-mediated apoptosis in CRC. According to bioinformatic data, *BAX* expression increased significantly in COAD tissues. Accordingly, these data show that the plant is effective experimentally.

MYC is generally associated with cell proliferation, depending on the cell cycle. There are data indicating that apoptosis is induced by MYC. While the increase in MYC levels due to the increase in growth factors causes the cell to proliferate, apoptosis can be observed in cells with reduced MYC in which growth factors are limited. Accordingly, the idea that MYC can induce apoptosis in cancer is exciting.[49] In this study, application of only *Allium polyanthum Schult* extract in CRC significantly reduced *MYC* expression due to combination therapy. In this case, it may be possible to say that the functioning of many mechanisms related to the cell cycle and apoptosis has changed. According to bioinformatic data, *MYC* gene expression was observed to increase in COAD tumors. The effects of *Allium polyanthum Schult* extract on the expression of apoptosis genes in CRC cells should be investigated more comprehensively.

Conclusion

Allium polyanthum Schult extract significantly reduced the expression of identified apoptosis genes. It is thought that the application of this plant extract alone in CRC cells

is due to its anti-apoptotic properties. It is predicted that the combination of *Allium polyanthum Schult* applied with docetaxel may have the effect of inducing apoptosis. The effects of this plant on apoptosis mechanisms in CRC cells should be investigated more extensively.

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Migration of Dental Implant into The Sinus and Secondary Odontogenic Maxillary Sinusitis: Case Report

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Case Report

History

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ABSTRACT

Due to the close relationship between the maxillary sinus and the posterior maxillary tooth apices, oro-antral fistula and maxillary sinusitis may occur after interventional procedures performed in this region. In this case, a 53-year-old male patient placed an implant for his left second premolar tooth 7 months ago, but migration of the implant into the maxillary sinus has been detected. Nasal endoscopic examination revealed edema and hyperemia at the level of the left maxillary sinus ostium, but no foreign body was observed. A hyperdense image was reported on paranasal sinus tomography, narrowing the left maxillary sinus ostium and creating obstruction in the osteomeatal unit. Surgical intervention was planned for the patient, implant material was removed, and the maxillary sinus was cleared of inflamed tissues by the functional endoscopic sinus surgery was performed under general anesthesia. With this case that we present to the literature, we aimed to draw attention to odontogenic factors in the etiology of maxillary sinusitis and to raise awareness for the diagnosis and treatment of the disease.

Keywords: Dental implant, odontogenic maxillary sinusitis, migration of foreign body, endoscopic sinüs surgery

Dental İmplantın Sinüse Migrasyonu ve Sekonder Odontojenik Maksiller Sinüzit: Olgu Sunumu

Olgu Sunumu

Süreç

Geliş: 08/11/2024
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ÖZET

Maksiller sinüs ile posterior maksiller diş apeksleri arasındaki yakın ilişki nedeniyle bu bölgeye yapılan girişimsel işlemler sonrası oro-antral fistül ve maksiller sinüzit gelişebilmektedir. Bu olguda, 53 yaşında bir erkek hastaya 7 ay önce sol 2. premolar dişine implant uygulanmış, ancak implantın maksiller sinüse migrasyonu tespit edilmiştir. Nazal endoskopik incelemede, sol maksiller sinüs ostiumu düzeyinde ödem ve hiperemi saptanmış, ancak yabancı cisim izlenmemiştir. Paranazal sinüs tomografisinde, sol maksiller sinüs ostiumunu daraltan ve osteomeatal ünitede obstrüksiyon yaratan hiperdens bir imaj rapor edilmiştir. Hastaya cerrahi müdehale planlanmış, genel anestezi altında fonksiyonel endoskopik sinüs cerrahisi uygulanarak implant materyali çıkarılmış ve maksiller sinüs enflame dokulardan arındırılmıştır. Literatüre sunduğumuz bu olguyla; maksiller sinüzit etiyolojisindeki odontojenik faktörlere dikkat çekmeyi, hastalık tanı ve tedavisi için farkındalık oluşturmayı amaçladık.

Anahtar Kelimeler: Dental implant, odontojenik maksiller sinüzit, yabancı cisim migrasyonu, endoskopik sinüs cerrahisi

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Introduction

Approximately 60% of iatrogenic sinusitis, which is included in the etiology of acute, chronic and recurrent sinusitis, can be seen after dental treatments, and at least 45% of these can be seen after surgical treatments (surgical tooth extraction, sinus lift or implant surgery).[1,2] Despite anatomical diversity, posterior maxillary teeth, especially second premolars, first and second molars, are frequently in contact with the maxillary sinus floor. This relationship between the maxillary sinus and the posterior maxillary tooth apices occurs through the cortical bone layer and/or sinus mucosa. Because of this close relationship, sinusitis may develop as a result of oro-antral fistula and migration of prosthetic treatment materials into the maxillary sinus after interventional procedures in this region.[3,4] Foreign bodies in the sinus may cause irritation of the sinus membrane, slowing/stopping of mucociliary activity due to obstruction of the ostium, foreign body reaction and finally maxillary sinusitis.[2-6]

Our aim is to evaluate the endoscopic treatment of odontogenic maxillary sinusitis (OMS), which developed secondary to implant material crossed into the maxillary sinus after prosthetic treatment in the left maxilla posterior region, with the perspective of literature.

Case Report

A 53-year-old male patient was accepted as a case with no active complaints, referred to our ear, nose and throat department from the oral, dental and maxillofacial surgery clinic. The patient had an implant applied to the left maxillary second premolar tooth 7 months ago, but during follow-up examinations, it was determined that the implant was not in place. It was determined that the dental implant had migrated into the maxillary sinus in the orthopantomogram (Figure 1).

The patient was informed about the planned procedure and study, and written and verbal consent was obtained.

In the detailed ear, nose and throat examination, anterior rhinoscopic examination revealed that the nasal septum was subluxed in the caudal part and deviated to the right. Additionally, hypertrophy and degeneration of bilateral inferior turbinates, hypertrophy of the left middle turbinate, and increased seromucous secretion in the left nasal cavity were detected. During the oral cavity examination, it was observed that the area of the implant was covered with mucosa. No signs of oroantral fistula were detected.No additional pathological condition was detected other than postnasal discharge during oropharyngeal examination.

In the 0° and 45° nasal endoscopic examination performed using a rigid endoscope, edema and hyperemia were observed at the left maxillary sinus ostium level in addition to the anterior rhinoscopic findings, but no foreign body was detected.

