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PREFACE

It has been shown that the academic and scientific developments will concern all health sector employees. I would like to say that I benefited from readers valuable information and criticism while editing the book and translating some sections. Many studies have shown that some substances significantly increase the risk of cancer developments and its incidence such as, stomach cancer, hepatocellular carcinoma and multiple myeloma. While they significantly reduce the risk of other cancers such as colorectal cancer, adult acute lymphoblastic leukemia and childhood acute lymphoblastic leukemia.

Our main purpose is to organize this book for medical students, physicians and young researchers on current issues in health.

We would like to thank our colleagues who patiently provided consultative contributions throughout our efforts to write the book.

Editor Prof. Dr. Nihayet BAYRAKTAR

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CHAPTER 1

POSSIBLE ROLE OF N6-METHYLADENOSINE PATHWAY IN THE PATHOGENESIS OF CRIMEAN-CONGO HEMORRHAGIC FEVER

Assistant of Professor Doctor Badel İNCE

INTRODUCTION

Crimean-Congo Hemorrhagic Fever (CCHF) is a virulent tick-borne viral zoonotic disease. The disease is caused by Crimean-Congo Hemorrhagic Fever Virus (CCHFV). Threatens public health due to acute and potentially fatal human infection. According to 2019 data, CCHF is considered endemic or potentially endemic in approximately 50 countries in Europe, Africa and Asia (Table 1.) (Nasirian, H. 2019). This virus can be transmitted to humans and animals by ticks. The epidemiology of Crimean-Congo Hemorrhagic Fever may vary depending on factors such as the prevalence of infected ticks, the presence of infected animals, and human contact with these animals. Transmission through unprotected contact with sick people or nosocomial transmission is also possible. A case reported in Turkey in 2023 also reported human-to-human sexual transmission (Sümer, B. et al. 2023). The virus is generally transmitted to humans through the attachment of infected ticks of the Hyalomma marginatum species or

through contact with the blood or infected tissues of animals with viremia. For this reason, it spreads parallel to the geographical distribution of this tick species. It has also been reported to cause sporadic infections in travelers visiting these areas. Agricultural workers, especially those involved in animal husbandry and those living in rural areas, are most at risk. The disease usually begins with flu-like symptoms such as fever, headache, muscle aches, and fatigue. In more severe cases, hemorrhagic syndrome, liver damage and organ failure may occur. It is important for people with CCHF to seek medical help quickly. Treatment usually includes supportive care and aims to relieve the patient's symptoms. Prevention methods include avoiding ticks, limiting contact with infected animals and paying attention to hygiene rules.

Table 1. Geographic distribution of Crimean-Congo hemorrhagic fever disease

Crimean-Congo hemorrhagic fever virus has been detected in more than 50 countries in Asia, Europe and Africa (Graphic designed by @Puku.Geo).

Many hemorrhagic fever diseases, caused by many other viruses, have been observed in different parts of the world for centuries. The oldest history of hemorrhagic syndrome in the literature was described in present-day Tajikistan in the 12th century. Smallpox and yellow fever, which in the past showed rapid bleeding symptoms, are the most common hemorrhagic viral diseases, causing countless cases and thousands of deaths for centuries. CCHF had not yet been identified when, during the summers of 1944 and 1945, more than 200 cases of severe, acute, febrile illness with severe bleeding symptoms occurred in the USSR in the steppe region of Western Crimea. At first it was called "Acute infectious toxicosis". Most of the cases were among people in the military units of the Soviet Union who helped regional people during the harvest. Later it turned out that a similar disease has been known for many years in other parts of the USSR, especially in the Central Asian Republics. It was understood that the same syndrome was also identified in the regions of the USSR bordering the Black Sea and the Caspian Sea, Bulgaria and Yugoslavia. In 1956, it was reported that a virus antigenically indistinguishable from the Congo virus was isolated from a febrile patient in the Belgian Congo. Virus strains were isolated from blood samples of patients with acute illness and from the tick Hyalomma marginatum marginatum. Crimean hemorrhagic fever virus strains were later shown to be closely related antigenically and biologically to Congo fever virus. The common antigenic structure between Eurasian Crimean Hemorrhagic Fever strains and Asian and

African strains of Congo virus led to the virus being referred to as Crimean Hemorrhagic Fever-Congo Virus and subsequently Crimean-Congo Hemorrhagic Fever Virus (Ergönül, Ö. 2006). Further research showed that the virus was widespread in East and West Africa. Hazara, another virus isolated in Pakistan and Upper East Region of Ghana was also shown to be related in the following years (Addo, S. et al. 2024).

CCHFV has the widest geographical range among tick-borne viruses affecting human health and is the second most common among medically important arboviruses, after dengue viruses. Ticks are obligate bloodsucking arthropods and are observed in every region of the world. During the winter months when domesticated animals with tick infections are in intense contact, it is possible for animal to animal transmission and serve as a multiplier host. The incidence of the disease increases with seasonal epidemics, especially in June and July, as the weather gets warmer. The relationship between CCHF and ticks first gained importance as a result of the emergence of the disease in Crimea in 1944-45 and the isolation of the agent from ticks. Although it has been reported that 31 tick species belonging to the Ixodidae and Argasidae families can be vectors of the virus, the vector potential of all of them has not been demonstrated. In order for the tick to be fully considered a vector, in addition to the isolation of the agent, the tick must also have the ability to transmit the virus to susceptible animals and to acquire it from viremic animals. In addition, some species carry the virus both transovarially and transtadially, while others can only carry it transtadially. Today, it is accepted that the main vectors of the

disease are Hyalomma marginatum marginatum, H.m.rufipes and H.anatolicum anatolicum. However, in some countries where Hyalomma species are not present, the agent is Ixodes ricinus, Dermacentor spp., Rhipicephalus spp. and Boophilus annulatus, shows that the vector potential of other ticks should also be considered.

The incubation period after an infective tick bite is between 7-12 days. Initial symptoms include sudden onset of high fever (>38°C), headache, myalgia, back pain, stomach pain, vomiting, and petechiae. As the disease progresses, various hemorrhagic findings such as ecchymosis, epistaxis, hematuria and gastrointestinal bleeding may occur. Fever is persistent but may be recurrent and sometimes biphasic; It resolves with crisis or lysis after 8 days. The infected patient's face and neck are red and edematous, the conjunctiva and pharynx are injected, and the soft palate is edematous. The mouth is dry and there is a bad odor in the breath. Patients are depressed and sleepy. A fine petechial rash usually begins on the trunk and then covers the entire body. In approximately 50% of cases, the liver is enlarged but the respiratory system is unaffected. In the early stages of the disease, a hemorrhagic enanthem occurs on the soft palate and uvula. Other signs of bleeding, including hematemesis and melena, occur in more than 75% of patients by about the fourth or fifth day. Leukopenia and severe thrombocytopenia are common. From time to time, large purpuric areas appear, caused by blood leaking under the skin. Bleeding occurs with decreasing frequency from the nose, gums, buccal mucosa, stomach, uterus, intestines and lungs. Stomach and nose bleeding often lead to

death. Central nervous system involvement occurs in 10–25% of cases and generally portends a poor prognosis; include neck stiffness, arousal, and coma. However mortality rate varies by region, usually ranges from 5% to 50% due to shock, secondary blood loss, or other intervening infections. Factors affecting mortality include old age, weakness of the immune system, failure to diagnose the disease early, failure to initiate treatment early, the patient's general health condition and capacity to respond to treatment. These factors play an important role in determining the course and severity of the disease (Jannath, S., & Islam, M. R. 2024).

According to current taxonomic studies; Crimean-Congo hemorrhagic fever virus (CCHFV), known as Orthonairovirus haemorrhagiae, belongs to the genus Orthonairovirus within the family Nairoviridae and order Bunyavirales (Kuhn, JH. 2021). The CCHFV virion contains three single-stranded RNA segments with negative directional orientation. Nucleoprotein (NP) and RNA-dependent RNA polymerase (RdRp; L protein) protect RNA by surrounding RNA segments and forming ribonucleoprotein complexes (RNPs). When ribonucleoprotein (RNP) complexes form, they are surrounded by a protective envelope originating from the host cell's membrane. This envelope is covered with special glycoproteins known as Gn and Gc. CCHFV consists of three genomic segments: small (S), medium (M), and large (L). The S segment is responsible for encoding NP in an open reading frame, while small nonstructural protein (NSs) is encoded in an open reading frame in the reverse direction. The M segment is quite

complex as it encodes a glycoprotein precursor (GPC) that is processed by host proteases. This process results in the production of a GP160/85 domain, which is then processed into a mucin-like domain (MLD) and GP38. In addition, the M segment encodes the Gn and Gc glycoproteins as well as the intermediate nonstructural protein (NSm). The L segment of CCHFV, which is significantly larger than other bunyaviruses, encodes the viral RNA-dependent RNA polymerase (RdRP) and the ovarian tumor-like protease (OTU) at the N terminus (Vasmehjani, A.A. 2024).