In the coronal, axial and sagittal sections of the paranasal sinus computed tomography (PNSCT) requested by our clinic, a hyperdense image (probable metallic foreign body) at the level of the left maxillary sinus infundibulum, which significantly narrowed the left maxillary sinus ostium, and a soft tissue structure that almost completely filled the left maxillary sinus (probable OMS) secondary to the obstruction caused by this foreign body in the osteomeatal unit were reported (Figures 2,3,4).

Surgical intervention was planned for the patient and the procedure was performed under general anesthesia. Left functional endoscopic sinus surgery, left middle concha bullosa resection, and dental implant removal were performed. During the left uncinectomy performed after the left middle concha bullosa resection, a large amount of purulent material was encountered and an aspirate culture was taken from this material. During the enlargement of the maxillary sinus ostium, a foreign body located in the submucosal region at the level of the infundibulum was detected and successfully removed (Figures 5 and 6). The maxillary sinus was cleared of edematous and inflamed tissues and tissue sampling was performed for histopathological examination. Microscopic examination of the aspirate revealed gram-positive cocci, but no specific microorganism could be identified in the culture. Histopathological evaluation was reported as chronic sinusitis. No complications occurred during the surgical procedure. The patient was referred to the clinic where the implant was placed to complete the remaining dental treatment.

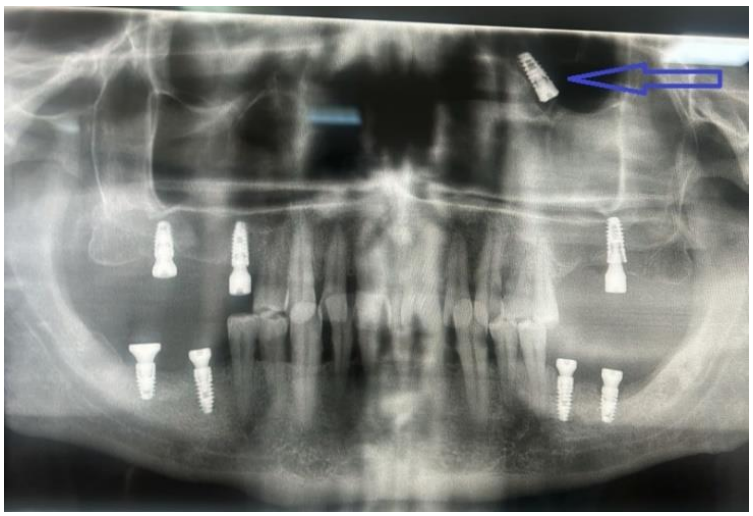

Figure 1. Orthopantomogram showing dental implant (blue arrow) migrating into the left maxillary sinus

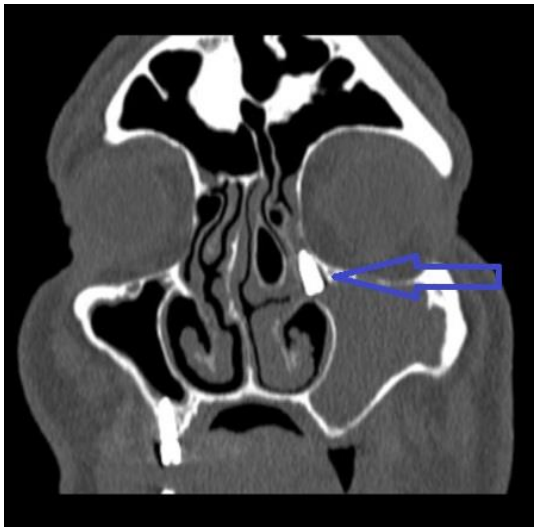

Figure 2. Coronal section of PNSCT showing the dental implant (blue arrow) obstructing the sinus ostium and causing odontogenic maxillary sinusitis, along with a concha bullosa of the left middle turbinate

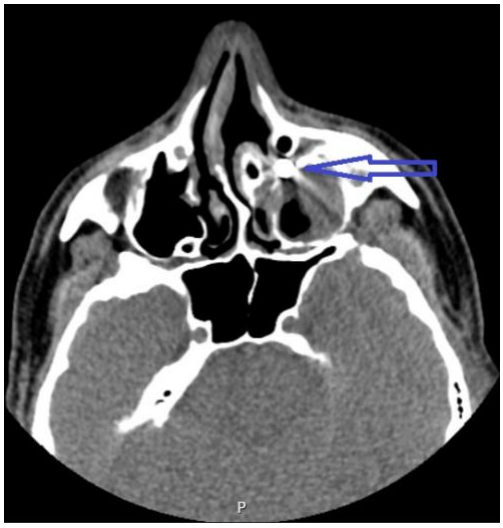

Figure 3. Axial section of PNSCT showing the appearance of the dental implant (blue arrow)

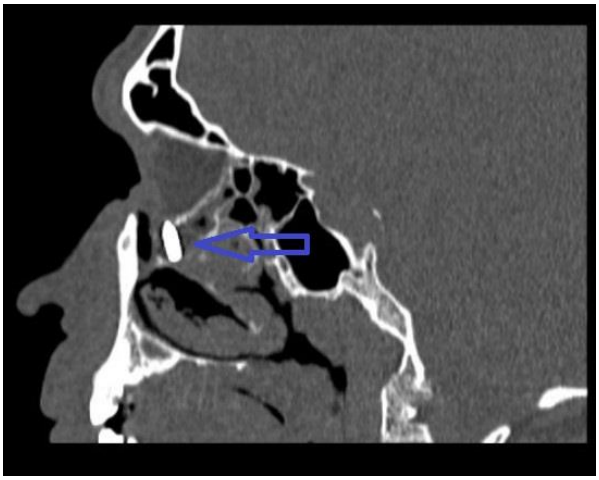

Figure 4. Sagittal section of PNSCT showing the appearance of the dental implant (blue arrow)

Figure 5. Dental implant (approximately 1 cm in length) removed from the left maxillary sinus

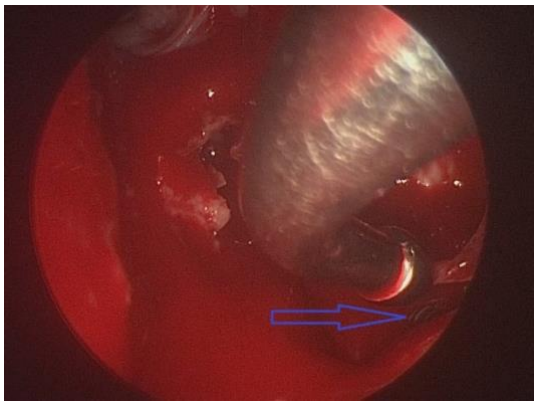

Figure 6. Intraoperative endoscopic view of the dental implant (blue arrow) that fell to the floor of the left maxillary sinus

Discussion

The definition of OMS was first described by Prof. William H. Bauer in 1943, and since then, it has become a widely recognized condition in both dentistry and otolaryngology.[7] OMS is a subtype of sinusitis that accounts for 10-15% of all maxillary sinusitis cases and 30% of unilateral maxillary sinusitis cases. Despite being described nearly 100 years ago, it remains a relatively underexplored etiology in the literature compared to other etiological factors.[8,9,10] The maxillary sinus is the first paranasal sinus to develop during the intrauterine period and has an average volume of 15-20 mL. This sinus reaches full development with the eruption of the permanent teeth between the ages of 12 and 14. The relationship between the maxillary sinus and dental-periodontal structures is a continuous and dynamic process, influenced by both physiological and pathological changes. The thin cortical bone thickness of the maxillary sinus floor and the extension of the roots of the first and second molars, as well as the second premolar teeth into the sinus, are key evidence of the anatomical variability observed in the etiology of OMS.[11]

The etiology is primarily attributed to iatrogenic causes such as periodontal diseases, dental abscesses, or implant complications.[7-9] It is often an underdiagnosed condition due to the presence of classic sinusitis symptoms that overlap with other forms of sinusitis. Suspected cases require a thorough patient history and appropriate radiological imaging for diagnosis.[12] Unlike non-odontogenic sinusitis, OMS is typically characterized by unilateral involvement and foul-smelling discharge. Additionally, other differences include its predominantly anaerobic polymicrobial nature in terms of microbiology, as well as the resistance of rhinosinusitis to traditional medical treatments due to the ongoing dental pathology.[8] Diagnosis requires multidisciplinary collaboration, and paranasal computed tomography scans are the gold standard.[13] OMS typically involves anaerobic polymicrobial infections and resistant biofilms.[8] Because oral antibiotics alone are inadequate, treatment involves a combination of dental interventions and sinus surgery.[13] When dental and sinus issues are addressed together, the success rate can reach 90-100%.[14] However, OMS is often misdiagnosed and underreported, which necessitates increased awareness among both otolaryngologists and dentists.[8] Our patient received both parenteral antibiotic therapy (amoxicillin + sulbactam and metronidazole), endoscopic sinus surgery with nasal passage irrigation, and extraction of the dental implant. Antibiotic therapy was completed over 14 days. With the appropriate treatment approach, the risk of potential sinusitis complications was reduced in our patient. Serious infections, including fungal sinusitis, may arise in the long term as a result of dental implant migration.[15] No fungal lesions were observed either among the PNCT findings or as a significant lesion during the intraoperative examination in our patient. In the differential diagnosis of OMS, factors such as dental impaction and dental implant migration can lead to significant changes in the anatomy and function of the sinuses. However, this condition does not always cause symptoms; some impacted teeth may remain asymptomatic. Therefore, patient-specific evaluation and treatment strategies are required. A personalized approach ensures the effective management of OMS and the prevention of complications.[16] Treatment approaches vary depending on the cause and severity, but generally involve addressing the dental source and managing the sinusitis. Surgical options include functional endoscopic sinus surgery, the Caldwell-Luc approach, and intraoral techniques.[17] Antibiotic therapy and sinus irrigation are commonly used as adjunctive treatments.[18] A multidisciplinary approach involving dentists and otolaryngologists is crucial for the successful management and prevention of OMS.[10]

Conclusion

With the case we present in the literature, we aim to draw attention to the odontogenic factors in the etiology of maxillary sinusitis and to increase awareness about the diagnosis and treatment of the condition.

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This case report abstract was presented as an e-poster at the 45th National ENT Congress between 23-27 October 2024.

Authorship Contribution

The concept was developed by A.A. The design was carried out by A.A and K.T.S. Supervision was provided by D.E. Resources were managed by A.A. Materials were prepared by K.T.S. Data collection was conducted by B.A and D.E. Analysis was performed by K.T.S and B.A. Literature review was carried out by A.A and K.T.S. Writing was done by A.A and B.A. Critical review was provided by A.A.

Conflicts Of Interest Statement

The authors declare no conflict of interest.

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Ectopic Decidua Mimicking Metastatic Lesions and Peritoneal Tubercules; Deciduosis

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Case Report

History

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ABSTRACT

Aim: Decidual cell groups outside the endometrium, "ectopic decidua" or "deciduosis", are commonly seen in the ovary and cervix. However, peritoneal localization is rare. Peritoneal deciduosis is usually an incidental histological finding in the form of white-yellow nodules resembling tuberculosis. Peritoneal deciduosis is macroscopically similar to peritoneal carcinomatosis and peritoneal tubercles, and microscopically may pose diagnostic difficulties with deciduoid mesothelioma, metastatic carcinoma and metastatic malignant melanoma. The purpose of this case report is to evaluate the clinical and histopathological features of ectopic decidua, which was detected as an incidental finding in the peritoneum and omentum during a cesarean section in our clinic, as it may mimic malignancy.
Case: Multiple gray-white colored, raised nodules, the largest of which was 1 cm, were observed in the bladder peritoneum and omentum during laparotomy. Biopsies were taken from the peritoneum and omentum for histopathological examination. The pathological diagnosis was reported as deciduosis.
Conclusion: Although these lesions that we encountered during our laparotomies resemble metastatic malignant lesions and peritoneomental tubercles macroscopically, ectopic decidual tissue due to pregnancy is a benign lesion and resolves without any treatment in the postpartum period, and therefore should be kept in mind in the differential diagnosis when we encounter such lesions.

Keywords: Deciduosis peritonei, Differential Diagnosis, Ectopic decidua.

Metastatik Lezyonları ve Peritoneal Tüberkülleri Taklit Eden Ektopik Desidua; Desiduozis

Olgu Sunumu

Süreç

Geliş: 07/10/2024
Kabul: 16/12/2024

ÖZET

Amaç: Endometrium dışındaki desidual hücre grupları olan "ektopik desidua" veya "desiduozis" over ve serviksteyaygın görülür. Ancak peritoneal lokalizasyon nadirdir. Periton yerleşimli desiduozis genellikle tüberküloza benzeyen beyaz sarı nodüller şeklinde rastlantısal histolojik bulgulardır. Periton yerleşimli desiduozis makroskopik olarak peritoneal karsinomatozis ve peritoneal tüberküllerle benzerdir, mikroskobik olarak desiduoid mezotelyoma, metastatik karsinom ve metastatik malign melanom ile tanısal zorluk oluşturabilir. Bu olgu sunumunun amacı kliniğimizde yapılan sezaryen ameliyatı sırasında periton ve omentumda tesadüfi bir bulgu olarak saptanan ektopik desidua olgusunun maligniteyi taklit edebilmesi nedeniyle klinik, histopatolojik özelliklerinin değerlendirilmesidir.
Olgu: Laparotomide mesane peritonu ve omentumda en büyüğü 1 cm.lik multipl gri-beyaz renkli yüzeyden kabarık nodüller izlendi. Histopatolojik inceleme için periton ve omentumdan biyopsiler alındı. Patolojik tanı desiduozis olarak raporlandı.
Sonuç: Laparotomilerimiz sırasında karşılaşabildiğimiz bu lezyonlar makroskobik olarak metastatik malign lezyonlara ve periton-omental tüberküllere benzese de, gebeliğe bağlı ektopik desidual doku benign bir lezyondur ve postpartum dönemde herhangi bir tedaviye gerek kalmadan düzelir ve bu nedenle bu tarz lezyonlar ile karşılaştığımızda ayırıcı tanıda akılda tutulması gerekir.