Although the use of ribavirin is essential in the treatment protocol, its effectiveness is a matter of debate. Current research focuses on monoclonal antibodies, antiviral drugs and mRNA vaccine studies in the treatment of the disease (Hawman, D.W. et al 2024). New treatment strategies are being tried, such as drug redesign with favipiravir, chloroquine, and chlorpromazine. Ongoing studies are exploring the efficacy of various antiviral drugs. Researchers have been identifying key signaling pathways that, when blocked, can limit the reproduction of CCHFV. This approach offers hope for developing effective antiviral treatments (Tahir, M. et al. 2021, Ferraris, O. 2015, Odhar, H. 2021, Hawman, DW. 2018). Researchers from the United States Army Medical Research Institute of Infectious Diseases (USAMRIID) discovered a protective antibody target called glycoprotein 38 (GP38), which shows promise in protecting against CCHFV (Garrison et al. 2024). Scientists have identified important targets for monoclonal antibody therapy. Antibodies targeting the

nucleocapsid protein (NP) have shown potential in providing substantial protection in mouse models, even against lethal strains of CCHFV (Li, L. 2024). Efforts are being made to develop vaccines against CCHFV. Experimental vaccines have shown efficacy in animal models, including cynomolgus macaques, providing a basis for future vaccine development for human use. ChAdOx2 vaccine was the first promising CCHFV vaccine in animals and clinical trials were initiated in the United Kingdom (Saunders, JE. 2023.) These advancements are crucial as CCHFV continues to be a significant threat, especially in regions where the virus is endemic. The development of effective treatments and vaccines is essential to control outbreaks and reduce mortality rates associated with the disease.

It is known that individual differences are effective in the clinical course and treatment response of viral diseases. Differences in immune response in different individuals infected with the same virus are associated with patients' survival rates. Variations in genes that regulate the immune response, epigenetic modifications and posttranscriptional modifications in these genes are especially important. For this reason, genes and epigenetic modifications that regulate the immune response stand out among the factors affecting the prognosis of Crimean-Congo Hemorrhagic Fever (CCHF) disease. For example; Various alleles of HLA (Human Leukocyte Antigen) genes can affect the immune response to CCHFV virus. Some HLA alleles may provide a more effective immune response to the virus, while others may be associated with a weaker response. Genetic variations of

pro-inflammatory and anti-inflammatory cytokines may influence the course of the disease. Genetic variants of cytokines such as TNF-α, IL-6 and IL-10 are especially important. Interferon (IFN) response genes play a critical role against viral infections. Variations in these genes can affect the body's response to the virus and therefore the severity of the disease. Genes associated with apoptosis and cellular stress response may affect the prognosis of the disease by regulating intracellular replication of the virus and cell death. Also METTL3, METTL14, FTO, and YTHDF1-3 genes which are associated with m6A RNA Methylation, can affect the stability and immune response of viral RNA through m6A modifications. Variations in these genes can alter the effectiveness of immune cells and the course of the disease. These genetic factors play an important role in determining the prognosis of CCHF by affecting the severity of the disease, recovery time and risk of death.

Post-transcriptional epigenetic modifications have been shown to play an important role in many physiological processes and the development of diseases. N6-methyladenosine (m6A) is the methylation of the adenosine N6 position and is the most common post-transcriptional modification seen in eukaryotic mRNA. M6A is known to modulate the expression of genes that regulate cellular processes including cell renewal, differentiation, invasion and apoptosis. m6A modulates the eukaryotic transcriptome. It performs this role by regulating the "splicing", localization, translation and stability of mRNA. M6A methylation process is carried out by m6A

methyltransferases called writers, m6A is removed by demethylases (erasers) and recognized by reader proteins that regulate RNA metabolism. The future of the modified transcriptome is determined by these processes (Batista, PJ. 2017). M6A is established by the methyltransferase complex simultaneously with transcription. This complex consists of the METTL3 catalytic subunit and other accessory subunits such as METTL14, WTAP, VIRMA, RBM15 and ZC3H13. METTL14 forms a stable complex with METTL3 and plays a key role in substrate recognition. Wilms tumor 1 associated protein (WTAP) localizes the METTL3-METTL14 heterodimer and initiates its catalytic activity (Ping, XL. et al. 2014). ALKBH5 and FTO proteins, which are M6A demethylases, function by using Fe+2 as a cofactor and α-ketoglutarate as a co-substrate and remove m6A. It is also known that FTO and ALKBH5 demethylases are involved in the control of the mRNA splicing mechanism (Zhao, X. et al. 2014, Zheng, G. et al. 2013). "Readers" recognize and bind m6A methyl profiles, which determines the fate of the target mRNA. YTHDF1 enhances mRNA translation and protein synthesis by interacting with initiation factors. YTHDF2 induces degradation of transcripts by selectively binding m6A-modified mRNA and recruiting them to mRNA degradation sites. YTHDF3, in turn, enhances RNA translation by interacting with YTHDF1 and promotes RNA degradation by associating with YTHDF2.

The importance of posttranscriptional epigenetic modifications in the immune system's response to viral diseases has been

demonstrated by numerous studies conducted in recent years. These studies suggest that N6-methyladenosine (m6A) is associated with both innate and adaptive immune responses, affecting immune recognition, cell fate decisions, cytokine production, and immune cell functions during infections and diseases. m6A modification may affect mRNA stability and translation efficiency. This is critical for the ability of immune cells to respond quickly. When the virus and the host first encounter each other, a race begins between the epigenetic modification mechanisms in both genomes. N6-Methyladenosine pathway may play a role in determining the winner of this race. The outcome of this struggle determines the survival success of the host. The clinical severity of the disease may also be proportional to this epigenetic modification race (Arslan, B. et al. 2023). N6-methyladenosine (m6A) modifications play a critical role in regulating inflammatory responses in a complex interaction with the immune system. The m6A pathway is involved in the control of inflammation by regulating the stability and translation of mRNA of pro-inflammatory cytokines (e.g., TNF-α, IL-6). In this process, components of the m6A pathway such as METTL3 and YTHDF2 play an important role. m6A modifications are of great importance in the functions of innate immune cells (e.g., macrophages, dendritic cells). The responses of these cells to infections and other pathogens are shaped under the influence of m6A regulatory proteins. For example, m6A can increase the stability of interferon mRNA, which plays a critical role in regulating antiviral responses. m6A modification can regulate innate immune responses. For example, m6A modification of viral RNAs can affect the response of host cells to viral

infections. Some studies show that m6A may prevent viral RNA from being recognized by the immune system. m6A modifications are of great importance in the functions of innate immune cells (e.g., macrophages, dendritic cells). The responses of these cells to infections and other pathogens are shaped under the influence of m6A regulatory proteins. For example, m6A can increase the stability of interferon mRNA, which plays a critical role in regulating antiviral responses. The development and functions of adaptive immune cells, such as T and B cells, are also affected by m6A modifications. In particular, the differentiation and functions of T cells are under the regulatory influence of m6A methylation. METTL3 deficiency may negatively affect T cells' differentiation and mounting an effective response. The roles of the genes involved in this pathway on the immune system have been partially revealed by some studies.

METTL3 (Methyltransferase-like 3) gene plays an important role in the RNA methylation process by catalyzing N6-methyladenosine (m6A) modification with the enzyme it encodes. This modification regulates processes such as mRNA stability, translation, and splicing. METTL3 works with other proteins such as METTL14 and WTAP to form the m6A methyltransferase complex and adds methyl groups to adenosine bases in mRNA (Barajas, JM. et al. 2022). In summary, it is of critical importance in RNA biology and gene regulation. METTL3 gene also plays an important role in regulating the immune response. Regulates NK cell function, cytokine signaling, T cell activation, immune microenvironment, macrophage activation and

polarization (Song, H. et al. 2021). In particular, it plays an important role in the activation of pro-inflammatory macrophages (M1) (Liu, Y. et al. 2019). This process is critical for a rapid and effective response to infections. Additionally, it provides an effective immune response against infection and inflammation by regulating the production of pro-inflammatory cytokines (e.g. TNF-α, IL-6) (Zhang, X. et al. 2021, Wen, L. et al. 2022). Involved in proliferation, differentiation, function of T cells and signaling of T cell receptors (TCR) (Yao, Y. et al. 2021). It affects the humoral immune response by taking part in the regulation of genes necessary for the development of B cells and antibody production (Grenov, A.C. et al. 2020). During viral infections, host cells' recognition of viral RNA, m6A methylation of viral genomes play a critical role in disease pathogenesis by regulating viral replication and antiviral response (Fiorentino, F. et al. 2023, Qiu, W. et al. 2021). With current studies, analysis of the m6A methylation pattern of mRNAs of hemorrhagic fever viruses such as Crimean-Congo hemorrhagic fever virus, Junín virus (JUNV) (CCHFV), and Ebola virus (EBOV) has shown that they are methylated by METTL3. METTL3 has been demonstrated to be important for RNA synthesis and protein expression, as well as its interaction with the viral nucleoprotein (Wendt, L. et al. 2023).