Anahtar Kelimeler: Kelimeler: ayırıcı tanı, desiduozis, ektopik desidua

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Introduction

"Ectopic decidua" or "deciduosis", groups of decidual cells outside the endometrium, are commonly seen in the ovary and cervix.[1-4] Deciduosis is a physiological phenomenon of pregnancy.[5-7] It is thought to result from progesterone-induced metaplasia of the coelomic stroma and is usually completely reversible (four to six weeks postpartum).[5] It has been detected in approximately 90% of patients who underwent laparotomy during pregnancy.[7] However, peritoneal localization is rare.

Peritoneal deciduosis (PD) is usually an incidental histological finding in the form of white-yellow nodules resembling tuberculosis.[1,8] PD is similar to peritoneal carcinomatosis and peritoneal tubercles macroscopically, and may pose diagnostic difficulties with deciduoid mesothelioma, metastatic carcinoma, and metastatic malignant melanoma microscopically.[1,3,8-10] The aim of this case report is to evaluate the clinical and histopathological features of ectopic decidua, which was detected as an incidental finding in the peritoneum and omentum during a cesarean section in our clinic, because it can mimic malignancy.[4,5]

Case

A 31-year-old patient with a second pregnancy underwent a cesarean section at 39 weeks of gestation due to a previous cesarean section indication. The patient had a medical history of Hashimoto's thyroiditis, Rh incompatibility, and a cesarean section 7 years ago. Multiple gray-white colored, raised nodules, the largest of which was 1 cm, were observed in the bladder peritoneum and omentum at laparotomy. Biopsies were taken from the peritoneum and omentum for histopathological examination. The patient's postoperative period was uneventful.

Pathology

The macroscopy of the tissue sample was reported as 1 piece of tissue with irregular appearance, gray-white color, 1x0.5x0.4 cm in size. Immunohistochemical analysis was performed using Ventana Brand Benchmark Ultra model automatic device. CD68: Positive in histiocytes, PanCK: Negative. The definitive diagnosis was reported as deciduosis.

Discussion

Ectopic decidua or deciduosis is most commonly seen in the ovary, cervix, uterine serosa, lamina propria of the tube uterina, while it is rare in the peritoneum, omentum, appendix, diaphragm, liver, spleen, para-aortic-pelvic lymph nodes, and renal pelvis.[8,12-16] Peritoneal ectopic decidua is found incidentally in biopsy materials taken during operations such as tubal pregnancy, elective tubal ligation, cesarean section, and appendectomy. However, as in our case, it can also present without symptoms or with symptoms such as hemoperitoneum,[11,17,18] pain mimicking appendicitis,[15,19] hydronephrosis or hematuria due to renal pelvic involvement, or with life-threatening complications such as mechanical ileus.[14,16] Most cases of ectopic decidua are related to normal pregnancy, as in our case.[16,17] It is said to be a result of the exaggerated response of the endometrium to progesterone during pregnancy. The fact that the lesion resolves when the hormonal stimulus ends also supports this theory. However, it has also been reported that in cases of deciduosis found in non-pregnant or postmenopausal women, this condition is associated with an active corpus luteum or adrenal cortex that secretes progesterone.[9] In conclusion, although these lesions that we encounter during our laparotomies may macroscopically resemble metastatic malignant lesions and peritoneomental tubercles, ectopic decidual tissue due to pregnancy is a benign lesion and resolves without any treatment in the postpartum period, and therefore should be kept in mind in the differential diagnosis when we encounter such lesions.[4]

Figure 1A-B: In the sections of the material obtained as a result of the biopsy performed from the peritoneum, there are cells with large eosinophilic cytoplasm and thin chromatin, some with vacuolization, scattered singly within the loose stroma (blue arrow). Mitosis and necrosis are not observed. These cells were not stained by PanCK immunohistochemistry.

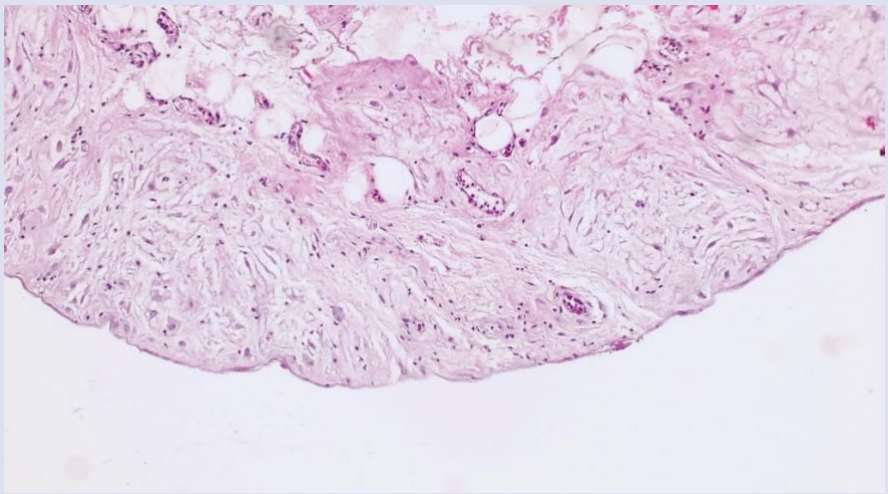

Figure 1,A. (HE X100)

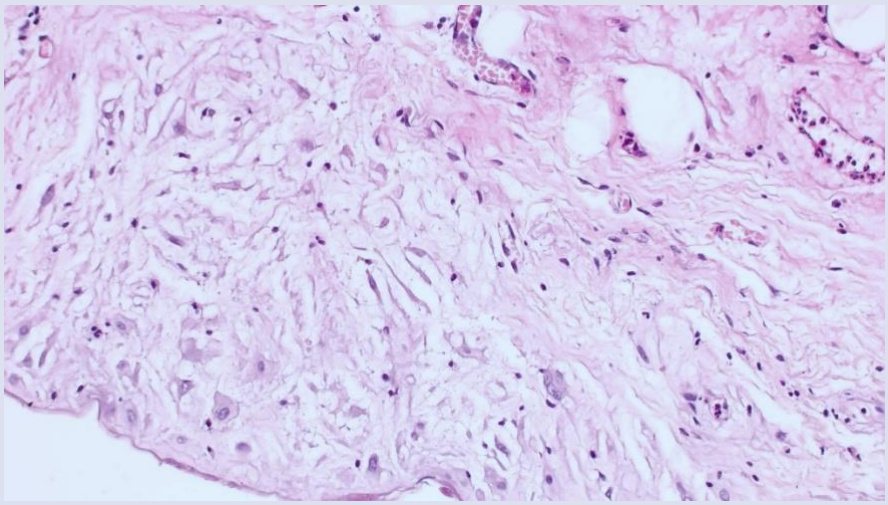

Figure 1,B. (HE X200)

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Meta-analysis of Odds Ratios for the COMT Gene rs737865 SNP for Schizophrenia

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Meta-analysis

History

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ABSTRACT

Objective: The collection of methods that enable combining the findings of several independent studies on the topic of interest with appropriate statistical methods is called meta-analysis.
Schizophrenia is a complex psychiatric disease linked to many environmental and genetic factors and affects up to 1% of the world's population.
A candidate gene for schizophrenia susceptibility is the catechol-O-methyltransferase (COMT) gene.
This study aimed to combine the odds ratios obtained from different studies according to the rs737865 SNP (single nucleotide polymorphism) of the COMT gene for schizophrenia by meta-analysis.

Material and Method:
Publications written in English were scanned with the keywords "COMT and schizophrenia" in Web of Science, Pubmed, and Google Scholar databases until October 2024. Common odds ratio estimates were obtained with the help of appropriate meta-analytic methods under different genetic models for 17 studies that met the inclusion criteria, as well as 8 and 4 studies for Asians and Caucasians, respectively, by race. STATA 14 program was used for all analyses.

Results: Under different genetic models applied to seventeen studies, only carriers of the CC genotype were found to have a higher risk for schizophrenia than carriers of the T (TT+CT) allele [OR=1.133 (95% CI=1.008 - 1.273)]. In subgroup analyses according to race, no risk was found for Asians. In contrast, for Caucasians, it was found that carriers of the C (CC+CT) allele had an increased risk of schizophrenia compared to those with TT genotype [OR=1.586 (95% CI=1.349 - 1.865)].

Conclusion: With the help of applied meta-analytic methods, overall estimates were obtained for odds ratios (OR) obtained from independent studies under different genetic models. However, it is thought that the association between COMT gene rs737865 SNP and schizophrenia should be examined with studies conducted in larger groups homogeneous in terms of ethnicity.

Keywords: Meta-analysis, COMT, odds ratio, schizophrenia

Şizofreni İçin COMT Geni rs737865 SNP Odds Oranlarının Meta Analizi

Meta Analiz

Süreç

Geliş: 24/12/2024
Kabul: 28/12/2024

ÖZET

Amaç: İlgilenilen konuda yapılan birden çok bağımsız çalışmanın bulgularını uygun istatistiksel metotlarla birleştirmeyi sağlayan metotlar topluluğuna meta analizi adı verilir.
Şizofreni, birçok çevresel ve genetik faktörle bağlantılı karmaşık bir psikiyatrik hastalıktır ve dünya nüfusunun %1'ini etkilemektedir. Katekol-O-Metiltransferaz (COMT) geni şizofreniye yatkınlık için aday bir gen olarak görülmektedir. Bu çalışmada, şizofreni için COMT geninin rs737865 SNP'ne (tek nükleotit polimorfizmi) göre farklı çalışmalardan elde edilen odds oranlarının meta analizi ile birleştirilmesi amaçlanmıştır.

Yöntem: Yazım dili İngilizce olan yayınlar "COMT ve şizofreni" anahtar kelimeleri ile Ekim 2024 tarihine kadar Web of Science, Pubmed ve Google Akademik veri tabanlarında ile taranmıştır. Dâhil olma kriterlerini sağlayan 17 çalışma ve ayrıca ırka göre Asyalı ve Beyaz ırklar için sırasıyla 8 ve 4 çalışma için farklı genetik modeller altında, uygun meta analitik yöntemler yardımıyla ortak odds oranı kestirimleri elde edilmiştir. Tüm analizlerde STATA 14.0 programı kullanılmıştır.

Bulgular: On yedi çalışmaya uygulanan farklı genetik modeller altında, sadece CC genotipine sahip olanların T (TT+CT) alleli taşıyıcılarına göre şizofreni için risk taşıdığı bulunmuştur [OR=1,133 (%95 G.A.=1,008 – 1,273)]. Irka göre yapılan alt grup analizlerinde ise Asyalı ırk için bir riskten söz edemezken Beyaz ırk için C (CC+CT) alleli taşıyıcılarının TT genotipine sahip olanlara göre şizofreni riskini arttırdığı bulunmuştur [OR=1,586 (%95 G.A.=1,349 – 1,865)].

Sonuç: Uygulanan meta-analitik yöntemler yardımıyla bağımsız çalışmalardan elde edilen odds oranları (OR) için farklı genetik modeller altında tümel kestirimler elde edilmiştir. Bununla birlikte, etnik köken açısından homojen daha büyük gruplarda yapılan çalışmalar ile COMT geni rs737865 SNP'i ile şizofreni arasındaki bağın incelenmesi gerektiği düşünülmektedir.

Anahtar Kelimeler: Meta analiz, COMT, odds oranı, şizofreni

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Introduction

Today, most diseases are known to be related to genetic factors. Schizophrenia affects 1% of the world's population and is approximately 80% inherited. Due to its function and location, the catechol-o-methyltransferase gene is a strong candidate gene for schizophrenia.[1]

Numerous case-control studies have been conducted to determine the association between the COMT gene rs737865 polymorphism and schizophrenia, but the results have been inconstant; some studies have concluded that there is a significant association between the polymorphism and schizophrenia, while others have concluded that there is no association. In the majority of studies, no significant association was found.

In this case, the researcher needs research synthesis to make a decision. Meta-analysis, one of the most effective research synthesis methods, helps us now. Meta-analysis is one of the most frequently used research syntheses.[2,3] Meta-analysis is a statistical method that combines the results of several independent studies on a particular question, explains the differences in those results, and makes the results more reliable.[4] This study aimed to determine the association between COMT gene rs737865 SNP and schizophrenia using the meta-analysis method.

Materials and Methods

Literature search

Publications written in English were scanned with the keywords “COMT” or “catechol-O-methyltransferase” and “schizophrenia" in Web of Science, Pubmed, and Google Scholar databases until October 2024.