METTL14 is part of the N6-methyladenosine (m6A) printer complex and plays a critical role in the formation of m6A modifications. It plays a role in various mechanisms in the immune response. METTL14 works together with METTL3 to add m6A

modification to mRNAs. This modification regulates gene expression in immune cells by increasing the stability of mRNA. For example, stabilization of mRNAs of pro-inflammatory cytokines contributes to the rapid and effective execution of the immune response. METTL14 plays an important role in the differentiation and functions of T cells. Deficiency of METTL14 may negatively affect the differentiation process and memory of T cells. In particular, the capacity of T cells decreases and they cannot mount an effective immune response. METTL14 also plays a role in innate immune responses. For example, the expression of interferons produced in response to viral infections can be regulated through METTL14. METTL14 may also affect the functions of antigen-presenting cells such as dendritic cells. Pro-inflammatory cytokine production by dendritic cells and antigen presentation to T cells can be regulated through m6A modifications. METTL14 also plays a role in immune surveillance processes. Modifications of m6A regulated through METTL14 may affect the ability of immune cells to recognize and kill cancer cells. METTL14 is a critical enzyme that affects many aspects of the immune response. mRNA methylation plays a role in processes such as T cell function, innate immune responses, antigen presentation, and immune surveillance. This indicates that METTL14 is an important target in regulating the immune system and fighting diseases. While some studies suggest that the METTL14 gene regulates the immune response through m6A RNA modification and plays an important role in fighting viruses, tumor immunity and hematopoiesis, other studies indicate that low METTL14 expression may reduce immune cell infiltration, leading

to poor prognosis (Zhu, X. et al. 2021). While some studies suggest that METTL14 promotes tumorigenesis in EBV-associated cancers, other studies suggest that METTL14 has antiviral capacity and may interfere with negative-sense single-stranded RNA virus infections (Lang, F. et al. 2019).

The WTAP (Wilms' tumor 1-associated protein) gene forms the m6A methylation complex together with METTL3 and METTL14 proteins. WTAP helps localize this complex correctly within the nucleus and efficiently carry out m6A modification. WTAP may affect the translation of viral mRNA through regulation of m6A methylation. This affects the production of viral proteins and therefore viral particle formation (Selberg, S. et al. 2021). It is involved in the activation of macrophages and M1 (pro-inflammatory) and M2 (anti-inflammatory) macrophage polarization (Long, H. et al. 2022, Liang, L. et al. 2022). Its regulatory role in these processes is important in controlling inflammatory responses and the progression of viral infections. It regulates the production of pro-inflammatory and anti-inflammatory cytokines, so it is critical for balancing immune responses during infection and inflammation. Like METTL3, it plays an important role in the differentiation and activation of T cells and can modulate TCR signaling and T cell proliferation. They have a role in the development, activation and antibody production processes of B cells in the regulation of the humoral immune response. Interferons are the immune system's first line of defense against viral infections. WTAP has also been reported to affect interferon-alpha response and IFN-I signaling (Ge, Y.

et al. 2021). It has been supported by recent research that it plays important roles in viral infections (Srinivas, K.P. et al. 2021, Sacco, M.T. et al. 2022).

The FTO (Fat Mass and Obesity-Associated) gene is one of the enzymes that removes (demethylates) N6-methyladenosine (m6A) modification and has various roles in the immune response. FTO affects the stability and translation of mRNA by removing m6A modifications. This may alter the ability of immune cells to respond quickly. For example, removal of m6A modifications on the mRNAs of some inflammatory cytokines can affect the production and release of these cytokines. FTO plays a role in regulating inflammatory responses. Removal of m6A modifications can modulate inflammatory responses by affecting the expression and production of pro-inflammatory cytokines. This may affect the immune response to infections or inflammatory diseases. FTO regulates the function and differentiation of T cells. Removal of m6A modifications may affect the ability of T cells to activate and mount an effective immune response. In particular, it can lead to changes in the functions of certain subsets of T cells (e.g., T regulatory cells). FTO regulates the energy requirements and functions of immune cells by affecting cellular metabolism. Removal of m6A modifications may affect the energy metabolism of immune cells and, accordingly, immune responses. The FTO gene regulates the immune response through various mechanisms by removing N6-methyladenosine (m6A) modifications. It plays critical roles in processes such as mRNA stabilization, inflammatory responses, T cell

function, metabolic regulation, and cancer immune surveillance. This suggests that FTO is an important target for the immune system and the fight against disease. Studies suggest that the FTO gene has various roles in the immune response, such as regulating macrophage inflammatory response, influencing NK cell immunity, promoting cancer stem cell self-renewal and immune escape (Hu, Z. et al. 2022, (Kim, S. et al. 2023, Gu, X. et al. 2020).

YTHDF (YTH Domain Family) genes play important roles in the immune response and specifically function as proteins that recognize m6A modifications. These proteins determine the fate of the mRNA; that is, it affects the stability, translation, and degradation of mRNA. Findings regarding some specific roles of YTHDF proteins in the immune response include: YTHDF proteins recognize m6A-tagged mRNAs and regulate the stabilization or degradation of these mRNAs. This allows immune cells to respond quickly and effectively. These proteins regulate cellular responses to viral infections. m6A modifications may control virus replication by affecting the stability and translation of viral RNA. This may increase the immune system's ability to recognize and destroy viruses. YTHDF proteins regulate inflammatory responses by stabilizing the mRNAs of pro-inflammatory cytokines and other immune regulatory molecules. This modulates the immune response in cases of infection or inflammatory disease. YTHDF proteins affect the differentiation and maturation of hematopoietic stem cells. This can have a direct impact on the production and functions of immune cells. In adaptive immune

responses, especially the activation and functions of T and B cells can be regulated by YTHDF proteins. This supports the formation of long-term immune memory and the development of specific responses to antigens. YTHDF genes support defense mechanisms against infections by regulating various aspects of the immune response. These proteins, which influence the stability, translation and degradation of mRNA, play a role in critical processes such as inflammatory responses, antiviral defense and adaptive immune responses. YTHDF1 increases translation of m6A-tagged mRNAs. It promotes binding to ribosomes and protein synthesis. It supports rapid immune response, especially by regulating the production of inflammatory cytokines. It recognizes m6A modifications and accelerates mRNA degradation. It controls mRNA stability and half-life. It plays a role in controlling inflammatory responses and preventing excessive immune responses. It both increases mRNA translation and regulates its degradation. It modulates mRNA metabolism by cooperating with YTHDF1 and YTHDF2. It supports defense mechanisms against infections by regulating the gene expression of immune cells (Feng, J. et al. 2024, Zong, X. et al. 2021, Zhang, Y. et al. 2018).

The ALKBH5 (AlkB homolog 5) gene is one of the N6-methyladenosine (m6A) demethylase enzymes and affects the stability, translation and localization of mRNA by removing m6A modifications on mRNA. Its role in the immune response is as follows: ALKBH5 increases the stability of these mRNAs and regulates their translation by removing m6A modifications on mRNA. This affects the capacity

of immune cells to produce certain proteins. ALKBH5 plays an important role in the activation and differentiation of T cells. It regulates the ability of T cells to mount an effective immune response. ALKBH5 modulates inflammatory responses by regulating the expression of pro-inflammatory cytokines. This may affect the immune response during infections or inflammatory diseases. It regulates the immune response against viral infections. It may affect virus replication and antiviral responses of immune cells by removing mRNA modifications. The role of ALKBH5 in these processes suggests that it is an important regulator of the immune system and disease fighting (Liu, Y., & Cao, X. 2023, Wei, C. et al. 2022). The VIRMA (Vir-like m6A methyltransferase associated) gene is involved in writing N6-methyladenosine (m6A) modifications and plays an important role in the immune response. Here are some roles of the VIRMA gene in the immune response: VIRMA enables m6A modifications to be added to mRNA. These modifications affect the stability, translation and localization of mRNA and thus regulate the functions of immune cells. m6A modifications regulate the activation and functions of immune cells, enabling a rapid and effective response to infections. VIRMA's role in this process increases the effectiveness of immune cells. It regulates inflammatory responses by making m6A modifications on the mRNAs of pro-inflammatory cytokines and other immune regulatory molecules. This allows control of the immune response during infection or inflammatory diseases. VIRMA also plays a role in antiviral immune responses. It controls virus replication by affecting the modification of the RNA of viruses and modulates the antiviral responses of immune

cells. These functions indicate that VIRMA plays an important role in the effectiveness of the immune system and in fighting diseases (Jiang, X. et al. 2021, Gu, Y. et al. 2023).

The role of the RBM15 (RNA Binding Motif Protein 15) gene in the immune response is associated with its ability to regulate RNA metabolism. RBM15 regulates m6A methylation, which in turn affects the stability, translation and degradation of mRNA. RBM15 is particularly involved in the activation and differentiation of T and B cells. This increases the sensitivity and effectiveness of the immune response. RBM15 regulates inflammatory responses by stabilizing the mRNAs of pro-inflammatory cytokines. This allows control of the immune response during infections or inflammatory diseases. RBM15 may affect virus replication and antiviral responses of immune cells by regulating the modification of viral RNA. This increases the immune system's capacity to fight viruses. With these functions, RBM15 plays an important role in protecting against infections by regulating the effectiveness and sensitivity of the immune system (Zhao, Z. et al. 2022, Matsuda, A. et al. 2011).

The ZC3H13 (Zinc Finger CCCH-Type Containing 13) gene plays an important role in regulating N6-methyladenosine (m6A) modification and thus may influence the immune response. ZC3H13 serves as a component of the m6A printer complex and is involved in the methylation of mRNAs. These modifications regulate mRNA stability, translation, and degradation. m6A modifications enable a rapid and effective immune response against infections by regulating

the activation and functions of immune cells. ZC3H13 plays a role in the control of inflammatory responses by regulating the mRNAs of pro-inflammatory cytokines. This may help prevent immune system overreactions. It may be effective in defending against viral infections by regulating virus replication and antiviral responses of immune cells. With these functions, ZC3H13 plays an important role in protecting against infections and inflammatory diseases by increasing the effectiveness of the immune system (Fu, M., & Blackshear, P. 2016, Gong, P. et al. 2020, Wu, S. et al. 2022).

Conclusion

In conclusion, the m6A pathway has a broad spectrum of effects on various components of the immune system, and the regulatory role of these modifications is critical in shaping both innate and adaptive immune responses. A better understanding of this pathway may contribute to the development of new treatment strategies for immune system diseases. These studies suggest that the N6-methyladenosine pathway has potential applications in the treatment and diagnosis of immunological diseases, in personalized immunotherapy strategies, and as a biomarker and therapeutic target of inflammatory diseases by regulating immune responses and altering immunoregulatory networks (Liu C. et al 2021; Lou X. et al. 2021; Shulman Z. et al, 2020). The effect of the N6-Methyladenosine (m6A) pathway on Crimean-Congo Hemorrhagic Fever (CCHF) is associated with interactions between the virus and the immune response. CCHF virus contributes to the pathogenesis of the disease by affecting various signaling pathways in

the host cell. m6A modifications regulate the stability and expression of the virus' RNA, thus may influence virus replication and immune evasion. Inhibitors or regulators of these modifications may affect the activity of the virus in the host cell and the immune response, thereby altering the clinical severity of the disease.

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CHAPTER 2

THE ROLES OF MTHFR GENE POLYMORPHISMS ON VARIOUS DISEASES

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INTRODUCTION

The 656 amino acid MTHFR enzyme, which is a dimeric protein consisting of 70-77 kDa subunits and encoded by the *5, 10-methylenetetrahydrofolate reductase (MTHFR)* gene localised at the 1p36.22 locus, 2.2 kb in size and consisting of 11 exons, is involved in the folate pathway (Hmimech et al., 2016; Levin & Varga, 2016; Liew & Das Gupta, 2014). MTHFR enzyme is accountable for maintaining the balance between methionine and homocysteine in order to prevent cellular dysfunction (Raghubeer et al., 2021). The MTHFR enzyme provides the process of converting homocysteine into methionine by remethylation. With the catalysis of this enzyme, 5, 10-methylenetetrahydrofolate is converted to 5'-methyltetrahydrofolate. Vitamin B12 acts as a cofactor in this reaction (Liew & Das Gupta, 2014). Circulating primary folate is in the form of methyltetrahydrofolate. Methionine amino acid is the precursor of S-adenosyl methionine (5'-SAM) which is the major methyl donor in the body (Levin & Varga, 2016). Homocysteine can be converted to methionine, the methyl donor of homocysteine, by methionine synthase

(MS) under the cofactor of vitamin B12 or by transsulfuration to cysteine under the cofactor of vitamin B6. This pathway is also critical in the methylation of nucleotide/DNA, which is important in the regulation of DNA synthesis. This cycle is called the methionine cycle (Figure 1).

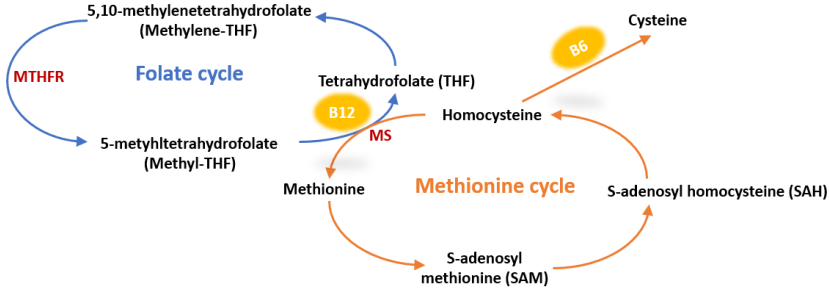

Figure 1. Folate and Methionine Cycles

In the *MTHFR* gene, C677T and A1298C polymorphisms are predominantly observed. In the general population, at least one variant is observed in 60-70% of individuals (Long & Goldblatt, 2016). The C677T polymorphism is the variant (rs1801133) that occurs when the thymine (T) nucleotide replaces the cytosine (C) nucleotide at position 677 of the 4th exon of the *MTHFR* gene (Chita, Tudor, Christodorescu, Nicoleta Buleu, et al., 2020). The C677T polymorphism, which is the most common single point polymorphism in the *MTHFR* gene, results in a missense mutation with the conversion of the valine amino acid at position 222 of the enzyme to alanine amino acid. Since this missense mutation is in the binding site of flavin adenine dinucleotide (FAD), the cofactor of the enzyme, it causes the enzyme to be thermolabile and

facilitates its separation from its cofactor. When homozygous TT genotype is present, the enzyme activity decreases by 50-60% at 37°C and 65% at 46°C in vitro, whereas it is in the intermediate range in heterozygous CT genotype. When folate intake is insufficient in individuals with homozygous genotype, there is a tendency to increase in plasma homocysteine levels (Hmimech et al., 2016; Y.-M. Yang et al., n.d.). The A1298C polymorphism (rs1801131), which is another common polymorphism, is an adenine-cytosine transposition at position 1298 in exon 7 of the *MTHFR* gene and results in the conversion of the glutamine amino acid at position 429 of the C-terminal regulatory domain of the enzyme into valine amino acid. This polymorphism also leads to a decrease in the activity of the enzyme (Soleimani-Jadidi et al., 2022). While the CC genotype of the A1298C polymorphism leads to a 30% loss in enzyme function, the AC heterozygous genotype leads to a 15% loss in enzyme function. Both C677T and A1298C co-heterozygosity cause a 50-60% decrease in enzyme activity. In general, A1298C mutation weakly affects MTHFR activity, homocysteine and folate levels compared with C677T mutation (Y. Li et al., 2020).

Many studies have demonstrated a correlation between MTHFR polymorphisms and various disorders. In this review, the effects of these polymorphisms in various diseases will be discussed.

MTHFR and Cancer

The incidence of cancer, which is characterised by the proliferation of cells that manage to escape endogenous control

mechanisms, increases due to folate deficiency and polymorphisms in enzymes involved in the folate pathway, such as the MTHFR enzyme. In a comprehensive meta-analysis (200,699 controls and 150,086 cases from 446 studies) by Xie et al has been reported that MTHFR polymorphisms significantly increased to breast cancer, gastric cancer, hepatocellular carcinoma and multiple myeloma risk, while significantly decreased colorectal cancer, adult acute lymphoblastic leukemia (AALL) and childhood acute lymphoblastic leukemia (CALL) risk (Xie et al., 2015).

Breast cancer

In a study involving breast cancer patients, the C677T CT genotype was correlated with a reduced risk and homozygous alleles of both polymorphic regions (TT genotype for C677T; CC genotype for A1298C) were not observed (Floris et al., 2020). In a study by Omran et al, it was reported that both the percentage of MTHFR C677T T alleles and the percentage of MTHFR A1298C C alleles were higher in breast cancer patients than in the control group and were statistically significant (Omran et al., 2021). In another study, there was no association between the risk of breast cancer and MTHFR C677T and A1298C polymorphisms, but interestingly, MTHFR A1298C polymorphism was reported to have a preventive effect against breast cancer (Paula D' et al., n.d.). These genetic variations can result in a reduction in the methyl donor SAM, resulting in DNA hypomethylation and sequential activation of carcinogenic oncogenes (Omran et al., 2021).