We also checked the bibliographies of key studies to find further relevant studies. As there was no detailed information on how effect sizes were calculated, we attempted to contact the authors.

The flowchart in Figure 1 describes the process used to select the studies that were included in the meta-analysis.

Inclusion-Exclusion Criteria

Case-control studies including patients diagnosed according to DSM-IV diagnostic criteria were considered as inclusion criteria. Studies including family (family-based, trio, sibling) data, patients with additional mental illness or any comorbidity, head trauma, violent tendencies, Alcohol and/or drug abuse were excluded. We found 17 studies examining the association between the r737865 SNP for the COMT gene and schizophrenia that met our criteria. In the first application, the results of 17 studies shown in Table 1 were combined by meta-analysis.

In the second application, the results of 8 and 4 studies were combined for Asian and Caucasian races, respectively, among the studies examining the association between rs737865 SNP for COMT gene and schizophrenia.

Meta-Analysis

In this study, to examine the association between COMT gene rs737865 SNP and schizophrenia, as an effect size the odds ratio (OR) was used. OR can only take positive values and indicates the risk of a factor on an outcome. An exposure to the factor is not risky if OR=1, it increases the risk if OR>1, and it decreases the risk for the outcome of interest if OR<1.

When calculating the ORs, we used each study's genotype and allele frequencies. In some studies (C and T) allele frequencies were also given, for studies in which only genotype distributions were presented (CC, CT, TT), we calculated the allele frequencies. Following the purpose of the study, odds ratios obtained based on genotype frequencies and allele frequencies were combined with appropriate meta-analytical methods.

To decide whether to use the fixed or random effect model for combining the study results, the heterogeneity of the effect sizes between the studies was evaluated. For this purpose, Q statistic and I^2 values were utilized. If the Q statistic resulted as $p<0.05$, it was regarded as statistically significant.[30] When I^2 is greater than 50%, it is classified as large heterogeneity.[30] If there was the absence of heterogeneity or moderate heterogeneity between studies; the common OR was calculated with the Mantel-Haenszel fixed-effects model (MH). Otherwise, the DerSimonian-Laird random-effects model method (DSL) was used.

For the 17 studies considered for the first application, the Der Simonian-Laird method was used under the random effect model since the effect sizes were heterogeneous among the studies.

In the 8 and 4 studies considered in the second application, it was found appropriate to use fixed effect models since homogeneity between studies was provided for both Asian and Caucasian races.

For both applications, the odds ratio point estimate and 95% confidence interval of each study and the common estimation obtained as a result of meta-analysis are presented with a forest plot. A funnel plot, in which the logarithm of the point estimates is plotted against their standard errors, was used to visualize publication bias. In addition, Egger's test was implemented to

evaluate the publication bias. Trim and fill analysis was performed to eliminate publication bias. For all analyses, the significance level was considered 0.05. Statistical analyses and graphical representations were performed using the STATA 14.0 software (Stata Corporation, College Station, Texas, USA).

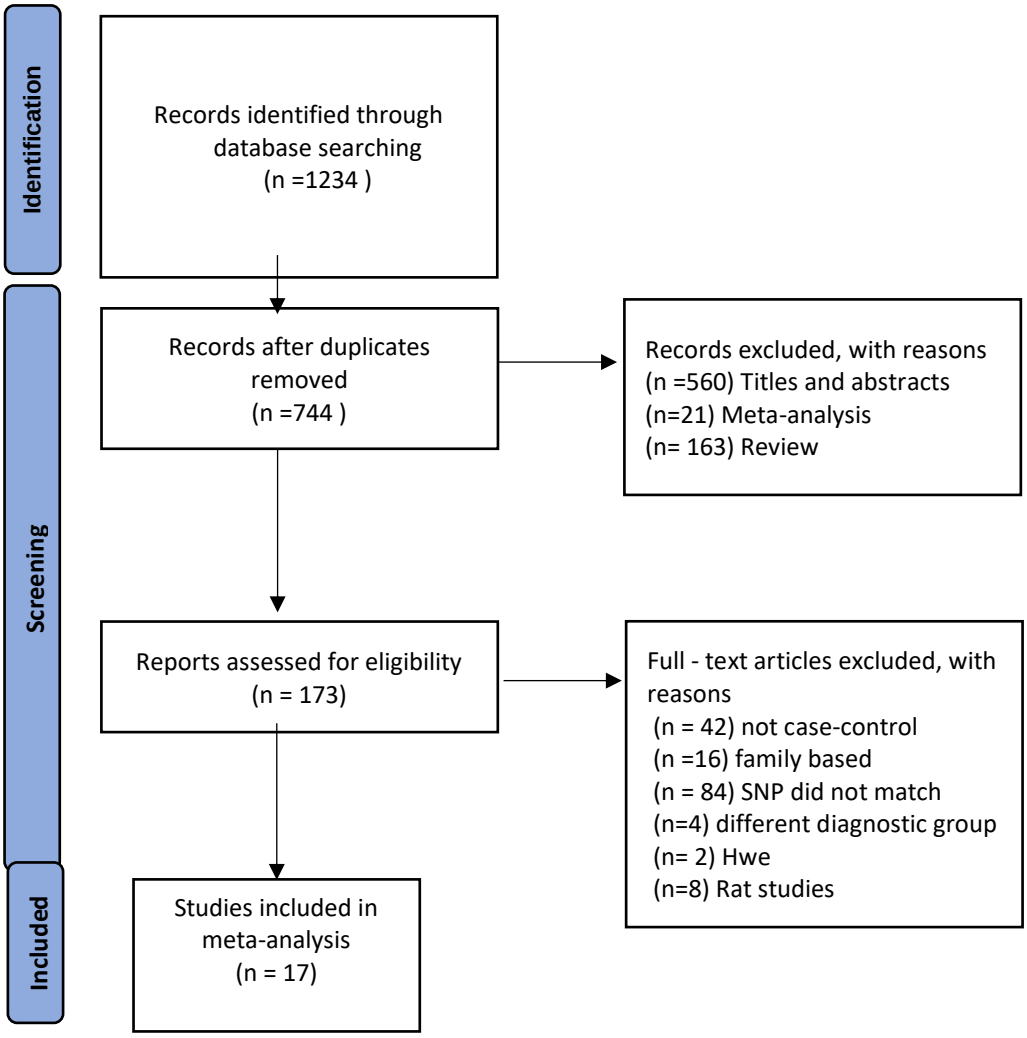

Figure 1. Flow chart of the selection process of the studies included in the meta-analysis

The literature search identified 17 case-control studies that met the inclusion criteria, enrolling 7664 cases and 10235 controls. Of the 17 studies, 8 involved Asian populations and 4 Caucasian populations. The OR estimates for these studies were pooled using appropriate meta-analytic methods. Table 1 gives the characteristics of the included studies.