Hepatocellular carcinoma

The role of MTHFR polymorphisms in hepatocellular carcinoma remains controversial, but one study has shown that the A1298C polymorphism may be an important biomarker, while the C677T polymorphism is ineffective in the development of cancer risk (Su, 2019). Another meta-analysis reported that C677T polymorphism is a protective factor for hepatocellular carcinoma (Zhang et al., 2020). In a meta-analysis reviewing the association between hepatocellular cancer and MTHFR gene polymorphisms, it was concluded that C677T polymorphism increased the risk of HCC in the Chinese population, whereas A1298C polymorphism decreased the risk of hepatocellular carcinoma (B. Wang et al., 2021)Additionally, another study demonstrated a correlation between the presence of C677T and rs9651118 (C>T) polymorphisms with an increased susceptibility to hepatocellular cancer (Zhang et al., 2019).

Lung cancer

The possible role of MTHFR polymorphisms has also been examined in terms of the risk of developing lung cancer, the most frequently diagnosed cancer worldwide with a high mortality rate. In two different studies, the C677T polymorphism was linked to an increased susceptibility to developing lung cancer, while A1298C was not associated with lung cancer susceptibility (Y. Yang et al., 2016; Zhong et al., 2019). In another study, it was stated that sex may play a vital role in cancer risk together with MTHFR polymorphisms. For example, the presence of MTHFR C677T polymorphism in the female

sex has been shown to reduce the risk of developing lung cancer and some other types of cancer. It has been shown that the risk of lung cancer is higher in women with the MTHFR A1298C homozygote mutant compared to the wild type, while there is no association in men (Almutairi et al., 2021; Shi et al., 2005). In contrast to this study, Tong et al found that the presence of MTHFR C677T polymorphism may play a role in the occurrence of lung cancer and adenocarcinoma in Chinese females. Conversely, the presence of MTHFR A1298C polymorphism decreases the likelihood of developing lung cancer and adenocarcinoma (Tong et al., 2018). In addition, there are studies showing that the presence of MTHFR C677T and A1298C polymorphisms does not have a statistically significant association with the risk of developing lung cancer (Pérez-Ramírez et al., 2018; Y. Yang et al., 2016).

The presence of homozygous genotypes of MTHFR C677T and A1298C is linked to elevated amounts of homocysteine, which can result in DNA hypomethylation and an increased likelihood of developing cancer. Nevertheless, the decline in enzyme activity leads to elevated levels of thymidine and 5,10-methylenetetrahydrofolate, hence promoting enhanced DNA synthesis and repair. Thus, MTHFR polymorphisms are considered to be a safeguard against tumor formation (ZHAO et al., 2013).

MTHFR and Cardiovascular Diseases

Cardiovascular diseases (CVDs), involving conditions affecting the heart and blood arteries, are the primary cause of mortality on

worldwide (Cardiovascular Diseases, n.d.). CVDs can be classified as coronary heart disease, peripheral arterial disease, cerebrovascular disease and venous thromboembolism (Flora & Nayak, 2019; Olvera Lopez et al., 2023). The majority of the progression of CVDs is of atherosclerotic origin (Flora & Nayak, 2019). Atherosclerosis is a pathological condition that affects the arteries and aorta, leading to reduced or blocked blood flow as a result of narrowing of the blood vessels (Olvera Lopez et al., 2023). Although CVDs have a significant mortality rate, effectively identifying and reducing the main risk factors may significantly decrease the prevalence of CVDs worldwide (Flora & Nayak, 2019).

Hyperhomocysteinemia has both vasculotoxic and neurotoxic effects. These effects are neurodegeneration, neuroinflammation, pro-oxidation and prothrombotic/proatherogenic (Hayden & Tyagi, 2021). Hyperhomocysteinemia, which can be caused by MTHFR polymorphisms, has been shown to be toxic to cardiac cells in experimental animal models (P.-F. Liu et al., 2020) . This makes it possible to explain the C677T polymorphism in particular as a possible hereditary risk factor for cardiovascular diseases.

In a study based on the number of atherosclerotic vessels, the C677T TT genotype was found to be more common in people with more than one atherosclerotic vessel and the presence of this genotype may be an independent risk factor (Cai et al., 2023). C677T T allele frequency and TT genotype distributions were found to be significantly higher in CVD patients (Raina et al., 2016). A significant association

was found between coronary artery disease risk and C677T polymorphism in the Chinese population (L. Li et al., 2022; H. Wang et al., 2022). The presence of the C677T polymorphism was found to have a strong and statistically significant association with the development of carotid artery atherosclerosis (A. Li et al., 2020). Although significant hyperhomocysteinemia was observed in young individuals with coronary artery disease, independent of C677T and A1298C polymorphisms, high homocysteine was also associated with C677T polymorphism (Shivkar et al., 2022). The A1298C CC allele was significantly associated with peripheral artery disease, but the C677T polymorphism did not exhibit any association with peripheral artery disease (Yalım et al., 2020).

In a study by Hou et al investigating the effect of MTHFR C677T polymorphisms in patients with acute cerebral infarction and acute myocardial infarction, it was shown that C677T polymorphism did not directly contribute to the development of acute myocardial infarction/ acute cerebral infarction, but C677T TT genotype caused hyperhomocysteinaemia and low folic acid levels and these two parameters played a role in the development of acute cerebral infarction/acute myocardial infarction.(Hou et al., 2023; Lupi-Herrera et al., 2019). Another study showed that female patients with fatal myocardial infarction had higher A1298C C allele frequency than non-fatal patients. However, when males and all patients with myocardial infarction were evaluated together, no significance was found (Söderström et al., 2023)

In children with primary hypertension, the C677T T allele was found to be higher and to increase serum homocysteine levels. It has also been shown that TT genotype is an independent risk factor in children with hyperhomocysteinemia and may be associated with the severity of early target organ damage (H. Wang et al., 2022). The C677T polymorphism is a risk factor for both hypertension and cardiovascular diseases (Katsa & Gil, 2022; H. Wu et al., 2022). In addition to C677T, some studies have shown that A1298C is also significantly associated with hypertension risk (Y. Liu et al., 2023; F. Yang et al., 2022). However, there are also studies that are found to be meaningless (S. Liu et al., 2019). Another polymorphism in the MTHFR gene, rs17367504 (A>G) minor allele G allele, has been shown to be a protective allele against nongestational hypertension and preeclampsia (Thomsen et al., 2017). In one study, the rs17367504 polymorphism AA genotype was shown to be more frequent in individuals with high blood pressure, while another study showed that it was not associated with hypertension (Xi et al., 2013). The presence of the C677T polymorphism in individuals diagnosed with non-valvular atrial fibrillation has been found to be linked to many cardiovascular comorbidities, including , heart failure, dyslipidemia, hypertension, and type 2 diabetes mellitus. However, the A1298C polymorphism is specifically linked to dyslipidemia. This study further emphasized the correlation between the C677T polymorphism and the severity of stroke (Chita, Tudor, Christodorescu, Buleu, et al., 2020). C677T and A1298C polymorphisms have been linked to congenital heart disease in both Asians and Caucasians (A. Xu et al., 2018). The C677T T allele has

been associated with congenital heart disease, especially in Asians (P.-F. Liu et al., 2020)

Thrombosis has been widely recognized as a significant risk factor associated with hyperhomocysteinaemia. Therefore, it has been suggested that MTHFR polymorphisms, which may lead to impaired homocysteine metabolism, may lead to vascular thrombotic events such as venous thromboembolism (Hayden & Tyagi, 2021; Simonenko, 2019)After acute myocardial infarction and stroke, venous thromboembolism is the most prevalent vascular illness (Simonenko, 2019). The presence of C677T and A1298C polymorphisms was found to be associated with an increased risk of cerebral venous sinus thrombosis, which accounts for less than 1% of all strokes (Gogu et al., 2020). According to the findings of Zeng et al, the presence of the C677T polymorphism may potentially play a role in the pathogenesis of pulmonary embolism and could serve as a potential biomarker for venous thromboembolism (Lupi-Herrera et al., 2019; Zeng & Zeng, 2019). The C677T polymorphism was additionally observed to exhibit an association with the occurrence of deep vein thrombosis (J. Xu et al., 2019).

The findings of a meta-analysis investigating the impact of MTHFR polymorphisms on haemorrhages revealed that the presence of the C677T T allele may influence the susceptibility to cerebral hemorrhage, whereas the presence of the A1298C C allele may influence the susceptibility to intraventricular haemorrhage (F. Wang et al., 2021). Increased concentrations of homocysteine in the plasma lead

to the self-oxidation of homocysteine, resulting in the generation of peroxides and free oxygen radicals facilitated by metal ions. These reactive species subsequently inflict harm upon the integrity and functionality of endothelial cells. The impairment of vascular endothelial cells can result in a reduction in the release of prostaglandin and endothelium-derived relaxing factor, causing an alteration in the equilibrium of vasomotor factors in the patient. Consequently, this imbalance can initiate the occurrence of thrombosis and cardiovascular disease (J. Xu et al., 2019).

MTHFR and Diabetes Mellitus

Diabetes mellitus (DM) is a chronic metabolic disorder defined by high levels of glucose in the blood. This disease can cause significant damage to the heart, blood vessels, kidneys, eyes and nerves over a period of time. Type 2 diabetes (T2DM) is the most widespread form of diabetes and mostly impacts the adult population. This is a result of the body's inadequate or unresponsive reaction to insulin. It is caused by an insufficient or resistant response to insulin by the body. In economies of all income levels, the prevalence of T2DM has grown significantly over the past three decades and is still rising (Diabetes, n.d.).

There are studies examining the effect of MTHFR polymorphisms in gestational diabetes, which can occur in approximately 7% of all pregnancies and can lead to serious maternal and fetal complications. A meta-analysis has shown that he presence of the C677T T allele has been linked with a higher likelihood of

developing gestational diabetes, particularly in Asians, but the A1298C polymorphism is not linked to the risk of developing this defected pregnancy condition (Chen et al., 2022). A study conducted by Pathak et al. investigated the MTHFR C677T polymorphisms in individuals newly diagnosed with T2DM and control subjects. The results of the study showed that the MTHFR C677T polymorphism has a significant impact in the susceptibility to developing T2DM. This study also examined the expression of the MTHFR gene and found that individuals with the C677T CT heterozygous and TT mutant genotypes had reduced MTHFR expression (Pathak et al., 2022). A study conducted in Palestine examined the MTHFR C677T polymorphism in patients with T2DM, both with/out dyslipidemia. The study found that individuals with the TT genotype had lower diastolic blood pressure in T2DM patients without dyslipidemia, and higher levels of HDL cholesterol in T2DM patients with dyslipidemia (Elqadi et al., 2021). Another study showing that the T allele may have a protective effect was shown by Kucukhuseyin et al in T2DM patients with coronary heart disease. The study showed that individuals with the CC genotype had elevated levels of triglycerides, LDL, and diastolic blood pressure in comparison to individuals with the CT and TT genotypes (Kucukhuseyin et al., 2013). Wu et al found that the MTHFR 677T allele was found to be higher in diabetic patients with coronary heart diseases than those without. Additionally, this study demonstrated that individuals with diabetes who had homocysteine levels ≤15 μmol/L were more likely to develop coronary heart disease, but MTHFR polymorphisms had no effect on the incidence of coronary heart disease

in diabetic patients with homocysteine levels >15 μmol/L (K. Wu et al., 2021). A study by Lu et al showed that for MTHFR A1298C, individuals carriers of 1298AC + CC had a lower risk of T2DM (Lu et al., 2022). Another polymorphism, the rs17367504 polymorphism, has been linked to an increased risk of peripheral vascular disease in T2DM, particularly in AA genotype carriers (Y.-T. Liu et al., 2021). In patients with diabetic retinopathy, one of the advanced complications of diabetes, left atrial diameter was found to be higher in C677T T allele carriers. However, no significant association was found with other cardiac parameters neither C677T nor A1298C polymorphisms (Alcântara et al., 2023).

MTHFR and Neurodegenerative Diseases

Neurodegenerative disorders (ND) are a group of brain-related disorders, such as Alzheimer's Disease (AD) and Parkinson's Disease (PD), that are characterized by a gradual loss of neurons. The incidence of NDs is on the rise, and the most significant risk factor is age. However, research indicates that both hereditary and environmental factors have an equal role (Dugger & Dickson, 2017; Gitler et al., 2017; Lamptey et al., 2022). The majority of older people with memory loss had mutations in the MTHFR gene (Román et al., 2019).

Alzheimer's Disease

Epigenetic factors such as DNA methylation and miRNA, and polymorphisms are very important in the pathogenesis of late-onset AD patients. Patients with neurodegenerative diseases are increasingly

showing impaired methylation of mitochondrial DNA (Stoccoro et al., 2020). The MTHFR enzyme is critical for the production of SAM, a methyl donor essential for epigenetic processes (Román et al., 2019). MTHFR C677T polymorphism has been reported to be associated with an increased risk of AD (Rai, 2017). In a study investigating the relationship between variations in genes related to folate metabolism, specifically MTHFR C677T and A1298C, none of these gene variations were shown to have a statistically significant link with late-onset AD patients (Stoccoro et al., 2020). According to a study, the A1298C CC genotype was significantly more common in individuals with late-onset AD compared to the control group, and the MTHFR 1298C allele increased the risk of AD by 1.5-fold. It has also been reported that the MTHFR 677TT genotype reduces the age of onset of AD by 5 years (Durmaz et al., 2019). The MTHFR 677TT genotype has been shown to increase vulnerability to late-onset AD in Italian population in a meta-analysis; however, more research is required to determine whether this genotype may be utilized as a non-invasive biomarker (Zuin et al., 2021). A study investigating the connection between MTHFR polymorphisms and the risk and age at which Alzheimer's disease (AD) develops revealed that the occurrence of the C677T T allele was notably higher in AD patients. However, no statistically significant difference was identified in the A1298C and A1793G alleles. When the genotype distribution was analyzed, it was shown that both C677T and A1298C were significantly different in AD patients compared to controls. Additionally, it was reported that individuals with the C677T polymorphism developed AD earlier (Jiang et al.,

2021). The CC allele of the C677T polymorphism has been shown to play a protective role against AD, whereas the CT and TT genotypes have been shown to have no effect on AD risk (Bouguerra et al., 2022). In several previous studies, it was stated that the A1298C polymorphism may be a protective feature for AD disease, in the Indian and Japanese population. Only Japanese AD patients had the protective effect of the A1793G polymorphism validated (Jiang et al., 2021). According to a subgroup analysis based on ethnicity, the C677T polymorphism was found to be linked to AD in Asians, but not in Caucasians (Hua et al., 2011).

Parkinson Disease

The MTHFR gene has been identified as a candidate gene associated with a higher likelihood of developing Parkinson's disease (PD), although the findings from various investigations indicate conflicting results. A meta-analysis of 19 research revealed that there is no significant correlation between the C677T polymorphism and the risk of Parkinson's disease (PD) in the overall population when considering five genetic models. However, when examining different ethnic groups, it was shown that the C677T TT variant is much more common in Caucasians compared to Asians. When the association between A1298C polymorphism and PD risk was evaluated with the same analysis methods, the C allele was found to be significant in the total population with five genetic models, but no statistical significance was observed when evaluated by ethnicity (L. Liu et al., 2018). According to Wu et al, they found a correlation between the MTHFR

C677T polymorphism and an elevated risk of PD, but A1298C was not associated with increased PD risk in both Asians and Caucasians (Y.-L. Wu et al., 2013). One study showed a positive correlation between cognitive decline in PD and hyperhomocysteinemia, but MTHFR C677T and A1298C polymorphisms were not associated with PD (Periñán et al., 2023).

Although there is evidence suggesting that MTHFR C677T and A1298C polymorphisms could contribute to Multiple Sclerosis (MS), a significant neurological disease (Cakina et al., 2019), there are also conflicting studies indicating the contrary (Chorąży et al., 2018). The presence of the C allele of the MTHFR A1298C polymorphism is associated with an increase in saccade delay in patients diagnosed with spinocerebellar ataxia type 2 (SCA2), another neurodegenerative disease. However, it was no association with between disease risk and A1298C genotype or allele frequencies. Furthermore, no correlation was seen between the C677T polymorphism and SCA2 (Almaguer-Mederos et al., 2020).

In conclusion, MTHFR C677T polymorphism has the potential to be an important risk factor for ND diseases, especially AD.

MTHFR and Coronavirus Disease-19

The Coronavirus Disease-19 (COVID-19) emerged in 2019 and was officially classified as a pandemic by the World Health Organization (WHO) in March 2020. It has exhibited a diverse range of clinical outcomes. The influence of genetic predisposition on COVID-

19 morbidity and death was anticipated (Tekcan et al., 2023). It has been shown that the MTHFR C677T polymorphism is strongly correlated with mortality from COVID-19 infection and thus may modulate incidence and severity (Ponti, Pastorino, et al., 2021; Ponti, Roli, et al., 2021). There are also studies with opposite results. (Kose et al., 2023; Lapić et al., 2022). In a study, it was shown that the C677T T allele and CT genotype were more common in intensive care unit patients and thus may have an impact on the clinical course of COVID-19 in the Turkish population. It was also reported that C allele and CC genotype were higher in PCR positive patients, while CT genotype was more common in computed tomography positive patients (Tekcan et al., 2023).