Table 1. Characteristics of the populations from studies included in the meta-analysis

First Author	Study Year	Population	n		
			Case	Control	Total
Shifman	2002	Israil	714	2849	2965
Lee	2005	Korean	320	379	641
Funke	2005	Caucasion-Usa	394	467	597
Yu	2007	Han Chinese	241	290	484
Nunokawa	2007	Japanese	399	440	779
Martorell	2008	Caucasion-Spain	585	615	1070
Okochi	2009	Japanese	1118	1100	2031
Gupta	2009	Southern Indian	398	241	591
Chien	2009	Taiwanese	124	112	221
Park	2009	Korean	354	396	682
Chen	2011	Han Chinese	434	442	819
Wright	2012	South Africa	238	240	461
Maria	2012	Greek	108	97	189
Acar	2015	Turkish	96	100	172
Higashiyama	2016	Japanese	1854	2137	1101
Dean	2016	European Han Chinese	75	73	172
Matsuzaka	2017	Mixed	212	257	396

Table 2. The findings of meta-analyses performing different genetic models for all studies

Genetic model	I^2	p_Q	Method	OR (95% C.I.)	p_Z	p_E
T versus C [17]	43.2	0.030	DSL	1.007 (0.934 – 1.087)	0.854	0.236
TT versus (CC+CT) [17]	41.6	0.037	DSL	1.002 (0.909 – 1.104)	0.972	0.855
(TT+CT) versus CC [17]	**22.4**	**0.194**	**MH**	**1.133 (1.008 – 1.273)**	**0.036**	**TF**
TT versus CC [17]	26.6	0.150	MH	1.133 (1.000 – 1.283)	0.050	**TF**

Note: The numbers in parentheses indicate the number of studies included in the meta-analysis. p_Q: p-value for Q test; p_z: p-value for Z test; p_E: p-value of Egger's test; TF: Trim and Fill analysis

When the Egger test was carried out to assess the bias in the publications, if the test result showed publication bias, a trim and fill analysis was performed (Table 2).

The pooled odds ratios with 95% CIs did not show a statistical association between the COMT gene rs737865 SNP and the risk of schizophrenia except for the (TT+CT) vs CC genetic model (Table 2). It was found that carriers of the CC genotype have a higher risk for schizophrenia than carriers of the T (TT+CT) allele [OR=1.133 (95% CI=1.008 - 1.273)].

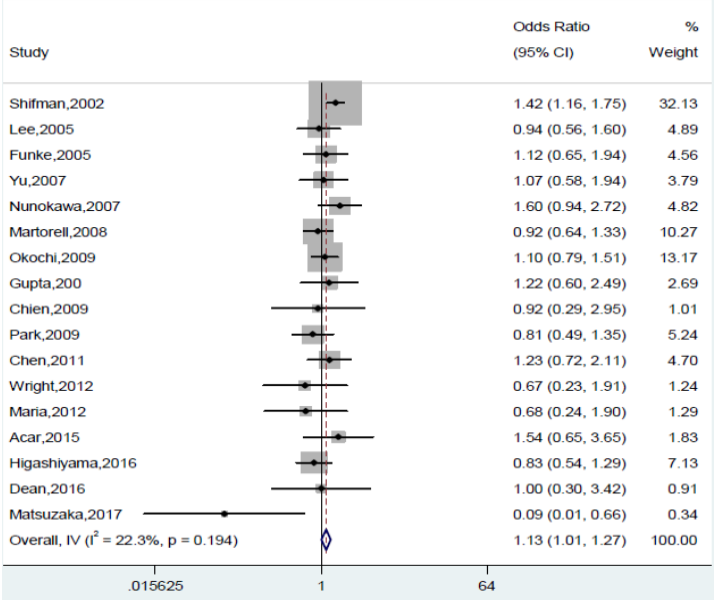

Figure 2. Forest plot for rs737865 (TT+CT) versus CC genetic model for all studies

In forest plots (Figure 2 and 3), the squares and horizontal lines indicate study-specific OR and 95% CI. The area of the squares corresponds to the study-specific weight of the study. The diamond shows the pooled OR and 95% CI.

In the stratified analysis according to race (Table 3), there was no heterogeneity between studies for Asian and Caucasian subgroups under any genetic model (I^2=0, p>0.05). While no risk was found for Asians, for Caucasians it was found that carriers of the C (CC+CT) allele had an increased risk of schizophrenia compared to those with TT genotype [OR=1.586 (95% CI=1.349 - 1.865)] (Figure 3).

Table 3. Results of meta-analyses for race subgroups based on different genetic models.

	Genetic model	I^2	p_Q	Method	OR (95% C.I.)	p_Z
Asian	T versus C [8]	0	0.890	MH	0.997 (0.927 – 1.073)	0.946
	TT versus (CC+CT) [8]	0	0.701	MH	0.984 (0.898 – 1.079)	0.735
	(TT+CT) versus CC [8]	0	0.654	MH	1.043 (0.877 – 1.241)	0.633
	TT versus CC [8]	0	0.797	MH	1.035 (0.866 – 1.238)	0.705
Caucasian	T versus C [4]	0	0.679	MH	0.989 (0.871 – 1.123)	0.864
	TT versus **(CC+CT)** [4]	0	0.804	MH	**1.586 (1.349 – 1.865)**	**<0.001**
	(TT+CT) versus CC [4]	0	0.600	MH	0.999 (0.758 – 1.315)	0.992
	TT versus CC [4]	0	0.577	MH	0.985 (0.737 – 1.317)	0.920

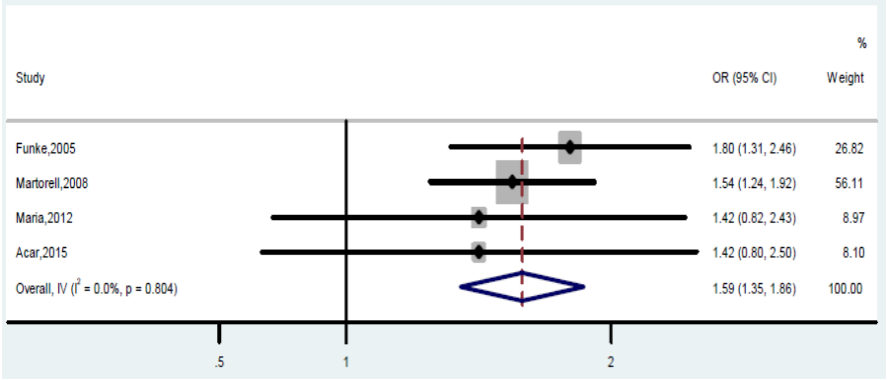

Figure 3. Forest plot for rs737865 TT versus (CC+CT) genetic model for Caucasians.