Thrombosis and coagulopathy are commonly seen in COVID-19 patients, especially those with a severe clinical course. Since high homocysteine levels are also a factor that activates the coagulation cascade, polymorphisms in MTHFR, one of the genes that may cause increased homocysteine levels, may contribute as a potential genetic factor (Abu-Farha et al., 2020). The C677T CC genotype was found to be higher in patients with long COVID-19 (da Silva et al., 2023). Ponti et al demonstrated that elevated homocysteine levels serve as a prognostic indicator for the outcomes of Covid-19 patients who are admitted to the hospital (Ponti, Roli, et al., 2021). Another previous study also reported that hyperhomocysteinemia was predictive for lung progression with computed tomography imaging.

Highlights

- In some diseases, MTHFR polymorphisms do not directly affect the risk of the disease, but these polymorphisms contribute to the pathogenesis of the diseases by elevating serum/plasma homocysteine levels.

- In conclusion, C677T than A1298C polymorphism causes predisposition to various cancer, cardiovascular diseases, neurodegenerative diseases (especially AD). In severe COVID-19 patients, C677T T allele frequency was higher.

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CHAPTER 3

EFFECTIVENESS OF EVIDENCE-BASED MODULE TO IMPROVE PATIENT'S OUTCOME USING THE BRADEN SCALE

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INTRODUCTION

Evidence based guidelines are a crucial part of pressure ulcer control, and nurses‘ knowledge and practice should be in line with them to ensure the safety of immobilized hospital patients. There are several strategies for improvement that can be implemented to ensure that nurses have an adequate understanding of evidence based guidelines and their importance in pressure ulcer control. One such strategy is the incorporation of evidence based guidelines into nursing curricula. This ensures that nurses are educated from the start of their career on the importance of pressure ulcer control and the protocols that must be followed in order to ensure the safety of immobilized hospital patients (Sucu and Kilic, 2022).

Moreover, it is considered as a quality health care indicator given by the hospitals. Redness, tenderness, swelling, and a discharge that mimics pus are some of the signs of a pressure ulcer. Another symptom of a pressure ulcer is pain. People who are confined to places where they cannot move around, such as hospitals or nursing homes, have a greater risk of becoming infected. The most common risk factors that were mentioned were prolonged stays in the intensive care unit (ICU), less frequent repositioning, a history of cardiovascular disease or diabetes, and old age. (De Oliveira et al. 2017; Wurzer et al. 2018) are some citations that can be looked up. Finding out who is at the greatest risk for developing a pressure ulcer is the first step in developing a plan to prevent them.There are many different interventions that can be used to prevent pressure ulcers and eliminate friction and shear (Porter, et al., 2018; Saghaleini, et al., 2018). Some of these interventions include various types of support surfaces (such as integrated bed systems, cushion overlays, and mattresses), nutritional supplements, repositioning, skin care (such as dressing and incontinence management), and topical creams (Porter, et al., 2018).

Pressure ulcers can be a serious issue for hospitalized patients, particularly those who are immobilized. Their prevention and treatment require nurses to possess a high level of knowledge and practice in order to ensure patient safety. Evidence-based guidelines have been shown to be an effective tool to improve nurses ‘knowledge and practice in pressure ulcer control. These guidelines provide nurses with evidence-based recommendations that can be used to identify best practices and the most effective strategies to prevent, detect and treat pressure ulcers.

Implementing these guidelines can lead to improved patient outcomes, greater patient satisfaction and improved patient safety (Kottner et al., 2019).

Similar to that Research on PUs in Pakistan has mostly focused on patients who have suffered from Neuro trauma and spinal cord injuries. Therefore, we have come to the conclusion that despite the prevalence of PUs in the United States, there are not enough data to provide an appropriate evaluation of the damage they inflict or the financial burden they impose on patient health and safety. This is the conclusion that we have reached. We have created guidelines for the prevention and treatment of PUs based on the results of scientific investigations; however, these guidelines are not consistently used in clinical settings. Based on the results of scientific studies, it is possible that the lack of understanding regarding pressure ulcer (PU) care among medical personnel, patients, and their loved ones is to blame. PU stands for pressure ulcer. Even though PU care is a team effort, nurses play a significant part in the process, and ulcer avoidance should be one of the nursing staff's primary priorities (Qazi et al., 2022).

The presumption that every patient has the potential to become infected with a pressure ulcer underlies the methodology used to calculate the risk of pressure ulcers (National Institute for Healthand Care Excellence ([NICE, 2015). A risk assessment scale has been established for the purpose of identifying persons who are at risk and the circumstances that can contribute to pressure ulcers so that preventative action can begin. It cannot be overstated how important it is to conduct risk assessments while taking into account the current state of the patient.

Assessment of the patient'srisk of developing a pressure ulcer must take place upon admission to tertiary care facilities, as well as every 48 hours thereafter, and particularly following any change in the patient's mobility or neurological status. Over the past four decades, numerous instruments for determining the risk of developing pressure ulcers have been developed. The majority of these tools provide numerical representations of the risk scores. The Braden Scale is the most common method among these for estimating the likelihood of developing a pressure ulcer (Jansen, Silva and Moura, 2020).

There is empirical evidence to support the assumption that a certain degree of skill .The Braden scale is comprised of six different sub-measurements that determine the extent of prolonged and rigorous pressure, as well as the tissue's tolerance to pressure. Every sub-measurement includes a descriptive title, in addition to a brief explanation of its individual characteristics. There are five different sub-measurements, and their values range from 1 (the least favourable) to 4 (the most favourable). The friction and shear sub- measurements are ranging from 1 to 3. The maximum possible score is 23 points while the lowest possible score is 6 points. A decreased numerical score describes greater risk for pressure ulcer (Lima-Serrano et al., 2018).

The sensitivity and specificity of the Braden scale have been shown by several investigations. Two separate studies have separately shown that a cutoff score of 16 delivers complete sensitivity while showing specificity ranging from 64 percent to 90 percent. The validity of the Braden scale was confirmed by further research, which found that it had a reliability value of 0.9929.

The predictive efficacy of the Braden scale for predicting the incidence of pressure ulcers was restricted in a meta-analysis that included 17 studies.AUC values for the SROC have been found to be more than 0.7 in the investigations by Wei et al. (2020), Griswold et al. (2017), and Park and Lee (2016).

A pressure ulcer is a wound that can be found in the skin as well as the layers of tissue that are directly under it. They occur most frequently over bony surfaces and are caused by ischemia injury that is either prolonged or repeated and does not provide the tissues the time to heal. The risk of patients developing pressure ulcers while in the hospital is a significant concern for medical staff(Awali, Nagshabandi and Elgmail, 2018). The development of a large number of clinical regimens for the prevention and treatment of pressure ulcers is on-going (Dalvand, Ebadi and Gheshlagh, 2018). When healthcare providers put these guidelines into practise, it makes it easier to treat patients who have pressure ulcers and prevent them from getting them in the first places. Guidelines for pressure ulcers have the potential to encourage nurses to put their knowledge into practice. There is a connection between the amount of experience that nurses have with non- traditional approaches and the number of times that they break from the regular operating protocols. It's great to see such a strong link between the two factors. (Etafa et al., 2018).

Methods

The research design chosen a quantitative quasi-experimental study method. The study was conducted in two tertiary cares private hospitals

in Lahore, Pakistan—Shalamar Hospital Lahore (SHL) served as the intervention group, while Fatima Memorial Hospital (FMH) functioned as the control group. The study focused on registered nurses (RN) of any gender employed in specified departments and adult immobilized patients of any gender admitted to the cited departments. The sampling method employed was a Two-Stage cluster sampling method, which is a statistical technique used to select samples from populations organized into clusters. The study included a sample size of 64 for each group (intervention and control).

Data Collection

Data collection utilized structured questionnaires to gather socio-demographic information from patients, and the Braden scale was employed to assess pressure ulcers. The Braden scale encompassed three phases: the first phase assessed patients on admission day, the second phase on the 5th day of hospitalization, and the third phase on the 10th day of hospitalization. This comprehensive methodology was employed to systematically investigate and evaluate the impact of the intervention on the occurrence of pressure ulcers in the designated hospital settings.

Results

Effectiveness of IMPUPM to Improve Patients Outcome

The results of a repeated measures ANOVA displays means (M) and standard deviations (SD) for three different levels of a variable (BS1, BS2, BS3) across two different groups (Interventional and Control). Its

significant differences between the three levels of the variable and shows a significant main effect for the variable ($F(2,252) = 3.86$, $p < .001$, $\eta 2 = .056$). This indicates that there are significant differences between at least two of the means for the three levels of the variable.

The effect size ($\eta 2$) is .056, which is a small effect size.

This suggests that while the difference between the means is statistically significant, the practical significance of the difference may be relatively small.

The p-value is less than .001, which is a very small probability. This indicates that the results are highly statistically significant, and that it is highly unlikely that the observed differences occurred by chance (Table 1).

Table 1. Effectiveness of IMPUPMN to Patients Outcome

	BS1		**BS 2**		**BS 3**				
Variables	*M*	*SD*	*M*	*SD*	*M*	*SD*	*F(2,252)*	*P*	η^2
Interventional	14.154.15		15.81	3.98	17.40	3.86	7.37	>0.001	.056
Control	14.902.25		15.39	3.78	14.64	4.63			

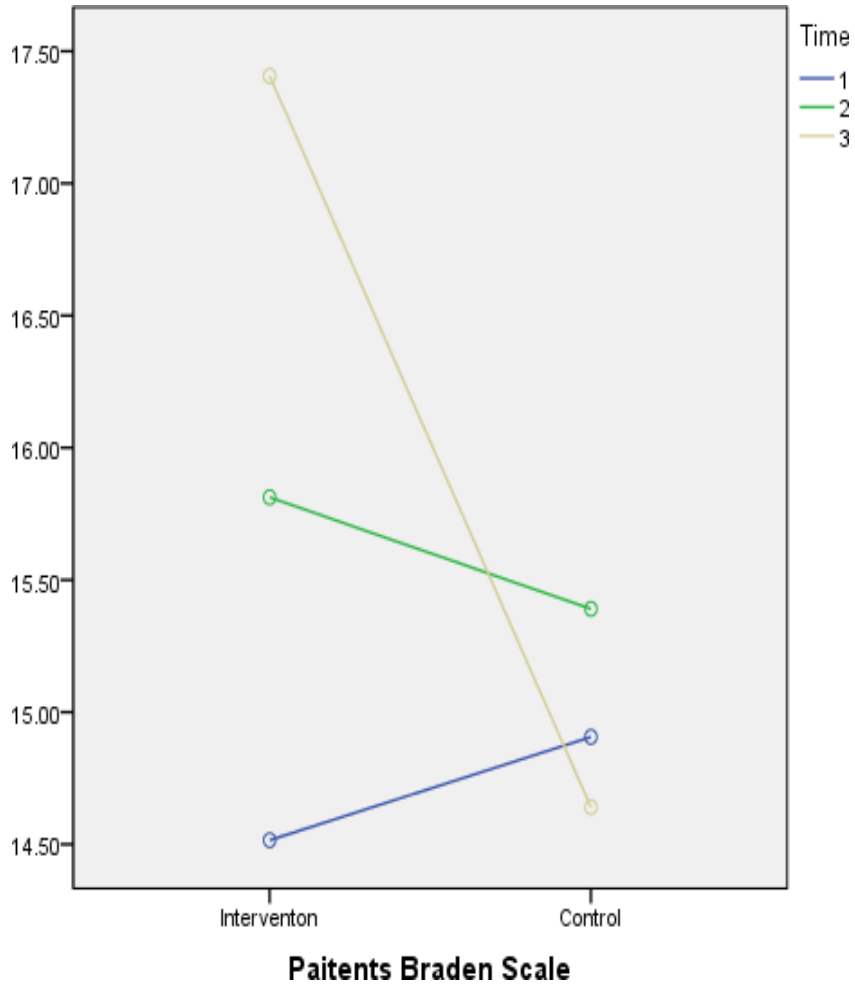

Fig 1. Braden Scale

The pressure ulcer development was determined through skin inspection, and the risk assessment for developing pressure ulcers was conducted using the Braden scale. The p- value was less than .001, indicating that the results are highly statistically significant association of intervention (Figure 1).

Post-Hoc Multiple Comparison (Bonferroni Test) Effectiveness of IMPUPMN to Improve PPUPM

The results of the Post-Hoc Multiple Comparison (Bonferroni Test) suggest that the intervention of IMPUPMN has a statistically significant effect on improving PPUPM (prevention of pressure ulcer in perineal area) compared to the control group.

The mean difference between the intervention group and the control group was significant for all three comparisons.

There is a statistically significant difference between BS1 and BS2 based on the fact that the p-value is lower than 0.001, which indicates a mean difference of 1.594. This suggests that there was a statistically significant increase in PPUPM improvement in the intervention group as compared to the control group. The calculated difference in mean between BS2 and BS3 was 1.656, and the threshold of significance was lower than 0.001.

It would seem that those in the intervention group had a considerably lower rate of PPUPM improvement than those in the control group did. Similarly, for BS1, BS3, the mean difference was 3.250 with a p-value of less than 0.001, indicating that When comparing the intervention group to the control group, there was a statistically significant rise in PPUPM. P-values of all three comparisons were less than the Bonferroni-corrected alpha level of 0.047 which indicates that the findings are statistically significant even after adjusting for multiple comparisons. Overall, the results of the Post- Hoc Multiple Comparison

(Bonferroni Test) suggest that the IMPUPMN intervention is effective in improving PPUPM compared to the control group (Table 2).

Table 2. Post-Hoc Multiple Comparison (Bonferroni Test) Effectiveness of IMPUPMN to Improve PPUPM

	Intervention		Control	
	Mean difference	**P-value**	**Mean difference**	**P-value**
BS1* BS2	1.594*	<0.001	0.047	>1.000
BS2* BS3	1.656*	<0.001	0.484	>1.000
BS1*BS3	3.250*	<0.001	0.531	>1.000

Effect of intervention on patient's outcome:

It seems that the intervention had a positive effect on patient outcomes. Specifically, the intervention group had a reduced number of patients at risk for developing pressure ulcers compared to the control group. This suggests that the instructions given to nurses in the intervention group were effective in preventing pressure ulcers from forming. The use of the Braden Scale tool to assess patient condition is also a positive sign, as it is a widely recognized tool used to identify patients at risk for pressure ulcers. Patients in both the intervention and control groups can be evaluated using this instrument, allowing for more precise comparisons of intervention efficacy.

Overall, the intervention had a positive impact on patient outcomes, specifically in reducing the number of patients at risk for developing pressure ulcers.

Discussion

The development of PUs was determined through skin examination and risk assessment using the Braden scale. The p- value was less than0.005, indicating that the association between the intervention and the results is highly statistically significant. Overall, it appears that the intervention had a positive impact on patient outcomes, particularly in reducing the number of patients at risk for developing PUs. The Braden Scale achieved a greater equilibrium between its sensitivity and its specificity in this research, demonstrating that it is an improved risk prediction instrument for the patients in this particular group.

In a study of (Gaspar et al., 2022) it was found that Socioeconomic status and clinical characteristics varied significantly across the groups. Scores on the Braden Scale as a whole improved between the first and second evaluations for all groups with the exception of Cluster 4 (the least at risk). If individuals are incorrectly labeled as "low risk," efforts to identify those at higher risk may be misplaced. For medical professionals to effectively predict the risk for PUs, a greater grasp of the whole Braden scale is necessary. Its urge increased efforts to pinpoint potentially risky patients on both a regional and national scale. Just like that in the study of (Suma K et al., 2021) it was found the largest organ in the body and its surface is the skin.

A patient's skin condition is indicative of the kind of care they will get throughout their hospital stay, making proper skin care an essential part of providing excellent medical care. PUs, one of the most physically crippling diseases of the 20th century, will raise healthcare expenses. Students whose lessons were organized according to a curriculum saw the most significant gains in knowledge. A systematic education programme improved nursing students' understanding of the Braden scale's ability to predict PUs, the research revealed.

Implications of the study

It has been shown that lowering the incidence of pressure ulcers in bedridden hospital patients will improve patient safety. Pressure ulcers and their consequences, such as infections and sepsis, may be avoided with the support of nurses who are well-versed in the recommendations and who follow them to the letter.

Conclusion

In the present study, the Braden scale was found to be an efficient measure to assess the risk of pressure ulcers in hospitalized immobilized patients. It is recommended that all hospitalized immobilized patients be assessed using the Braden scale upon admission as well as during their hospital stay.

Strength of Study

Effective intervention: The intervention module was highly effective in improving nursing knowledge and practice related to PUPM, which can

provide strong evidence for the potential benefits of the intervention and can help inform future practice and policy decisions.

Adequate sample size: The sample size was calculated based on power analysis, which can increase the chances of detecting a true effect if one exists, and can enhance the statistical validity of the study results.

Trained nursing faculty: The data collection and intervention were carried out by trained nursing faculty, which can increase the reliability and consistency of the intervention and data collection process.

Recommendations

Local hospital authorities should consider using the Risk Assessment Tool (Braden Scale) to assess pressure ulcer risk in all hospitalized patients. The use of a validated risk assessment tool like the Braden Scale can help identify patients who are at higher risk of developing pressure ulcers and allow for timely interventions and prevention strategies. This can potentially reduce the incidence of pressure ulcers in hospitals and improve patient outcomes.

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