Discussion

The COMT gene is a strong candidate for schizophrenia susceptibility. It is a likely candidate gene because of the enzyme's role in dopamine metabolism and the chromosomal location of 22q11. Many studies in different populations around the world have produced conflicting results regarding the COMT gene and schizophrenia. This study aims to investigate the association between COMT gene rs737865 SNP and schizophrenia. We examine this relationship by performing a meta-analysis that includes case-control studies.

We found that having CC genotype have a higher risk for schizophrenia than T (TT+CT) allele carriers (OR=1.133, 95% C.I=1.008 – 1.273). Similarly, a meta-analysis included 10 studies conducted by Okochi et al. found having CC genotype increases the risk of schizophrenia (OR=1.155, 95% C.I=1.025 – 1.303).[16]

Also, in our meta-analysis based on four studies which were carried out on Caucasians, the pooled OR (1.586, 95% C.I=1.349 – 1.865) estimation indicated that having a C (CC+CT) allele increases the risk of having schizophrenia compared to TT homozygous genotype. These results suggest that T allele might have a protective effect. However, when we compared T allele carriers with C allele carriers, we found no statistically significant association between allele frequencies and schizophrenia.

Oppositely, in Wright et.al's study they found that the C allele is protective against schizophrenia in an African population.[6]

In the study by Shifman et al., rs737865 SNP was found to be highly associated with schizophrenia in Ashkenazi

Jews and affects both sexes but in different ways. They reported that the CC genotype is associated with susceptibility to schizophrenia in males and the TT genotype is protective in females.[4]

Another case-control study reported no association between COMT haplotype (rs4680, rs6267, rs737865, rs4633, rs6269) and schizophrenia risk in the Korean population.[8] Also, no significant association was found between COMT polymorphisms including six SNPs (rs737865, rs740603, rs4633, rs6267, rs4680, rs165599) and schizophrenia in the Korean population.[19]

A case-control study of five functional polymorphisms (rs2075507, rs737865, rs6267, rs4680, and rs165599) in the Japanese population was carried out by Nunokawa et al. (399 schizophrenia patients and 440 controls). Neither COMT haplotypes nor polymorphisms were shown to be significantly linked to schizophrenia.[5] Another research of eight SNPs in the Japanese population, including rs737865, rs6267, rs4680, and rs165599, found no evidence of a significant association between schizophrenia and COMT polymorphisms or haplotypes.[11]

In the Chinese population studies by Yu et al. and Chien et al., no association was found between haplotype (rs737865, rs4680, rs165599) and risk of case and control groups. Similarly, Chen et al. concluded that the COMT gene haplotype (rs2075507 - rs737865 - rs933271) would not cause schizophrenia risk and psychopathological symptoms in a Chinese population study.[12,18] In an additional Chinese population study Dean et al. did not find an association between COMT genotype SNPs (rs4680 or rs4818, as well as rs165519 and rs737865) and schizophrenia in a case-control study. [17]

In a case-control study in a Turkish population, no association was found between COMT gene rs737865, rs4680, and rs165599 polymorphisms, and schizophrenia.[14] However, Maria et al. demonstrated an association between the COMT gene and schizophrenia in a Greek population. Although no significant results were obtained individually, haplotype analysis showed that haplotypes 2 (rs737865 - rs165599) and 3 (rs737865, rs4680 and rs165599) were highly associated with schizophrenia.[25]

According to Funke et al., the rs737865 SNP is not associated with schizophrenia, bipolar disorder, schizoaffective disorder, or major depressive disorder in the population of the United States. For all included psychiatric diseases, however, haplotype analysis produced statistically significant results (278A/G; rs737865; Val108/158Met; rs165599).[9]

Martorell et al. found no evidence for an association between the COMT gene and schizophrenia.[7] Their study included the 3 most commonly studied SNPs, rs737865, rs4680 (Val/Met), and rs165599, which are highly related to schizophrenia in Ashkenazi Jews.[4]

There is no evidence that the COMT gene is linked to schizophrenia, according to Martorell et al.[7] The three most often researched SNPs that are strongly linked to schizophrenia in Ashkenazi Jews (rs4680, rs737865, rs165599) were included in their analysis.[4]

In a case-control study, Gupta et al. constructed a haplotype analysis containing seven SNPs (rs3788319, rs737865, rs6269, rs4818, rs4633, rs4680, rs165599) of the COMT gene were found to be associated with schizophrenia in the Indian population.[20] According to Shifman et al., COMT haplotype of three SNPs (rs737865, rs4680, and rs165599) and schizophrenia were significantly associated. Similarly, Gupta et al. reported that their investigation revealed a strong association between schizophrenia and this three-marker haplotype.[20]

A study of an African population by Wright et al, which investigated 14 SNPs (including rs737865, rs165599, and rs4680, etc.) in the COMT gene, a significant association was found between schizophrenia and rs737865 and rs2020917 polymorphisms.[6]

However, in a mixed population, Matsuzaka et al. assessed the contribution of three COMT SNPs (rs737865, rs165599, and rs4680) to schizophrenia and discovered a strong association between the rs737865 genotype and schizophrenia. They concluded that the CC genotype had a protective effect and CT had a risk effect.[24]

Conclusion

In conclusion, some of the studies with different populations and different sample sizes found a significant association between SNP rs737865 and schizophrenia, while others found nonsignificant results. In our meta-analysis, the pooled OR obtained from 17 independent studies led us to conclude generally CC genotype is associated with the disease. However, according to ethnicity the risky allele or genotype can be changed. So, we think it would be better to investigate the association between the SNPs and the disease for different ethnicities separately. Because, when we constructed the meta-analysis for population subgroups, the heterogeneity between studies disappeared. Also, differences in linkage disequilibrium (LD) between populations suggest that different haplotypes, rather than SNPs, are associated with schizophrenia in different populations.[28,30]

Nevertheless, the association between schizophrenia and the COMT gene rs737865 single-nucleotide

polymorphism or haplotype analysis needs to be confirmed by appropriately designed researches with larger sample sizes.

Ethics Committee Approval

It is a meta-analysis study and the data of the studies included in the research are open access.

Author Contributions

Concept – HGB, GH Design HGB, GH ; Supervision HGB.; Materials -HGB,GH ; Data Collection and/or Processing - GH; Analysis and/or Interpretation -GH; Literature Review - GH.; Writing – GH,HGB; Critical Review – GH.

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Compliance with ethical standards

Conflict of interest: The authors declare no conflict of interest.

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